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Molecular and computational approaches to identification of genes underlying complex traits

A dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy

in

Molecular Pathology

by

Martin L. Jirout

Committee in charge:

Professor Nicholas J. Schork, chair
Professor Mark P. Kamps
Professor Fred Levine
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Professor Daniel T. O'Connor

2008

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Chair

University of California, San Diego

2008

DEDICATION

To my wife Zuzana.

EPIGRAPH

A conclusion is the place where you got tired of thinking.

TABLE OF CONTENTS

Signature Page iii
Dedication iv
Epigraph v
Table of Contents vi
List of Abbreviations xi
List of Figures xii
List of Tables xiv
Acknowledgements xv
Vita xviii
Abstract xx

CHAPTER 1 COMPLEX QUANTITATIVE TRAIT LOCUS ANALYSIS: AN INTRODUCTION AND HISTORICAL PERSPECTIVE 1

INTRODUCTION 2
MENDELIAN vs. COMPLEX TRAITS 2
QUANTITATIVE GENETICS AND STATISTICAL GENOMICS 4
A HISTORICAL PERSPECTIVE 6
THE HERITABILITY OF COMPLEX TRAITS 9
MODEL ORGANISMS AND EXPERIMENTAL CROSSES 10
THE HXB/BXH PANEL OF RAT RECOMBINANT INBRED STRAINS 12
QUANTITATIVE TRAIT LOCI AND QTL MAPPING 13
NOVEL STRATEGIES FOR QTL GENE IDENTIFICATION 15
DISSERTATION OUTLINE 16

CHAPTER 2 GENETIC STRUCTURE OF THE HXB/BXH PANEL OF RI STRAINS 18

PREFACE 19
ABSTRACT 19
INTRODUCTION 20

MATERIAL AND METHODS 23
Animals 23
Markers 23
Genotyping the RI strains for the *Dbh*, *Pnmt* and *Chga* genes 23
Distribution of non-framework markers on the framework map 24
Pairwise correlation matrix 24
Linkage map building with the single nucleotide polymorphism data 25
Other statistical analyses 25
RESULTS 25
A genetic linkage map consisting of 648 non-redundant loci 25
Genome-wide interactions between loci. 26
A SNP-based linkage map 26
An integrated SNP and microsatellite map. 27
Progenitor strain allele distribution in the HXB/BXH RI panel 27
Genetic Similarity Matrix and Allele Sharing 28
Detectability of a QTL depends on the number of RI strains and the magnitude of the QTL effect 29
DISCUSSION 30
Overview 30
New microsatellite and SNP-based linkage maps 31
The genetic structure of the HXB/BXH RI strains 33
Correlated, but physically unlinked loci 34
Power limitations for mapping in the HXB/BXH RI strains 35
Conclusions 35
CHAPTER 3 DELINEATION OF A REGULATORY GENETIC NETWORK CONTROLLING CATECHOLAMINE BIOSYNTHESIS IN THE SPONTANEOUSLY HYPERTENSIVE RAT 43
PREFACE 44
ABSTRACT 44

INTRODUCTION 45
MATERIAL AND METHODS 50
Animals 50
Tissues for gene expression profiling, biochemical profiling, and qRT-PCR validation 50
Gene expression profiling 51
Biochemical profiling 51
Validation of microarray data by qRT-PCR 52
Microarray analysis and pathway annotation 53
Blood pressure measurement 53
Statistical analysis and heritability calculation 54
Normalized Ratio (*NR*) 54
QTL analysis 55
Definition of *cis*- and *trans*-acting eQTLs 55
DNA extraction for re-sequencing 56
Polymorphism discovery at the *Pnmt* and *Dbh* genes in the SHR and BN.*Lx* strains 56
Pyrosequencing 58
Sequence analysis 59
Candidate gene promoter/reporter transfection assay 59
Pnmt alternative splicing assay 61
RESULTS 62
Gene expression differences in hypertension candidate genes 62
Enzymatic activity of Dbh and Pnmt in adrenal medulla 63
Adrenal catecholamine content 63
Correlations among biochemical, physiological and gene expression phenotypes 64
Mapping QTLs for chromaffin cell-expressed genes 65
QTLs for adrenal *Dbh* transcript, Dbh activity and DA concentration cluster on RNO 3p12 66
Genetical genomics analysis of adrenal dopamine concentration 66
Regulation of *Pnmt*, *Chga* and *Vmat1* from the *Pnmt* locus on chromosome 10q31 67
Polymorphism discovery in the *Dbh* and the *Pnmt* genes 68

In-vitro studies on *Dbh* and *Pnmt* promoter polymorphism function 69
Analysis of QTLs previously mapped to genomic regions supporting the *Dbh* and *Pnmt* QTLs 70
Pnmt alternative splicing.. 71
DISCUSSION.. 71
Overview.. 71
Dbh regulation and dopamine concentration in the adrenal gland 72
Regulation of Pnmt activity and gene expression: Chga and Vmat1 co-regulation 75
Conclusions and perspectives 78
ACKNOWLEDGEMENTS.. 79
CHAPTER 4 THE IDENTIFICATION OF *TRANS*-ACTING GENES THAT INFLUENCE THE ABUNDANCE OF MULTIPLE TRANSCRIPTS SIMULTANEOUSLY.. 100
PREFACE... 101
ABSTRACT 101
INTRODUCTION 102
MATERIAL AND METHODS.. 103
RI strains and tissues.. 103
Adrenal tissue gene expression dataset.. 104
Probe set selection for the final dataset.. 104
Statistical analysis and heritability calculation 105
Mapping of eQTLs.. 106
Definition of a *cis*-acting vs. *trans*-acting eQTL 106
Detection of eQTL clusters... 106
Multivariate distance-matrix regression analysis (MDMR) 107
RESULTS 110
The *cis*-eQTL/*trans*-eQTLs ratio depends on the genome-wide threshold 110
Heritability (H^2) of gene expression as a predictor of eQTL effect................. 112

Median heritability of gene expression differs for *cis*- and *trans*-eQTLs 112
Clustering of *cis*- and *trans*-eQTLs 113
Clustering *trans*-eQTLs 113
Testing the *trans*-eQTL clusters by MDMR 114
A *cis*-eQTL cluster on RNO20 117

DISCUSSION 117
Global gene expression 118
Discussion of the pre-mRNA processing genes 120
Discussion of the *cis*-eQTL cluster on RNO20 121
Conclusions and perspectives 122

ACKNOWLEDGEMENTS 123

CHAPTER 5 CONCLUSIONS AND PERSPECTIVES: SIGNIFICANCE OF THE PRESENTED RESULTS AND FUTURE DIRECTIONS 145

SIGNIFICANCE OF THE PRESENTED RESULTS 146
THE FUTURE OF COMPLEX TRAIT ANALYSIS 148

APPENDICES 151

Appendix 1 : Marker strain distribution patterns 152
Appendix 2 : RT-PCR primers 165
Appendix 3 : Primers for re-sequencing in rat *Dbh* 166
Appendix 4 : Primers for re-sequencing in rat *Pnmt*. 168
Appendix 5 : Polymorphism discovery (re-sequencing) in rat *Dbh* 169
Appendix 6 : Polymorphism discovery (re-sequencing) in rat *Pnmt* 207
Appendix 7 : Primers for SNP genotyping at the *Dbh* and *Pnmt* loci 212
Appendix 8 : Primers for the *Dbh* and *Pnmt* promoter/reporter constructs 213

GLOSSARY OF TERMS 214

REFERENCES 222

LIST OF ABBREVIATIONS

Brown Norway rat containing a segment with the *Lx* allele from the PD rat, BN.*Lx*

Diastolic Blood Pressure, DBP

DOPA Decarboxylase, Ddc

Dopamine Beta-Hydroxylase, Dbh

Multivariate Distance Matrix Regression, MDMR

Normalized Ratio, NR

Phenylethanolamine N-Methyltransferase, Pnmt

Quantitative Trait Locus, QTL

Physiological Quantitative Trait Locus, pQTL

Expression Quantitative Trait Locus, eQTL

Quantitative Trait Nucleotide, QTN

Recombinant Inbred Strains, RI Strains

Designation of rat chromosomes (abbreviation of *Rattus Norvegicus*), RNO

Reverse Transcriptase Polymerase Chain Reaction, RT-PCR

RI Strains Derived By Crossing Of The SHR/Ola And BN.*Lx*/Cub, HXB/BXH

Systolic Blood Pressure, SBP

Single Nucleotide Polymorphism, SNP

Spontaneously Hypertensive Rat, SHR

Standard Deviation, SD

Strain Distribution Pattern, SDP

Tyrosine Hydroxylase, Th

LIST OF FIGURES

Figure 1–1 The production of RI strains by repeated sibling mating. 17

Figure 2–1 Linkage map of the rat derived from the HXB/BXH RI strains. 36

Figure 2–2 Genome-wide interactions between loci. 39

Figure 2–3 Progenitor allele distribution among the HXB/BXH RI strains. 40

Figure 2–4 Pair-wise genetic similarity matrix of the HXB/BXH RI strains. 41

Figure 2–5 Ability to detect QTLs as a function of RI panel size and QTL effect magnitude. 42

Figure 3–1 Hypertension candidate genes: heritability of gene expression in the RI strains and differential expression in the SHR vs. BN.*Lx* progenitors. 81

Figure 3–2 Chromaffin cell genes involved in catecholamine biosynthesis, storage, secretion and degradation. 82

Figure 3–3 Adrenal gene expression levels of genes vital for catecholamine biosynthesis and storage: Affymetrix data verified by RT-PCR. 83

Figure 3–4 Enzymatic activity of dopamine-beta hydroxylase (Dbh) and phenylethanolamine N-methyltransferase (Pnmt) in adrenal tissue. 84

Figure 3–5 Concentration of catecholamines in adrenal tissue. 85

Figure 3–6 *Cis*-acting expression quantitative trait locus for *Vamp1* adrenal transcript. 86

Figure 3–7 Co-localization of physiological and expression QTLs on the short arm of chromosome 3. 87

Figure 3–8 Co-localization of physiological and expression QTLs on chromosome 10. 88

Figure 3–9 Single nucleotide polymorphism discovery at the *Dbh* locus in SHR vs. BN.*Lx* strains. 89

Figure 3–10 Sequence polymorphism discovery at the *Pnmt* locus in SHR vs. BN.*Lx* strains. 90

Figure 3–11 Functional studies on the single nucleotide polymorphisms identified in the *Dbh* promoter. 91

Figure 3–12 Functional studies on the single nucleotide polymorphisms identified in the *Pnmt* promoter....92

Figure 4–1 Adrenal transcriptome map....123

Figure 4–2 Heritability of transcript abundance vs. eQTL genetic effect. 125

Figure 4–3 Clustering of *trans*-eQTLs....126

Figure 4–4 Clustering of *cis*-eQTLs on RNO 20....127

Figure 4–5 MDMR analysis of the *trans*-eQTL cluster #1 on RNO 1....128

Figure 4–6 MDMR analysis of the *trans*-eQTL cluster #2 on RNO 3....129

Figure 4–7 MDMR analysis of the *trans*-eQTL cluster #3 on RNO 8....130

Figure 4–8 MDMR analysis of the *trans*-eQTL cluster #4 on RNO 11....131

Figure 4–9 MDMR analysis of the *trans*-eQTL cluster #5 on RNO 17....132

Figure 4–10 A *trans*-eQTL cluster model....133

LIST OF TABLES

Table 3–1 Biochemical phenotypes measured in adrenal tissue homogenate and plasma. 93

Table 3–2 Correlations among biochemical, physiological and gene expression phenotypes. 94

Table 3–3 Expression QTL mapping results for chromaffin cell-expressed genes with heritable transcript levels in the HXB/BXH RI strains. 95

Table 3–4 *Cis*-eQTLs mapped to the short arm of chromosome 3. 96

Table 3–5 Analysis of the *Dbh* region for known physiological QTLs. 97

Table 3–6 Analysis of the *Pnmt* region for known physiological QTLs. 98

Table 4–1 Number of eQTLs detected in the HXB/BXH adrenal tissue espression dataset. 134

Table 4–2 RNO 1 *trans*-eQTL cluster genes. 135

Table 4–3 RNO 3 *trans*-eQTL cluster genes. 136

Table 4–4 RNO 8 *trans*-eQTL cluster genes. 137

Table 4–5 RNO 11 *trans*-eQTL cluster genes. 138

Table 4–6 RNO 17 *trans*-eQTL cluster genes. 139

Table 4–7 RNO 20 *cis*-eQTL cluster genes. 140

Table 4–8 *Cis*-eQTL genes located within the *trans*-eQTL cluster regions used as predictors in the MDMR analysis. 141

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Publications

The results contained in Chapter 3 of this dissertation were presented as a poster (Jirout ML, Friese RS, Mahapatra NR, Mahata M, Mahata SK, Pravenec M, Kren V, Hubner N, Aitman TJ, Ziegler MG, Schork NJ, O'Connor DT. *Independent genetic mechanisms downregulate genes involved in catecholamine biosynthesis, storage and secretion in the spontaneously hypertensive rat.* American Society of Human Genetics, San Diego, CA, October 23-27, 2007), as an oral presentation (Jirout ML, Friese RS, Mahapatra NR, Mahata M, Taupenot L, Mahata SK, Kren V, Zidek V, Fischer J, Maatz H, Ziegler MG, Pravenec M, Hubner N, Aitman TJ, Schork NJ, O'Connor DT. *Discovery of genetic regulatory network controlling catecholamine biosynthesis in the spontaneously hypertensive rat.* Rat Genomics & Models, Cold

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I was the primary author of the results contained in all chapters of this dissertation. The co-authors listed on the above publications facilitated and/or supervised the research that formed the basis for these publications.

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2) Conti LH, Jirout M, Breen L, Vanella JJ, Schork NJ, Printz MP. *Identification of quantitative trait Loci for anxiety and locomotion phenotypes in rat recombinant inbred strains.* Behav Genet 2004;34(1):93-103.

3) Jirout M, Krenova D, Kren V, Breen L, Pravenec M, Schork NJ, Printz MP. *A new framework marker-based linkage map and SDPs for the rat HXB/BXH strain set.* Mamm Genome 2003;14(8):537-46.

4) Printz MP, Jirout M, Jaworski R, Alemayehu A, Kren V. *Genetic models in applied physiology. HXB/BXH rat recombinant inbred strain platform: a newly enhanced tool for cardiovascular, behavioral, and developmental genetics and genomics.* Journal of Applied Physiology 2003;94(6):2510-2522.

5) Jaworski RL, Jirout M, Closson S, Breen L, Flodman PL, Spence MA, Kren V, Krenova D, Pravenec M, Printz MP. *Heart rate and blood pressure quantitative trait loci for the airpuff startle reaction.* Hypertension 2002;39(2 Pt 2):348-52.

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2. Jirout M, Krenova D, Kren V, Printz MP. *New framework marker based genetic linkage map and strain distribution pattern of polymorphic markers for HXB/BXH set of RI strains.* Satellite Symposium on the Genetics of Experimental and Human Hypertension V: From Mendel to Humans , Brno, Czech Republic, June 21-22, 2002.

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2. Jirout ML, Friese RS, Mahapatra NR, Mahata M, Mahata SK, Pravenec M, Kren V, Hubner N, Aitman TJ, Ziegler MG, Schork NJ, O'Connor DT. *Independent genetic mechanisms downregulate genes involved in catecholamine biosynthesis, storage and secretion in the spontaneously hypertensive rat.* American Society of Human Genetics, San Diego, CA, October 23-27, 2007.

3. Jaworski RL, Jirout M, Conti LH, Breen L, Printz MP. *Novel QTL for the startle response to airpuff stimulus in recombinant inbred rat strains.* Experimental Biology 2003, San Diego, CA, April 11-15, 2003 (published in: Faseb Journal 2003;17(4):A494-A494).

4. Jaworski RL, Jirout M, Closson S, Breen L, Printz MP. *A quantitative trait locus for the orienting bradycardia in response to airpuff startle.* 55th Annual Fall Conference and Scientific Meeting of the Council for High Blood Pressure Research, Chicago, IL, September 22-25, 2001(published in: Hypertension 2001;38(3):481-481).

ABSTRACT OF THE DISSERTATION

Molecular and computational approaches to identification of genes underlying complex traits

by

Martin L. Jirout

Doctor of Philosophy in Molecular Pathology

University of California in San Diego, 2008

Professor Nicholas J. Schork, Chair

Understanding the genetic architecture of complex traits is of great interest to the biomedical community. HXB/BXH recombinant inbred (RI) strains, derived from the spontaneously hypertensive rat (SHR) and normotensive Brown Norway (BN.*Lx*), are an important genomic resource for complex trait analysis by means of genetic linkage mapping. The power and accuracy of quantitative trait locus (QTL) analysis critically depends on the quality of the genetic map. To maximize the potential of the HXB/BXH RI strains for complex trait mapping, the latest available genotype information was used to construct a new genetic linkage map. Further, gene expression profiling and biochemical phenotyping in the adrenal glands of the HXB/BXH rats was performed to address the possible link between the dysregulated

catecholamine biosynthesis in the SHR and the development of hypertension. Expression levels and enzyme activities of the two main catecholamine biosynthetic enzymes, Dbh and Pnmt, were found to be regulated from their genic regions (i.e., in *cis*). *Pnmt* re-sequencing revealed promoter polymorphisms, which resulted in a decreased response of the transfected SHR promoter to glucocorticoid stimulation. Dbh activity was negatively correlated with systolic blood pressure in RI strains, and Pnmt activity was negatively correlated with heart rate. These heritable changes in enzyme expression suggest primary genetic mechanisms for regulation of catecholamine action and blood pressure control in the SHR. In a separate analysis, genetic determinants of gene expression in the adrenal gland were explored. The adrenal transcriptome assayed via microarrays was subjected to expression quantitative trait locus (eQTL) mapping. Significant clustering of *trans*-eQTLs was observed, implying that groups of genes are jointly regulated from a single locus. A novel multivariate distance-matrix regression analysis (MDMR) method was applied to identify *cis*-eQTL genes whose expression profiles strongly correlate with those of the *trans*-eQTL cluster genes. The resulting genes, *Rbm16* and *Prp4b*, are involved in pre-mRNA processing and as such present leading candidates for further studies aimed at better understanding of the quantitative genetics of gene expression. In conclusion, an important genomic resource was enhanced and then utilized to identify genetic loci controlling key aspects of catecholamine physiology, and differences in global gene expression.

Chapter 1

Complex Quantitative Trait Locus Analysis:

An Introduction and Historical Perspective

INTRODUCTION

The majority of the genetics and genomics research is focused on the identification of genes involved in human disease. While the genetic basis of a great number of overtly Mendelian, monogenic diseases have been elucidated, finding genes that underlie complex disease phenotypes and using this information to understand their genetic architecture remains a major challenge. Complex diseases, such as hypertension, diabetes, or cancer account for the majority of the health burden in developed countries: thus the identification of the genes underling complex disease phenotypes could have enormous positive biomedical and public health impact. Not only can better diagnostics be developed from a knowledge of which inherited variations contribute to a particular disease, but rational therapeutic strategies can also be devised through the insights obtained into molecular pathways that contribute to complex disease development. In this dissertation, I describe and implement a strategy for identification of genes influencing complex phenotypes using a unique animal model population.

I first describe the historical precedents for genetic studies of complex quantitative phenotypes. This introduction will shed light on the evolution of ideas in the field of genetics and genomics and put into context the experiments I pursued, which are then described in the ensuing chapters.

MENDELIAN vs. COMPLEX TRAITS

Most traits and diseases observed or measured in human and experimental populations are controlled by multiple genetic and non-genetic factors. The final

phenotypic value of these traits is a result of a *complex* interplay of many genes and environmental stimuli, resulting in a continuous distribution of phenotypic values in a population. Such traits are termed *complex traits*. Historically, complex traits were regarded as the opposite of simple or *monogenic traits*, also known as 'Mendelian' traits.

Typical Mendelian trait has a categorical distribution in a population and follows a characteristic pattern of inheritance (dominant, recessive, X-linked). More than 1800 Mendelian disorders have been identified in human so far, most of them quite rare, nevertheless causing a severe disruption of normal function[1].

The very basic nature of the molecular genetic architecture of a Mendelian trait (i.e., one affected gene → one affected protein → simple pathophysiologic mechanism → disease) makes it more amenable to identification of the causative gene via relatively simple statistical and molecular approaches. This fact, fueled by the hope that one may be able to find treatments and implement preventative measures upon discovering the genetic basis of a disease, led many researchers to focus on the genetic dissection of Mendelian traits, in spite of the reality, that monogenic diseases are comparatively infrequent and affect only a small fraction of the world's population. In fact, virtually all common contemporary diseases contributing the greatest burden to public health, such as hypertension, diabetes, and cancer, fall into the category of complex, multifactorial traits[2]. Nevertheless, the successes and lessons learned from the study of Mendelian traits contributed significantly to our understanding of the

genetic basis of disease in general inspired researchers to consider the genetic architecture of more complex traits[1,3-5].

Mendelian and complex traits are no longer regarded as fundamental opposites, but rather as two ends of a continuum ranging from overtly monogenic, through oligogenic (i.e., involving a few genes) to polygenic (i.e., involving a great many genes), each possibly influenced by additional environmental and gene-gene interaction components[6,7]. Interestingly, the statistical apparatus used to describe and analyze simple monogenic and complex traits is not different in principle since it is largely based on extensions of simple correlation and regression techniques. The identification and characterization of genes that influence polygenic, quantitative traits using statistical analysis strategies forms the basis for the discipline of *quantitative genetics*[8].

QUANTITATIVE GENETICS AND STATISTICAL GENOMICS

Quantitative genetics is a branch of genetics research that employs statistical and probabilistic principles to identify the genetic and non-genetic components of a complex trait and to describe them in statistical terms[8,9]. It is an extension of simple Mendelian principles to complex traits. Quantitative genetics assumes that complex traits result from the combined effect of multiple underlying genes, each segregating according to Mendel's laws and being affected to varying degrees by the environment, thus resulting in a continuous distribution of phenotypic values.

Statistical genomics is a relatively new research discipline that considers the evaluation of entire genomes in order to identify and characterize genetic factors that

influence a particular trait. Statistical genomics integrates moderns statistical and bioinformatics techniques with more traditional Mendelian, quantitative, population and molecular genetics techniques. One ancillary goal of statistical genomics is to learn about the structure, function and evolution of genomes as these factors influence phenotypic variation. However, the central issue in statistical genomics is to find regions of the genome, or loci, which control the phenotypic value of a particular trait. These loci are termed *quantitative trait loci* or QTLs. The theory and methods for identifying QTLs in experimental as well as natural populations include single locus analysis, multi-point (interval) analysis, composite interval mapping and other more advanced techniques, such as multiple interval mapping[10]. In order to identify loci that influence phenotypes, statistical genomics researchers must also deal with problems such as, e.g. linkage grouping, genetic marker ordering, and linkage map construction, linkage map merging, screening genetic markers for association with a disease and searching for genes using linkage disequilibrium[11]. Other statistical approaches include resampling techniques, such as permutation analysis and bootstrapping, and simulation of segregating populations.

In summary, quantitative genetics and statistical genomics provide a set of methods and tools to identify, characterize, and quantify relationships between observed phenotypes and the underlying genotype. The general presumption for quantitative traits is that the observed continuous distribution of phenotypic values is a result of a combined effect of many underlying genes and the environment.

A HISTORICAL PERSPECTIVE

The science of *quantitative genetics* has been around since the early 20th century. It developed in response to a major controversy between two schools of thought about the mechanisms by which evolutionary changes occur, and by which phenotypic traits are inherited, while variation in the population is maintained. The contrast between the obvious need of a species to adapt in order to achieve the best fit to its environment on the one hand, and the concept of passing an invariant trait from a parent to an offspring on the other, presented a problem that took several decades to reconcile and which ultimately resulted in a synthesis called the *modern quantitative genetic theory* [8,12].

The growing popularity of Darwin's theory of evolution[13], which put forward the notion of natural selection as the main variation-shaping force, resulted in the founding of the *Biometrical school of though*t (F. Galton, K. Pearson, W.F.R. Weldon), which viewed evolution of the species as a result of a gradual changes coupled with natural selection (i.e., *gradualism*). The Biometricians focused on statistical studies of phenotypic variation. Quantitative methods developed by this school out of the need to describe and analyze continuously distributed characters laid the foundations for a great deal of modern statistical theory. However, the biometricians provided no real explanation for the transmission of hereditary traits from parent to offspring.

The rediscovery of Mendel's work[14] offered such explanation, but was initially interpreted in support of evolution by sudden leaps (i.e., *saltationism*) and in direct

contrast to Darwin's gradualism. The *Mendelian school* (W. Bateson, C.B. Davenport) believed that evolution was driven by the appearance of new mutations with large effects (macromutations), thus giving rise to variation in discrete characters. Mendel's experiments with peas[15] revealed that such characters were transmitted in a mathematically predictable manner. His laws of independent segregation and assortment of heritable 'factors' went on to become key concepts in the development of genetics. T.H. Morgan was the first to apply Mendel's laws to biology and inquire about the ultimate biological nature of the Mendelian 'factors'[16,17]. Based on breeding experiments in the fruit fly, his student A. Sturtevant constructed the first genetic map of a chromosome[18] and thus paved the way for *linkage analysis*, which subsequently became one of the most important tools of quantitative genetics. In the interim, in order to clarify the distinction between the genetic factors and the actual observed value of a trait for an individual, terms like *gene*, *genotype* and *phenotype* were coined[19].

Gradually it became apparent that the observations of biometry can be interpreted in accordance with Mendel's laws by means of a rigorous statistical approach[20]. The debate further led to the emergence of the *multiple-factor hypothesis*, which postulated that the variation exhibited by continuous characters can be maintained by the independent segregation of multiple factors (i.e. genes). By the 1930s geneticists and statisticians largely resolved their differences and the *modern theory of evolution of Mendelian systems* was produced[21]. The mechanism that produces evolutionary change and phenotypic variation is now believed to be a

combination of natural selection and gradual change of allele frequencies in the population (genetic drift), simultaneously acting upon genetic variation, as created by recombination, mutation and gene flow. This modern synthesis thus introduced a connection between two important concepts: *genes as units of evolution* (which can be recombined and mutated) and *selection as the mechanism of evolution*.

The next big step to understanding the complexity of genome function was the finding that DNA is the material harboring heritable factors[22], followed by the discovery of the DNA structure and its genetic implications[23]. Another vital piece to the puzzle was added when the nature of the genetic code became understood[24]. The subsequent rapid technological advances in molecular biology led to the identification of genomic features that could be used as markers, such as genes, repeats, RFLPs, SNPs, etc. Such markers were essential for the construction of genetic maps of model organisms[25], and these in turn allowed for high resolution linkage mapping[26]. Linkage analysis proved an indispensable tool for genomic investigation, especially for annotating the genome with information on physiological function and disease[27].

The emergence of the whole genome sequencing capability permanently changed the landscape by providing the ultimate navigation tool to genomes[28-31] and heralded the post-genome era whose chief goal no longer is the *molecular anatomy* of the genome itself but rather how the genome influences *molecular physiology* and *molecular pathology*.

In the current era, researchers are at a unique juncture in the history of biological and medical sciences. Researchers have at their disposal a vast collection of

data on the genomic structure of individual species. In addition, insights into how genomes function are continually being produced. Using microarrays and similar high-throughput techniques, we will continue to accumulate enormous amount of data on transcription, translation, glycosylation, etc. in different tissues, across species, and both in health and disease.

Statistics and genetics (and later genomics) are in many ways branches of the same intellectual pedigree, since the level of mutual influence of the two disciplines has been considerable. Problems first identified by the early population geneticists accelerated the development of statistics as an independent discipline, which in return never ceased to be one of the important tools for understanding genetic relationships and genomic functions.

THE HERITABILITY OF COMPLEX TRAITS

The extent to which genotype determine phenotype is expressed by the degree of genetic determination, or *heritability* (H^2). Heritability is an important quality to be ascertained for a complex trait whose genetic underpinnings we wish to study, since it is rational to look for genes for just those traits that are in fact genetically determined.

The genetics of complex traits is based on the study of their *variation* in a population, with respect to the degree of relatedness of the studied individuals. The amount of *variation* is measured and expressed as the *variance* (the mean of the squared deviations from the population mean). The extent of phenotypic variation (phenotypic variance, V_P) can be view as a result of the genetic differences (genotypic variance, V_G) and differences in the environment (environmental variance, V_E):

$$V_P = V_G + V_E \tag{1}$$

Genotypic variance can be subdivided into additive genetic effect (V_A), dominant genetic effect (V_D) and interaction effect (V_I). Hence

$$V_G = V_A + V_D + V_I \tag{2}$$

Heritability is the extent to which the genotypes determine phenotypes, or

$$H^2 = V_G / V_P \tag{3}$$

This means in practical terms that if the genetic determination of a trait is large, closely related individuals will display trait values of similar magnitude.

Heritability is also likely to be affected by the place, which the measured quantitative trait occupies in the hierarchy of *gene* → *message* → *protein (intermediate phenotype)* → *pathway* → *end phenotype*. The lower in the hierarchy, i.e. closer to the DNA, the (molecular) trait lies, the more heritable it is likely to be. Exploration of the genetic determinants of gene expression and intermediate (biochemical) phenotypes has become an important approach to complex trait elucidation[32,33].

MODEL ORGANISMS AND EXPERIMENTAL CROSSES

Model organisms have been utilized for over a century to understand biological processes[34]. Inbred strains of rodents, especially mouse and rat, have been used to study the contribution of genes in the pathogenesis of the disease process[35]. This strategy is based on the notion of common descent of all living organisms and the conservation of metabolic, signaling and developmental pathways, with the caveat that phenotypic correlates may reflect different pathogenetic processes.

The mouse and rat model systems have several advantages over higher mammals, including lower maintenance cost, shorter generation time, existence of numerous genetically well-defined lines that differ in phenotypic characteristics and, especially compared to human, fewer ethical constraints with respect to breeding and experimentation[36]. With the availability of the human, mouse and rat genome sequences and the resulting identification of the homologous regions between species, it is now possible to translate results from one species to another, using comparative genomics strategies.

A good example involves the rodent models of essential hypertension. More than 30 years ago, researchers began selectively breeding rats to produce models with high, normal and low blood pressures. Many hypertensive rat strains have been produced ranging from the strains such as the spontaneously hypertensive rat (SHR) and stroke-prone spontaneously hypertensive rat (SHRSP) to strains where high dietary salt is necessary to induce hypertension such as the Dahl salt-sensitive. These models also exhibit end-organ damage phenotypes similar to those seen in human essential hypertension, including left ventricular hypertrophy, stroke and renal failure. Experimental models of hypertension allowed for investigation of factors associated with regulation of blood pressure and genetic basis of hypertension, as well as for development of targeted interventions aimed at decreasing not only blood pressure but also target organ injury[37].

The disease models are, of course, only approximations of pathologic states in humans; therefore results obtained from animal studies need to be cautiously evaluated

before comparisons are drawn towards the human. Nonetheless, the phenotypic contrast of the different inbred strains can be exploited by developing genetic crosses – segregating populations – in which the co-segregation of phenotypes and genotypes can be studied and analyzed (see below). There are several principal types of populations used for genetic mapping: recombinant inbred (RI) strains, F_2 intercross, backcross, congenic strains[38], chromosome substitution strains[39], advanced intercross[40], heterogeneous stock[41], etc. These experimental populations of mouse and rat facilitate a more profound understanding of the role of specific genes in the determination of both monogenic and multifactorial traits, and allow us to study the genotype-phenotype relationship in ways that are, for a wide variety of reasons, difficult to apply in humans.

The genetic dissection of complex traits still remains one of the most important challenges facing science and medicine today. Over the last 20 years, segregating populations derived from established inbred strains of the mouse or rat have become the main tools for pursuing this avenue of research.

THE HXB/BXH PANEL OF RAT RECOMBINANT INBRED STRAINS

One of the most widely used sets of model organisms derived from a cross is the HXB/BXH rat recombinant inbred (RI) strain panel, which is the focus of the work presented in this dissertation. These strains were produced as a model system for genetic and correlation analysis of spontaneous hypertension and other risk factors for cardiovascular disease[42]. The HXB/BXH recombinant inbred strains represent an

inbred, genetically fixed, random breeding F2 progeny, derived from F1 progeny, which in turn was obtained through gender-reciprocal crossing of the two progenitors – the spontaneously hypertensive rats (SHR/OlaIpcv) and normotensive Brown Norway (BN.*Lx*/Cub) rats (Figure 1–1). Of the surviving 31 RI strains, 29 strains are utilized in the presented work. The HXB set of RI strains, denoted as 1H, 3H, etc., originates from crossing SHR female and BN.*Lx* male progenitors, while the BXH set denoted as 2B, 3B, etc., originates from crossing BN.*Lx* females and SHR males. The original sets of RI strains are maintained at Charles University (BXH), the Czech Academy of Science (HXB).

QUANTITATIVE TRAIT LOCI AND QTL MAPPING

Quantitative trait loci (QTLs) are genomic segments that harbor genes controlling the variation of a quantitative trait in a population. QTL mapping seeks to identify these genomic segments. With the sequence of many organisms nearly complete, QTL mapping turned into a powerful way in which the known genomic sequence can be annotated with information that relates to complex biological processes[27].

Complex traits are controlled by multiple QTLs, contributing to the trait's heritability in an uneven fashion. The notion of the genetic architecture of complex traits is changing along with the increasing power to detect QTLs with very small effects. The dilemma is to decide whether heritability arises from a very large number of QTLs with a very small effect or from a few QTLs with a modest effect. Because of

the inability to detect small effect QTLs in the past, researchers were more likely to detect large effect QTLs, giving them more prominence then they deserved.

A recent study showed that most QTLs explain between 1% and 5% of the phenotypic variance[41]. This may be surprising, but it seems to be consistent with the model of exponential distribution of allelic effects, which proposes many small effect QTLs alongside a few QTLs with respectively increasing effect[43].

There are two major approaches to finding complex trait-controlling genes: *linkage analysis* and *association studies*. Linkage and association can provide complementary information. Association studies examine relationships between alleles and phenotypes in a population of unrelated individuals, whereas linkage analysis is used to scrutinize relationships between loci and phenotypes in a pedigree of individuals with known degree of relatedness. Linkage analysis provides an unbiased and comprehensive search across the whole genome. It requires fewer markers (several hundreds to a few thousand), for which it examines their co-segregation with the studied trait (or disease phenotype). By contrast, association studies require that the marker and the disease gene not merely be linked across a few generations, but they must be in linkage disequilibrium, i.e. inherited together throughout the population despite the many generations that have elapsed from the common ancestor. Because the chromosomal segments exhibiting linkage disequilibrium in weakly and possibly arbitrarily related individuals are much smaller, hundreds of thousand of markers will be required for a whole genome scan involving

such population. It is therefore beneficial when association studies can focus on predetermined candidate regions, e.g. QTL interval resulting from a linkage study.

The HXB/BXH panel of rat recombinant inbred strains lends itself particularly well to linkage analysis. However, there are some limitations as to the power and precision, with which a trait can be mapped even using the finest mapping techniques; a QTL usually harbors about a hundred genes, which have to be further investigated. Some novel strategies can be brought to bear on the problem of causative gene identification.

NOVEL STRATEGIES FOR QTL GENE IDENTIFICATION

The advances in QTL mapping made possible by the development of high resolution maps and the availability of the genomic sequence shifted the focus from the identification of a QTL-bearing chromosomal segments to the problem of narrowing a QTL interval down to a single gene (i.e., quantitative trait gene, QTG), or even a single nucleotide polymorphism (i.e., quantitative trait nucleotide, QTN). Several strategies for mapping and cloning the quantitative trait genes have been put forth and implemented in the recent years[44]. Of those, gene expression profiling coupled with linkage analysis is especially of relevance to the present work.

Gene expression profiling coupled with linkage analysis combines linkage mapping to locate a QTL for a physiological trait (pQTL), with subsequent expression QTL (eQTL) mapping for expression profiles of genes located within the pQTL support interval. This strategy has been termed *genetical genomics* in the literature[45]. This information provides an insight into the contribution of each gene present in the

pQTL to the examined physiological phenotype[46]. Expression QTL mapping can thus facilitate the problem of sifting through a large number of genes in search for the best candidates to be tested in subsequent validation experiments.

DISSERTATION OUTLINE

As the title of the dissertation suggests, I have pursued a series of experiments and studies aiming at identification of genes underlying complex traits in in a model population – the HXB/BXH recombinant inbred strains – by means of a wide spectrum of molecular and computational methods.

Chapter 2 describes the genetic structure of the HXB/BXH RI strains and explores the limitations to map quantitative trait loci in this population as a starting point for all subsequent genetic analyses.

Chapter 3 deals with mapping of genetic determinants for the regulation catecholamine biosynthesis in the chromaffin cell of the adrenal gland, combining transcriptional profiling and biochemical phenotyping with traditional QTL mapping techniques to discern underlying regulatory mechanisms.

Chapter 4 deals with the genetics of gene expression on a whole-genome scale, as opposed to the pathway-focused approach presented in Chapter 3, with the aim to characterize the heritable properties of the adrenal transcriptome and to identify loci influencing transcript levels of multiple genes (i.e. the “master regulators”).

Chapter 5 provides a review of the results presented here, discusses the limitations of the current approaches to quantitative trait dissection and comments on future directions relevant research can take.

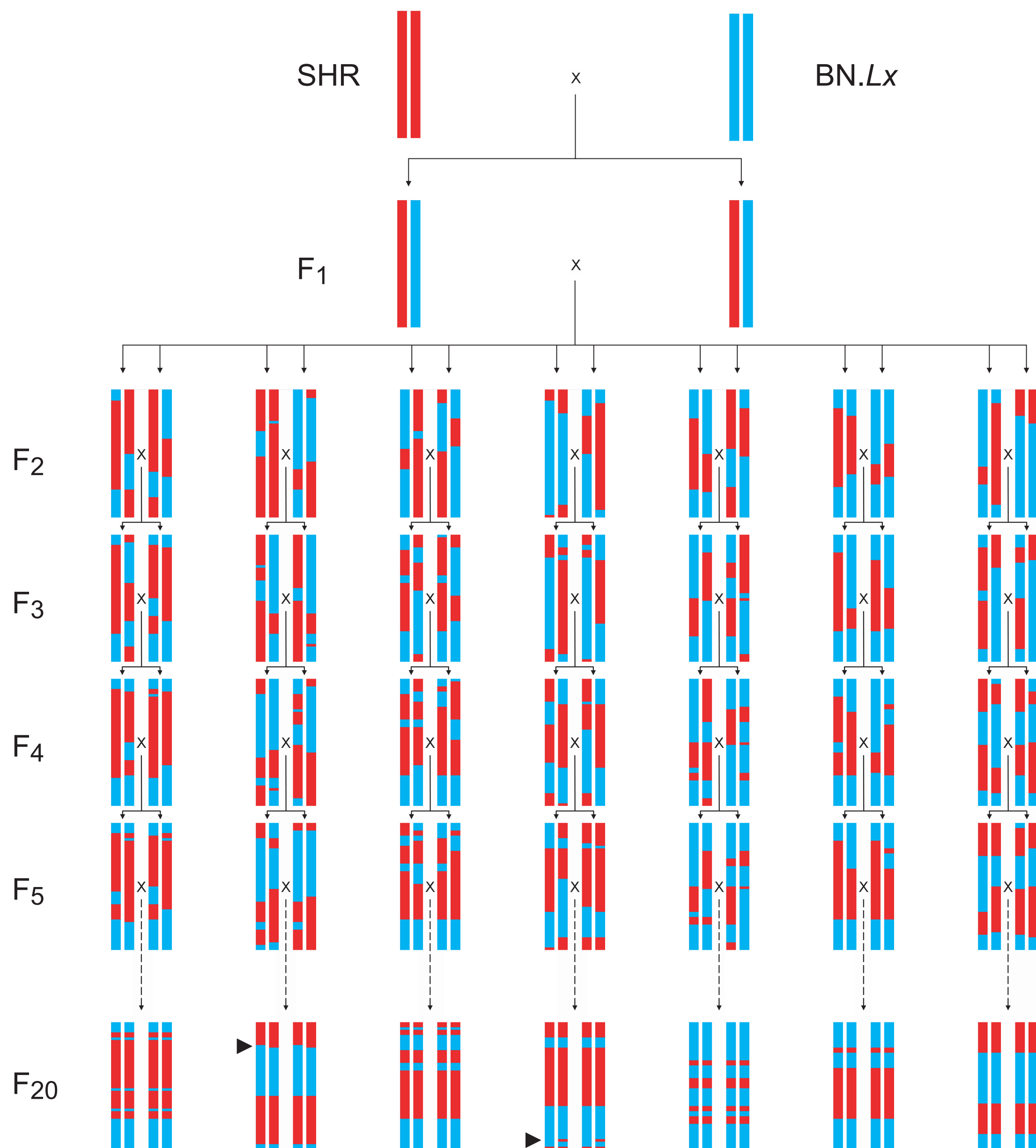


Figure 1-1 The production of RI strains by repeated sibling mating. Crossing two inbred strains is followed by repeated sibling mating to produce a panel of inbred strains whose genome is a mosaic of the two parental genomes. This figure is the result of a computer simulation, which considered fifty segregating loci over twenty generations of brother-sister mating. Note that some residual heterozygosity can still be found in F_{20} (black arrowheads), which is representative of the in vivo situation, because complete homogenization of the RI strain genomes is reached only asymptotically.

Chapter 2

Genetic structure of the HXB/BXH panel of RI strains

PREFACE

This chapter builds on my earlier work[47] on the construction of a framework-marker based map of the rat HXB/BXH set of RI strains. The following chapter deals with additional development of the microsatellite-based maps and also includes recently genotyped single nucleotide polymorphisms in order to comprehensively characterize the HXB/BXH genomic resource.

ABSTRACT

Comprehensive characterization of the genetic structure of a mapping population is a prerequisite for successful detection and localization of quantitative trait loci. A genetic linkage map of the HXB/BXH set of RI strains and a description of the genetic structure of this model population is presented here. First, a framework map was developed, which was subsequently used to integrate all available markers into a contiguous genetic linkage map. The resulting map consists of 648 non-redundant loci. Second, the marker order was tested by building a pairwise correlation matrix of the markers. A clear diagonal line of identity is present, which validates the marker order in the map. Detailed scrutiny of the correlation matrix also revealed several highly correlated, but physically unlinked genomic regions. This finding is important for interpretation of QTL results, as it may provide insight into potential false positive linkages or allele effect studies. Further, in an effort better to describe the genetic structure of the HXB/BXH RI strains, a set of single nucleotide polymorphism data was also considered. This data permitted further genetic characterization, including strain independence and genetic similarity among the

individual strains. Of the total of 3234 SNPs available, 804 SNPs were informative (non-redundant with respect to the adjacent loci) and represent the number of unique recombination points throughout the whole RI set. This number of recombination events is comparable to the one detected in the analysis of the microsatellite markers.

INTRODUCTION

The power and resolution of QTL mapping in RI strains by means of linkage analysis depends on the number of RI strains used and on the density of genetic markers for which the RI set has been genotyped. Marker loci displaying polymorphism in the progenitor strains, when analyzed across the whole RI strain set, provide information on the distribution of the parental alleles in the RI strains. Analysis of single locus in this manner provides insight into the *strain distribution pattern* (SDP) for individual loci. Examination of the degree of concordance of SDPs of adjacent marker loci is the basis for ascertaining potential interlocus linkage and detection of potential recombination events. The proportion of discordant alleles between two adjacent marker SDPs can easily be converted to centimorgan distance, using a mapping function, such as Haldane's or Kosambi's functions[10]. The distance within which linkage can be detected on either side of a marker with a probability of 95%, known as the *swept radius*, increases along with the number of RI strains. It has been estimated that for a population the size of the HXB/BXH set (originally 36 strains) the swept radius equals to ~ 8 cM. This number is critical for the construction of genetic linkage framework maps, because maps with intervals larger than the swept radius are not likely to be contiguous and will not capture all recombination events.

By examining the SDPs and linkages among three or more loci, their relative chromosomal order can be determined by rearranging them so as to minimize the total number of recombinations within the whole linkage group. The order that minimizes the number of putative recombination events can then be taken as the most likely order of the markers. By working with SDPs and groups of markers in this manner, one can develop an optimal (at least with respect to the number of observed recombination events) map of markers across the entire genome. However, trying to resolve the order of loci solely by statistical means can prove challenging, especially when resolving the order of loci within and across very small distances, or over distances greater than the swept radius.

The original characterization of the HXB/BXH RI strain marker locus map was gradually developed and refined over several years in the form of SDP analyses of polymorphic mini- and microsatellite markers and RFLPs [42,48,49]. Despite this effort, a contiguous linkage map was lacking and the genetic structure of the set was only partially defined. This deficiency compromised linkage studies and hindered the power and precision of more sophisticated QTL mapping studies.

In response to this insufficiency, we reported in an earlier work the construction of a new framework marker-based linkage map and SDPs for the HXB/BXH RI strain panel[47]. To avoid the ambiguities in the placement of certain markers we decided to use an F2 based integrated genetic and radiation hybrid map [50] as a reference from which we could arrange more recently genotyped markers manually by using marker positions from this high density radiation hybrid physical

map. The marker order in the resulting framework map was then verified computationally using the statistical strategies discussed. The original map was constructed using DNA from all original 36 RI strains, although five were by that time extinct, but nevertheless their DNA contributed valuable information about recombination points. The resulting framework map in this initial analysis consisted of 245 microsatellite markers contiguously covering the whole genome, with an average between-marker distance of ~ 8 cM.

In this work I take advantage of the original framework marker-based map for the construction of a higher density map, which also encompasses the majority of previously genotyped markers[42]. Integrating these markers into a larger and more comprehensive map not only increases QTL mapping precision, but also allows for a more precise positioning of the older markers, some of which are no longer included in the current rat genome resources.

Further, I compared the above microsatellite-based map with a dataset of SNP-based SDPs, which was recently completed[51] and made available by Dr. Norbert Hübner (Max Delbrück Center, Berlin). This comparison of genetic and physical maps allows for additional validation of the locus positions in the genetic map. Comprehensive analysis of the SNP dataset led to several important observations about the utility of the HXB/BXH RI set as well as its limitations. Integration of the microsatellite markers, for which the precise genomic position (Mbp) is known, with the non-redundant SNP markers, yields the most comprehensive information on the genetic structure of the HXB/BXH RI strains available to date.

MATERIAL AND METHODS

Animals

HXB/BXH recombinant inbred (RI) strains were produced by inbreeding between F2 generation resulting from a sex-reciprocal cross of two highly inbred strains: BN (BN.*Lx*/Cub) and SHR (SHR/Ola)[42,52]. In this study, 29 RI strains were used. For background information on the HXB/BXH RI strains see Chapter 1, page 12.

Markers

For the present analyses, marker information derived from 29 HXB/BXH RI strains typed for 1061 microsatellite markers was used. This genotype information was generated previously[42,47-49]. No additional genotyping for microsatellite markers was necessary. New SNP genotype data consisting of 3234 SNPs typed in 29 strains[51] were utilized for additional analyses.

Genotyping the RI strains for the *Dbh*, *Pnmt* and *Chga* genes

Precise positioning of *Dbh*, *Pnmt* and *Chga* genes on the linkage map was important for the studies described in Chapter 3. In order to accomplish that, polymorphism discovery was conducted (see Chapter 3, page56). The progenitor strains (BN vs. SHR) were found to exhibit single nucleotide polymorphisms in *Dbh* (T−551G) and *Pnmt* (T−529C), and a simple repeat polymorphism in *Chga* (oligo-Gln repeat in exon 5, 287-331bp, BN.*Lx*:15 vs. SHR:8 repeats; data not shown). Using these polymorphisms as markers, alleles were typed by pyrosequencing for *Dbh* and

Pnmt (see Chapter 3, page 58) and by polyacrylamide gel electrophoresis for *Chga* (data not shown) in each RI strain to ascertain the SDPs for the respective genes.

Distribution of non-framework markers on the framework map

Previously genotyped markers[42,48,49] were integrated into the HXB/BXH framework map[47] using the Map Manager QTX software[53]. Four subsequent rounds of searches for the best position match for the non-framework markers were performed, each with decreasing stringency (probability of false positive linkage), relative to the previous step, from $P = 10^{-6}$ to $P = 10^{-3}$. In this manner, first markers to be placed were those showing the strongest linkage to the HXB/BXH framework. During the following 3 steps the remaining markers were tested for linkage and placed within the framework map, gradually relaxing the stringency criterion. Finally, no additional markers could be placed with probability of false positive linkage lesser that $P = 10^{-3}$. The resulting map was then examined for redundancy, and adjacent markers with the same SDPs were eliminated as non-informative. Chromosomal ideograms were generated with the Windows QTL Cartographer computer program[54].

Pairwise correlation matrix

648 marker SDPs were subjected to correlation analysis using MatLab 7.0 (The MathWorks, Inc., 2004) to compute the correlation coefficient for each marker SDP pair. The results were plotted as a 2-dimensional matrix. Each axis represents linearized rat genome, with the 648 markers ordered with respect to their position in the genetic map.

Linkage map building with the single nucleotide polymorphism data

3234 SNPs typed in 29 RI strains[51] were ordered according to their Megabase (Mbp) position in the genome. Adjacent markers with identical SDPs were eliminated as non-informative. Data were handled with the Map Manager QTX software[53].

Other statistical analyses

Statistical calculations (e.g., correlation analyses) were performed in MatLab 7.0 (The MathWorks, Inc.), MS Excel 2003 (Microsoft Inc.) and SPSS 10.0 (SPSS Inc.).

RESULTS

A genetic linkage map consisting of 648 non-redundant loci.

The distribution of ~ 800 previously genotyped markers onto the HXB/BXH framework map of 245 markers, and subsequent elimination of non-informative markers, yielded a linkage map consisting of 648 distinct markers (Figure 2–1). Positions of the *Dbh*, *Pnmt* and *Chga* genes, which were added to the map explicitly to facilitate analyses described in Chapter 3, are highlighted in yellow. The map spans 2365 cM over the 20 rat autosomal chromosomes, with an average between-marker interval of ~3.7 cM. Due to insufficient marker data, the sex chromosomes were not included in the final map. This new map provides a dense and contiguous coverage of the rat genome, representing a great enhancement of the QTL detection power over the previously available linkage maps for the HXB/BXH RI strain set. All genotypes used in this map are included in Appendix 1.

Genome-wide interactions between loci.

A correlation matrix (genetic map validation matrix) for the HXB/BXH RI strain set was generated and represented as a heatmap (Figure 2–2). The matrix involves the SDPs for each of the 648 markers used in the above described the linkage map. The predominance of high level of positive correlation (red) along diagonal line (i.e., the line of identity) is clearly evident and constitutes a dominant feature of the matrix, thus validating the marker order in the integrated map. There are, however, also several other regions in the matrix that show a high positive (red) and high negative (blue) correlation between physically remote marker SDPs. This may be attributable to the modest number of strains in the HXB/BXH RI panel and the chance associations between marker SDPs that results from the small sample size. Another explanation could be that the linked genomic regions harbor alleles that are functionally coupled and their co-segregation is vital to the strain survival.

A SNP-based linkage map

The total of 3234 SNP SDPs present in the SNP dataset[51] were ordered according to the physical (Mbp) genomic location. After collapsing adjacent SNPs with perfectly concordant SDPs into one locus, a new subset containing 804 informative, non-redundant loci was produced. A 3-fold increase in coverage density (3234 SNPs vs. 1061 microsatellites) yielded about 25% boost in identification of unequivocal recombination points (804 SNPs vs. 648 microsatellites).

An integrated SNP and microsatellite map.

In another attempt to recover as much information about genetic recombination points in the HXB/BXH RI set, as possible, 347 microsatellites (of the set of 1061), for which the genomic (Mbp) location of was known, were merged with the 3234 SNP SDPs. Again, markers were ordered according to their physical genomic location and redundant loci eliminated. The resulting merged map file consists of 1047 SNP and microsatellite markers and is at present the most comprehensive map available for the HXB/BXH RI strains. However, bearing in mind that the markers in the merged file come from different sources and were generated at different time in different laboratories, using different methodologies, it is quite possible that some mapped recombination point are due to error.

Progenitor strain allele distribution in the HXB/BXH RI panel

To compute the proportion of progenitor genomes in the individual strains, the dataset consisting of 3571 merged SNPs and microsatellites, including the redundant loci were used. Using only the non-redundant SDPs to calculate the estimates of progenitor strains alleles among the RIs, would be correct only if recombinations were distributed evenly and randomly. Otherwise, there would be a risk of overrepresentation of the regions, where recombination occurs, and consequently underrepresentation of regions that may contain longer stretches of progenitor DNA without recombination. Therefore, the computation was based on the known physical positions of all markers. Distances spanned between same genotypes were counted as such; distances spanned between different genotypes (recombination points) were split

between the two. The totals for SHR and BN.*Lx* progenitor alleles were than compared for each strain and percentages calculated.

The distribution of SHR and BN.*Lx* alleles among the individual RI strains can be rendered as a “genetic mosaic” (Figure 2–3), which offers a practical visualization of the genetic structure of the HXB/BXH RI set. In theory, each strain should receive an equal amount of its genome from each of the progenitor strains. We found that the HXB/BXH genomes consist, on average (± SD), of 51% (± 7) of SHR and 49% (± 7) of BN.*Lx* alleles. However, the fraction of SHR and BN.*Lx* genomes transmitted to the individual RI strains varies continuously from about one-third to two-thirds (Figure 2–4, bottom two rows). This information may be useful for analyses in which the influence of an overall genetic background is assessed. Genetic background information, as proxy for the combined effect of multiple small effect QTLs, can be included as a predictor of phenotype in multiple regression analyses, along with marker SPDs[55].

Genetic Similarity Matrix and Allele Sharing

The merged map file with all 3581 SNP and microsatellite markers was used to compute the amount of genome the RI strains share among each other. Marker information was used to determine regions of same parental origin (bins), essentially genome segments between recombination points, and the amount of genome sharing was then computed based on the length of these segments, rather than merely on the marker genotypic information, to account for the non-uniform distribution of markers. A pair-wise similarity matrix was computed (Figure 2–4). The 29 RI strains can be

paired up in 406 different ways. The similarity matrix shows the degree of sharing for each such pair. The HXB21 and HXB22 display the lowest degree of allele sharing (33%), whereas HXB3 and HXB15 exhibit the highest degree of genome similarity (87%). It is interesting to compare these extremes with an estimate computed with the framework markers only [47]. The same pairs of strains shared 38 and 78%, respectively. It appears that by using more markers and by counting distances between markers, rather than markers, the extremes gained even more prominence. However, the average amount of allele sharing among the RI strains remained the same (Figure 2–4, histogram in the inset). The average (± SD) allele sharing among the RI strains is 50.5% (± 6.1).

Detectability of a QTL depends on the number of RI strains and the magnitude of the QTL effect

The power to detect a locus in QTL mapping contexts using the HXB/BXH RI strains was assessed. Figure 2–5 presents the results and exposes some limitations of the HXB/BXH RI set, having to do with its relatively small strain number. The relationship between the number of strains (n) and the detectable QTL effect size (V_{QTL}) is given by Equation (4)[44,56]. The genome-wide significance threshold (α) and power ($1-\beta$) are represented as Z-scores, or cutoff points for α and $1-\beta$ in the standard normal cumulative distribution.

$$n = \frac{(1 - V_{QTL})(Z_{1-\alpha/2} + Z_{1-\beta})^2}{V_{QTL}} \qquad (4)$$

To obtain the genome-wide significance threshold, the single-locus threshold needs to be divided by the number of loci to correct for multiple comparisons. For ~ 1000 markers and a local $\alpha = 0.05$, the genome wide $\alpha = 0.0005$[57]. If the power is set to $(1-\beta) = 0.8$, and Equation 4 solved for n = 29 (the number of HXB/BXH RI strains), it turns out that for a QTL to be detected in the HXB/BXH RI set, the proportion of phenotypic variance attributable to that QTL needs to be more than 40%. The implication of this result is that, if all due statistical stringency is applied, the HXB/BXH RI set is suitable chiefly for detection of QTLs with larger effect.

DISCUSSION

Overview

Recombinant inbred strains are one of the best resources for genetic mapping[44,58]. Once established, an RI strain set is a renewable resource consisting of strains with genetically fixed genomes. Determining the parental origin of alleles at as many loci as possible in each of the RI strains, and defining the genetic structure of the whole RI panel, is critical to the ability to correlate phenotypes with genotypes and thus to detect and localize quantitative trait loci.

The HXB/BXH RI set has a special place among the genomics resources as the largest *rat* RI strain set produced to date. Originally created as a model system for analysis of spontaneous hypertension and other cardiovascular disease risk factors[59-62], it has also been instrumental for mapping a variety of other traits, such as morphological, reproductive, metabolic and behavioral phenotypes [63-68]. The new

maps and strain analyses presented here will enhance the use of the rat HXB/BXH RI strains and permit utilization of their full potential.

There are two major determinants of the ability to detect QTLs in an RI population: marker density and RI strain number[56]. The present study focuses on making the most of the genotypic data accumulated thus far by means of correct marker positioning and redistributing to obtain high density maps.

New microsatellite and SNP-based linkage maps

To improve the utility of the HXB/BXH RI strains for complex trait analysis and to provide a reference a tool to locate older markers to which some formerly published QTLs were linked, the available microsatellites were redistributed over the previously reported framework map[47], yielding a linkage map with 648 unique SDPs. In a separate analysis, attempting to position the SDPs with the best attainable accuracy, the newly obtained single nucleotide polymorphism data were merged with the microsatellite data, for which the exact physical (Mbp) location was known. 1,047 unique SDPs have been ascertained.

In the context of the integrated analysis of microsatellite and SNP markers, it is interesting to note that SNP data set used was the result of the single most extensive genotyping effort in the HXB/BXH undertaken to date. It is thus somewhat surprising that the number of recombination points captured provides only a small incremental benefit over the existing microsatellite-based data. Thus, despite extensive SNP genotyping, it appears that the genetic structure of RI strains, and most of the

information on recombination points in their genomes, was sufficiently captured even by the original microsatellite marker-based map[47].

It is believed that the number of recombinations in an RI set usually significantly exceeds the number of those detected, largely because of insufficient marker density[69]. The total number of observed recombinations is decreased by a failure to discover them in sparsely mapped regions and increased by genotyping errors, as well as errors in marker ordering. The latter is a concern especially with microsatellites, since the order of SNP is unambiguously defined by their physical (Mbp) location. Present results, however, suggest that at least in the case of the HXB/BXH RI strain set, the actual number of recombinations is unlikely to be significantly higher then the ~1000 detected in the merged set. If true, this would bring the total expected number of recombinations down to about a half compared to previous estimates. Therefore further genotyping in the HXB/BXH RI panel would be only of limited benefit. The only way to increase the number of detectable recombination points would be to expand the HXB/BXH RI panel by producing additional RI strains.

It is unlikely that a recombination would take place at the exact same locus in multiple RI strains, unless there was a special cause for such occurrence (e.g. a recombination hot spot). An ideal RI strain map for the purpose of QTL detection would therefore consist of markers ordered according to their physical location, whose SDPs would only be discordant in one strain-genotype at a time relative to their neighbors. In this way, uncovering of recombination events would be maximized and

the full potential attainable with an RI set could be mustered. However, as the results of the present study suggest, the benefit of additional genotyping is likely to be fairly small, and the amount of additional genotyping necessary to uncover all existing recombination points would be excessive. Therefore, an increase in the number of RI strains, rather than a more extensive genotyping, would be the best way to improve the utility of the HXB/BXH RI strain set.

It is necessary to emphasize that the main purpose of the genetic linkage map presented here is to provide a tool for detection and location of quantitative trait loci. The usefulness of the presented map is exclusive to the HXB/BXH RI panel. Other possible objectives for uses of this genetic linkage map, such as determining the gene order or distance between genes are best served using other resources, such as the genomic sequence for the *Rattus norvegicus* (Ensembl, UCSC Genome Browser, etc.).

The genetic structure of the HXB/BXH RI strains

Besides map construction, the genotypic information obtained was used to analyze the overall genetic structure of the HXB/BXH RI strain set. We compared the degree of allele sharing among all strains, and computed the fraction of the progenitors' alleles in each individual strain. It is an important finding that the parental alleles are distributed evenly among the HXB-BXH RI strains and that, apart from the exceptions discussed above, the allele sharing among the strains is on average 50%, as expected for a randomly segregating population. As a sibling-like population, the RI strains are expected to share around a half of the alleles among each other. It is important that there are no major deviations from this expectation to insure that no

clustering or grouping of strains occurred. Non-randomness and strain clustering with respect to genotype across the strains can negatively affect the QTL detection.

Correlated, but physically unlinked loci

Also of relevance to the utility of the HXB/BXH RI strains is the existence of correlated, but physically unlinked loci. The situation, in which two loci on different chromosomes have congruent SDPs and therefore erroneously suggest that they are linked, can have an impact on the detection and correct positioning of a QTL. The most probable explanation of this phenomenon lies with the small number of RI strains in the HXB/BXH set. In a biallelic mapping population, such as the RI strains, the number of possible different SDPs equals 2^n, where *n* is the number of strains. When the number of RI strains is small, the total number of possible different SDPs is comparable to the number of loci for which the RI strains are genotyped, and a high degree of SDP concordance between unlinked loci is regularly observed. To a lesser extent, this poses a problem even for medium-sized populations, such as the HXB/BXH RI strain set. Correlation, both positive and negative, between distant loci can cause a QTL to be located incorrectly, and in the case of a negative correlation the direction of the allelic effect of a QTL can be obscured. The pairwise correlation matrix (Figure 2–2) provides a means to uncover correlated genomic regions in order to aid in the interpretation of QTL mapping results.

Power limitations for mapping in the HXB/BXH RI strains

Because of the polygenic nature of complex traits, when mapping QTLs for such traits, one would expect to find multiple QTLs, with varying proportion of phenotypic variance attributable to each one of them. The notion of the genetic architecture of complex traits is changing along with the increasing power to detect QTLs with very small effects. Because of the inability to detect small effect QTLs in the past, researchers were more likely to detect large effect QTLs, giving them more prominence then they deserved. However, it turns out that the greater the mapping power, the larger the detected proportion of small effect QTLs[41]. Such QTLs explain between 1% and 5% of the phenotypic variance, which is consistent with the model of exponential distribution of allelic effects. Small effect QTLs can only be mapped in RI strain panels numbering hundreds of strains (Figure 2–5). For the HXB/BXH RI strain set the detectable QTL should explain ~ 40% of the phenotypic variance, thus mapping is possible only for large effect QTLs. Most of the traits, for which a QTL can be detected, will therefore have closer to oligogenic or Mendelian traits.

Conclusions

It can be thus concluded that the greatest limitation of the HXB/BXH RI set is the relatively small size of the set, which not only makes is difficult to discover all existing recombination points and causes some non-linked loci to appear correlated, but also hinders the ability to localize QTLs with a higher precision, and makes it near impossible to detect small effect QTLs, which are key to understanding of the genetic structure of quantitative traits.

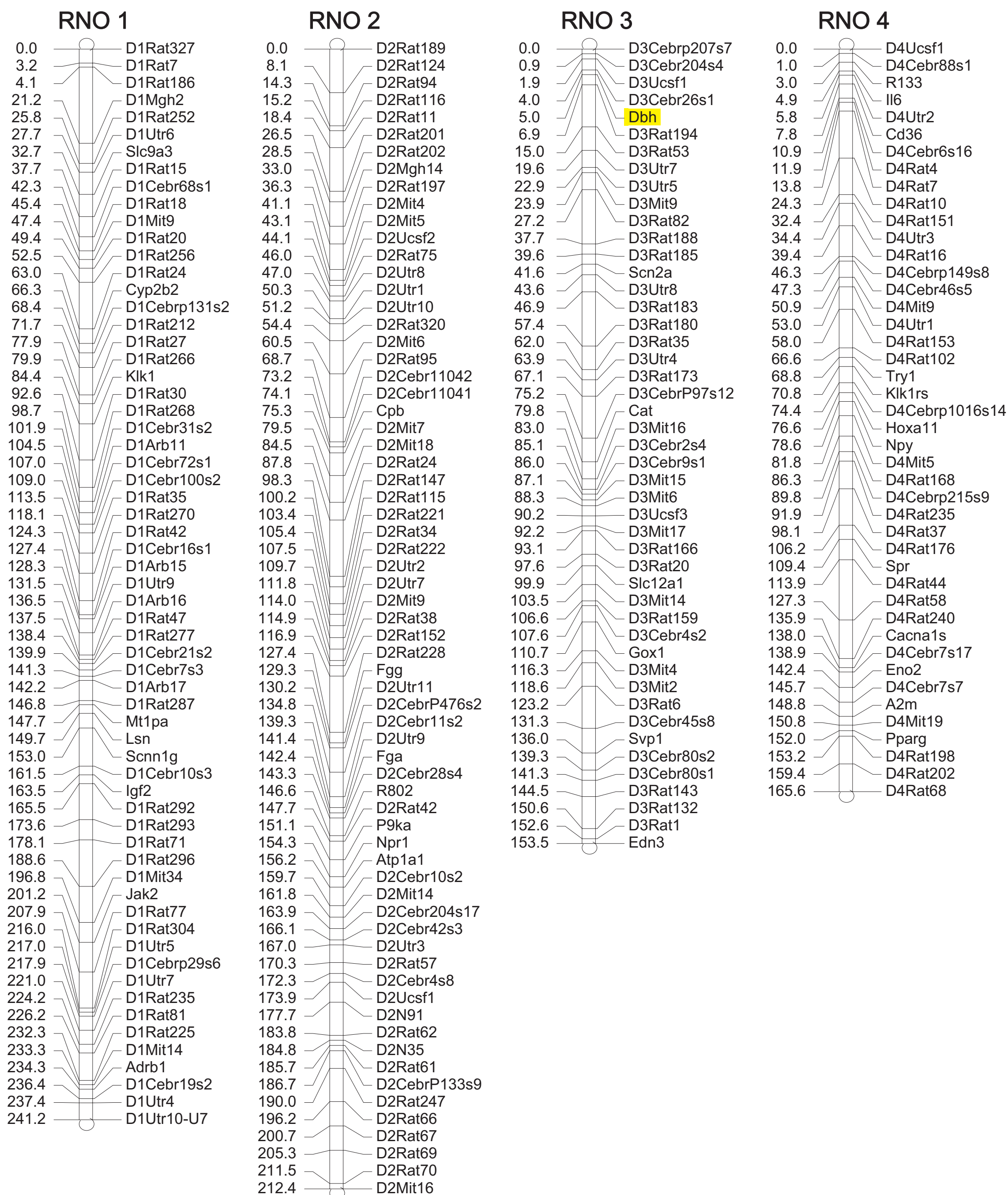


Figure 2-1 Linkage map of the rat autosomes derived from the HXB/BXH RI strains. The map was created by positioning ~ 800 non-framework markers on the HXB/BXH framework map of 245 markers (see Methods). After eliminating non-informative markers, the resulting map consists of a total of 648 markers contiguously covering all 20 rat autosomal chromosomes with an average between-marker distance of ~ 3.7 cM.

(Continued.)

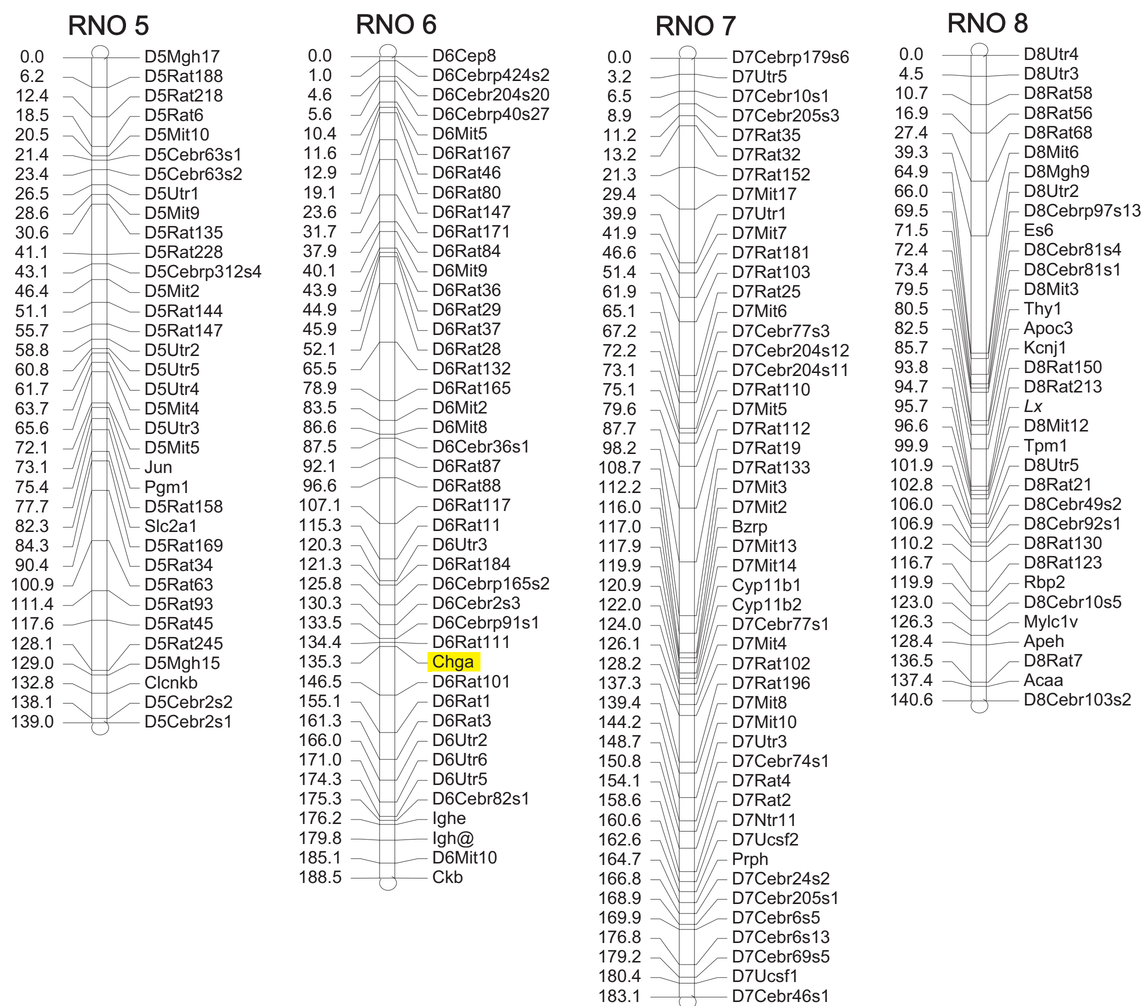


Figure 2-1 Linkage map of the rat autosomes derived from the HXB/BXH RI strains. (Continued.)

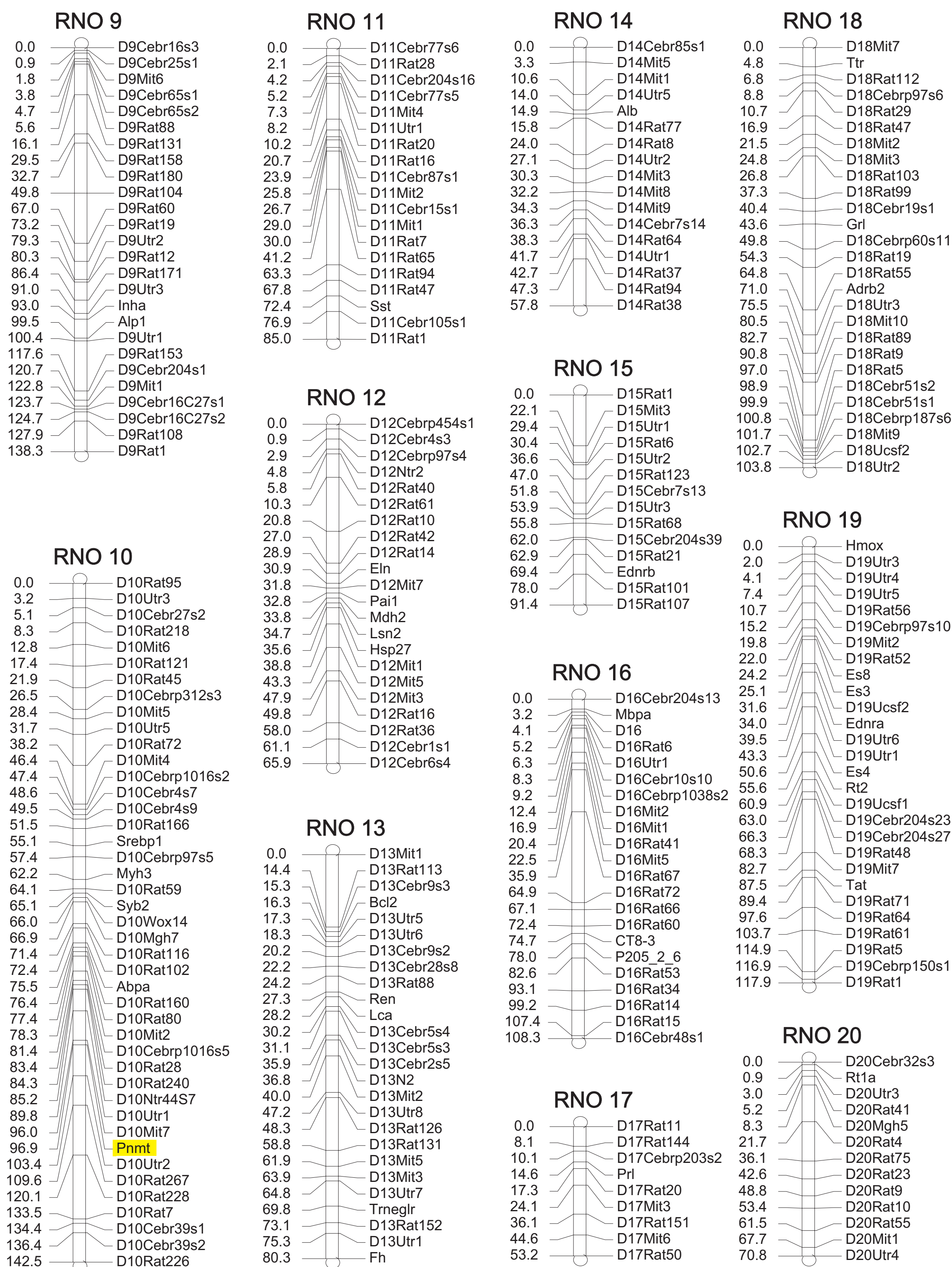


Figure 2-1 Linkage map of the rat autosomes derived from the HXB/BXH RI strains. (Continued.)

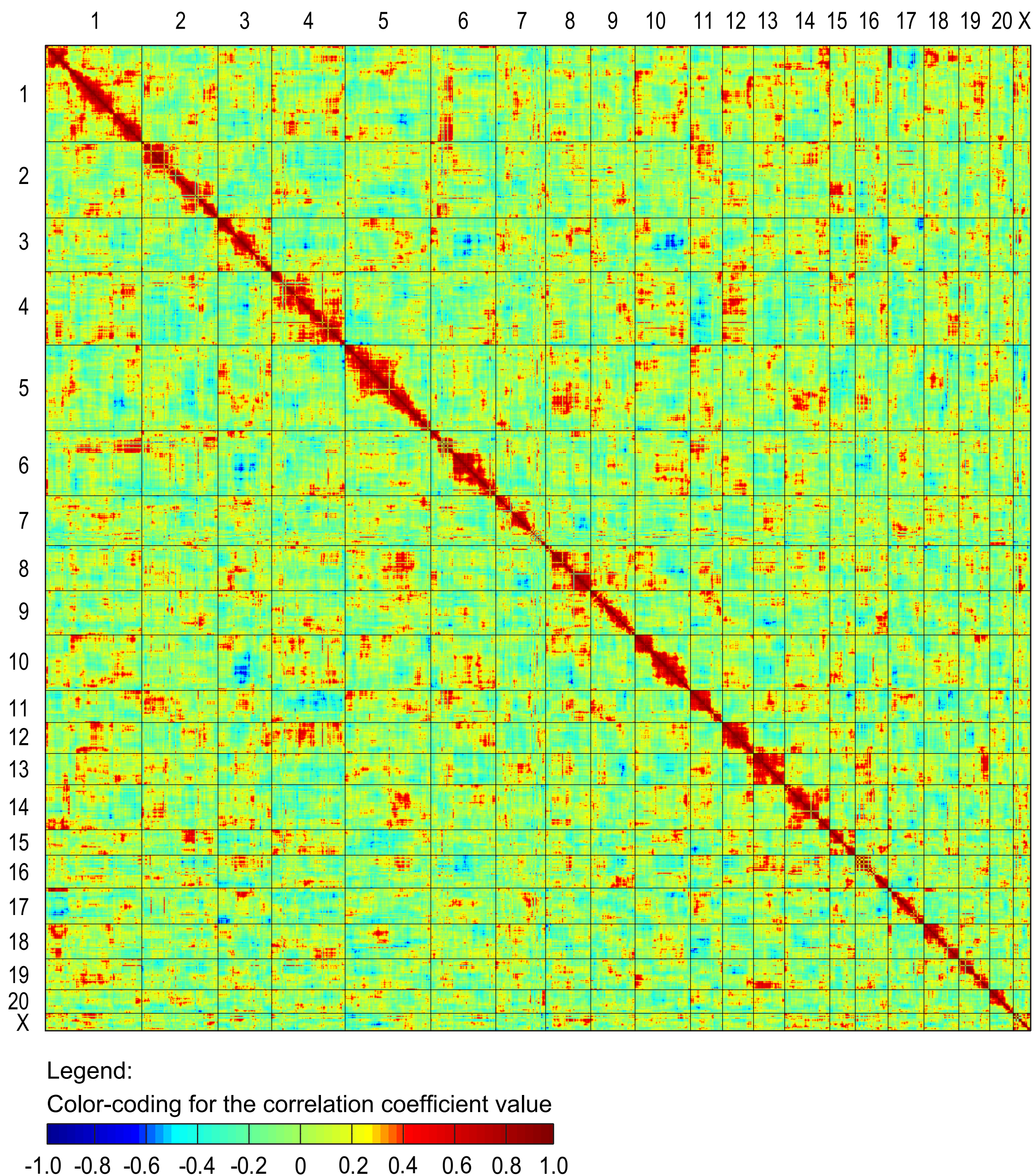


Figure 2-2 Genome-wide interactions between loci. Heat map representation showing the Pearson product moment correlation coefficient for all pairwise combinations of marker SDPs. Strong correlations are expected principally along the diagonal, which is the line of identity. Several other regions in the matrix also show a high positive (red) or negative (blue) correlation between physically remote markers. Highly correlated regions on different chromosomes may reflect networks of functionally linked genes, whose co-segregation is positively (or negatively) selected for during the inbreeding process. Such domains of linkage disequilibrium, however, pose a potential problem for precisely localizing QTLs. Numbers outside the matrix refer to chromosomes.

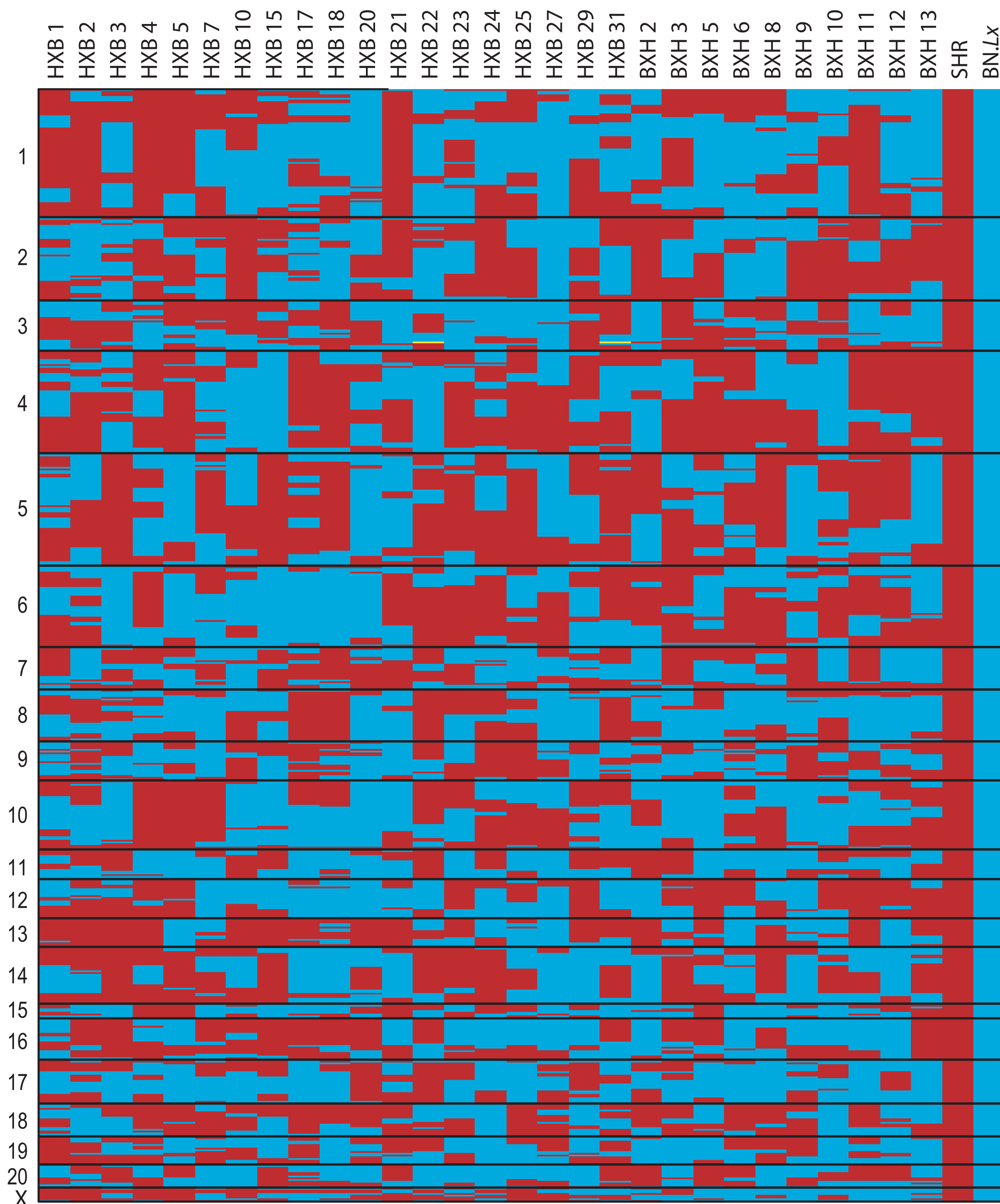
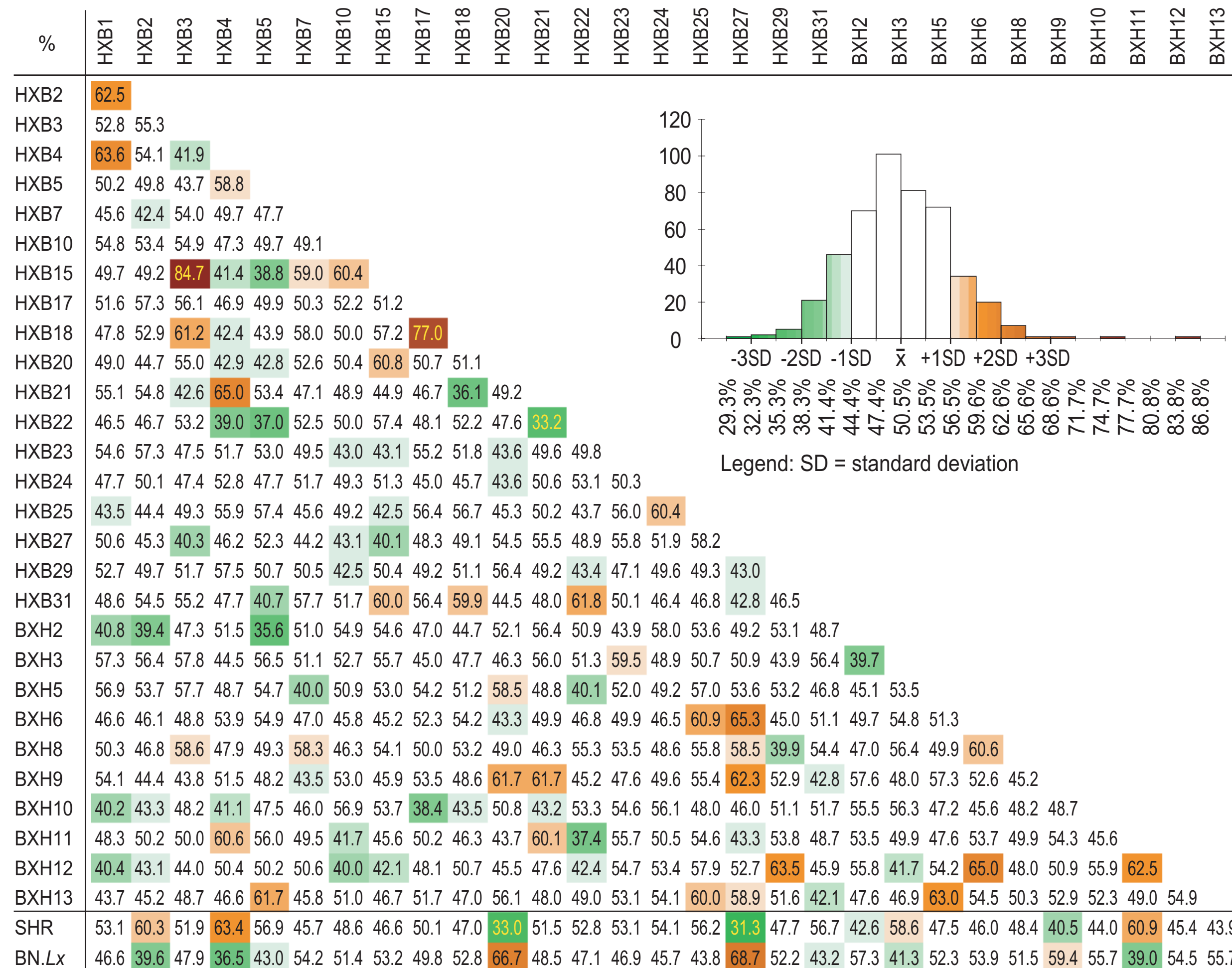


Figure 2-3 Progenitor allele distribution among the HXB/BXH RI strains. This figure is based on merged genotypic data, containing 1047 non-redundant SNPs and microsatellites with known physical location. Red and blue rectangles denote genomic segments of SHR or BN.*Lx* origin, respectively. Each column represents the linearized genome of one RI strain. Horizontal lines separate chromosomes. Yellow denotes unknown genotype.



%	HXB1	HXB2	HXB3	HXB4	HXB5	HXB7	HXB10	HXB15	HXB17	HXB18	HXB20	HXB21	HXB22	HXB23	HXB24	HXB25	HXB27	HXB29	HXB31	BXH2	BXH3	BXH5	BXH6	BXH8	BXH9	BXH10	BXH11	BXH12	BXH13
HXB2	62.5																												
HXB3	52.8	55.3																											
HXB4	63.6	54.1	41.9																										
HXB5	50.2	49.8	43.7	58.8																									
HXB7	45.6	42.4	54.0	49.7	47.7																								
HXB10	54.8	53.4	54.9	47.3	49.7	49.1																							
HXB15	49.7	49.2	84.7	41.4	38.8	59.0	60.4																						
HXB17	51.6	57.3	56.1	46.9	49.9	50.3	52.2	51.2																					
HXB18	47.8	52.9	61.2	42.4	43.9	58.0	50.0	57.2	77.0																				
HXB20	49.0	44.7	55.0	42.9	42.8	52.6	50.4	60.8	50.7	51.1																			
HXB21	55.1	54.8	42.6	65.0	53.4	47.1	48.9	44.9	46.7	36.1	49.2																		
HXB22	46.5	46.7	53.2	39.0	37.0	52.5	50.0	57.4	48.1	52.2	47.6	33.2																	
HXB23	54.6	57.3	47.5	51.7	53.0	49.5	43.0	43.1	55.2	51.8	43.6	49.6	49.8																
HXB24	47.7	50.1	47.4	52.8	47.7	51.7	49.3	51.3	45.0	45.7	43.6	50.6	53.1	50.3															
HXB25	43.5	44.4	49.3	55.9	57.4	45.6	49.2	42.5	56.4	56.7	45.3	50.2	43.7	56.0	60.4														
HXB27	50.6	45.3	40.3	46.2	52.3	44.2	43.1	40.1	48.3	49.1	54.5	55.5	48.9	55.8	51.9	58.2													
HXB29	52.7	49.7	51.7	57.5	50.7	50.5	42.5	50.4	49.2	51.1	56.4	49.2	43.4	47.1	49.6	49.3	43.0												
HXB31	48.6	54.5	55.2	47.7	40.7	57.7	51.7	60.0	56.4	59.9	44.5	48.0	61.8	50.1	46.4	46.8	42.8	46.5											
BXH2	40.8	39.4	47.3	51.5	35.6	51.0	54.9	54.6	47.0	44.7	52.1	56.4	50.9	43.9	58.0	53.6	49.2	53.1	48.7										
BXH3	57.3	56.4	57.8	44.5	56.5	51.1	52.7	55.7	45.0	47.7	46.3	56.0	51.3	59.5	48.9	50.7	50.9	43.9	56.4	39.7									
BXH5	56.9	53.7	57.7	48.7	54.7	40.0	50.9	53.0	54.2	51.2	58.5	48.8	40.1	52.0	49.2	57.0	53.6	53.2	46.8	45.1	53.5								
BXH6	46.6	46.1	48.8	53.9	54.9	47.0	45.8	45.2	52.3	54.2	43.3	49.9	46.8	49.9	46.5	60.9	65.3	45.0	51.1	49.7	54.8	51.3							
BXH8	50.3	46.8	58.6	47.9	49.3	58.3	46.3	54.1	50.0	53.2	49.0	46.3	55.3	53.5	48.6	55.8	58.5	39.9	54.4	47.0	56.4	49.9	60.6						
BXH9	54.1	44.4	43.8	51.5	48.2	43.5	53.0	45.9	53.5	48.6	61.7	61.7	45.2	47.6	49.6	55.4	62.3	52.9	42.8	57.6	48.0	57.3	52.6	45.2					
BXH10	40.2	43.3	48.2	41.1	47.5	46.0	56.9	53.7	38.4	43.5	50.8	43.2	53.3	54.6	56.1	48.0	46.0	51.1	51.7	55.5	56.3	47.2	45.6	48.2	48.7				
BXH11	48.3	50.2	50.0	60.6	56.0	49.5	41.7	45.6	50.2	46.3	43.7	60.1	37.4	55.7	50.5	54.6	43.3	53.8	48.7	53.5	49.9	47.6	53.7	49.9	54.3	45.6			
BXH12	40.4	43.1	44.0	50.4	50.2	50.6	40.0	42.1	48.1	50.7	45.5	47.6	42.4	54.7	53.4	57.9	52.7	63.5	45.9	55.8	41.7	54.2	65.0	48.0	50.9	55.9	62.5		
BXH13	43.7	45.2	48.7	46.6	61.7	45.8	51.0	46.7	51.7	47.0	56.1	48.0	49.0	53.1	54.1	60.0	58.9	51.6	42.1	47.6	46.9	63.0	54.5	50.3	52.9	52.3	49.0	54.9	
SHR	53.1	60.3	51.9	63.4	56.9	45.7	48.6	46.6	50.1	47.0	33.0	51.5	52.8	53.1	54.1	56.2	31.3	47.7	56.7	42.6	58.6	47.5	46.0	48.4	40.5	44.0	60.9	45.4	43.9
BN.*Lx*	46.6	39.6	47.9	36.5	43.0	54.2	51.4	53.2	49.8	52.8	66.7	48.5	47.1	46.9	45.7	43.8	68.7	52.2	43.2	57.3	41.3	52.3	53.9	51.5	59.4	55.7	39.0	54.5	55.7

Figure 2-4 Pair-wise genetic similarity matrix of the HXB/BXH RI strains. The numbers in the matrix represent the percentage (%) of shared genome among RI strains. The average (± SD) allele sharing is 50.5% (± 6.1%). The progenitors are included in the bottom two rows. Colored background indicates pairs that share less (green) or more (brown) than the average, defined as mean ± 1 SD. White background indicates an average sharing. Inset shows a histogram of genome sharing among RI strains.

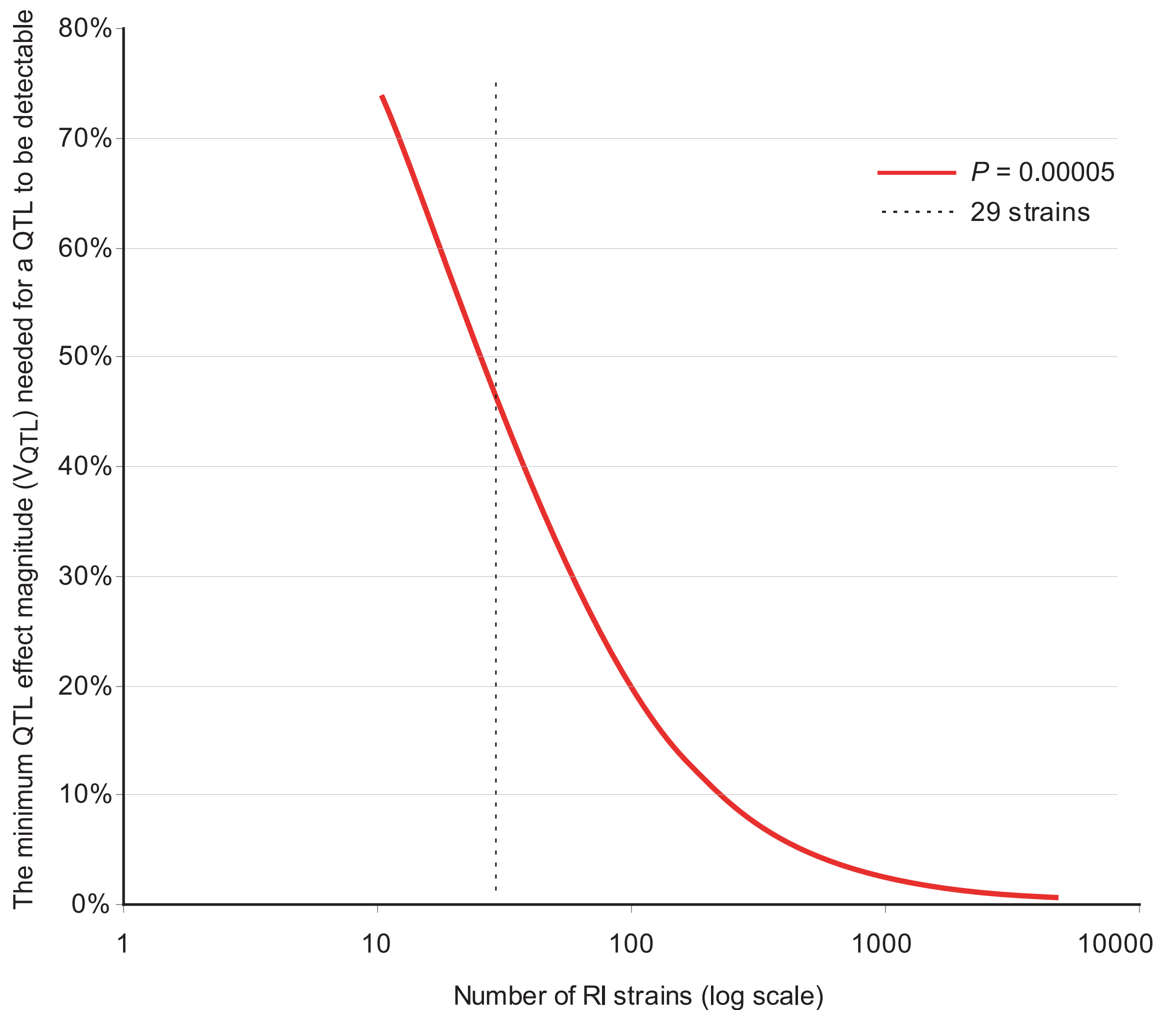


Figure 2-5 Ability to detect QTLs as a function of RI panel size and QTL effect magnitude. V_{QTL} denotes the percentage of phenotypic variance attributable to the QTL, i.e. the effect magnitude. Power is set to 0.80. The genome-wide corrected significance level $\alpha = 0.05$ corresponds to nominal significance level $\alpha = 0.00005$ when using a map consisting of 1000 markers. If all due statistical stringency is applied, for a QTL to be detectable with enough confidence its V_{QTL} needs to be greater than 40% .

Chapter 3

Delineation of a regulatory genetic network controlling catecholamine biosynthesis in the spontaneously hypertensive rat

PREFACE

The material in this chapter was presented as a poster at the American Society of Human Genetics meeting in San Diego, CA, October 23-27, 2007, presented as an oral presentation at the Rat Genomics & Models meeting in Cold Spring Harbor, NY, December 6-9, 2007, and submitted as a scientific paper to Nature Genetics.

ABSTRACT

Understanding the regulation of genes involved in catecholamine metabolism is crucial for elucidating the pathogenesis of hereditary hypertension. To this end, integration of transcriptional and biochemical profiling, coupled with expression and physiological quantitative trait locus mapping (eQTL and pQTL) was pursued in the adrenal tissue of the HXB/BXH recombinant inbred (RI) rat strains, derived from spontaneously hypertensive rat (SHR) and Brown Norway (BN.*Lx*) progenitors. We find simultaneous downregulation of the transcription of five genes in the catecholamine biosynthesis/storage/release pathway in the SHR and identify *cis*-acting eQTLs for *Dbh*, *Pnmt* and *Vamp1* whose gene products are key regulators of catecholamine biosynthesis and secretion. Enzyme activities of Dbh and Pnmt correlated with gene expression and are also regulated in *cis*. We also detected *trans*-regulated expression of *Vmat1* and *Chga* (both involved in catecholamine storage) with co-localization of both of these *trans*-eQTLs to the *Pnmt* gene locus. *Pnmt* re-sequencing revealed several promoter polymorphisms, which result in a decreased response of the SHR *Pnmt* promoter to glucocorticoids compared to that of the normotensive BN.*Lx* strain. Of physiological significance, Dbh enzyme activity was

negatively correlated with systolic blood pressure and Pnmt enzyme activity with heart rate. The finding of *cis* regulation of several of the key genes and enzymes in catecholamine synthesis and secretion, in young rats, and the correlation of enzymatic activities with blood pressure and heart rate suggest that these heritable changes in gene expression delineate a regulatory genetic network for the regulation of blood pressure and heart rate in this widely studied model of essential hypertension.

INTRODUCTION

Essential hypertension is one of the leading causes of premature cardiovascular morbidity and mortality. In developed countries more than 20% of the adult population is clinically hypertensive[70]. The complications of hypertension include stroke, myocardial infarction, congestive heart failure and end-stage renal disease. Hypertension is also associated with abdominal obesity, blood lipid disorders, glucose intolerance and insulin resistance, but the exact nature of the association between the two disorders remains unclear[71]. Within a decade, largely due to economic progress in the developing countries and the resulting lifestyle changes, hypertension is projected to become the most common risk factor for death and disability worldwide[72,73].

Essential hypertension is a paradigmatic complex multifactorial trait with many genetic and non-genetic (i.e., environmental) determinants. It has been estimated that genetic determinants contribute up to 40% of the blood pressure variation among individuals[74]. The non-genetic factors include caloric and salt intake, alcohol consumption, physical activity, smoking, etc. The separation and characterization of each of these determinants is difficult, since the effects of any one of them may be

obscured by the effects of the others. Possible ways to overcome this difficulty include the study of *intermediate phenotypes*, which are biochemical or subclinical parameters likely to reflect early changes in the physiological pathways[75], and the study of *gene transcript abundance*, which may reveal perturbations at levels even closer to the DNA[45]. A different approach involves the study of experimental hypertension in *animal models*, such inbred strains of rodents, especially the rat[35]. There are several hypertensive inbred rat strains, each mimicking certain aspect of human essential hypertension, which can help separate individual subtypes and thus reduce the significant problem of heterogeneity of the phenotype in humans[76]. Other advantages of rodent models include better control of the environmental factors (e.g., diet), possibility of invasive blood pressure measurements (e.g., telemetry) and unrestricted access to tissues for analyses, such as biochemical tests or gene expression profiling[34]. The spontaneously hypertensive rat (SHR), in particular, has been extensively used for studying the genetic basis of hypertension[77].

The catecholaminergic system plays a key role in the regulation of blood pressure, especially on a short timescale[78], but sustained increase in sympathoadrenal activity can result in chronic elevation of systemic blood pressure[79]. The sympathoadrenal system acts by release through exocytosis of transmitters - including catecholamines - from secretory vesicles of postganglionic sympathetic axons into the neuroeffector junctions or chromaffin cells into the bloodstream, where transmitters exert their effect on target cells, such as vascular smooth muscle, cardiomyocytes, and endothelial cells. The principal physiologically active catecholamines, dopamine

(DA), norepinephrine (NE) and epinephrine (EPI) are formed by hydroxylation, decarboxylation and methylation of the initial precursor amino acid tyrosine by tyrosine hydroxylase (Th), DOPA decarboxylase (Ddc), dopamine beta-hydroxylase (Dbh) and phenylethanolamine N-methyltransferase (Pnmt). Peripherally active catecholamines are produced mainly in the adrenal medulla and the postganglionic fibers of the sympathetic nervous system. Each of the three principal physiologically active catecholamines has a unique set of functions. DA is the immediate metabolic precursor to NE and EPI. NE is primarily a neurotransmitter of the peripheral sympathetic nervous system but is also found in the blood, mostly through escape from synapses of the sympathetic system[80]. NE causes blood pressure elevation, predominantly through an increase in peripheral vascular resistance. EPI acts as a neurotransmitter in the central nervous system and a hormone in the blood circulation. In the periphery, EPI is secreted by the neuroendocrine chromaffin cells of the adrenal medulla. The majority (~90%) of chromaffin cells are EPI secreting. EPI plays a crucial role in the acute stress reaction (i.e., fight-or-flight response). When released into the bloodstream, epinephrine binds to both alpha and beta adrenergic receptors throughout the body and causes numerous effects, such as increase of heart rate, stroke volume and blood sugar, sweating, mydriasis, vasodilatation in skeletal muscle, and vasoconstriction in the skin and intestine. Thus, sympathetic ganglia along with the adrenal medulla are the source of the stress-elicited rise in circulating catecholamines. All three principal catecholamines are rapidly inactivated after release, in part by the

liver, which is rich in both catecholamine degrading enzymes, catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO).

Excessive sympathoadrenal activity is implicated in the pathogenesis of both essential[81,82] and acquired[83] hypertension, in humans[84,85] and experimental animals[86], and adrenergic receptor antagonists are a mainstay of antihypertensive therapy[87,88]. Increased sympathoneuronal activity was observed in normotensive humans with family history of hypertension[82], as well as young spontaneously hypertensive rats (SHR)[89-91]. The present study focuses specifically on the adrenal medulla as an integral part of the sympathoadrenal system, and its possible role in the pathogenesis of essential hypertension. Implicating evidence comes from studies showing that adrenal demedullation in young SHR results in a decrease in blood pressure in the adult[92-94], but the exact mechanism by which this occurs is not well established. Understanding mechanisms that influence catecholamine biosynthesis, storage, secretion and degradation in the adrenal medulla of young SHRs may give clues as to which processes are dysregulated early in the development of hypertension, before full manifestation of the disease, and may shed light on factors that mediate disease susceptibility.

Previous studies focusing on catecholamine biosynthetic enzyme activities in the adrenal medulla of the SHR showed inconsistent results, possibly owing to the different normotensive controls used in the experiments. Th was in turn reported to be decreased[90,95,96], unchanged[97] and increased[98,99] in young SHR. However, studies involving adult SHR report increased Th activity when compared to age and sex

matched controls[95,100]. Dbh, Pnmt and Ddc activities were decreased in the young SHR[95,101], but unchanged in the adult SHR[95]. Because of the apparent correlation of enzyme activities of Th, Dbh and Pnmt, some authors suggested that these genes may be co-regulated by a single locus[102].

In order to characterize the effect of genetic variation on catecholamine physiology, we integrated gene expression profiling and biochemical phenotyping with linkage mapping. We used the HXB/BXH panel of recombinant inbred (RI) strains, derived from the SHR and the normotensive BN.*Lx* inbred progenitor strains[52] (the derivation of the HXB/BXH RI strains is also described in Chapter 1, page 12), and used widely for genetic dissection of phenotypes segregating in this panel. Young (6 week old) males were used for all experiments to avoid confounding the results by the consequences of fully developed hypertension or by the effects of female sex hormones. We first quantified the variability across 29 RI strains and the two progenitor strains of transcript levels of chromaffin cell-expressed genes that are responsible for crucial aspects of catecholamine biology. We then collected biochemical phenotypes reflecting the protein products (i.e., enzymes) of these genes, as well as the intermediates synthesized by them. Subsequently, linkage mapping was performed for both sets of phenotypes (i.e., transcript levels and biochemical traits). Several quantitative trait loci (QTLs) were detected, which suggested a regulatory network of *cis*- and *trans*-acting loci. Positional candidates from QTL regions were re-sequenced in the progenitor strains to discover specific DNA sequence variants which were subsequently tested for their functional significance by transfection. Correlation

of key catecholamine biosynthetic enzymes with systolic blood pressure and heart rate was established.

MATERIAL AND METHODS

Animals

HXB/BXH recombinant inbred (RI) strains were produced by inbreeding between F2 generation resulting from a sex-reciprocal cross of two highly inbred strains: BN.*Lx* (BN.*Lx*/Cub) and SHR (SHR/Ola)[42,52] (see Chapter 1, page 12). In this study, 29 RI strains (HXB and BXH) at F_{60} were used. Animals were housed in an air-conditioned animal facility and allowed free access to standard laboratory chow and water. All experiments were done in agreement with the Animal Protection Law of the Czech Republic (311/1997) and were approved by the Ethics Committee of the Institute of Physiology, Czech Academy of Sciences, Prague. Males were killed by cervical dislocation at 6 weeks of age. At this age, hypertension is only incipient and therefore this increases the likelihood of separating the causative pathogenetic mechanism from the consequential effects of fully developed hypertension.

Tissues for gene expression profiling, biochemical profiling, and qRT-PCR validation

The animal material originated from two separate tissue harvests: 1) adrenal glands harvested for global gene expression profiling, and 2) adrenal glands harvested for biochemical phenotyping and RT-PCR verification of gene expression profiling data. Both sets consisted of tissue harvested from the same HXB/BXH RI strains, but

on two separate occasions, and thus from different individual members of the respective RI strains. The sets were similar in size: 29 RI strains, 2 progenitor strains, 4-6 individuals per strain.

Gene expression profiling

HXB/BXH RI strains were profiled for gene expression in adrenal tissue using the Affymetrix Gene Chip array RAE 230A (Affymetrix, Santa Clara, California, USA). The original experimental design is discussed in Hübner et al.[32] and Petretto et al.[103]. We used a total of 124 microarrays: four animals × 31 (i.e., 29 RI strains + progenitor strains). cRNA was labelled and run on RAE 230A Affymetrix GeneChip arrays (number of transcripts 15,923). Gene expression summary values for Gene Chip data were computed using the Robust Multichip Average algorithm[104]. The adrenal dataset has been submitted to ArrayExpress and an ArrayExpress identifier is pending.

Biochemical profiling

Adrenal glands were harvested, immediately frozen and stored at −80ºC. From each pair of adrenal glands per harvested animal, one adrenal was used for biochemical phenotyping and the other was used to extract mRNA for RT-PCR validation of microarray data. Adrenal glands for biochemical phenotyping were homogenized in 1.9 ml of 10 mM MES buffer (pH=6.0) using a Tissuemizer (Tekmar, Cincinnati, OH). Frozen adrenals were placed into precooled (4ºC) buffer, homogenized, spun at 13 000 G for 1 minute to clear debris, the supernatants divided into aliquots and placed immediately on dry ice to freeze. Aliquots were stored at −80ºC. The following

analyses were performed in aliquots of adrenal homogenates: Dbh spectrophotometric enzymatic activity assay[105], Pnmt enzymatic activity assay[106], radioenzymatic catecholamine assay based on O-methylation[107,108], corticosterone assay (competitive immunoassay, Assay Designs, Inc., Ann Arbor, MI, USA); in plasma, catecholamines (dopamine, norepinephrine, epinephrine) were measured by radioenzymatic assay based on O-methylation[107,108]. Results were normalized to mg wet weight or mg protein.

Validation of microarray data by qRT-PCR

Total RNA was prepared from the freshly frozen adrenal glands of the progenitor strains (median yield: 55 μg/one adrenal gland). RNA was extracted by the RNAzol (guanidinium thiocyanate) method (TelTest, Friendswood, TX), followed by RNAse-free DNAseI (Qiagen, Valencia, CA) treatment (to eliminate residual genomic DNA). Integrity of the RNA was confirmed by the appearance of 28S and 18S rRNA bands on ethidium bromide-stained gels. Total RNA was quantitated using a Ribogreen Quantitation kit (Molecular Probes - Invitrogen). First strand cDNA was prepared from 1 μg of total RNA template by reverse transcription with the "SuperScript™ first-strand synthesis system for RT-PCR", using SuperScript II reverse transcriptase (Invitrogen; Carlsbad, CA), and random hexamer primers. Samples were randomized. RT-PCR was performed using real-time TaqMan technology with a Sequence Detection System, model 7700 (Perkin Elmer) and fluorescent plate reader, using the Amplifluor™ universal detection system (Serologicals Corporation; Norcross, GA). Quantitative RT-PCR primers for Th, Ddc,

Dbh and Pnmt were designed using Primer Express V.2.0 (PE Applied Biosystems, Foster City, CA, USA). For primer detail see Appendix 2. Normalization was performed by quantitating the endogenous 18S rRNA and transcript abundance expressed as fold modulation over 18s rRNA.

Microarray analysis and pathway annotation

Affymetrix RAE 230A GeneChip data were analyzed for the differentially expressed genes in the progenitor strains (SHR and BN.*Lx*) using parametric (*t*-test) and as well as non-parametric (Mann-Whitney U) tests. Pathways, in which the differentially expressed genes are involved, were identified using Kyoto Encyclopedia of Genes and Genomes (KEGG, www.genome.jp/kegg) and annotated with Gene Map Annotator and Pathway Profiler (GenMAPP 2.1, www.genmapp.org)[109].

Blood pressure measurement

Arterial blood pressure and heart rate were measured using radiotelemetry in 12 week old unanesthetized, unrestrained males from the progenitor SHR and BN.*Lx* strains and from RI strains (N=6-8 males per strain). All rats were allowed to recover for at least 7 days after surgical implantation of radiotelemetry transducers (Data Sciences International, Inc) before the start of blood pressure recordings. Pulsatile pressures were recorded in 5-second bursts every 10 minutes throughout the day and night and 12- and 24-hour averages for systolic and diastolic arterial blood pressures were calculated for each rat for a one week period. The results from each rat in the same group were then averaged to obtain the group means.

Statistical analysis and heritability calculation

Phenotype data were checked for outliers using a method described by Grubbs[110] and optimized for 3-7 observations, corresponding to the number of individual rats within each strain. Data from all ~150 samples were grouped according to strain resulting in 31 groups (29 RI strains + 2 progenitors). *T*-tests were performed to detect significant differences between the progenitor strains. Heritability (H^2) for gene expression profiles and biochemical phenotypes was calculated using a technique that has been designed for use in RI strains that corrects for the inbreeding incurred during the RI strain production[111]. Because H^2 calculated in a traditional way (V_G/V_P) tends to be overestimated when compared to H^2 obtained from F2 population, to obtain an F2 comparable estimate, the H^2 was calculated as $(V_P - V_E)/(V_P + V_E)$. Pearson's product-moment correlation and Spearman's rank correlation coefficients were computed to assess the degree of correlation among phenotypes.

Normalized Ratio (*NR*)

NR is used here to compare biochemical phenotype means and gene expression profile means for the two progenitor strains, the SHR and the BN.*Lx*. It is a variant of the simple fold-change value, e.g., [SHR]/[BN.*Lx*], but it transforms the values between 0 and 1 into values between –INF(infinity) and –1 by inverting them and multiplying by negative one. It is calculated as follows: if [SHR] ≥ [BN.*Lx*], then *NR* = [SHR]/[BN.*Lx*]; if [SHR] < [BN.*Lx*], then *NR* = –[BN.*Lx*]/[SHR]. The main advantage of *NR* is its symmetry about zero.

QTL analysis

Revised linkage map of the HXB/BXH RI set (Chapter 2) with new SNP genotypes[51] was employed to perform genome-wide scans to detect QTLs for measured biochemical phenotypes using QTL Cartographer[112] and Map Manager QTX[53], and to detect expression QTLs using QTL Reaper[113] software packages. Permutation analysis[114] was carried out to assess the probabilistic significance of the linkages and to correct for multiple testing across genetic markers to obtain a genome-wide corrected *P*-value. To estimate the size of the chromosomal segment that, with a 95% confidence, contains detected QTLs (95% CI), two complementary strategies were implemented: the 2-LOD support interval method[115] and the bootstrap test[116]. The bootstrap test estimates a confidence interval by creating multiple bootstrap datasets by randomly choosing strains with replacement from the original RI set; each bootstrap dataset is then used for QTL mapping, and the location of the strongest QTL for each set is recorded. Here, 200 bootstrap datasets per phenotype were used. These locations are summarized in a histogram that shows the size of the region in which the QTL would be expected to be found.

Definition of *cis*- and *trans*-acting eQTLs

Cis-eQTLs were defined as eQTLs that map within 10 Mbp of the physical location of the probe set on the genomic sequence (20 Mbp total window size). Other eQTLs were defined as acting in *trans*. Physical locations of probe sets were downloaded from the UCSC Genome Browser website <http://genome.ucsc.edu>.

DNA extraction for re-sequencing

The progenitors of the HXB/BXH recombinant inbred strains, the Brown Norway rat (BN.*Lx*/Cub) and the Spontaneously Hypertensive Rat (SHR/Ola) were used. Liver was collected from one male per each strain at 6 weeks of age. Progenitor strain DNA was extracted from liver tissue using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) and adhering to the manufacturer's protocol. To eliminate residual RNA, samples were treated with RNase A (Qiagen, Valencia, CA).

Polymorphism discovery at the *Pnmt* and *Dbh* genes in the SHR and BN.*Lx* strains

The progenitors of the HXB/BXH recombinant inbred strains, the Brown Norway rat (BN.*Lx*/Cub) and the Spontaneously Hypertensive Rat (SHR/Ola) were used. Liver was collected from one male per each strain at 6 weeks of age. Progenitor strain DNA was extracted from liver tissue using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) and adhering to the manufacturer's protocol. To eliminate residual RNA, samples were treated with RNase A (Qiagen, Valencia, CA). Primer3[117] web-based application was used to design PCR primers for amplification of ~800 bp-long overlapping segments, spanning all exons, exon/intron borders, ~1.75 kbp of proximal promoter, and ~1.75 kbp of the 3' [downstream] sequence of the *Dbh* gene. However, in the case of *Pnmt*, the extent of re-sequencing was limited by the extent of known genomic sequence (gb: X75333.1; gi: 414186) in this locus, resulting in only ~1000 bp upstream and ~100 bp downstream segment re-sequenced. Primer sequences are listed in Appendix 3 for *Dbh* and Appendix 4 for *Pnmt*. Initial amplification to

enhance the target sequences was carried out in 25µl PCR reactions containing 25 ng genomic DNA, 2 mM MgCl2, 10 mM Tris HCl, 200 µM dNTP, 0.5 U Amplitaq Gold DNA Polymerase (PE Applied Biosystems, Foster City, CA, USA) and 50 pmol of each primer. PCR was performed by Peltier Tetrad Thermal Cycler (MJ Research, Watertown, MA). The Touchdown Profile program (MJ Research) was used, which begins at annealing temperatures of 66 °C and runs down to 50 °C at 1 °C/cycle for the first 16 PCR cycles, followed by a uniform three-step amplification profile (94 °C denaturing step for 30 s, 50 °C annealing step for 30 s, 72 °C extension step for 30 s) for another 24 cycles, finally holding at 10 °C. Enzymatic purification (Exo-SAP) was then pursued using Exonuclease I (Fermentas Inc., Ontario, Canada) and Shrimp Alkaline Phosphatase (Fermentas Inc., Ontario, Canada). 15µl of each PCR product was mixed with 0.225 µl of Exonuclease I (20 U/µl), 1.2 µl of SAP (1 U/µl) und 4.35 µl of water. The Exo-SAP program (MJ Research) was used, which runs at 37 °C for 30 min., followed by 15 min. at 85 °C. The cycle-sequencing reaction was performed following the Big Dye Terminator Version 3.1 (PE Applied Biosystems, Foster City, CA, USA) protocol on 2.5 µl of the template (Exo-SAP product), using either the forward or the reverse primer for the Big Dye amplification origin. The cycle-sequencing reaction was subsequently performed with both primers to achieve better coverage. The cycle-sequencing reaction program begun at 96 °C for 1 min., followed by a uniform three-step amplification profile (96 °C denaturing step for 10 s, 50 °C annealing step for 5 s, 60 °C extension step for 4 min.) for another 29 cycles, holding at 4 °C. Finally, the product of the cycle-sequencing reaction was purified on soaked

Sephadex G50-50 beads (Sigma-Aldrich, St. Louis, MO, USA), and placed in multiscreen filtration plates (Fisher Scientific, Pittsburgh, PA, USA). After spinning into clean plates, loading dye was added and samples were then loaded into a ABI PRISM 3100 capillary DNA Analyzer (PE Applied Biosystems) according to the manufacturer's instructions.

Pyrosequencing

Single nucleotide polymorphisms at *Dbh* T−550G and *Pnmt* T−529C were scored using pyrosequencing (Biotage, Charlottesville, VA, USA), performed according to described protocol[118]. Primers were designed using the PSQ Assay Design software (Biotage) and ordered from Sigma-Proligo (http://www.proligo.com). For primer sequences see Appendix 7. A PCR product from each sample was generated in a 10 µl reaction containing 1× AmpliTaq Gold Master Mix, 2 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA), 250 µ M dNTPs, 0.25 µM forward primer, 0.25 µM reverse primer, and 5 µl of bisulphite-modified sample DNA. The amplifications were carried out at 95 °C for 10 min, followed by a six-cycle touchdown PCR protocol of 95 °C for 1 min, 63 °C for 1 min with −1 °C for each cycle to 58 °C, and 72 °C for 1 min. This was followed by 44 cycles at 95 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and a 10 min extension at 72 °C. Single-stranded DNA of 10 µl of each PCR sample was generated following the PSQ 96 sample preparation guide using a vacuum filtration sample device following the manufacturer's instructions (Biotage). The single-stranded product was annealed to 0.4 µM of the sequencing primer placed at 85 °C for 2 min and cooled to room

temperature for 5 min. Pyrosequencing was performed on a PSQ96 HS 96A system (Biotage) with the Pyro Gold Reagent kit (Biotage) according to the manufacturer's instructions.

Sequence analysis

Output data from the ABI PRISM 3100 DNA Analyzer were read by the BioEdit sequence alignment editor (Ibis Therapeutics, Carlsbad, CA, USA). SHR and BN.*Lx* sequences were aligned and between-strain polymorphisms identified. MacVector (MacVector, Inc., Cary, NC, USA) was used to analyze the sequence for differences in transcription factor binding sites resulting from such polymorphisms.

Candidate gene promoter/reporter transfection assay

Construction of the promoter/reporter plasmids: Primer3[117] <http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi> was used to design PCR primers to amplify a ~1.1 kb fragment of *Dbh* proximal promoter, or a ~1 kb fragment of the *Pnmt* proximal promoter from genomic DNA of the SHR and BN.*Lx*. Computational sequence analysis of the *Dbh* and *Pnmt* promoters revealed the absence of SacI and XhoI restriction enzyme sites. Therefore, a SacI (5'-GAGCTC-3') or XhoI (5'-CTCGAG-3') restriction enzyme site was inserted in the 5'-end of the forward and reverse PCR primers to allow cloning. The resulting primer sequences are detailed in Appendix 8. The *Dbh* or *Pnmt* promoters were PCR amplified from SHR and BN.*Lx* genomic DNA and subsequently digested with SacI and XhoI. T4 DNA ligase (Invitrogen) was used to insert the promoter fragments between the SacI and XhoI

sites in the polylinker region of the firefly luciferase reporter vector, pGL3-Basic (Promega, Madison, WI), which lacks eukaryotic promoter and enhancer sequences, and contains the cDNA for firefly luciferase. Creation of single nucleotide polymorphism (SNP) variants of the Pnmt promoter/luciferase reporter constructs was accomplished using the QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). Correct insertion of the promoters was confirmed by DNA sequencing. Plasmid DNA for transfection was prepared and purified using the QIAfilter Plasmid Midi Kit (Qiagen, Valencia, CA).

Transfection of the promoter/reporter constructs: Rat PC12 pheochromocytoma cells [grown in DMEM high glucose (Invitrogen) with 5% heat-inactivated fetal bovine serum (Gemini Bioproducts, Woodland, CA), 10% heat-inactivated horse serum (Gemini Bioproducts), penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (0.292 mg/ml)] were transfected (at 50-60% confluence, 1 day after splitting 1:4) with *Dbh* or *Pnmt* promoter reporter plasmid DNA [1 μg supercoiled DNA per well; 12-well polystyrene plates (coated with poly-L-lysine; Sigma), 2.2-cm diameter wells, Corning Inc., Corning, NY] using the liposome method (Superfect; Qiagen). Cells were incubated with or without dexamethasone (different concentrations: 1nM, 10nM, or 100nM) (Calbiochem), 100 nM pituitary adenylate cyclase-activating peptide (PACAP) (ovine; Calbiochem), or 1 mM nicotine (Sigma). Cells were lysed 16 hours after transfection with lysis buffer (300 μL per well) [0.1 M phosphate buffer (K2HPO4 + KH2PO4) (pH 7.8), 1 mM DTT, and 0.1% Triton-X 100].

Luciferase reporter activity assay: The bioluminescent activity of luciferase in 80 μL of transfected cell lysates was determined using the AutoLumat LB 953 luminometer (EG&G Berthold, Nashua, NH) to measure light emission (incubation time = 0 seconds, measure time = 10 seconds, temperature = 25˚C) after addition of assay buffer [100 μl per sample; 100 mM Tris-acetate (pH 7.8), 10 mM Mg-acetate, 1 mM EDTA (pH 8.0), 3 mM ATP, and 100 μM luciferin (Sigma-Aldrich)]. As a control for varying cell number within individual wells, the total protein content was measured in the cell lysate using the Bio-Rad Protein Assay (coomassie blue dye absorbance shift; based on the Bradford method) (Bio-Rad, Hercules, CA). Luciferase activity in the cell lysate is expressed as the normalized ratio of (luciferase activity)/(total protein content) or (RLU/μg protein).

***Pnmt* alternative splicing assay**

Primer3[117] application was used to design forward (5'-CCTCAACAGGAGCATGGAC-3') and reverse (5'-GCTGGGGACTGTTACTTTATTAGG-3') PCR primers that target the first and last exons of the *Pnmt* gene, and amplify a 937-bp fragment of full-length Pnmt mRNA. PCR was performed using 10ng of adrenal, first-strand cDNA (SHR or BN.lx), 1.5 mM Mg2+, 0.2 μM forward primer, 0.2 μM reverse primer, 0.2 mM dNTPs, and 2.5 U HotStarTaq (Qiagen, Valencia, CA). PCR amplicons were separated by electrophoresis in a 0.8% agarose gel and visualized with ethidium bromide.

RESULTS

Gene expression differences in hypertension candidate genes

A total of 211 adrenal gene expression profiles of hypertension candidate genes from Affymetrix RAE230A were examined for heritable differences in transcript levels in the RI strains (see Figure 3–1). Candidate genes were grouped according to their function and/or pathway to which they belong. For each expressed gene, heritability (H^2), normalized ratio (*NR*) and *t*-test were calculated. 125 hypertension candidate genes were found expressed in the adrenal dataset, with 64 of those exhibiting heritable gene expression with $H^2 \geq 20\%$. Catecholaminergic genes stood out as the most represented group with 13 out of the 64 heritable transcripts (see Figure 3–2). Some of them exhibited significant progenitor differences (6 genes), and those were uniformly in the direction of underexpression in the SHR. Gene expression levels for all four catecholamine biosynthetic enzymes - *Th*, *Ddc*, *Dbh* and *Pnmt* - were highly heritable ($H^2 \geq 60\%$ for *Ddc*, *Dbh* and *Pnmt*, $H^2 \sim 50\%$ for *Th*) and, with the exception of *Pnmt*, also significantly underexpressed in the SHR. Other significantly SHR-underexpressed genes with heritable transcripts included vesicle-associated membrane protein 1 (*Vamp1*), neuropeptide Y (*Npy*) and catechol-O-methyltransferase (*Comt*). Gene transcript levels for catecholamine biosynthetic enzymes (*Th*, *Ddc*, *Dbh, Pnmt*) and granins (*Chga*, *Chgb*, *Scg2*) from the microarray data were validated by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) in tissues from a separate harvesting of age and sex- matched progenitors (see Figure 3–3). Significant differences were confirmed for *Th*, *Ddc* and

Dbh gene expression, with all three genes showing underexpression in SHR compared to BN.*Lx*.

Enzymatic activity of Dbh and Pnmt in adrenal medulla

To explore whether differences in mRNA levels across the RI panel translate into differences in enzyme activities, Dbh and Pnmt were assayed in adrenal gland homogenate (Figure 3–4). Results are summarized in Table 3–1. Compared to the BN.*Lx*, the activity of both enzymes was significantly decreased in the SHR. Dbh exhibited nearly a two-fold decrease ($NR = -1.8$, $P = 0.003$), with $H^2 = 46\%$ in the RI strains. Pnmt enzyme activity showed a smaller ($NR = -1.3$) but significant ($P = 0.001$) decrease in the SHR, with $H^2 = 34\%$.

Adrenal catecholamine content

Dbh and Pnmt are involved in the two final steps of catecholamine biosynthesis in the chromaffin cell. In order to investigate the effect of Dbh and Pnmt activity changes on catecholamine concentrations, their respective substrates and products, i.e. dopamine (DA), norepinephrine (NE) and epinephrine (EPI), were measured in adrenal tissue of the RI strains and their progenitors (Figure 3–5). Results are summarized in Table 3–1. SHR DA content was significantly increased ($NR = 1.4$, $P = 0.048$), whereas NE was significantly decreased ($NR = -1.4$, $P = 0.002$), when compared to the BN.*Lx*. EPI content did not show differences between the parental strains. Adrenal DA demonstrates much higher heritability ($H^2 = 63\%$) then NE, or EPI ($H^2 = 35\%$ and $H^2 = 36\%$, respectively). Corticosterone is known to stimulate the

gene expression of Pnmt, and to a lesser degree Dbh as well. Adrenal tissue corticosterone content was significantly different between the progenitors, with *NR* = 1.9 and *P* = 0.001. However, the H^2 of adrenal corticosterone content in the RI strains was only 13%.

Correlations among biochemical, physiological and gene expression phenotypes

To examine the extent to which biochemical phenotypes, transcript levels and cardiovascular physiological phenotypes are codetermined in the RI panel, Spearman's rank correlations (ρ) were performed. The results can be found in Table 3–1. Adrenal DA, NE and EPI were significantly correlated across the RI panel. Adrenal DA content was negatively correlated (ρ = −0.408, *P* = 0.031) with adrenal Dbh activity. Adrenal EPI correlated positively with adrenal Pnmt (ρ = 0.429, *P* = 0.023). *Dbh* transcript abundance (probeset ID 1370564_at) was significantly positively (ρ = 0.570, *P* = 0.002) correlated with the adrenal Dbh enzyme activity. There was also a significant negative (ρ = −0.551, *P* = 0.002) correlation between *Dbh* transcript abundance and adrenal tissue DA concentration. *Pnmt* transcript abundance (probeset ID 1371054_at) correlated positively (ρ = 0.436, *P* = 0.02) with adrenal Pnmt enzyme activity. Cardiovascular phenotypes were measured in the RI strains at age 12 weeks using telemetry. Dbh enzymatic activity was negatively correlated with systolic blood pressure (ρ = −0.476, *P* = 0.01). Pnmt enzyme activity was negatively correlated with heart rate (ρ = −0.383, *P* = 0.044), as was adrenal EPI (ρ = −0.550, *P* = 0.002) and DA (ρ = −0.382, *P* = 0.045).

Mapping QTLs for chromaffin cell-expressed genes

Gene transcripts (obtained from microarrays) of chromaffin cell-expressed genes with heritable transcript level variation were treated as quantitative traits and subjected to expression quantitative trait locus (eQTL) mapping. The results are summarized in Table 3–3. There were a total of six significant (including marginally significant) eQTLs (bolded *P*-values in Table 3–3). Uniformly for each of these QTLs, the SHR allele at the eQTL peak locus was associated with a decrease in transcript level. *Cis*- and *trans*-eQTLs were determined by examining the relative position of a gene and its associated eQTL. *Cis*-eQTLs were mapped for *Dbh*, *Pnmt* and *Vamp1*, whereas *trans*-eQTLs were detected for *Ddc*, *Vmat1 Chga* transcripts.

The *Vamp1 cis*-eQTL mapped to chromosome 4 at 152-173 Mbp (95% CI), with a peak LOD = 3.50 and $P = 0.072$, explaining ~ 43% of the transcript abundance variability (see Figure 3–6). *Ddc trans*-eQTL mapped to chromosome 14 at 30-68 Mbp (95% CI), with a peak LOD = 3.43 and $P = 0.040$, explaining 42% of the transcript abundance variability.

Results of integrative analysis of transcript abundance and biochemical parameters for *Dbh* and *Pnmt* are described in the following two subheadings. *Vmat1* and *Chga trans*-eQTLs co-localized with *Pnmt cis*-eQTL and are described together with *Pnmt*.

QTLs for adrenal *Dbh* transcript, Dbh activity and DA concentration cluster on RNO 3p12

A *Dbh cis*-eQTL mapped to chromosome 3 at 1-14 Mbp (95% CI), with a peak LOD = 5.58 and $P = 0.0061$, explaining 60% of the *Dbh* transcript variability (see Figure 3–7A, red curve). Dbh enzyme activity in adrenal tissue mapped to chromosome 3 at 1-12 Mbp (95% CI), LOD = 4.73 and $P = 0.0031$ (see Figure 3–7A, blue curve). The QTL explains 54% of the variability in Dbh enzyme activity (see Figure 3–7B, blue curve). The SHR allele at the peak locus was associated with a decrease in phenotypic value for *Dbh* transcript as well as Dbh enzyme activity. Adrenal DA concentration mapped to chromosome 3 at 2-14 Mbp (95% CI), with a peak LOD = 4.26 and $P = 0.0354$ (see Figure 3–7A, green curve). The QTL explains 50% of adrenal DA concentration variability and the SHR allele at the peak locus was associated with an increase in adrenal DA (see Figure 3–7B, green curve). It is important to note that the QTL confidence intervals for adrenal DA, Dbh activity, and *Dbh* transcript are nearly identical, and contain the *Dbh* gene (see Figure 3–7A+C).

Genetical genomics analysis of adrenal dopamine concentration

To follow-up on the adrenal DA QTL and search, in an unbiased manner, for a positional candidate gene that could explain the observed inter-strain differences, the concept of genetical genomics[45] was applied, seeking functionally-related *cis*-eQTL genes within the confidence interval for the adrenal DA QTL. Because the 95% CI for adrenal DA QTL covers the majority of the short arm of chromosome 3 (RNO 3p), all genes physically located on RNO 3p were considered in this analysis to prevent a type

II error owing to distortion in the genetic map due to positive crossover interference around centromeres[119]. Affymetrix RAE 230A microarray contains probes for 233 genes mapping to RNO 3p. Of those, 149 showed detectable expression in at least one RI strain. The remaining 84 genes were considered not expressed in the adrenal tissue of the RI strains and disregarded for further analysis. The transcript levels of the 149 expressed genes were then treated as quantitative traits and eQTLs for those transcripts were mapped. A total of 13 transcripts exhibited significant *cis*-eQTLs (Table 3–4), thus qualifying as potential positional candidate genes. These 13 candidate genes were then examined for functional relationships with catecholamine metabolism using publicly accessible databases PubMed <www.pubmed.gov> and Kegg Pathway <www.genome.jp/kegg/ pathway.html>. Emerging from this analysis, the most parsimonious candidate, which could directly explain the observed inter-strain differences in adrenal dopamine concentration, was *Dbh*, for which dopamine is the natural substrate. This is further supported by the significant negative correlation between *Dbh* transcript abundance and DA concentration (see Table 3–2).

Regulation of *Pnmt*, *Chga* and *Vmat1* from the *Pnmt* locus on chromosome 10q31

The *Pnmt cis*-eQTL is located on chromosome 10 at 81-101 Mbp (95% CI), with a peak LOD = 2.95, $P = 0.049$, and explains 36% of the *Pnmt* transcript variability (see Figure 3–8A, red curve). Pnmt enzyme activity also demonstrated linkage to chromosome 10 at 81-102 Mbp (95% CI), with a peak LOD = 3.97 and $P = 0.003$ (see Figure 3–8A, blue curve). The QTL explains 48% of Pnmt enzyme activity variability. For both the *Pnmt* transcript abundance and the Pnmt enzyme

activity, the SHR allele at the peak locus was associated with a decrease in trait value. Because of the bimodal distribution of the LOD scores (most likely due to linkage disequilibrium within RNO 10), bootstrap tests were also performed. The results suggested that the evidence for linkage was most pronounced in the more telomeric peak, which we therefore regarded as the most likely position for the QTL. The 95% CI for these LOD peaks contain the *Pnmt* promoter SNP T−529C (see Figure 3–8A+B).

Both *Vmat1* and *Chga* expression profiles mapped to the *Pnmt* region (see Figure 3–8A). *Vmat1 trans*-eQTL mapped to chromosome 10 at 82-101 Mbp (95% CI), with a peak LOD = 3.33, $P = 0.041$, and with 43% of transcript variability attributable to the eQTL. *Chga trans*-eQTL mapped to chromosome 10 at 80-104 Mbp (95% CI), with a peak LOD = 3.50 and $P = 0.093$, explaining 35% of the transcript abundance variability. Importantly, this confidence interval contains the *Pnmt* gene, marked by the promoter SNP T−529C, and is nearly identical with the confidence intervals for *Pnmt*, *Vmat1* and *Chga* eQTLs reported above. The SHR allele at the peak locus was associated with a decrease in transcript levels for both *Vmat1* and *Chga*.

Polymorphism discovery in the *Dbh* and the *Pnmt* genes

Systematic discovery of sequence polymorphism in *Dbh* and *Pnmt* between SHR and BN.*Lx* strains by re-sequencing yielded several single nucleotide polymorphisms (SNPs) and one insertion/deletion (in/del). The results are described

below. Numbers refer to the bp distance from the CAP site; nucleotide change is given as BN.*Lx* → SHR.

Re-sequencing in the *Dbh* gene (see Figure 3–9) revealed 8 variants, all of them SNPs: 3 in the promoter region (T−892G, T−885C and T−551G), 4 in the intronic regions (G2354A, T2513G, G4258T and A15184G), and 1 located downstream of the 3'UTR (G18530A). For the full *Dbh* sequence see Appendix 5.

Re-sequencing in the *Pnmt* gene (see Figure 3–10) showed 7 variants: 4 SNPs (T−529C, T−404C, C−396T and C−351T) and 1 in/del (−457 A/-) in the promoter region, 1 SNP in exon 1 (A209G, synonymous at codon 63, Ala63Ala), and 1 SNP in exon 3 (T1475C, coding for a non-synonymous amino acid change Val285Ala). For the full *Pnmt* sequence see Appendix 6.

All non-coding SNPs were located outside the highly conserved regions across species <http://genome.ucsc.edu>. The non-synonymous coding SNP T1475C identified in *Pnmt*, exon 3, alters the enzyme's last amino acid from valine to alanine. However, this substitution is outside the catalytic domains and is thus likely to be neutral[120].

In-vitro studies on *Dbh* and *Pnmt* promoter polymorphism function

Bioluminescent activity of luciferase was measured in rat PC12 pheochromocytoma cells transfected with *Dbh* or *Pnmt* promoter/ luciferase reporter plasmids, with or without stimulation by secretagogues. For each gene, two variants, which differed in the SNPs identified between SHR and BN.*Lx*, were tested and compared. *Dbh* promoter SNPs did not cause significantly different expression of the

luciferase reporter under several conditions (see Figure 3–11). *Pnmt* promoter SNPs, however, demonstrated a significant functional effect evident after stimulation with dexamethasone, resulting in a lesser increase of luciferase expression in the cells carrying the SHR promoter variant (see Figure 3–12A), congruent with the directional changes of the Pnmt biochemical phenotype. This was replicated in a separate experiment, proving a dose-dependent effect (see Figure 3–12B).

Analysis of QTLs previously mapped to genomic regions supporting the *Dbh* and *Pnmt* QTLs

To explore the extent to which cardiovascular phenotypes studied by others are linked to chromosomal regions delimited by the 95% confidence intervals for the *Dbh* and *Pnmt* QTLs reported here, the Rat Genome Database <http://rgd.mcw.edu> was searched. The results of this analysis are summarized in Table 3–5 for Dbh and in Table 3–6 for Pnmt.

In the *Dbh* region, a total of 17 QTLs overlap wholly, or in part, with the interval spanning 1-14 Mbp, which corresponds to the conflated 95% CIs for the three QTLs on chromosome 3 (Figure 3–7D). There are 14 cardiovascular QTLs (8 blood pressure, 4 cardiac mass, 1 heart rate, 1 for aerobic running capacity), 2 for alcohol consumption and 1 for body weight. QTL symbols, progenitor strains of the populations in which QTLs were mapped, genomic positions, LOD scores and/or *P*-values are detailed in Table 3–5.

In the *Pnmt* region, a total of 27 QTLs overlap wholly, or in part, with the interval 81 – 104 Mbp corresponding to the conflated 95% CIs for the four QTLs on

chromosome 10 (Figure 3–8C). There are 20 blood pressure QTLs, 4 cardiac mass QTLs, 2 stress response QTLs and 1 heart rate QTL. QTL symbols, progenitor strains of the mapping populations, genomic positions, LOD scores and/or *P*-values are detailed in Table 3–6.

Pnmt **alternative splicing**

Finding no difference in Pnmt expression levels combined with significant difference in Pnmt enzyme activity between SHR and BN.lx led us to testing the possibility of alternative splicing of the Pnmt pre-mRNA. However, no difference in length of Pnmt cDNA fragments was found by gel separation.

DISCUSSION

Overview

We have taken an integrative approach to the hereditary basis of complex traits, utilizing both gene expression and protein-level phenotypes to identify sequence variations that influence catecholamine biosynthesis and storage. Our study focused on heritable gene expression and biochemical traits of the sympathoadrenal system that may shed light on the genetic basis of cardiovascular pathology observed in the SHR. We utilized the HXB/BXH RI population to follow the segregation of these traits among strains of varying genetic makeup. The genetic determinants of regulatory networks in the chromaffin cell of the adrenal medulla were examined by linkage analysis. Through integration of gene expression profiling, biochemical phenotyping and quantitative trait locus mapping, *cis*-acting regulatory mechanisms were identified

for the *Dbh*, *Pnmt* and *Vamp1* genes (see Figure 3–2). Analysis of tissue catecholamine concentrations and enzyme activities corroborated these findings for *Dbh* and *Pnmt* and provided a more complete picture of relevant regulatory networks and the interplay between various genes involved in catecholamine biosynthesis. Genetic variants were discovered in *Dbh* and *Pnmt*, which were subsequently tested *in vitro* for functional effects. The *Pnmt* locus also appeared to *trans*-regulate two additional genes, *Chga* and *Vmat1*, both being functionally linked with *Pnmt*.

Vamp1 is a small integral membrane protein of secretory granules that plays a key role in membrane fusion and exocytosis[121]. The c*is*-mediated decrease in *Vamp1* expression described here is likely to result in changes in catecholamine secretion rate. To establish such effect, precise measurement of blood catecholamines would be required. In this study, we did not pursue this line of research. However, it is important to note that a decrease in *Vamp1* transcript was associated with the SHR allele – a common observation in other findings reported here.

Dbh regulation and dopamine concentration in the adrenal gland

Dbh transcript levels positively correlated with Dbh tissue activity (Table 3–2), suggesting that the source of the observed biochemical trait variation lies with differences in gene expression/mRNA stability, rather than enzyme structure. QTL analysis of both gene expression and biochemical data yielded highly significant, overlapping QTLs centered on the *Dbh* gene on chr 3 at 6 Mbp, implying that the differential gene expression is regulated in *cis* (see Figure 3–7). The SHR allele was associated with a lower value for both of these traits, accompanied by an increase in

DA, which is a substrate for Dbh. Viewed from a pathway perspective, increased DA can result either from an increase in quantity or turnover of the upstream enzymes (Th, Ddc), or from a decrease in quantity or turnover of the downstream enzymes (Dbh, Pnmt). Because DA concentration was negatively correlated with Dbh tissue activity, as well as with *Dbh* mRNA levels, the most parsimonious explanation of these findings is that decreased *Dbh* gene expression leads to decreased enzyme level, which in turn results in DA accumulation in the adrenal tissue of the SHR. This is further supported by a QTL for DA tissue concentration mapping to precisely the same region as the Dbh QTLs, but with an opposite allelic association. In addition, a search for dopamine metabolism-related *cis*-eQTL genes was performed within the dopamine QTL region, taking advantage of the adrenal gene expression profiling data (Table 3–4). This search yielded only one plausible candidate – *Dbh*. Furthermore, in the adrenals of the SHR the *Dbh* transcript abundance shows the lowest levels (by RT-PCR) of all catecholamine biosynthetic enzymes – about half the value for *Th* that is traditionally considered the rate-limiting step in catecholamine biosynthesis. In contrast, the *Dbh* and *Th* levels are comparable in BN.*Lx* (see Figure 3–3).

We therefore propose that lower Dbh in the young SHR presents a "bottleneck" in catecholamine biosynthesis, leading to DA accumulation (and catecholamine depletion) in adrenergic cells, which then contributes to the pathogenesis of hypertension. Consistent with this notion, adrenal Dbh activity and SBP correlate negatively in the RI strains. Even though re-sequencing (see Figure 3–9) and subsequent functional *in vitro* testing of discovered *Dbh* SNPs did not lead to

identification of a causative sequence variant (see Figure 3–11), the evidence for *Dbh* region involvement in the adrenal tissue Dbh enzyme activity, and the resulting DA concentration changes, is strong. In addition, the *Dbh* region is enriched in cardiovascular QTLs mapped in various crosses by multiple groups (see Figure 3–7D and Table 3–5), suggesting the presence of an as-yet-to-be-identified important cardiovascular regulatory variant in this immediate region.

The underlying functional mechanism of a strong *cis*-eQTL is likely to operate in all tissues where the gene is expressed[103]. This viewpoint is supported by the findings by others of low Dbh activity in young SHR not only in adrenal glands, but also in heart ventricle and spleen[122], as well as in the brain[123]. Low Dbh activity seems to lead to increased DA, accompanied by decreased NE. Increased brain DA was indeed described in young SHR[124]. Dopamine systems in the brain are known to be involved in central blood pressure control and in integrating limbic information with cardiovascular homeostasis[125]. Stimulation of the region of origin of the mesolimbic dopamine system in the brain, the ventral tegmental area, causes a long-lasting increase in blood pressure[126]. Therefore, an increase in central DA might lead to the development of hypertension. However, the other consequence of low central Dbh activity, i.e. the decrease in NE, can by itself cause a rise in blood pressure. NE has an inhibitory effect on blood pressure elevation in the nucleus tractus solitarii[127]; thus a decrease in NE may be involved in the development and progression of hypertension in the SHR. Decreased noradrenergic activity of sympathoinhibitory neurons in the

anterior hypothalamus may also contribute to exacerbations of hypertension that occur in SHR[128].

Borderline hypertensive humans, in concordance with our rat data, appear to be characterized by increased DA coupled with Dbh suppression[129,130]. Thus, genetically determined variation in Dbh activity, ultimately influenced by a promoter (enhancer) variant, may affect the DA/NE ratio in various tissues, including the basal ganglia, where such changes may have effects on central blood pressure regulation. Indeed, naturally occurring genetic variation at the human *DBH* locus can have profound effects upon blood pressure[131].

Regulation of Pnmt activity and gene expression: Chga and Vmat1 co-regulation

The neurotransmitter and neurohormone EPI is a physiologically active neuroregulator that assumes an important role in the stress response and is a major factor in the pathogenesis of cardiovascular and neuropsychiatric illnesses[132]. The mechanisms by which Pnmt, the biosynthetic enzyme of EPI, is genetically regulated have been of interest in an effort to better understand the pathogenesis of these disorders. The general *Pnmt* region on RNO 10 harbors many cardiovascular QTLs implying the involvement of the region in blood pressure control (see Figure 3–8C). In addition, significant difference in allelic frequencies of *PNMT* promoter SNPs were described between hypertensive and normotensive individuals, suggesting that genetic variation at the *PNMT* locus may play a role in the development of human essential hypertension[133,134].

Pnmt was previously examined as a hypertension candidate gene in the rat[135] but no polymorphisms were discovered between SHRSP and WKY in the *Pnmt* coding region, or the 5'- and the 3'-flanking regions. In the present study, we searched for polymorphisms between SHR and BN.*Lx* strains on the grounds of the observed differences in gene expression levels and enzyme activity, which mapped in *cis* (see Figure 3–8), thus implying a causative variant in (or close to) the *Pnmt* genic region. Positive correlation of *Pnmt* gene expression levels with Pnmt enzyme activity, the discovery of SNP polymorphisms in the promoter, and the lack of functionally significant polymorphism in the coding regions is consistent with changes of quantity, rather than quality of the enzyme and point in towards differences in transcriptional regulation. This is further supported by the discovery that promoter SNPs caused differential gene expression in response to glucocorticoid stimulation *in vitro* (see Figure 3–12). Glucocorticoids are the main regulators of *Pnmt* expression *in vivo*[136]. The intra-adrenal portal vascular system provides the medulla with uniquely high concentrations of glucocorticoids, which are needed to induce *Pnmt* expression resulting in EPI synthesis[137]. Despite the significant differences in Pnmt activity between the parental strains, we did not detect difference in the *Pnmt* transcript levels between the two parental strains in this study. However, there are clear *cis*-acting *Pnmt* transcript level-influencing allelic effects that segregate in the RI strains, as demonstrated by the significant *cis*-eQTL. Variation in the Pnmt locus is therefore likely to be functionally significant for determination of both, *Pnmt* transcription and Pnmt enzymatic activity in the SHR.

The mechanism by which a decrease in Pnmt contributes to hypertension is elucidated by EPI-deficient, Pnmt knock-out mice[138], which become hypertensive under stress, possibly due to decreased central activity of vasodepressor adrenergic neurons of the brainstem, coupled with diminished peripheral (EPI-mediated) vasodilatation. In the present study, we did not find significant correlation between Pnmt activity and blood pressure; however, adrenal Pnmt and its product EPI correlated negatively with heart rate.

An integrative finding of our study is mapping of *trans*-eQTLs for *Chga* and *Vmat1* to the *Pnmt* region. *Chga* is crucial for the formation of secretory granules, within which it stabilizes catecholamines in a storage complex[139]. *Vmat1* function[140] involves transporting DA into the secretory granule for hydroxylation by Dbh to form NE, which is then transported by Vmat1 out of the granule for methylation by Pnmt to form EPI, which is finally transported - again by Vmat1 - into the granule for storage, where it is stabilized by Chga (see Figure 3–2). Chga also participates in autonomic control of blood pressure though its fragment catestatin, which causes inhibition of cholinergic-stimulated catecholamine release. *Chga* knock-out results in hypertension in mouse[141], which is consistent with our results showing low *Chga* expression associated with the SHR allele. *Vmat1* has not so far been implicated in hypertension but was found associated with neuropsychiatric diseases[142,143].

Given the functional coupling of these three genes, it is plausible to put forward a hypothesis that *Pnmt*, *Chga* and *Vmat1* expression are regulated jointly by a variant within the *Pnmt* locus. Our eQTL mapping results implicate the *Pnmt* genomic

region as being central to this proposed co-regulation. Mapping the gene expression levels of *Pnmt*, *Chga* and *Vmat1* to the same locus (see Figure 3–8), as well as the uniform association of low gene expression with the SHR allele for all three genes, seems to corroborate such co-regulation.

Conclusions and perspectives

We present evidence for simultaneous downregulation of the transcription of five genes in the catecholamine biosynthetic pathway in the SHR. Decreased levels of catecholamine biosynthetic genes in the SHR have been described before[95]. The novelty of our study lies in the examination of the genetic basis of gene expression differences of these genes in the HXB/BXH RI strains and finding simultaneous downregulation of several key genes in the young SHR. Furthermore, we established that such downregulations are heritable traits and that decreases in transcript levels are associated with the SHR genotype in each case. We also measured activities of Dbh and Pnmt and found that enzyme activity mirrored gene expression in that they were regulated in *cis*, implying primary, genetically regulated changes in expression of these genes between SHR and BN.*Lx*. Because the tissues for microarray analysis came from different animals from the tissues for biochemistries and RT-PCR, the finding of co-localized expression and biochemical QTLs for Dbh and Pnmt can be regarded as a confirmation of the genetic underpinnings of these traits. Furthermore, the finding of differential gene expression for *Dbh*, *Pnmt* and *Vamp1* in young SHR supports control by inherited variants in the genic regions, rather then gene suppression in response to longstanding blood pressure increase. The downregulation

of these genes is therefore temporally independent of the development of hypertension, and may contribute to the pathogenesis of blood pressure elevation, possibly in part via central mechanisms, since catecholamines exert central vasodepressor actions in the brain stem[127,128]. These results suggest new approaches to characterizing the role of the sympathochromaffin system in essential hypertension.

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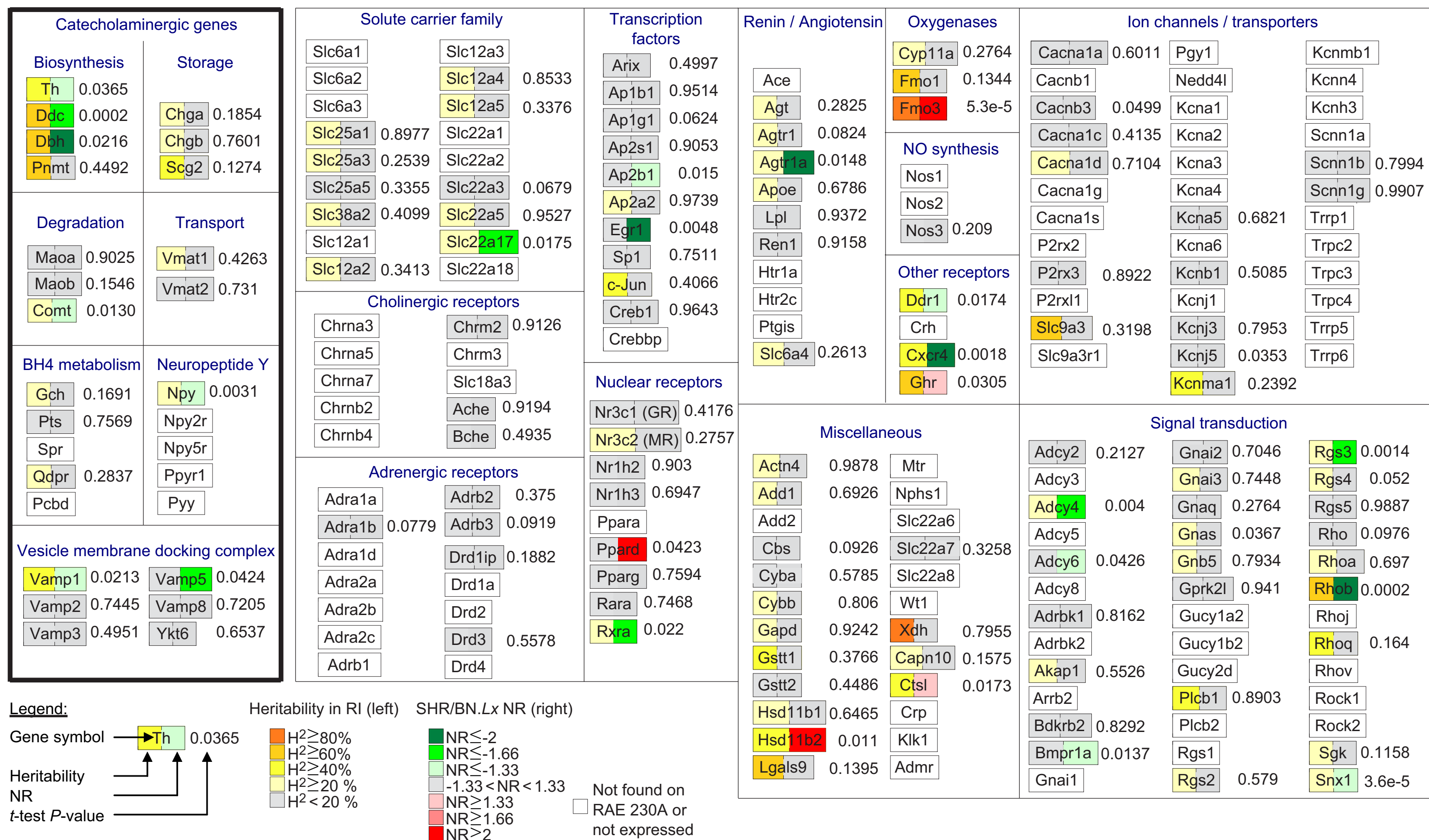


Figure 3-1 Hypertension candidate genes: heritability of gene expression in the RI strains and differential expression in the SHR vs. BN.*Lx* progenitors. Each box represents a candidate gene referred to by its official symbol. Two different parameters are color-coded: the transcript level heritability (H^2) as computed from the RI panel is shown on the left, and the normalized ratio (*NR*) for differences between the progenitors is shown on the right side of each box. Numbers next to boxes are *t*-test *P*-values obtained from progenitor data. Genes are grouped according to their function. Genes were selected with the help of the UCSD NHLBI PPG Program website <elcapitan.ucsd.edu/hyper/index.html>.

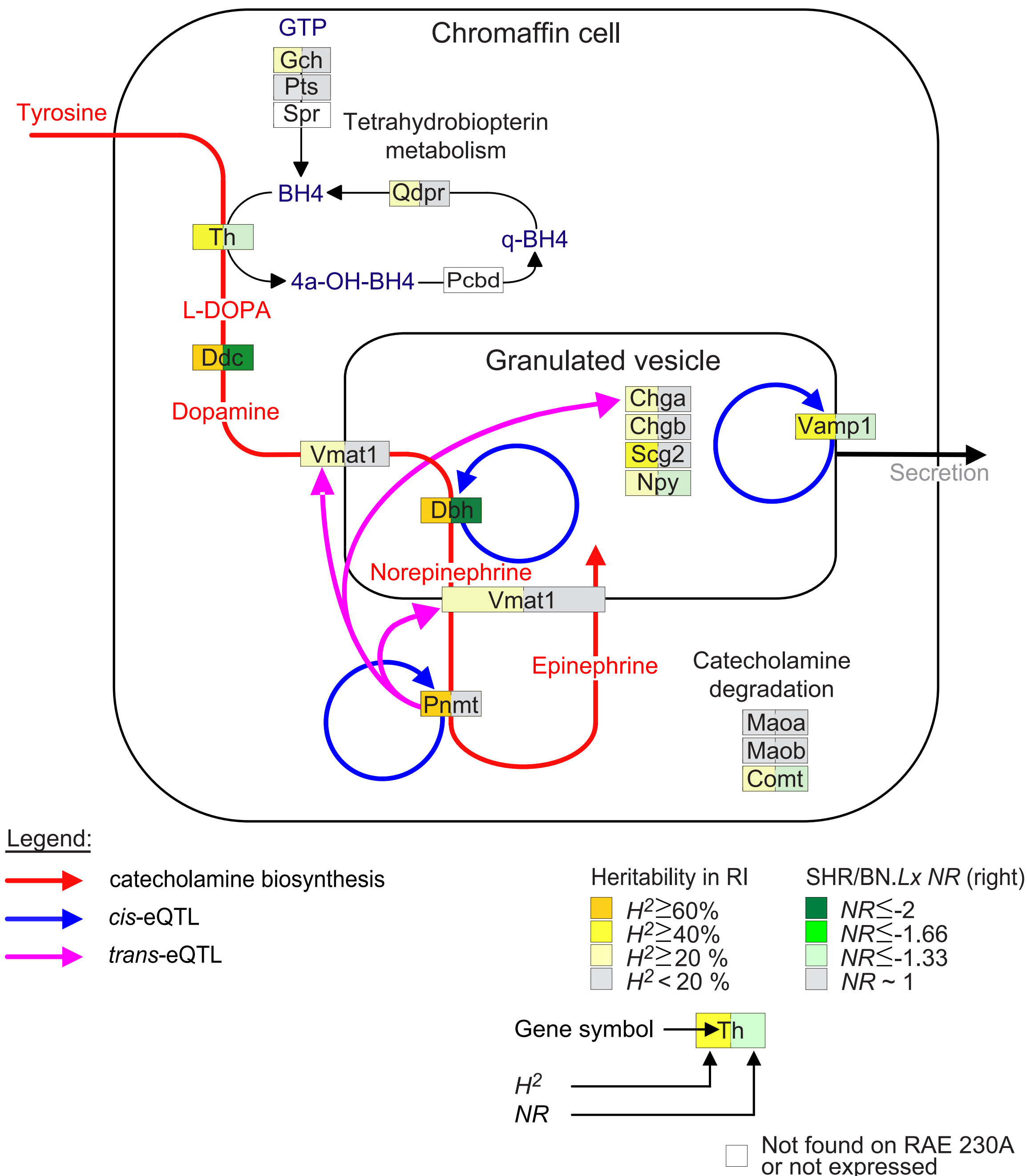
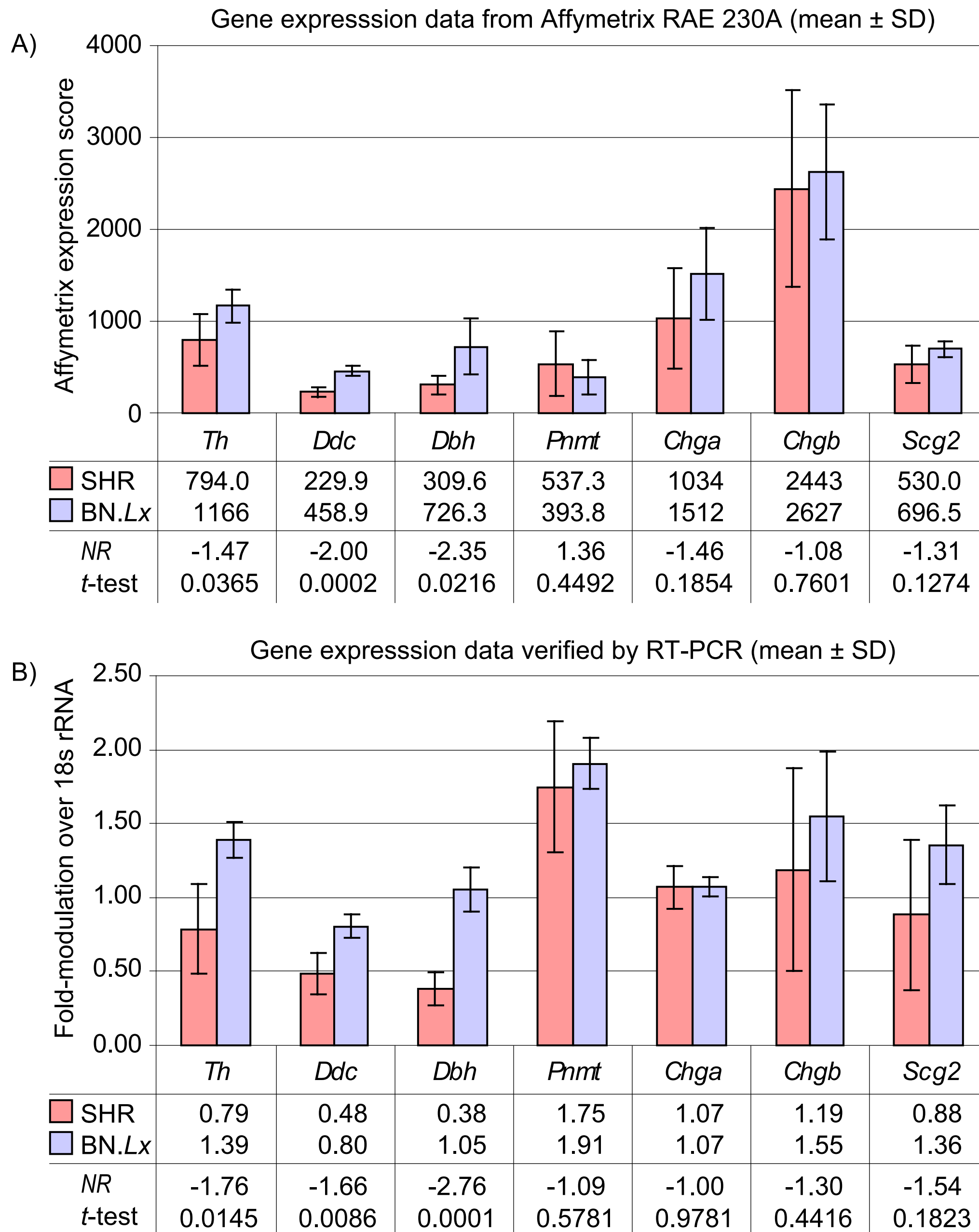


Figure 3-2 Chromaffin cell genes involved in catecholamine biosynthesis, storage, secretion and degradation. Important chromaffin cell-expressed genes and functional relationships between them are depicted. Two different parameters are color-coded: the transcript level heritability (H^2) as computed from the RI panel and the normalized ratio (*NR*) for differences between the progenitors is (on the left and right side of each box, respectively). The red curved arrow represents the catecholamine biosynthetic pathway, with the intermediates also in red. The blue curved arrows represent the detected gene regulatory networks from eQTL analysis, pointing from the regulator to the regulated gene.



A)	*Th*	*Ddc*	*Dbh*	*Pnmt*	*Chga*	*Chgb*	*Scg2*
SHR	794.0	229.9	309.6	537.3	1034	2443	530.0
BN.*Lx*	1166	458.9	726.3	393.8	1512	2627	696.5
NR	-1.47	-2.00	-2.35	1.36	-1.46	-1.08	-1.31
t-test	0.0365	0.0002	0.0216	0.4492	0.1854	0.7601	0.1274

B)	*Th*	*Ddc*	*Dbh*	*Pnmt*	*Chga*	*Chgb*	*Scg2*
SHR	0.79	0.48	0.38	1.75	1.07	1.19	0.88
BN.*Lx*	1.39	0.80	1.05	1.91	1.07	1.55	1.36
NR	-1.76	-1.66	-2.76	-1.09	-1.00	-1.30	-1.54
t-test	0.0145	0.0086	0.0001	0.5781	0.9781	0.4416	0.1823

Figure 3-3 Adrenal gene expression levels of genes vital for catecholamine biosynthesis and storage: Affymetrix data validated by RT-PCR. Data obtained from Affymetrix RAE 230A microarray (A), were subsequently validated by real time-PCR in tissues from different age and sex-matched genetically identical animals (B). Tables underneath the graphs provide the mean values for gene expression in each progenitor, normalized ratios (*NR*) computed from those means, and *t*-test *P*-values calculated from the progenitor strain data.

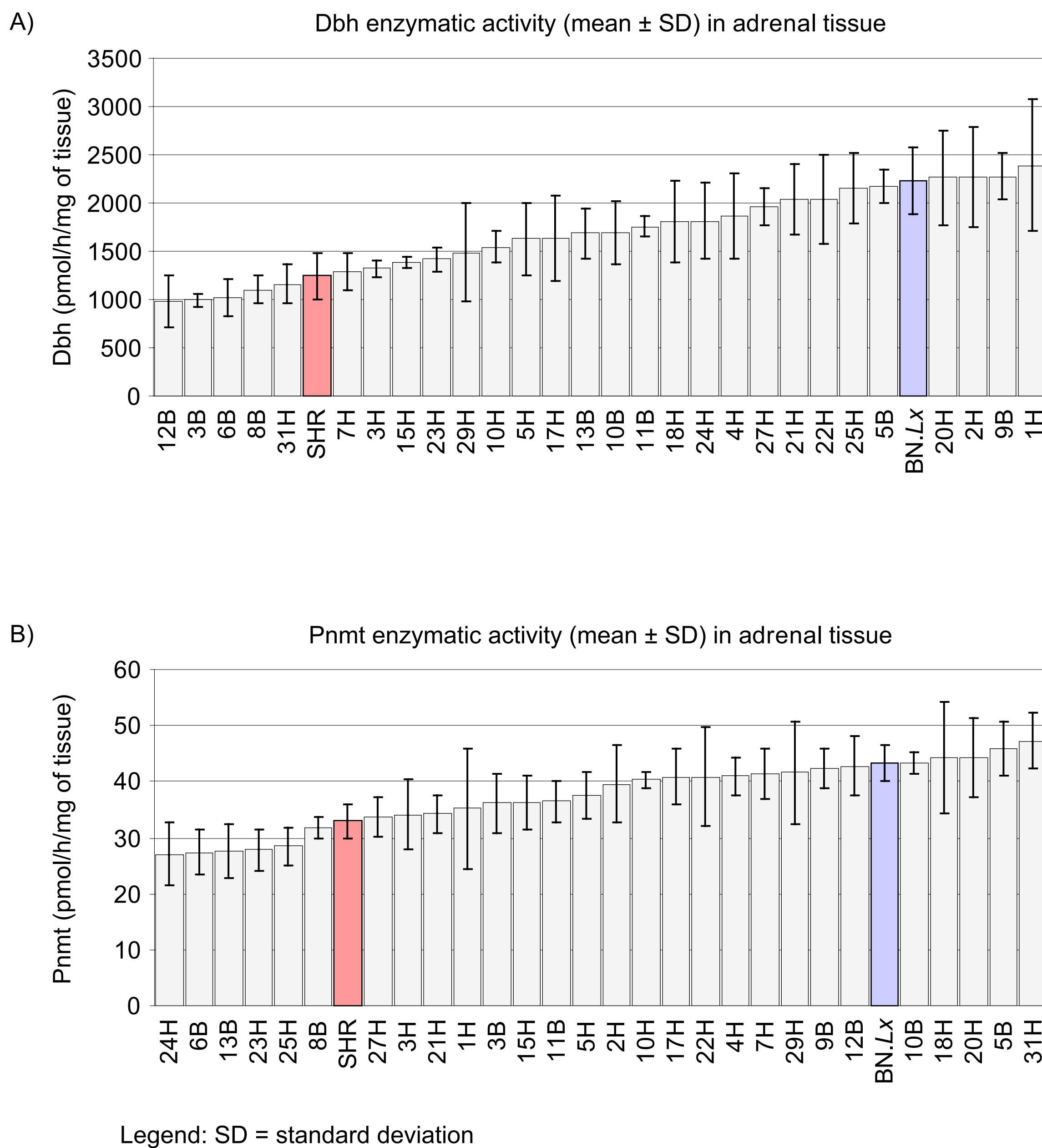


Figure 3-4 Enzymatic activity of dopamine-beta hydroxylase (Dbh) and phenylethanolamine N-methyltransferase (Pnmt) in adrenal tissue. Dbh (A) and Pnmt (B) enzymatic activity was measured in 29 RI strains and two progenitor strains. Strains are ordered left to right according to strain mean. Progenitor strains are highlighted in red (SHR) and blue (BN.*Lx*).

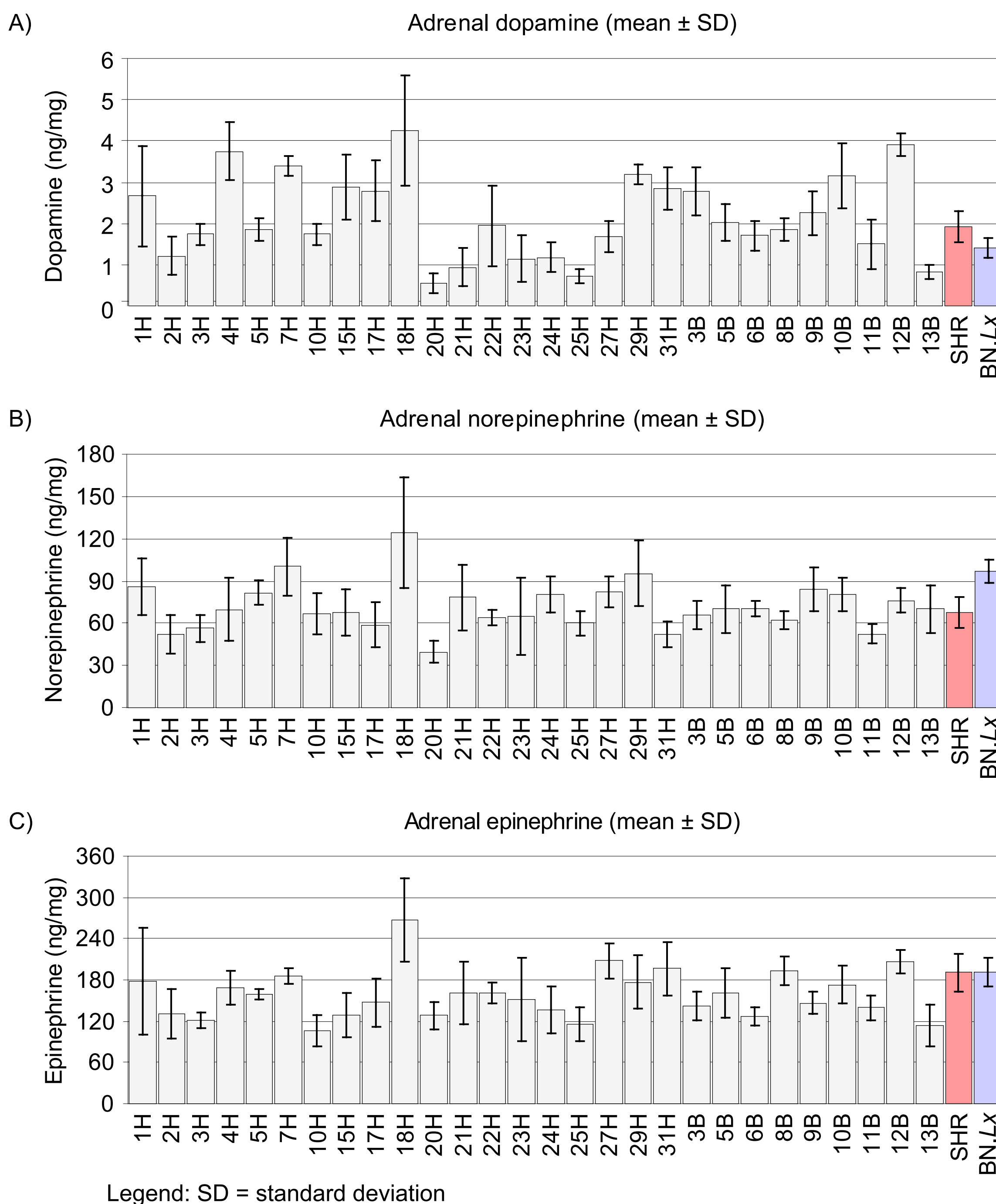


Figure 3-5 Concentration of catecholamines in adrenal tissue. Dopamine (A), norepinephrine (B) and epinephrine (C) concentration was measured in 29 RI strains and two progenitor strains. Bars represent strain means and error bars show standard deviations. Progenitor strains are highlighted red (SHR) and blue (BN.*Lx*).

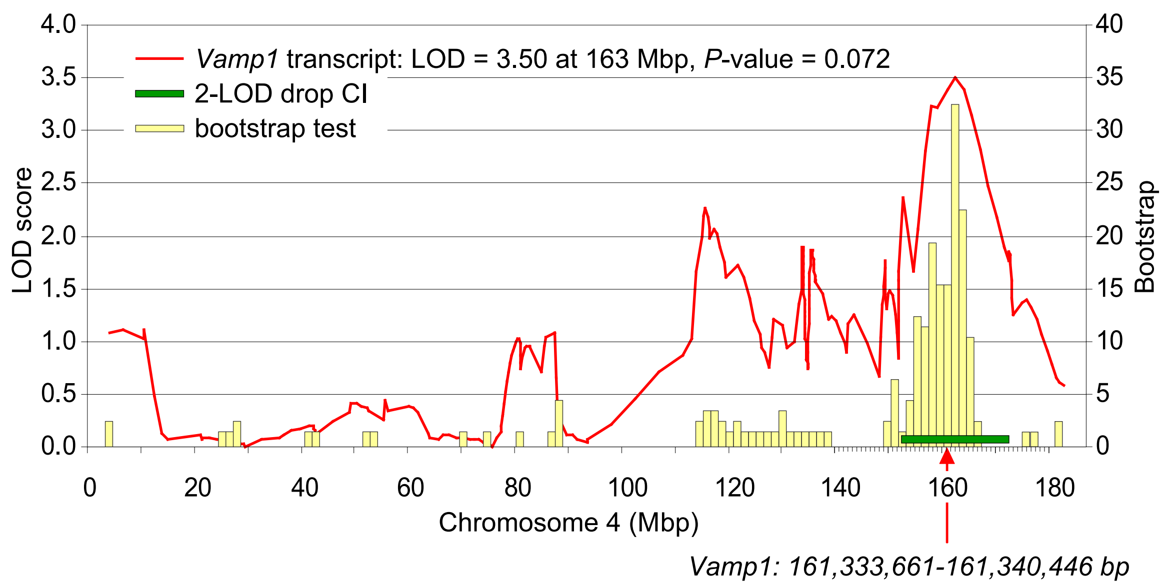


Figure 3-6 *Cis*-acting expression quantitative trait locus for *Vamp1* adrenal transcript. The figure shows the LOD plot for Vamp1 transcript abundance, which peaks over the Vamp1 gene physical location (LOD score = 3.50 and $P = 0.072$). The 95% CI was estimated by 2-LOD drop (green horizontal bar = 152-173 Mbp) and by bootstrap test (yellow vertical bars). The eQTL explains ~ 43% of the transcript abundance.

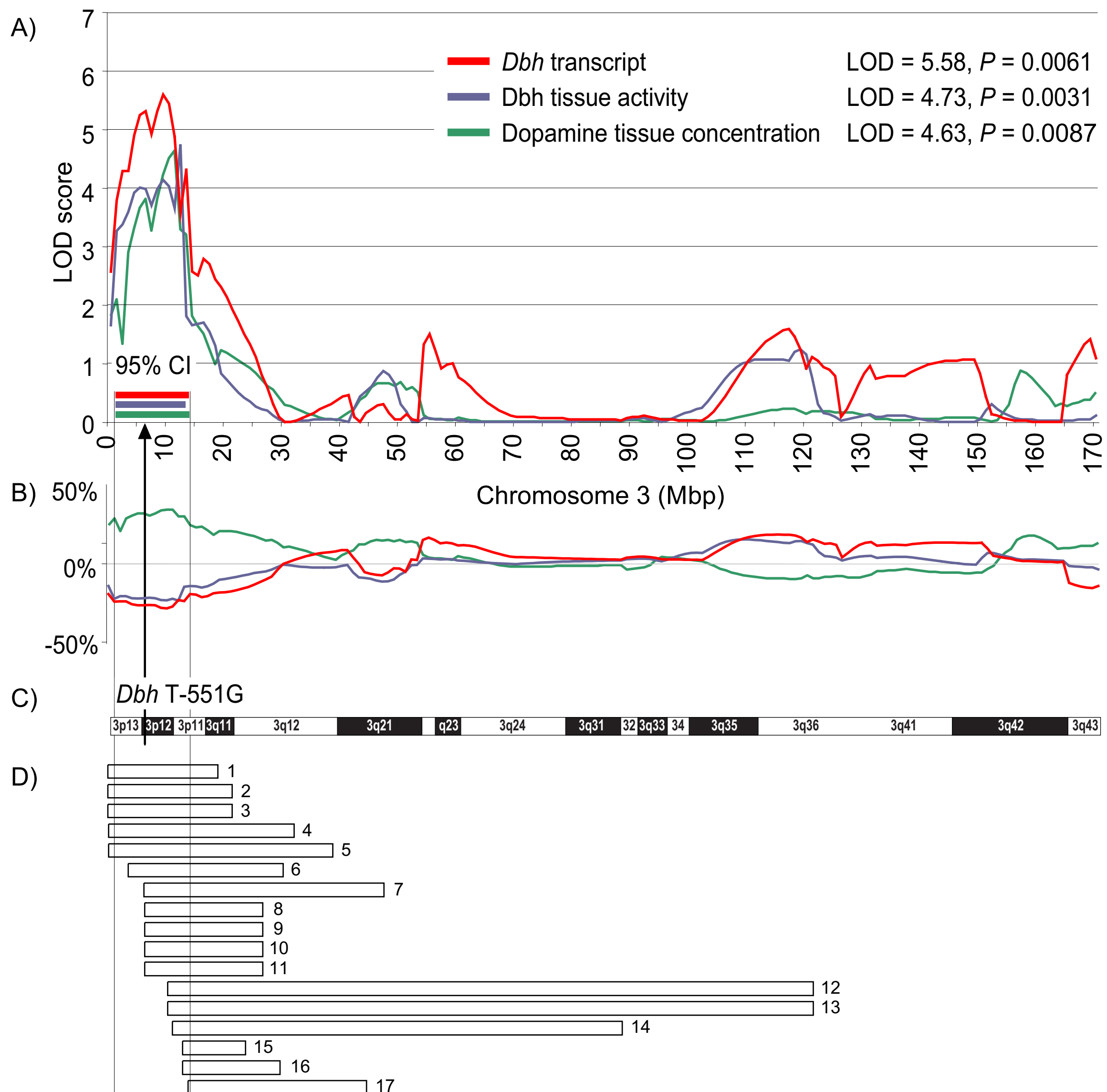


Figure 3-7 Co-localization of physiological and expression QTLs on the short arm of chromosome 3. A) LOD plots for dopamine tissue concentration, Dbh tissue enzymatic activity, and *Dbh* gene expression are shown. Peak LOD values are given in the inset. 95% confidence intervals (95% CI's), determined by 2-LOD drop, are shown beneath the peaks. B) Additive/directional effect of the SHR allele on each trait at different RNO 3 loci is expressed as % deviation from the overall trait mean. The arrow marks the location of the *Dbh* promoter SNP T-551G. C) Chromosome 3 idiogram is provided for reference. D) Combined cross analysis of previously mapped cardiovascular QTLs overlapping with the conflated 95% CI's for the three QTLs reported here. Each bar represents a previously localized physiological QTL (data from http://rgd.mcw.edu). Bar numbers refer to Table 3-5, where details for each QTL can be found.

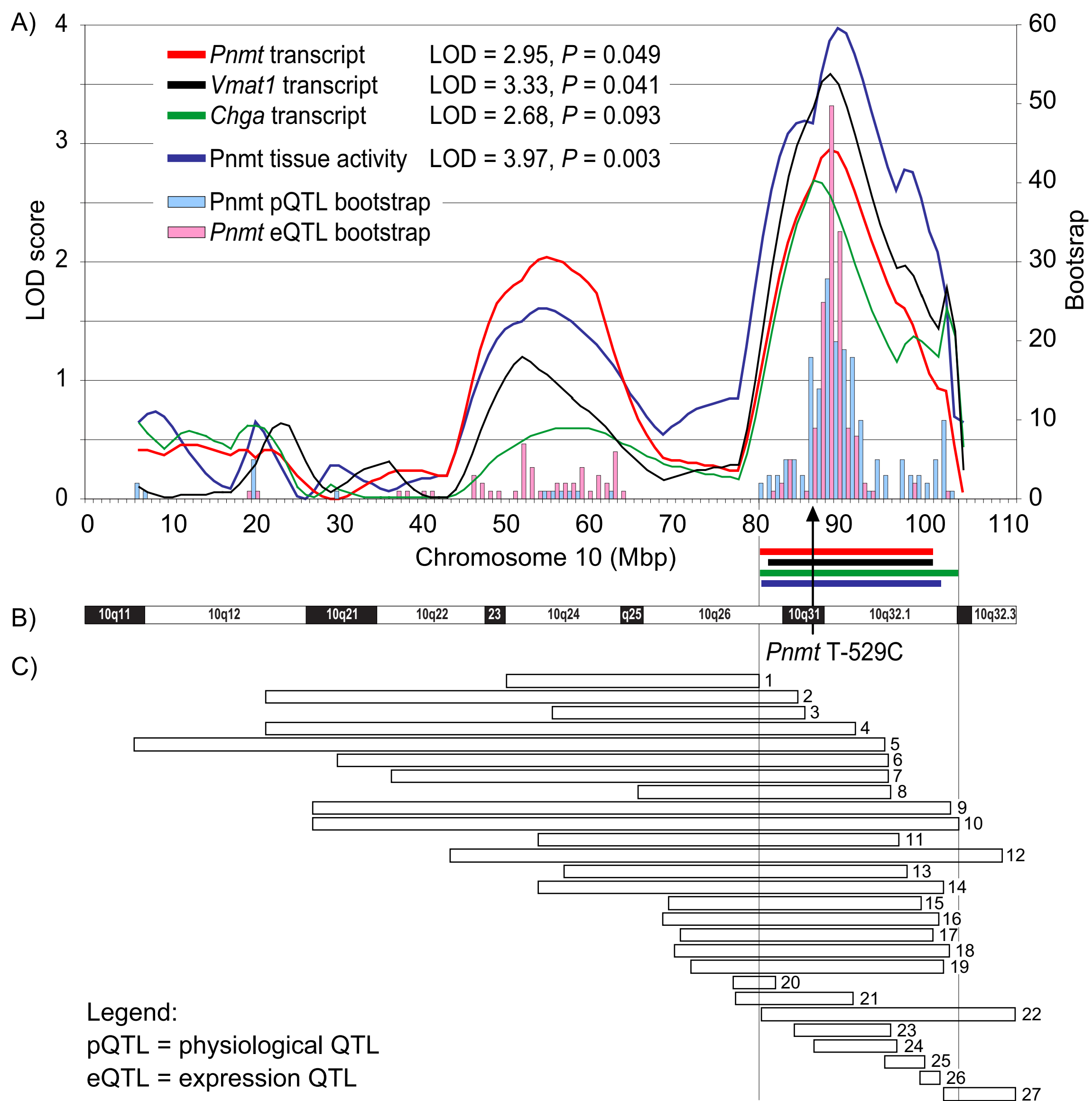


Figure 3-8 Co-localization of physiological and expression QTLs on chromosome 10. A) LOD plots for adrenal Pnmt tissue enzymatic activity and *Pnmt*, *Vmat1 and Chga* transcript abundance. Peak LOD values are given in the inset. Bootstrap test results (vertical bars) were used to estimate the 95% CI's for Pnmt tissue activity and *Pnmt* transcript abundance. Horizontal bars represent 2-LOD drop CI's; different traits are colored same as the LOD plots. SHR allele at the peak locus was associated with a trait value decrease for all four traits. The arrow points to the location of the *Pnmt* promoter SNP T-529G. B) Idiogram of rat chromosome 10 is provided for reference. C) Combined cross analysis showing previously mapped cardiovascular QTLs overlapping with the conflated 95% CI for the QTLs reported here. Each bar represents a physiological QTL (data from http://rgd.mcw.edu). Bar numbers refer to Table 3-6, where details can be found.

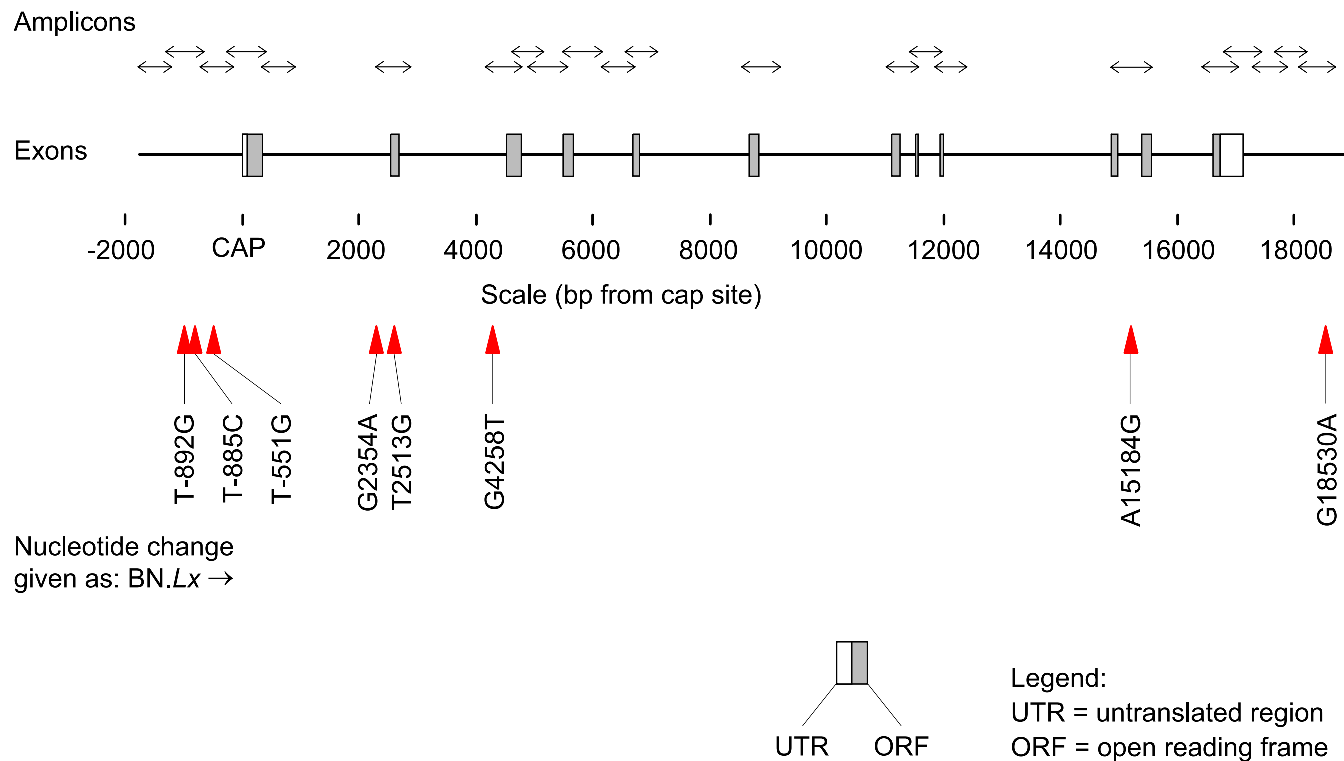


Figure 3-9 Single nucleotide polymorphism discovery at the *Dbh* locus in SHR vs. BN.*Lx* strains. The bp distances refer to the distance from the CAP (transcription initiation) site. Exons are rendered as boxes: grey portions are translated, while empty portions are 5'- and 3'-untranslated. The extent of each of the 22 amplicons (spanning each exon, exon/intron border, ~1.75 kbp of proximal promoter, and ~1.75 kbp of 3' [downstream] sequence) is represented by double-headed arrows. The solid red arrowheads indicate the SNP positions (all variants discovered were SNPs). The nucleotide change is given as BN.*Lx* → SHR.

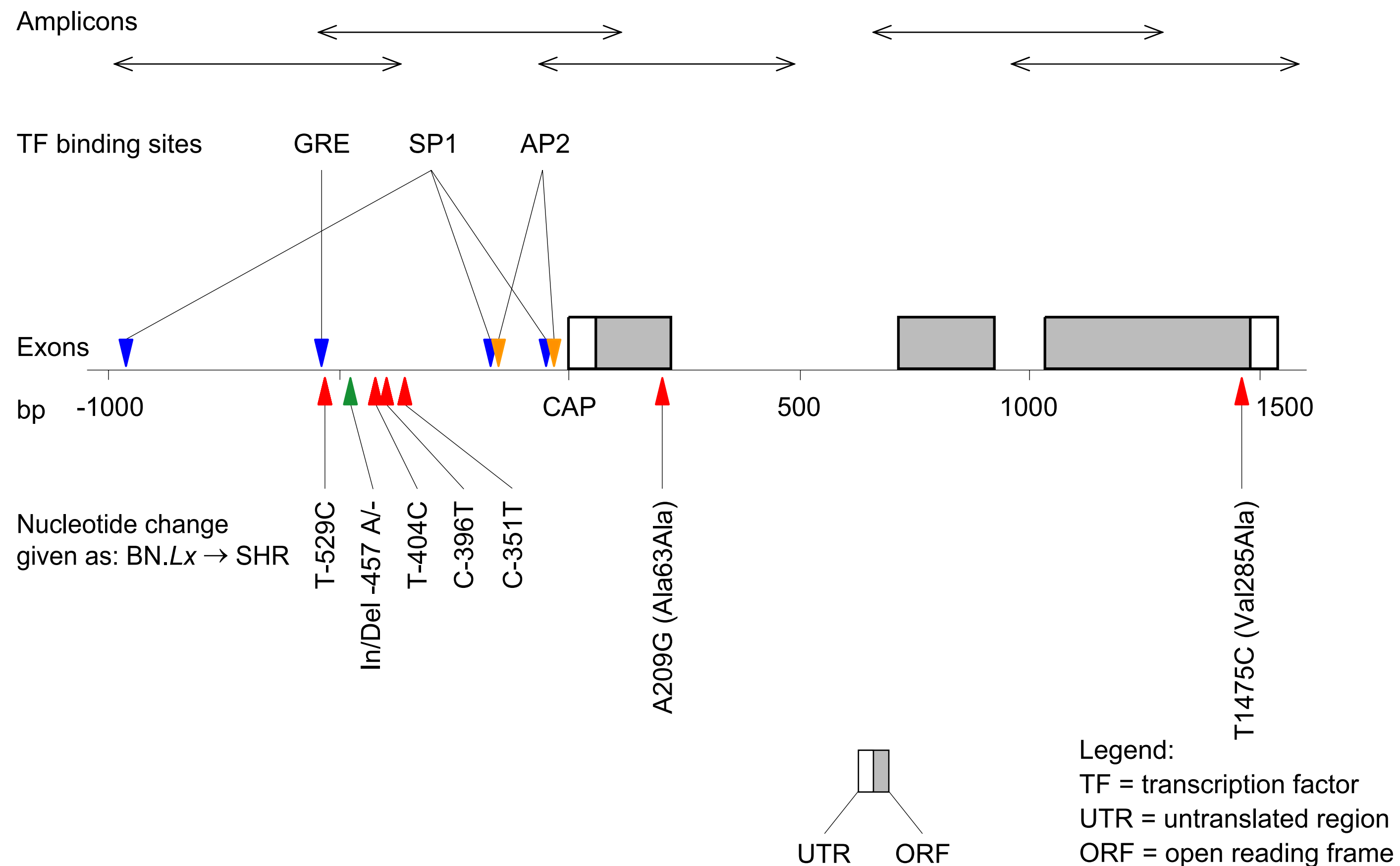
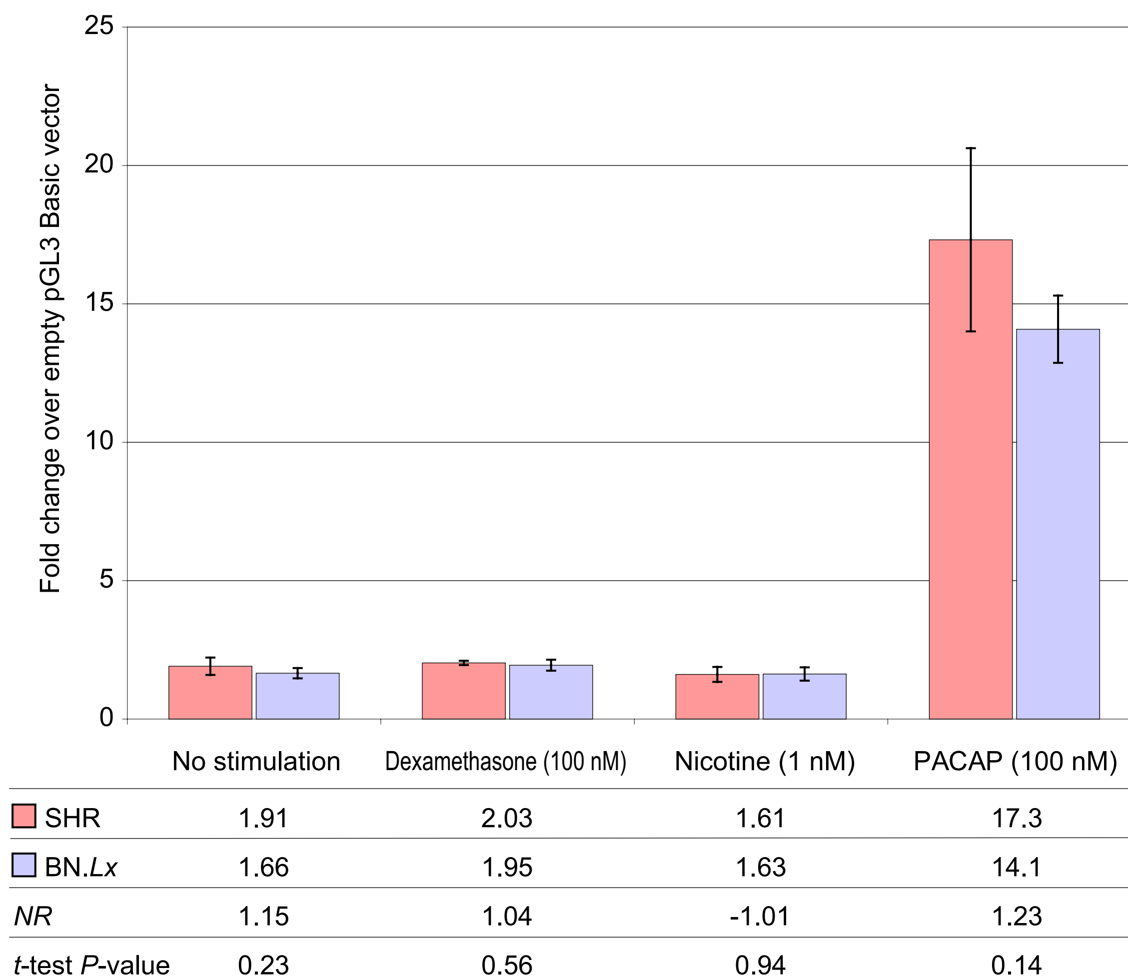


Figure 3-10 Single nucleotide polymorphism discovery at the *Pnmt* locus in SHR vs. BN.*Lx* strains. The bp distances refer to the distance from the CAP (transcription initiation) site. Exons are rendered as boxes: grey portions are translated, while empty portions are 5'- and 3'-untranslated. The span of each of the 6 amplicons is represented by double-headed arrows. The solid red arrowheads indicate the SNP positions, the green arrowhead indicates an insertion/deletion polymophism. The nucleotide change is given as BN.*Lx* → SHR. Amino acid changes in coding region SNPs are given in parentheses. Blue arrowheads indicate important trascription factor binding sites.



	No stimulation	Dexamethasone (100 nM)	Nicotine (1 nM)	PACAP (100 nM)
SHR	1.91	2.03	1.61	17.3
BN.*Lx*	1.66	1.95	1.63	14.1
NR	1.15	1.04	-1.01	1.23
t-test *P*-value	0.23	0.56	0.94	0.14

Figure 3-11 Functional studies on the single nucleotide polymorphisms identified in the *Dbh* promoter. Bioluminescent activity of luciferase was measured in rat PC12 pheochromocytoma cells transfected with ~1.1 kbp segment of *Dbh* promoter/luciferase reporter in pGL3-Basic vector (Promega) after 16 hour incubation with or without secretagogues (dexamethasone, PACAP and nicotine). Each experiment was conducted in 4 replicates, with luciferase results normalized to cell protein in each plate. Results are presented as fold-augmentation (by secretagogue) over the signal from cells transfected with a promoterless (empty) pGL3-Basic vector. No significant differences between SHR and BN.*Lx* were observed.

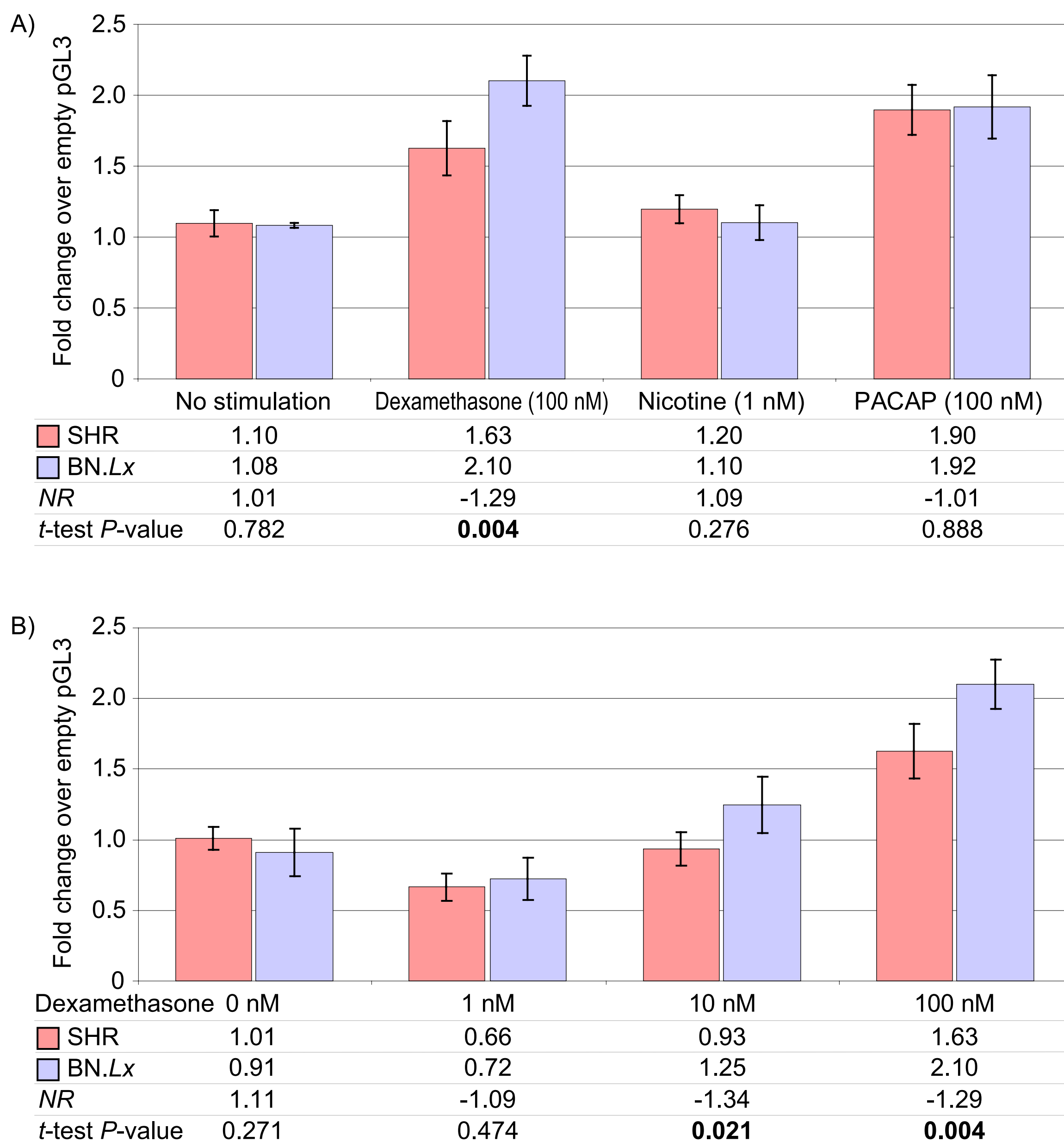


Figure 3-12 Functional studies on the single nucleotide polymorphisms identified in the *Pnmt* promoter. Bioluminescent activity of luciferase was measured in rat PC12 pheochromocytoma cells transfected with ~1 kbp segment of *Pnmt* promoter/luciferase reporter in pGL3-Basic vector (Promega) after 16 hour incubation with or without secretagogues (dexamethasone, PACAP and nicotine). Each experiment was conducted in 4 replicates, with luciferase results normalized to cell protein in each plate. Results are presented as fold-augmentation (by secretagogue) over the signal from cells transfected with a promoterless (empty) pGL3-Basic vector. No significant differences between SHR and BN.*Lx* were observed. (A). Only dexamethasone stimulation elicited significant differences in promoter activity. The experiment was then repeated with incremental doses of dexamethasone (B). Differences between SHR and BN.*Lx* promoters are dose-dependent and were observed starting at 10 nM dexamethasone concentration.

Table 3-1 Biochemical phenotypes measured in adrenal tissue homogenate. HXB/BXH RI strains and the progenitor strains, SHR and BN.*Lx*, were typed for catecholaminergic biochemical parameters in the adrenal gland and in plasma. The table shows progenitor strain means ± SD, *t*-test *P*-value for progenitor strain differences, normalized ratio (*NR*) to indicate the direction of the progenitor strain difference, and heritability (H^2) calculated from RI strain data.

Phenotype	SHR (mean ± SD)	BN.*Lx* (mean ± SD)	*SHR vs. BN.Lx t-test P-value*	$NR_{SHR/BN.Lx}$	*H^2 in RI strains*
DA (ng/mg)	1.92 ± 0.46	1.41 ± 0.23	0.048	1.4	63%
Dbh (nmol/h/mg prot)	69.4 ± 25.2	128.3 ± 11.3	0.003	-1.8	46%
NE (ng/mg)	67.6 ± 10.9	97.6 ± 8.07	0.002	-1.4	35%
Pnmt (pmol/h/mg)	33.0 ± 3.09	43.3 ± 3.29	0.001	-1.3	34%
EPI (ng/mg)	191 ± 27.8	191 ± 20.6	0.994	1.0	36%
Cort (IU/mg)	466 ± 263	244 ± 73.1	0.001	1.9	13%

Legend: DA = dopamine. NE = norepinephrine, EPI = epinephrine, Cort = corticosterone

Table 3-2 Correlations among biochemical, physiological and gene expression phenotypes. Spearman rank order correlations among cardiovascular phenotypes and adrenal biochemical and gene expression phenotypes were computed. Enzymes catalyzing the last two steps of catecholamine biosynthesis, Dbh and Pnmt, and their respective substrates and products (dopamine, norepinephrine and epinephrine) were assayed in adrenal tissue at the age of 6 weeks. *Dbh* and *Pnmt* gene expression levels were measured by Affy RAE230A at the age of 6 weeks. Cardiovascular physiological phenotypes were measured telemetrically at the age of 12 weeks. Correlation coefficients (ρ) and *P*-values (*P*) are given for all phenotype pairs. Significant correlations are flagged with asterisks (* = significant at the 0.05 level; ** = significant at the 0.01 level (2-tailed)).

Spearman's rho		DA	Dbh	NE	Pnmt	EPI	Dbh gene expression	Pnmt gene expression	SBP	HR
DA	ρ		-0.408*	0.423*	0.594**	0.633**	-0.551**	0.345	0.250	-0.382*
	P		0.031	0.025	0.001	0.000	0.002	0.072	0.199	0.045
	N		28	28	28	28	28	28	28	28
Dbh	ρ	-0.408*		0.095	-0.119	0.011	0.570**	0.046	-0.476*	0.050
	P	0.031		0.632	0.545	0.954	0.002	0.816	0.010	0.801
	N	28		28	28	28	28	28	28	28
NE	ρ	0.423*	0.095		0.059	0.463*	-0.330	-0.031	-0.257	-0.205
	P	0.025	0.632		0.764	0.013	0.086	0.875	0.187	0.295
	N	28	28		28	28	28	28	28	28
Pnmt	ρ	0.594**	-0.119	0.059		0.429*	-0.119	0.436*	0.094	-0.383*
	P	0.001	0.545	0.764		0.023	0.547	0.020	0.634	0.044
	N	28	28	28		28	28	28	28	28
EPI	ρ	0.633**	0.011	0.463*	0.429*		-0.386*	0.047	0.013	-0.550**
	P	0.000	0.954	0.013	0.023		0.042	0.814	0.949	0.002
	N	28	28	28	28		28	28	28	28
Dbh gene expression	ρ	-0.551**	0.570**	-0.330	-0.119	-0.386*		0.294	-0.148	0.187
	P	0.002	0.002	0.086	0.547	0.042		0.121	0.443	0.332
	N	28	28	28	28	28		29	29	29
Pnmt gene expression	ρ	0.345	0.046	-0.031	0.436*	0.047	0.294		0.209	-0.276
	P	0.072	0.816	0.875	0.020	0.814	0.121		0.277	0.147
	N	28	28	28	28	28	29		29	29
SBP	ρ	0.250	-0.476*	-0.257	0.094	0.013	-0.148	0.209		-0.079
	P	0.199	0.010	0.187	0.634	0.949	0.443	0.277		0.680
	N	28	28	28	28	28	29	29		29
HR	ρ	-0.382*	0.050	-0.205	-0.383*	-0.550**	0.187	-0.276	-0.079	
	P	0.045	0.801	0.295	0.044	0.002	0.332	0.147	0.680	
	N	28	28	28	28	28	29	29	29	

Legend:
Dbh = dopamine beta-hydroxylase, Pnmt = phenylethanolamine N-methyltransferase, DA = dopamine, NE = norepinephrine, EPI = epinephrine, SBP = systolic blood pressure, HR = heart rate

Table 3-3 Expression QTL mapping results for chromaffin cell-expressed genes with heritable transcript levels in the HXB/BXH RI strains. Genes with heritable transcripts (Figure 3-2, coded yellow/orange) were included in this table. Results for each transcript consist of LOD-peak position, eQTL type, LOD score and *P*-value. For the significant - including marginally significant - eQTLs (bolded *P*-values), the proportion of the variance in transcript level attributable to the eQTL is also given. The last column indicates chromaffin cell-expressed genes within the 95%CI of the mapped eQTLs.

Gene transcript					Mapped expression QTLs					
Transcript (Probe ID)	Gene symbol	Gene position (Chr:Mbp)	progenitor *t*-test *P*-val.	H^2 in RI	eQTL position (Chr:Mbp)	eQTL type	LOD	*P*-value	% variance attributable to eQTL	Genes of interest in the 95%CI
1387075_at	*Th*	1:203	0.0365	48%	8:80	*trans*	2.51	0.176		
1368064_a_at	*Ddc*	14:93	0.0002	70%	14:45	*trans*	3.43	**0.040**	42	
1370564_at	*Dbh*	3:6	0.0216	64%	3:6	*cis*	5.58	**0.006**	60	*Dbh*
1371054_at	*Pnmt*	10:87	0.4492	60%	10:90	*cis*	2.95	**0.049**	36	*Pnmt*
1387999_at	*Vmat1*	16:22	0.4263	23%	10:90	*trans*	3.33	**0.041**	43	*Pnmt*
1387235_at	*Chga*	6:127	0.1854	36%	10:87	*trans*	2.68	**0.093**	35	*Pnmt*
1373510_at	*Vamp1*	4:161	0.0213	53%	4:161	*cis*	3.50	**0.072**	43	*Vamp1*
1368826_at	*Comt*	11:85	0.0130	24%	4:149	*trans*	2.71	0.318		
1368034_at	*Chgb*	3:121	0.7601	25%	8:80	*trans*	3.11	0.124		
1368044_at	*Scg2*	9:79	0.1274	49%	5:16	*trans*	2.11	0.674		
1387154_at	*Npy*	4:78	0.0031	26%	17:78	*trans*	2.14	0.426		
1387221_at	*Gch*	15:23	0.1691	39%	19:48	*trans*	2.12	0.320		
1367695_at	*Qdpr*	14:71	0.2837	28%	10:99	*trans*	3.12	0.149		

Legend:
Probe ID = Affymetrix RAE 230A probeset ID
eQTL = expression QTL
H^2 = heritability calculated in the HXB/BXH RI strains
LOD = logarithm of odds
95%CI = 95% confidence interval for eQTL position

Table 3-4 *Cis*-eQTLs mapped to the short arm of chromosome 3. Adrenal transcripts were assayed by Affymetrix array RAE 230A. Genomic positions (bp) of the genes (left side), as well as of the corresponding eQTLs (right side), are detailed. Linkage significance was determined by permutation test and expressed as *P*-value. The entry criterion for a gene transcript to be reported in this table was a genomic location within 0-17 Mb, corresponding to the short arm of rat chromosome 3. The retention criterion was the presence of a significant ($P < 0.05$) eQTL within the same region.

Genes located on the short arm of chromosome 3, showing a *cis*-eQTL				Marker associated with the *cis*-eQTL		
Probeset ID	Name	Gene symbol (predicted)	Position (start bp)	Name	Position (start bp)	*P*-value
1372773_at	Neural proliferation, differentiation and control 1	*Npdc1*	3571499	*SNP 3_2*	3409637	$4x10^{-6}$
1377049_at	Neuropathy target esterase like 1	*Ntel1*	5122435	*SNP 3_4*	2671921	10^{-6}
1388912_at	RNA exonuclease 4 homolog (S. Cerevisiae)	*Rexo4*	5849797	*Dbh T-551G*	6052155	$2x10^{-5}$
1370564_at	**Dopamine beta-hydroxylase**	***Dbh***	**6052705**	***Dbh T-551G***	**6052155**	$\mathbf{10^{-4}}$
1372323_at	Sarcosine dehydrogenase	*Sardh*	6076165	*Dbh T-551G*	6052155	$2x10^{-5}$
1389816_at	Endonuclease G	*Endog*	9184282	*Dbh T-551G*	6052155	$2x10^{-4}$
1373537_at	Formin binding protein 1 (rapostlin)	*Fnbp1*	10091464	*D3Rat194*	10206859	$9X10^{-4}$
1389713_at	Similar to HLA-B associated transcript-2 isoform a	---	11292039	*SNP 3_8*	10585645	$4x10^{-6}$
1368267_at	Protein-O-mannosyltransferase 1	*Pomt1*	11348812	*SNP 3_8*	10585645	$3x10^{-3}$
1373331_at	Similar to Leucine rich repeat and sterile alpha motif containing 1	*(Lrsam1)*	11862794	*SNP 3_9*	11793545	$3x10^{-4}$
1372267_at	Proteasome (prosome, macropain) 26S subunit, non-atpase, 5	*(Psmd5)*	13861849	*SNP 3_9*	11793545	10^{-3}
1375687_at	RAB14, member RAS oncogene family	*Rab14*	14245692	*SNP 3_11*	14386227	$2x10^{-2}$
1370335_at	Disabled homolog 2-interacting protein	*Dab2ip*	14769583	*D3Rat53*	15057268	$6x10^{-3}$

Table 3-5 Analysis of the *Dbh* region for known physiological QTLs. The conflated 95% CI's for *Dbh* transcript, Dbh activity and dopamine concentration QTLs, and harboring the *Dbh* gene itself (RNO 3p:1-14 Mbp, see Figure 3-7A), was examined for known cardiovascular and cardiovascular-related physiological QTLs. The numbers in the first column correspond to the bars in Figure 3-7D. Progenitor strains used in the various crosses, in which QTLs were mapped, are shown. LOD scores and *P*-values are given where available.

Number in figure 3-7D	Cardiovascular pQTLs mapping to the *Dbh* region RNO 3p:1-14 Mbp. (source: http://rgd.mcw.edu)		Position (Mbp)		Progenitors of crosses in which QTL was mapped		LOD	*P*-val
	QTL symbol	QTL name (trait measured)	start	end	Progenitor 1	Progenitor 2		
1	*Cm10*	Cardiac mass (LV) QTL 10	0.00	19.01	SHR/FubRkb	SS/JrRkb	7.3	10^{-4}
2	*Bp15*	BP (salt loaded systolic) QTL 15	0.00	21.37	SHRSP	WKY	4.4	-
3	*Bp85*	BP (systolic) QTL 85	0.00	21.37	SHRSP/Izm	WKY/Izm	3.1	-
4	*Bp140*	BP (systolic) QTL 140	0.18	32.05	SHR/Snk	WKY/Snk	2.5	-
5	*Bp92*	BP (salt loaded systolic) QTL 92	0.18	38.71	SHR	SS/Jr	2.7	-
6	*Arunc3*	Aerobic running capacity QTL 3	3.48	30.25	COP/OlaHsd	DA/OlaHsd	3.3	-
7	*BpQTLcluster4*	BP (systolic) QTL cluster 4	6.27	47.58	SHR	BN	2.2	-
8	*Cm43*	Cardiac mass (BW adjusted) QTL 43	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	6.7	$2x10^{-5}$
9	*Bw56*	Body weight QTL 56	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	4.5	$3x10^{-8}$
10	*Cm46*	Cardiac mass (BW adjusted) QTL 46	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	6.6	$6x10^{-6}$
11	*Cm48*	Cardiac mass (BW adjusted) QTL 48	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	5.4	$4x10^{-4}$
12	*Bp264*	BP (mean arterial) QTL 264	10.27	121.62	HTG	LEW	4,0	-
13	*Bp251*	BP (mean arterial) QTL 251	10.27	121.62	HTG	LEW	2.8	-
14	*Hrtrt17*	Heart rate (salt loaded) QTL 17	11.14	88.65	SHRSP	WKY	3.8	-
15	*Alc19*	Alcohol consumption QTL 19	12.91	23.76	P	NP	4.4	-
16	*Alc8*	Alcohol consumption QTL 8	12.91	29.75	P	NP	5.9	$5x10^{-5}$
17	*Bp151*	BP (decreased) QTL 151	13.85	44.55	SS	LEW	-	$4x10^{-2}$

Legend: BP = blood pressure, BW = body weight, LV left ventricle, BN = Brown Norway, COP = Curtiss rat, DA = "d" blood group and agouti color, HTG = Prague hypertriglyceridemic (from WKY), LEW = Lewis rat, NP = Alcohol-nonpreferring, P = Alcohol-preferring, SHR = Spontaneously hypertensive rat, SHRSP = Stroke prone spontaneously hypertensive rat, SS = Salt Sensitive, WKY = Wistar Kyoto rat

Table 3-6 Analysis of the *Pnmt* region for known physiological QTLs. The conflated 95% CI's for Pnmt pQTL and *Pnmt*, *Chga* and *Vmat1* eQTLs, which also harbors the *Pnmt* gene itself (RNO 10q:81-104 Mbp, see Figure 3-8A), was examined for known cardiovascular and cardiovascular-related physiological QTLs. The numbers in the first column correspond to the bars in Figure 3-8C. Progenitor strains used in the various crosses, in which QTLs were mapped, are shown. LOD scores and *P*-values are given where available.

Number in figure 3-8C	Cardiovascular pQTLs mapping to the *Pnmt* region RNO 10q:81-104 Mbp. (source: *http://rgd.mcw.edu*)		Position (Mbp)		Progenitors of crosses in which QTL was mapped		LOD	*P*-val
	QTL symbol	QTL name (trait measured)	start	end	Progenitor 1	Progenitor 2		
1	*Bp12*	BP QTL 12	50.04	80.04	SS/Jr	LEW	6.3	10^{-4}
2	*Bp57*	BP QTL 57	21.51	84.56	MHS/Gib	MNS/Gib	5.0	-
3	*Bp71*	BP QTL 71	55.50	85.50	SS	LEW	-	$4x10^{-2}$
4	*BpQTLcluster9*	BP QTL cluster 9	21.51	91.48	SHR	BN	2.9	-
5	*Bp186*	BP QTL 186	5.92	94.98	SS/JrHsdMcwi	BN/SsNHsd	3.6	-
6	*Cm31*	CM QTL 31	29.97	95.37	SS/JrHsdMcwi	BN/SsNHsd	3.9	-
7	*Bp76*	BP QTL 76	36.42	95.38	SS/Jr	MNS	-	10^{-4}
8	*Bp87*	BP QTL 87	65.71	95.71	SHRSP/Izm	WKY/Izm	4.5	-
9	*Bp168*	BP QTL 168	27.09	102.70	SS/Jr	LEW	5.5	-
10	*Bp82*	BP QTL 82	27.09	103.67	SS/Jr	MNS	6.8	-
11	*Cm51*	CM QTL 51	53.79	96.59	SS/Jr	MNS	3.0	-
12	*Stresp5*	SR QTL 5 (corticosterone)	43.37	108.89	F344/NHsd	LEW/NHsd	3.0	$3x10^{-4}$
13	*Cm33*	CM QTL 33	56.93	97.59	LH/Mav	LN/Mav	2.8	-
14	*Bp1*	BP QTL 1	53.78	101.85	WKY	SHRSP	5.1	-
15	*Cm44*	CM QTL 44	69.26	99.26	WKY/Tkyo	SHRSP/Tkyo	4.8	$4x10^{-5}$
16	*Hrtrt21*	HR QTL 21	68.53	101.29	SHR/Ola	BN.*Lx*/Cub	2.4	-
17	*Bp72*	BP QTL 72	70.65	100.65	SS	LEW	-	-
18	*Bp249*	BP QTL 249	69.99	102.59	SS	MNS	-	10^{-4}
19	*Bp91*	BP QTL 91	71.96	101.96	SS	MNS	-	10^{-4}
20	*Bp150*	BP QTL 150	77.01	82.00	SS	LEW	-	10^{-4}
21	*Bp45*	BP QTL 45	77.25	91.18	SHR/Mol	BB/OK	23.2	-
22	*Bp9*	BP QTL 9	80.36	110.36	SS/Jr	MNS	4.8	10^{-4}
23	*Bp134*	BP QTL 134	84.26	95.69	SHRPS	WKY	-	10^{-3}
24	*Stresp7*	SR QTL 7 (catecholamines)	90.46	92.46	HTG	BN	3.52	-
25	*Bp149*	BP QTL 149	94.98	99.70	SS	LEW	-	10^{-4}
26	*Bp137*	BP QTL 137	99.11	101.47	SS	MNS	-	10^{-2}
27	*Bp250*	BP QTL 250	101.85	110.72	SS	MNS	-	10^{-4}

Legend: BP = blood pressure, CM = Cardiac mass, HR = heart rate, SR = stress response, BB = Diabetic strain from outbred Wistar rats, BN = Brown Norway, F344 = Fischer rat, HTG = Prague hypertriglyceridemic (from WKY), LEW = Lewis rat, LH = Lyon Hypertensive, LN = Lyon normotensive, MHS = Milan hypertensive strain, MNS = Milan normotensive strain, SHR = Spontaneously hypertensive rat, SHRSP = Stroke prone spontaneously hypertensive rat, SR = Salt Resistant (from a Sprague-Dawley outbred colony), SS = Salt Sensitive, WKY = Wistar Kyoto rat

Chapter 4

The identification of *trans*-acting genes that influence the abundance of multiple transcripts simultaneously

PREFACE

The results contained within this chapter have been presented as a poster at the Genomes, Medicine, and the Environment in San Diego, CA, October 8-10, 2007, and are being prepared for publication.

ABSTRACT

A novel multivariate distance-matrix regression (MDMR) method was applied to identify loci influencing the expression levels of multiple genes. Adrenal transcriptome obtained from the HXB/BXH rat recombinant inbred strains via microarrays was subjected to expression quantitative trait locus (eQTL) mapping. Of the total of 1085 significant eQTLs detected at the genome-wide P-value ≤ 0.05, 593 eQTLs mapped in *trans*, defined as more than 10 Mbp from the gene's physical location. Examination of the *trans*-eQTL distribution along the genome revealed a significant *trans*-eQTL clustering on chromosomes 1, 3, 8, 11, and 17. These clusters consisted of between 16 and 21 transcripts each, suggesting that specific *trans*-acting loci control the transcript abundance of the genes within these clusters. Several transcripts significantly predicted the variability within the detected clusters, among them genes involved in pre-mRNA processing, such as RNA binding motif protein 16 (*Rbm16*) located within the *trans*-eQTL cluster on chromosome 1, and pre-mRNA processing factor 4 homolog B (*Prpf4b*) located within the *trans*-eQTL cluster on chromosome 17. This example demonstrates the utility of the MDMR method for investigation of transcriptome regulation and underscores the importance of integrated data analytic approaches to understanding complex molecular genetic processes.

INTRODUCTION

Variation in gene transcription is an important contributor to phenotypic differences, including disease susceptibility. Gene transcript abundance is directly influenced by polymorphisms in *cis*-acting elements such as the promoters, as well as in the *trans*-acting factors such as the genes coding for proteins directly involved in mRNA synthesis and post-transcriptional modification. Genetic variants in some *trans*-acting factors tend to exhibit pleiotropic effects, i.e. they affect many transcripts simultaneously and therefore act as "master regulators" of a large number of genes. Genome-wide detection and characterization of the master regulators will improve our understanding of an important source of natural phenotypic variation.

In this study, we used an adrenal tissue gene expression dataset obtained from 29 rat recombinant inbred strains of the HXB/BXH rat panel[52] to identify loci influencing gene transcript variation by mapping *expression quantitative trait loci*, or eQTLs, on a genome-wide basis. We then focused on the genome-wide distribution of the eQTLs. Non-uniformity of eQTL distribution allowed us to define *cis*- and *trans*-eQTL clusters and hence potential master regulators.

A novel multivariate distance-matrix regression method (MDMR) was applied to the *trans*-eQTL clusters to detect and precisely localize genomic regions potentially harboring master regulators, as a prerequisite for future polymorphism discovery and functional studies in these regions. The MDMR analysis essentially works by combining the gene expression profiles of jointly regulated genes from each *trans*-eQTL cluster into one composite variable, which is subsequently regressed on

expression profiles of the *cis*-eQTL genes and on marker genotypes, which are taken as predictors from the master regulator candidate regions. *Cis*-eQTLs are an important resource for the identification of positional candidate genes[144]. This approach is designed to reduce the impact of noise (contained in the data for each gene expression profile) on the accuracy of eQTL positioning, while allowing the similarity between the co-regulated profiles to become the main parameter on which localization of the master regulator candidate regions is based. Considering marker genotypes as predictors in the MDMR analysis provides an additional means of interrogating the candidate regions and validating the results obtained with *cis*-eQTL gene expression profiles.

MATERIAL AND METHODS

RI strains and tissues

The set of 29 RI strains used in this study was produced by inbreeding between members of the F2 generation resulting from the cross of the two highly inbred strains BN.*Lx*/Cub and SHR/Ola[52] (see Chapter 1, page 12), designated here as BN.*Lx* and SHR. Rats were housed in an air-conditioned animal facility and allowed free access to standard laboratory chow and water. All experiments were performed in agreement with the Animal Protection Law of the Czech Republic (311/1997) and were approved by the Ethics Committee of the Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic. Animals were killed at 6 wk of age. Tissues from 4-6 males

of each RI strain were harvested, immediately frozen in liquid nitrogen, and stored at −80 °C.

Adrenal tissue gene expression dataset

HXB/BXH RI strains were profiled for gene expression in adrenal tissue using the Affymetrix Gene Chip array RAE 230A (Affymetrix, Santa Clara, California, USA). The original experimental design is discussed in Hübner et al.[32] and Petretto et al.[103]. We used a total of 128 microarrays: 4-5 animals × 31 (i.e., 29 RI strains + progenitor strains). cRNA was labelled and run on RAE 230A Affymetrix GeneChip arrays (number of transcripts 15,923). Gene expression summary values for Gene Chip data were computed using the Robust Multichip Average algorithm[104]. The adrenal dataset has been submitted to ArrayExpress and an ArrayExpress identifier is pending.

Probe set selection for the final dataset

Probe sets that did not reach $P \leq 0.05$ for gene expression quality assessment in at least one RI strain, as evaluated by the Affymetrix microarray suite version 5 software (MAS5), were removed to increase the ratio of true positives to false positives[145] and to avoid phantom eQTL detection for genes not expressed in the adrenal tissue. Further more, in order to avoid misclassification of *cis*- and *trans*-eQTLs, only genes that could be reliably located to unique genomic locations by using Ensembl[146] database were included. The resulting dataset used for analyses presented herewith consisted of 9931 adrenal tissue-expressed and genome-located transcripts.

Statistical analysis and heritability calculation

T-test*s* were performed using MS Excel 2004 (Microsoft, Redmond, WA) to appraise differences between the progenitor strains, the SHR and the BN.*Lx*.

Heritability (H^2) of gene expression was calculated using an approach designed for RI strains that corrects for the inbreeding incurred during the RI strain production[111]. Because H^2 calculated in a traditional way (V_G/V_P) tends to be overestimated when compared to H^2 obtained from F2 population, to obtain an F2 comparable estimate, the H^2 was calculated as $(V_P - V_E)/(V_P + V_E)$.

Normalized Ratio (*NR)* was used to compare gene expression profile means for the progenitor strains. It is a variant of the simple fold-change value, e.g., [SHR]/[BN.*Lx*], but it transforms the values between 0 and 1 into values between –INF(infinity) and –1 by inverting them and multiplying by negative one. It is calculated as follows: if [SHR] ≥ [BN.*Lx*], then *NR* = [SHR]/[BN.*Lx*]; if [SHR] < [BN.*Lx*], then *NR* = –[BN.*Lx*]/[SHR]. The main advantage of *NR* is its symmetry about zero.

Genetic effect of each eQTL was calculated as a ratio of *additive effect* to the overall trait average in the RI strains. Additive effect is an estimate of the absolute change in transcript abundance that would result from a substitution at the eQTL peak locus of allele of one type with that of another. Thus, the genetic effect value represents a proportion of change from the average RI value, if grouped by either of the progenitor alleles at the eQTL peak locus.

Mapping of eQTLs

Genome-wide linkage analysis was performed using the QTL Reaper[113] program. A set of 1,047 non-redundant genetic markers with known genomic positions (Mbp), which was produced by merging the framework marker-based map [47] with a newly typed SNP dataset[51] (see Chapter 2). For each transcript the likelihood ratio statistics for linkage was calculated. Permutation analysis [114] with 100,000 permutations was used to assess the probabilistic significance of the linkages and to correct for multiple testing across genetic markers to obtain a genome-wide corrected *P*-value. For each eQTL, allelic (additive) effect was calculated as the difference of means of two groups resulting from grouping of the RI strains according to the allele at the marker peak of linkage.

Definition of a *cis*-acting vs. *trans*-acting eQTL

Due to the limitations in the ability to precisely position eQTLs in this type of experimental cross, we arbitrarily define a *cis*-acting eQTL as having the peak of linkage within ±10 Mbp of the physical location of the corresponding gene, i.e. within the total window of 20Mbp (~ 15 cM), and a *trans*-acting eQTL as having the peak of linkage outside the 20 Mbp window on the same chromosome, or on a different chromosome from where the probeset is located.

Detection of eQTL clusters

Clustering of eQTLs was computed by counting eQTLs that map to the same genomic regions. Precise localization of eQTLs is often confounded by the

inaccuracies of the genetic map. A 10 Mbp sliding window was used to compensate for these inaccuracies. The frequency distribution of eQTL counts was plotted against the genomic position of the 10 Mbp window center. *Cis*- and *trans*-eQTLs were evaluated separately. To establish which eQTL clusters were significantly larger than expected by chance alone, normal distribution was estimated from the data and the significance threshold was set at $P \leq 0.01$.

Multivariate distance-matrix regression analysis (MDMR)

Multivariate distance-matrix regression analysis (MDMR), based on multiple regression analysis of multivariate symmetric distance matrices, was performed using the DISTLM v.5 computer program[147]. The methodology was first described by McArdle and Anderson[148] and its statistical properties were further explored by Zapala and Schork[149,150].

Description of the input data. The MDMR analysis requires two distinct inputs: the *predictor variables*, represented in this work by SNP genotypes or expression values of *cis*-eQTL genes, and the *response variables*, represented by the expression values of *trans*-eQTL genes. Input data are in the form of matrices. The following is a formal mathematical description of the matrices and contsructs used in the MDMR methodology. Boldface is used to indicate matrices or vectors in the following notation.

Let **Y** be an $N \times P$ matrix of response variables on N individuals (i.e., individual rat strains in the present setting) for P *trans*-eQTL genes. Let **X** be an $N \times Q$ matrix of predictor variables on N individuals for Q SNPs (or, alternatively, *cis*-eQTL

genes), whose relationship to the response variables is of interest. Matrices **Y** and **X** serve as input for the DISTLM program, which then performs a permutation test for the multivariate null hypothesis of no relationship between matrices **Y** and **X**, using permutations of the observations.

Distance matrix formation. To asses the level of cross-correlation of the gene expression profiles within each *trans*-eQTL cluster, a *similarity matrix* was computed from pairwise Pearson correlation coefficients. Let $\mathbf{R} = r_{ij}$ be an $N \times N$ similarity matrix harboring the correlation coefficients, r, for each pair of gene expression values to be reflected in the matrix. This similarity matrix can then be converted to a *distance matrix*. Let $\mathbf{D} = d_{ij}$ be an $N \times N$ matrix of distance measures d calculated from observation units of **Y**, where

$$d_{ij} = \sqrt{2(1 - r_{ij})} \tag{5}$$

This simple mathematical transformation results in a distance matrix with metric properties. Next, Gower's centered matrix[151] **G** is calculated from **D** in two steps. First, let $\mathbf{A} = a_{ij} = (-\tfrac{1}{2} d_{ij}^2)$, then, the matrix **A** is centered as follows:

$$\mathbf{G} = \left(\mathbf{I} - \frac{1}{n}\mathbf{11}'\right)\mathbf{A}\left(\mathbf{I} - \frac{1}{n}\mathbf{11}'\right) \tag{6}$$

where **1** is a N-dimensional column vector whose every element is 1 and **I** is an ($N \times N$) identity matrix.

Projection matrix formation. The projection matrix (or "hat" matrix)[152] **H** is used to estimate coefficients relating the predictor variables to response variables (i.e., the strain gene expression profiles whose similarities are reflected in **G**) in multiple-regression context. This matrix is calculated from the predictor values (i.e., SNPs or *cis*-acting genes thought to be the master regulators for the *trans* clusters) in matrix **X** as follows:

$$\mathbf{H} = (\mathbf{X'X})^{-1}\mathbf{X'} \tag{7}$$

Regression of the distance matrices on the projection matrix. An appropriate *F* statistic for assessing the relationship between the *Q* predictor variables and variation in the dissimilarities among the *N* subjects with respect to the *P* variables is

$$F = \frac{tr(\mathbf{HGH})}{tr[(\mathbf{I}-\mathbf{H})\mathbf{G}(\mathbf{I}-\mathbf{H})]} \tag{8}$$

where **H** is a hat matrix, **G** is Gower's centered matrix, and **I** is the identity matrix, formed as above.

Assessing statistical significance. The distribution of the *F* statistic defined in Equation 8 is complicated and its derivation for any particular distance matrix is unlikely to generalize to other distance matrices, especially with small sample sizes. Therefore, permutation tests were used to evaluate the probabilistic significance of an observed *F* statistic. *P*-values were computed by recalculating the *F* statistic for 100,000 random re-orderings (permutations) of the observations (i.e., the rows and columns of **G**), while keeping **X** and **H** constant.

The rationale behind the two groups of predictors. Initial eQTL mapping localizes the eQTLs into 10Mbp 'bins'. However it is impossible to establish from this mapping whether $eQTL_1$ which maps to SNP_1 and $eQTL_2$ which maps to an adjacent SNP_2, are in fact not regulated from same regulatory locus that is neither of those SNPs, but rather is linked to these SNPs. Using SNP marker genotypes as predictors we essentially attempt an even finer mapping utilizing a synthetic dependent variable based on the gene expression profiles of all genes within each of the *trans*-eQTL clusters. The regression portion of the MDMR analysis resembles composite interval mapping, in that the genotypes between known SNPs are imputed using the *'addinterval'* function in the QTL Reaper[113] program.

Using *cis*-eQTL gene expression profiles as predictors is a direct way of testing specific candidate genes. *Cis*-eQTL genes within *trans*-eQTL clusters are prime candidates for *trans*-acting master regulator candidate genes[144].

RESULTS

Of the 15923 transcripts represented on the RAE 230A rat microarray used to profile the HXB/BXH RI strain set, 9931 genes were both expressed and could be reliably localized to a unique genomic location. Only these 9931 localized and expressed genes were used to generate the results described herein.

The *cis*-eQTL/*trans*-eQTLs ratio depends on the genome-wide threshold

A total of 1085 eQTLs were detected (Table 4–1) at a genome-wide significance level of $P \leq 0.05$, established by 100,000 permutations. Of these,

492 (45.35%) mapped in *cis*, and 593 (54.65%) mapped in *trans*. However, with the use of an increasing level of stringency for genome-wide significance, the proportion of *cis*- and *trans*-eQTLs changed quickly in favor of *cis*-eQTLs. At the significance level of $P \leq 10^{-3}$ more than 90%, and at the $P \leq 10^{-5}$ a full 95% of all detected eQTLs were *cis*-acting (Table 4–1). A graphical representation of this trend can be seen on Figure 4–1, on which eQTL peak locations (Mbp) are plotted against their respective transcript gene locations (Mbp), at four different significance thresholds. The results essentially provide a map of adrenal transcriptomic regulation. At a low genome-wide *P*-value, most of the eQTL peaks line up along the diagonal, signifying an overrepresentation of the *cis*-eQTLs (Figure 4–1A). When relaxing the genome-wide *P*-value, a commensurate increase of the proportion of *trans*-eQTLs can be seen (Figure 4–1B+C). At the genome-wide $P \leq 0.05$, the number of *cis*- and *trans*-eQTLs is comparable (Figure 4–1D, Table 4–1). Vertical patterns, which correspond to *trans*-eQTL clusters (see below), are becoming more pronounced. Further relaxation of the genome-wide *P*-value ($P > 0.05$) leads to a preponderance of *trans*-eQTLs. The total number (regardless of the *P*-value) of *trans*-eQTLs in the dataset was 9044, compared to 887 *cis*-eQTLs (Figure 4–2A). Comparing these numbers with the numbers in Table 4–1, it is clear that a much larger proportion of the overall detected *cis*-eQTLs (492/887 = 55%) satisfies the $P \leq 0.05$ threshold than is the case for the *trans*-eQTLs (593/9044 = 7%).

Heritability (H^2) of gene expression as a predictor of eQTL effect

The distribution of H^2 for the abundance levels of different transcripts and its relationship to the eQTL genetic effect was examined. A scatter plot of heritability vs. eQTL effects reveals that the *trans*-eQTLs exhibit lower H^2 and a lower eQTL effect sizes, compared to the *cis*-eQTLs. This trend is even more pronounced if just the significant eQTLs are plotted ($P \leq 0.05$), (Figure 4–2B). Heritability of gene expression cannot serve as a direct predictor of eQTL genetic effect, but it clearly delineates its lower limit (Figure 4–2), especially for transcripts with $H^2 > 0.5$.

Median heritability (H^2_{median}) of gene expression differs for *cis*- and *trans*-eQTLs

We explored the relationship of H^2 of transcript abundance to eQTL detectability, i.e., the probability that the eQTL will be detected as significant ($P \leq 0.05$). With an increasing stringency for genome-wide significance of eQTLs, the median heritability (H^2_{median}) of the gene expression traits that still exceed the significance threshold also increased. The *cis*-eQTL transcripts displayed markedly higher median heritability ($H^2_{median} = 0.45$), compared to the *trans*-eQTL transcripts ($H^2_{median} = 0.18$) at the genome-wide significance level of $P \leq 0.05$. The differences in H^2_{median} between the two groups diminished at more stringent genome-wide significance levels, but there was a significantly lower number of *trans*-eQTLs that exceeded the stringent cut-off (Table 4–1).

Clustering of *cis*- and *trans*-eQTLs

The distribution of eQTLs across the genome was assessed by counting eQTLs within a 10 Mbp sliding window. *Cis*- and *trans*-eQTLs were evaluated separately. We found that eQTLs were not distributed evenly. Regions with relative eQTL paucity alternated with eQTL-rich regions. In addition, there were a total of five regions exhibiting an agglomeration of *trans*-eQTLs (Figure 4–3) and one exhibiting an agglomeration *cis*-eQTLs (Figure 4–4) that exceeded the cutoff for significant enrichment $P \leq 0.01$ (corresponding to the count of 15 eQTLs). We term these regions *trans*- and *cis*-eQTL clusters, respectively.

Clustering *trans*-eQTLs

Trans-eQTL clusters (TC) were defined by the regions into which the *trans*-eQTLs mapped and by the genes whose transcript levels appear to be regulated from these regions. TC1 mapped to RNO1: 37 – 47 Mbp and consisted of 16 gene transcripts (Table 4–2); TC2 was located on RNO3: 6 – 16 Mbp and contained 21 genes (Table 4–3); TC3 was found on RNO8: 5 – 15 Mbp and included 17 genes (Table 4–4); TC4 localized to RNO11: 25 – 35 Mbp comprising 16 genes (Table 4–5); finally, TC5 mapped to RNO17: 30 – 40 Mbp and consisted of 21 gene transcripts (Table 4–6).

All TCs were examined by the Promoter Analysis and Interaction Network Toolset (PAINT V 3.5) <http://www.dbi.tju.edu/dbi/tools/paint> for common transcription factor (TF) binding motifs. TF binding motifs which could explain co-

regulation of all transcripts within the TCs were not identified, suggesting the absence of a simple direct TF-based regulatory mechanism.

Testing the *trans*-eQTL clusters by MDMR

To try to determine the exact location of the *trans*-regulatory locus, we applied the MDMR method described above. This method allows one to combine the individual rat strain gene expression profiles of a set of genes (i.e., the *trans*-regulated genes) into a distance matrix which captures the degree to which these profiles are correlated. This matrix is then regressed on predictors such as genotypes or *cis*-acting gene expression values in order to find those that best explain the outcomes. An advantage of this approach is that errors that are inherent to every gene expression experiment can be mitigated by comparing multiple outcomes, thus enabling a more precise detection by means of enrichment in the overrepresented signal, while the signal from outliers is suppressed. In our analysis, we used two separate sets of predictors: the SNP genotypes (which were also used to map the eQTLs) and the *cis*-eQTLs gene profiles that were found within the 10Mbp window of the respective *trans*-eQTL clusters.

Evaluation of the results. The SNPs as predictors in effect test for position of the regulatory element, and the *cis*-eQTLs gene profiles test if specific candidate *cis*-eQTL genes are strongly correlated with the *trans*-eQTL genes in the matrix. Thus, a strong candidate gene should both lie under a peak for linkage between SNP genotypes and the *trans*-eQTL matrix, and it expression profile should exhibit a strong

correlation with the *trans*-eQTL expression profile similarity matrix. Below are the results for individual TCs.

MDMR results for TC1. Regressing the trans-eQTL gene similarity matrix on genotypes yielded significant (*P* = 0.00007) pseudo F-statistic peaking at 38-40 Mbp of RNO1, the peak SNP explaining 36% of the variation in the TC1 gene transcripts (Figure 4–5). Two cis-eQTL genes were found within the TC1 genomic region. TC1 gene expression similarity matrix was regressed on gene expression profiles of both genes (Table 4–8). *Rbm16* gene predicted 29% of the variation in the TC1 gene transcripts (*P* = 0.0003). The statistic for second gene, *Tctex1*, was not significant (*P* = 0.1).

MDMR results for TC2. Regression of the TC2 gene similarity matrix on genotypes did not produce a clear peak (Figure 4–6). A total of 16 *cis*-eQTL genes were mapped within the TC2 genomic region and all were tested for correlation with TC2 gene expression similarity matrix (Table 4–8). *Brd3*, *Rab14* and *1388624_at* exhibited the highest correlation with the TC2 genes.

MDMR results for TC3. Regressing the TC3 gene expression similarity matrix on genotypes yielded significant (*P* = 0.000011) pseudo F-statistic peaking at 6.5-9.5 Mbp of RNO8, the peak SNP explaining 29% of the variation in the TC3 gene transcripts (Figure 4–7). Four *cis*-eQTL genes were found within the TC3 genomic region. The TC1 gene expression similarity matrix was regressed on the specific gene expression profiles of these four genes. The probesets *1372805_at*, *1371763_at* and

1398460_at (unknown genes) were significantly correlated with the TC3 gene transcripts (Table 4–8).

MDMR results for TC4. Regressing the trans-eQTL gene expression similarity matrix on genotypes yielded significant (P = 0.000002) pseudo F-statistic peaking at 30-35 Mbp of RNO11, with the peak genotype explaining 41% of the variation in the TC4 gene transcript expression levels (Figure 4–8). A total of six significantly correlated *cis*-eQTL genes were found within the TC4 genomic region (Table 4–8). *Tmem50b* showed the strongest correlation (P = 0.00001) and predicted 45% of the variation in the TC4 gene transcript gene transcript expression levels. Three other genes (*Atp5o, Slc5a3* and *Cbr1*) and two probesets (unknown genes, *1390364_at* and *1370947_at*) were also highly correlated with the TC4 gene matrix (Table 4–8).

MDMR results for TC5. Regressing the trans-eQTL gene expression similarity matrix on genotypes yielded significant (P = 0.000102) pseudo F-statistic peaking at 34-38 Mbp of RNO17, the peak genotype explaining 23% of the variation in the TC5 gene transcripts (Figure 4–9). A total of five significantly correlated cis-eQTL genes were found within the TC5 genomic region (Table 4–8). *Prpf4b* showed the strongest correlation (P = 0.00001) and predicted 34% of the variation in the TC5 gene transcripts. Two other genes (*Bphl* and *Nqo2*) and two probesets (i.e., unknown genes, *1388884_at* and *1373672_at*) were also highly correlated with the TC5 gene matrix (Table 4–8).

No gene ontology enrichment was observed for genes within the five detected *trans*-eQTL clusters (i.e., gene expression profiles reflected used as response variables

in the MDMR analysis), suggesting simultaneous regulation of functionally unrelated genes by a general (pathway non-specific) cellular regulatory mechanism, consistent e.g. with pre-mRNA processing.

A *cis*-eQTL cluster on RNO20

Exploration of the distribution of *cis*-eQTLs within a 10 Mbp sliding widow yielded only one prominent *cis*-eQTL cluster at the genome wide significance of $P = 0.01$, mapping to RNO20p12 at 0-10 Mbp and consisting of 23 transcripts (Figure 4–4 and Table 4–7). The vast majority of these transcripts belong to the major histocompatibility complex (MHC) of the rat, known also as the RT1 complex. The remaining transcripts correspond to MHC-unrelated genes interspersed among the RT1 genes. The genomic arrangement of the MHC genes with the intervening MHC-unrelated genes appears to be conserved in human, rat and mouse[153]. The transcripts belonging to the *cis*-eQTL cluster display a wide spectrum of additive effects, ranging from high positive (72%) to low negative (−94%), and these differences exists even among physically close genes, suggesting independent transcription regulation of these genes (Table 4–7).

DISCUSSION

Analyzing the transcriptome of a tissue provides a glimpse into the functional state at the moment of harvest. Comparing transcriptomes from different tissues or strains, or from same strain under different environmental conditions, can yield insights into which genes are differentially expressed under specific circumstances.

Taking this paradigm one step further, one can utilize genetic linkage methodology to map loci controlling differential gene expression by assembling gene expression data on different individuals or strains that have been genotyped at various genetic loc[45]. This approach can potentially uncover new regulatory pathways and mechanisms that would not be ascertainable by the traditional transcriptome comparisons.

Global gene expression

A gene can be differentially expressed either due to variation in the genic region itself (*cis*-acting elements, detectable as *cis*-eQTLs), or due to variation in one or more of the many other genes that participate in the transcriptional process (*trans*-acting elements, detectable as *trans*-eQTLs). A key finding from this study is the difference in the statistical strength of the *cis*- and *tras*-eQTLs (Table 4–1). When no *P*-value filtering is applied, the majority of the detected eQTLs appear to map in *trans*. However, increasing the *P*-value stringency leads to an increase in the proportion of *cis*-eQTLs, and beyond certain *P*-value ($P \leq 0.0001$) the vast majority of eQTLs map in *cis*. The explanation of this phenomenon lies most probably in the complexity of the transcription regulation connected to the *trans*- and *cis*-acting elements. The *trans*-acting elements are likely pleiotropic and physically, as well as functionally, removed by several regulatory steps, each of which can be modified by other genetic factor, all of which ultimately weakens the effect of the mapped *trans*-eQTLs. However, the *cis*-factors operate at short distance and are most likely involved predominantly in transcription factor or microRNA binding, which has a direct effect on transcription levels or transcript stability.

It can be assumed that each *cis*-eQTL underlies at least one *trans*-eQTL, and that each *trans*-eQTL therefore has its controlling *cis*-eQTL. *Trans*-eQTL clustering can be seen as a reflection of the pleiotropy of some genes on the level of transcript abundance. Based on this assumption, one would expect to see the following general categories of genes among the candidates for *trans*-acting *cis*-eQTL genes: 1) transcription factors, 2) microRNAs, 3) functionally related genes that could elicit a regulatory (compensatory) response to one another, and 4) genes that are involved in vital cellular processes, such as energy metabolism, transcription, translation etc. A related observation supporting such interpretation may be the fact that the *trans*-eQTLs appear to form clusters, whereas the *cis*-eQTLs appear to be distributed in a much more uniform fashion across the genome, with one notable exception, the RT1 complex, which is discussed separately below.

The leading candidates as far as statistical and functional significance from the MDMR analysis of the adrenal transcriptome are two genes involved in pre-mRNA processing, *Rbm16* and *Prpf4b*, which significantly correlate with TC1 and TC5, respectively, and their molecular function is extremely relevant to the measured phenotype, i.e., mRNA abundance. These two genes are discussed below in detail. MDMR results with TC2 and TC3 failed to yield plausible candidates. TC4 data point to several possible candidate genes (*Tmem50b, Atp5o, Slc5a3 and Cbr*), some of which fall into the general categories mentioned above, but their functional link to mRNA abundance is rather unclear and indirect.

Discussion of the pre-mRNA processing genes

Rbm16 (RNA binding motif protein 16) is also known as CTD-binding SR-like protein rA8, or SR-like CTD-associated factor 8 (SCAF8[154]). Rbm16 binds the repetitive carboxy-terminal domain (CTD) of RNA polymerase II (Pol II), which is the enzyme that transcribes DNA into pre-mRNA. The transcription is coupled to pre-mRNA processing by the CTD. CTD-binding proteins like Rbm16 recognize it by means of conserved CTD-interacting domain and act with other CTD binding proteins to link transcription and pre-mRNA processing[155]. Rbm16 colocalizes in the cell nucleus with phosphorylated Pol II in sites of active transcription and is enriched in a nuclear matrix fraction known to contain proteins involved in pre-mRNA processing[156].

Prpf4b is a homolog of the pre-mRNA splicing factor *Prpf4*, whose function has been extensively studied in eukaryotes[157-160]. Prpf4 is a CDK-like kinase with homology to mitogen-activated protein kinases that associates with spliceosomal U4, U5 and U6 small nuclear ribonucleoproteins and likely acts as one of the kinases responsible for phosphorylation of spliceosomal components during the formation of active spliceosomes[159,161]. The importance of Prpf4 for pre-mRNA splicing can be demonstrated by the accumulation of unspliced pre-mRNA when Prpf4 function is affected[160]. But *Prpf4* has also been shown to interact with proteins involved in nuclear hormone-regulated chromatine remodeling such as Brg1 and N-CoR. Through these proteins, Prpf4 modulates histone deacetylase (HDAC)[158], which is responsible for removing the acetyl groups from histone tails, causing the histones to wrap more

tightly around the DNA, thus blocking access of transcription factors to DNA, leading to a non specific reduction in gene expression. It is known that pre-mRNA splicing occurs co-transcriptionally[162] (i.e., before the synthesis of the pre-mRNA is fully completed) and that the activation of transcription is coincident with chromatin remodeling[163] (i.e., wrapping and unwrapping of DNA, depending on the histone acetylation status). By interacting with a multitude of proteins involved in both splicing and transcription, Prpf4 provides a functional link between pre-mRNA splicing and transcriptional regulation and may therefore be crucial for coordination of the two processes[158].

Transcriptional regulation and pre-mRNA splicing are extremely complex processes that involve hundreds of proteins, RNA molecules and DNA sequences. This complexity prevents any generalization as to the direction of the allelic effect in the case of the results presented here and may help explain why some of the TC1 and TC5 transcripts show greater abundance associated with the SHR allele, whereas others exhibit higher levels associated with the BN allele. A model of how such antipodal allelic effects may arise is outlined in Figure 4–10.

Discussion of the *cis*-eQTL cluster on RNO20

Analysis of the genome-wide distribution of *cis*-eQTLs revealed a cluster of 23 cis-eQTL genes on 20p12. This chromosomal region harbors the RT1 major histocompatibility complex (MHC) of the rat and many of the *cis*-eQTL genes indeed belong to RT1 (Table 4–7). RT1 shares a great degree of homology with the human HLA and mouse H2 complexes. RT1 has long been a focus of intensive research

because of its central role in organ graft rejection and susceptibility to certain complex (e.g., autoimmune) diseases [153]. RT1 gene expression profiling revealed significant strain- and tissue-specific difference between BN and LEW strains of the rat [164] but a question remained unresolved of whether the cause of differential expression of RT1 genes lies within the MHC itself, or is regulated by genes outside of it (i.e., genetic background). The genomic and functional aspects of RT1 are still being investigated[165].

The results of this study suggest that a substantial proportion of the inter strain variation in RT1 gene expression can be explained by genetic variation within the complex itself. This is a significant finding also because of the known MHC association with disease. Expression level differences can hold a key to understanding some aspects of genomic and functional regulation of RT1 and its role in disease pathogenesis. Our results imply that association of MHC with diseases may depend on the amount of gene transcription as much as on the restriction of response to antigen.

Conclusions and perspectives

The results in this study describe an important part of the natural variation in gene transcription and the approaches outlined here may be used as a general tool for identification and investigation of disease candidate genes. MDMR method was applied to the problem of selection of master regulator candidate genes that underlie *trans*-eQTL clusters, which were detected by simple eQTL mapping. The results suggest that MDMR analysis can provide a useful addition to the more traditional eQTL mapping strategies. Moreover, using two sets of predictor variables, i.e. *cis*-

eQTL genes and SNPs, allowed us to interrogate each master regulator candidate region by two independent but complementary analyses. This provides a means for corroborating the findings if concordance of results for the two sets of predictor variables is found, as is the case for *Rbm16* and *Prpf4b*.

A caveat in this analysis strategy is that the only variation tested here is the transcript abundance, and thus we could not assess other sources of polymorphism. Also of note is the limited number of genes on the RAE230A microarray, thus allowing us to test only a subset of genes. This almost certainly results in omissions of important candidate genes potentially underlying the trans-eQTL clusters for which we did not detect a plausible candidate.

We demonstrated the utility of the MDMR method for investigation of transcriptome regulation. The MDMR analysis of adrenal eQTL data yielded two candidate genes that are extremely relevant to pleiotropic regulation of transcription. Further functional investigations of these candidate genes will be required to pinpoint the exact molecular mechanisms by which these "master regulators" operate.

ACKNOWLEDGEMENTS

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The material in this chapter was presented as a poster (Jirout ML, Heinig M, Hubner N, Pravenec M, Kren V, Aitman TJ, O'Connor DT, Schork NJ. *The identification of trans-acting genes that influence the abundance of multiple transcripts simultaneously.* Genomes, Medicine, and the Environment, San Diego, CA, October 8-10, 2007), and is being prepared for publication. I was the primary author of the results contained in all chapters of this dissertation. The co-authors listed on the above publications facilitated and/or supervised the research that formed the basis for these publications.

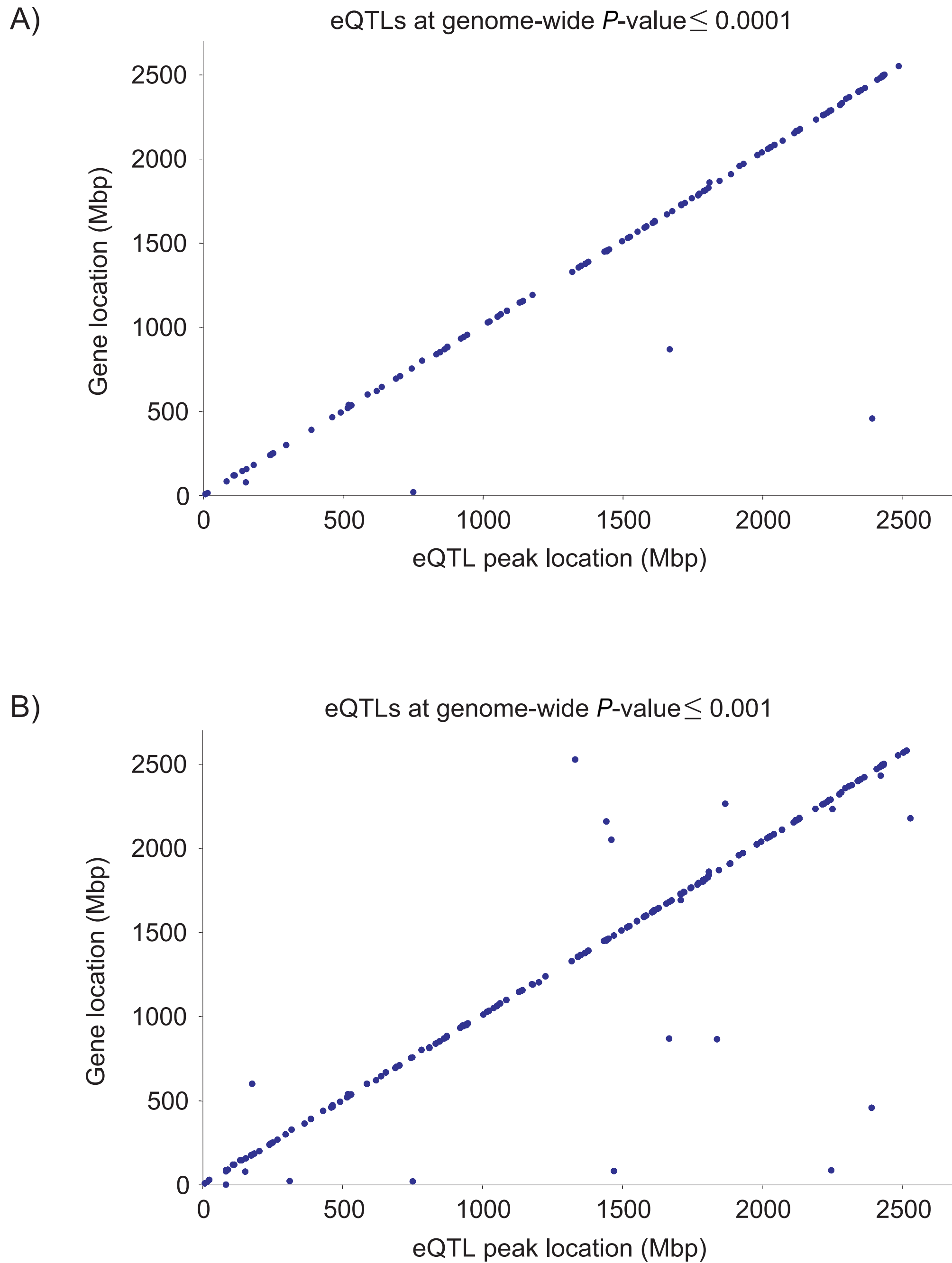


Figure 4-1 Adrenal transcriptome map. Global representation of the *cis*- and *trans*-acting eQTLs across the genome at different genome-wide *P*-values. A preponderance of *cis*-acting eQTLs (diagonal line) at high genome-wide significance levels is evident (A and B). With a relaxation of the genome-wide significance criterion, *trans*-eQTLs become increasingly more detectable (C), some forming vertical patterns (D), which correspond to *trans*-eQTLclusters. The clusters are better appreciated when *trans*-eQTLs are counted (per 10 Mbp moving window) and plotted (red curve in D).

(Continued.)

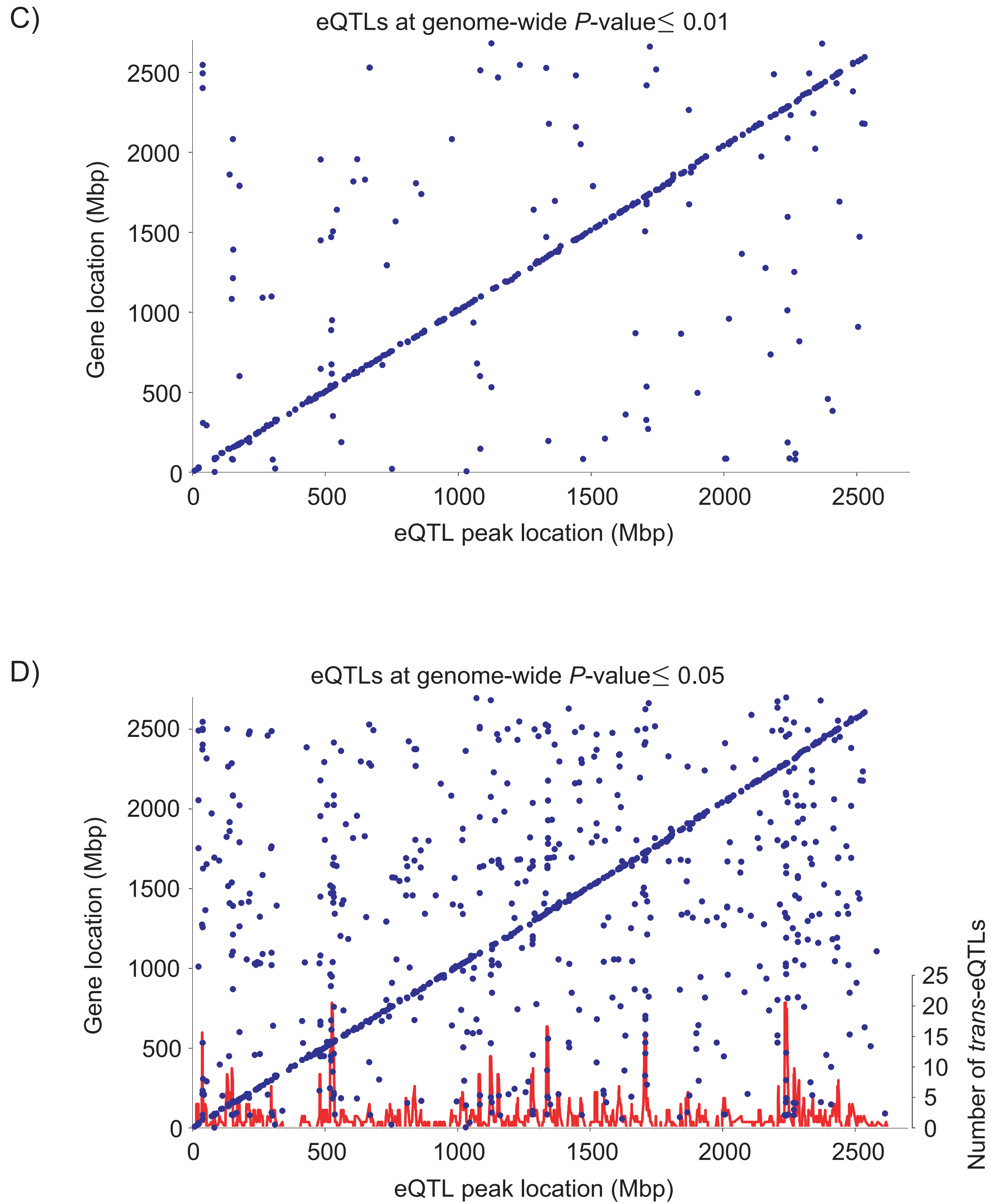


Figure 4-1 Adrenal transcriptome map. (Continued.)

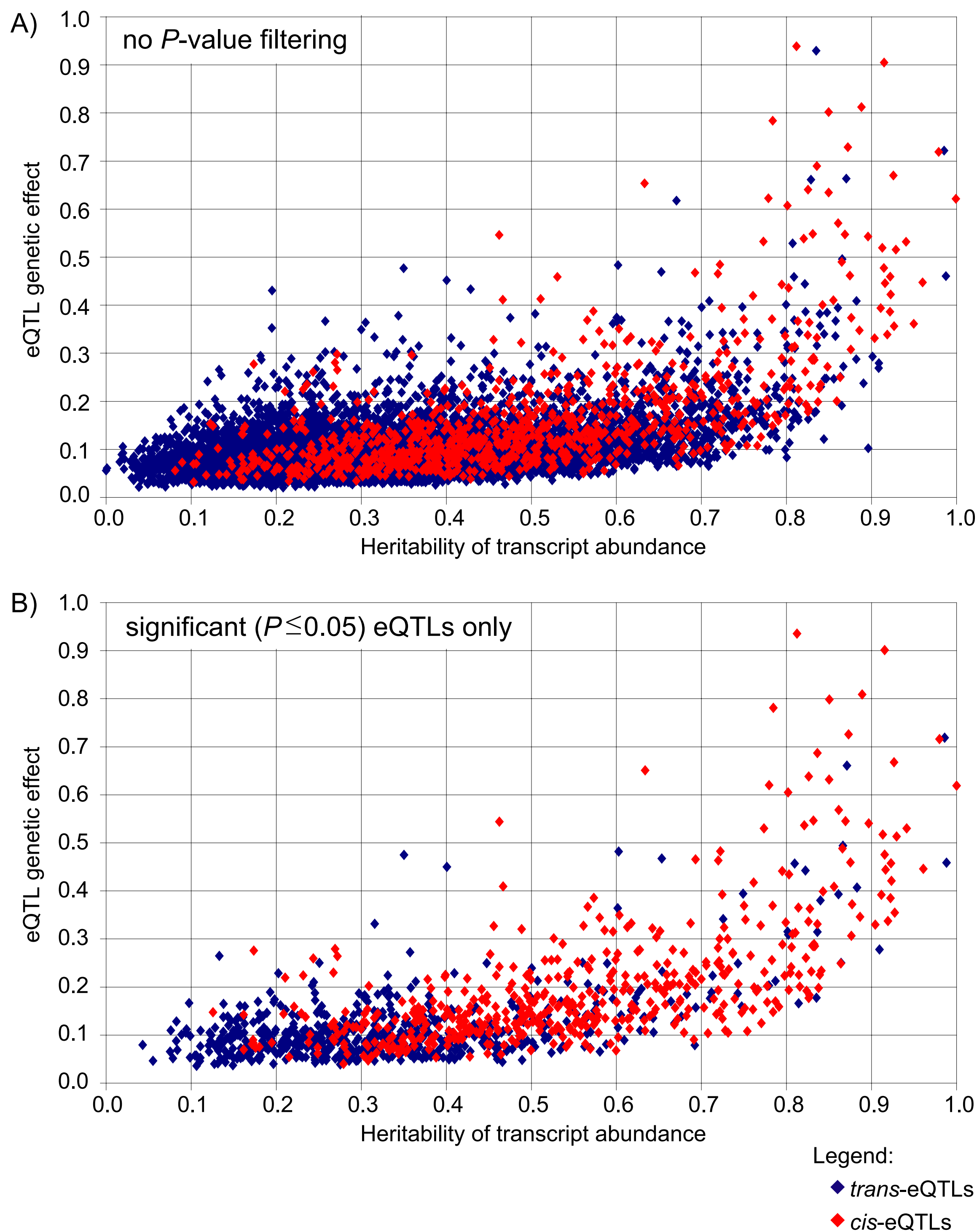


Figure 4-2 Heritability of transcript abundance vs. eQTL genetic effect. Heritability is on the abscissa and the genetic effect is on the ordinate (calculated as a ratio of the eQTL additive effect to the overall RI trait average). *Cis*- and *trans*-eQTLs are distiguished by color (*trans* = blue, *cis* = red). A) eQTLs for all expressed and localized genes regardless of the *P*-value. The total number of mapped eQTLs is 9931; 887 *cis*- and 9044 *trans*-eQTLs. B) eQTLs mapped at $P \leq 0.05$ (see Table 4-1).

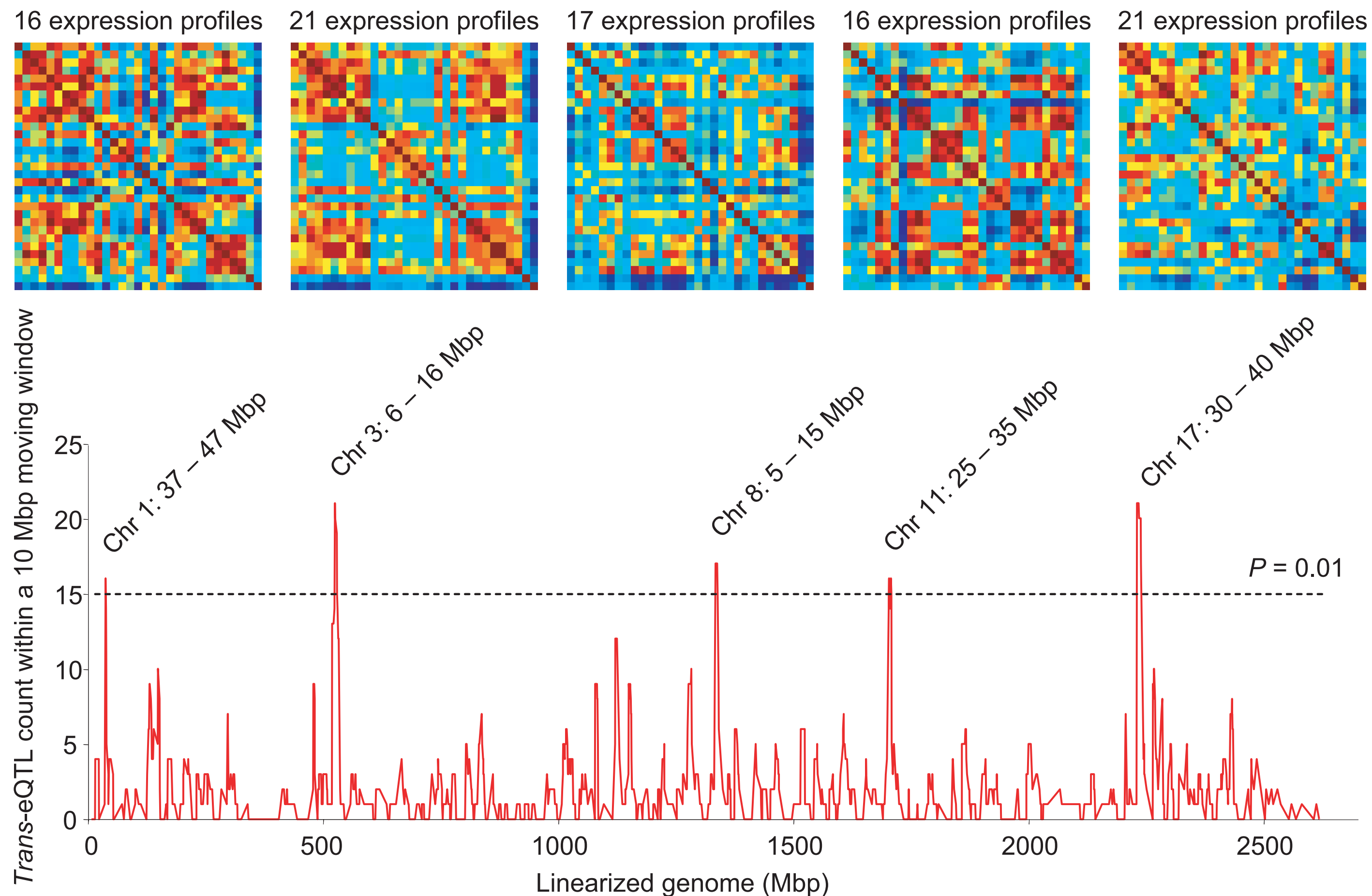


Figure 4-3 Clustering of *trans*-eQTLs. All *trans*-eQTLs detectable at genome-wide *P*-value$\leq$ 0.05 were counted using a 10 Mbp moving window. Red curve (same as in figure 4-1D) indicates cluster position (abscissa) and cluster size (ordinate). Threshold for cluster size significance ($P \leq 0.01$) is based on the frequency distribution of the *trans*-eQTLs counts. Heat maps above the cluster peaks represent similarity matrices formed from the cluster transcripts, and represent input for the MDMR analysis (see Methods).

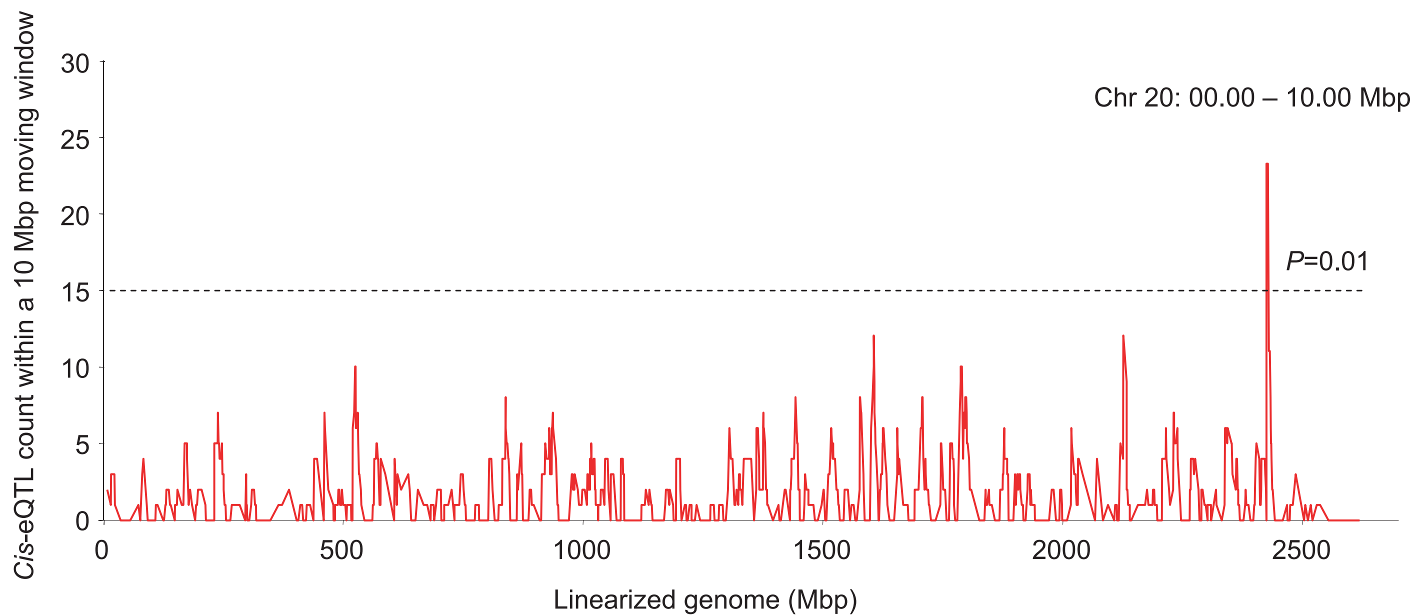


Figure 4-4 Clustering of *cis*-eQTLs on RNO 20. All *cis*-eQTLs, detectable at genome-wide *P*-value = 0.05, were counted using a 10 Mbp moving window. The red curve indicates cluster position (abscissa) and cluster size (ordinate). The cluster size significance threshold (*P* = 0.01) is based on the frequency distribution of the *cis*-eQTL counts. Significant clustering of 23 *cis*-eQTLs on chromosome 20 maps to the RT1 complex.

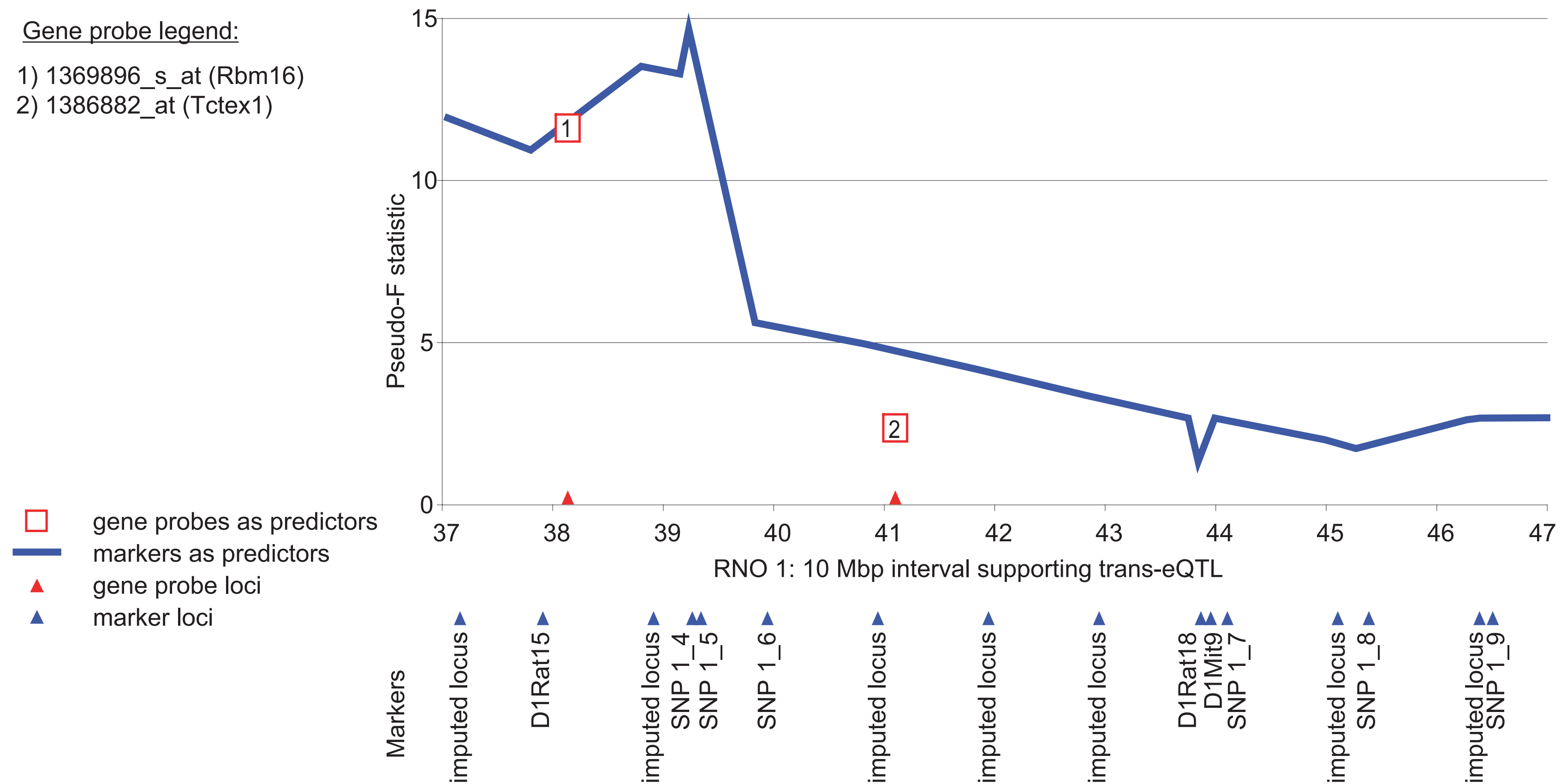


Figure 4-5 MDMR analysis of the *trans*-eQTL cluster #1 on RNO 1. 16 *trans*-eQTL genes, which mapped to the vicinity of the SNP 1_5 at a genome-wide *P*-value = 0.05 were included in the construction of a similarity matrix. Both genetic markers and *cis*-eQTL genes were then used as predictors of the variance captured by the matrix. The ordinate shows the pseudo-F statistic and the abscissa represents the 10 Mbp interval supporting the *trans*-eQTL cluster. Gene probes are numbered and detailed in the legend.

Gene probe legend:

1) 1370564_at (Dbh)
2) 1372323_at (Sardh)
3) 1372775_at (Brd3)
4) 1389816_at (Endog)
5) 1373537_at (Fnbp1)
6) 1389713_at
7) 1368267_at (Pomt1)
8) 1373100_at
9) 1390214_a_at (Ciz1)
10) 1388624_at
11) 1372759_at (Cdk9)
12) 1373331_at
13) 1375181_at
14) 1372267_at (Psmd5)
15) 1375687_at (Rab14)
16) 1370335_at (Dab2ip)

gene probes as predictors
markers as predictors
gene probe loci
marker loci

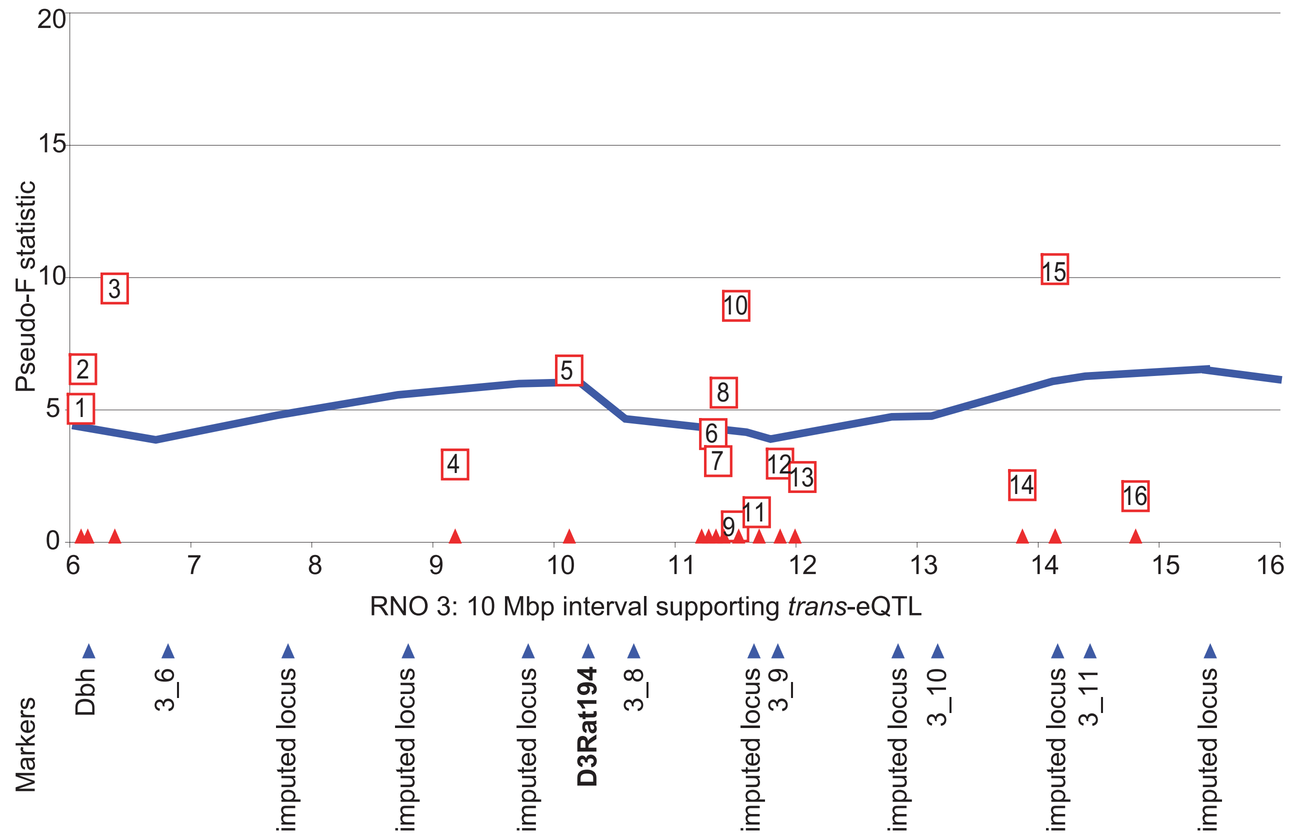


Figure 4-6 MDMR analysis of the *trans*-eQTL cluster #2 on RNO 3. 21 *trans*-eQTL genes mapping to the vicinity of the marker D3Rat194 at a genome-wide *P*-value = 0.05 were included in the construction of a similarity matrix. Both genetic markers and *cis*-eQTL genes were then used as predictors of the variance captured by the matrix. The ordinate shows the pseudo-F statistic and the abscissa represents the 10 Mbp interval supporting the *trans*-eQTL cluster. Gene probes are numbered and detailed in the legend.

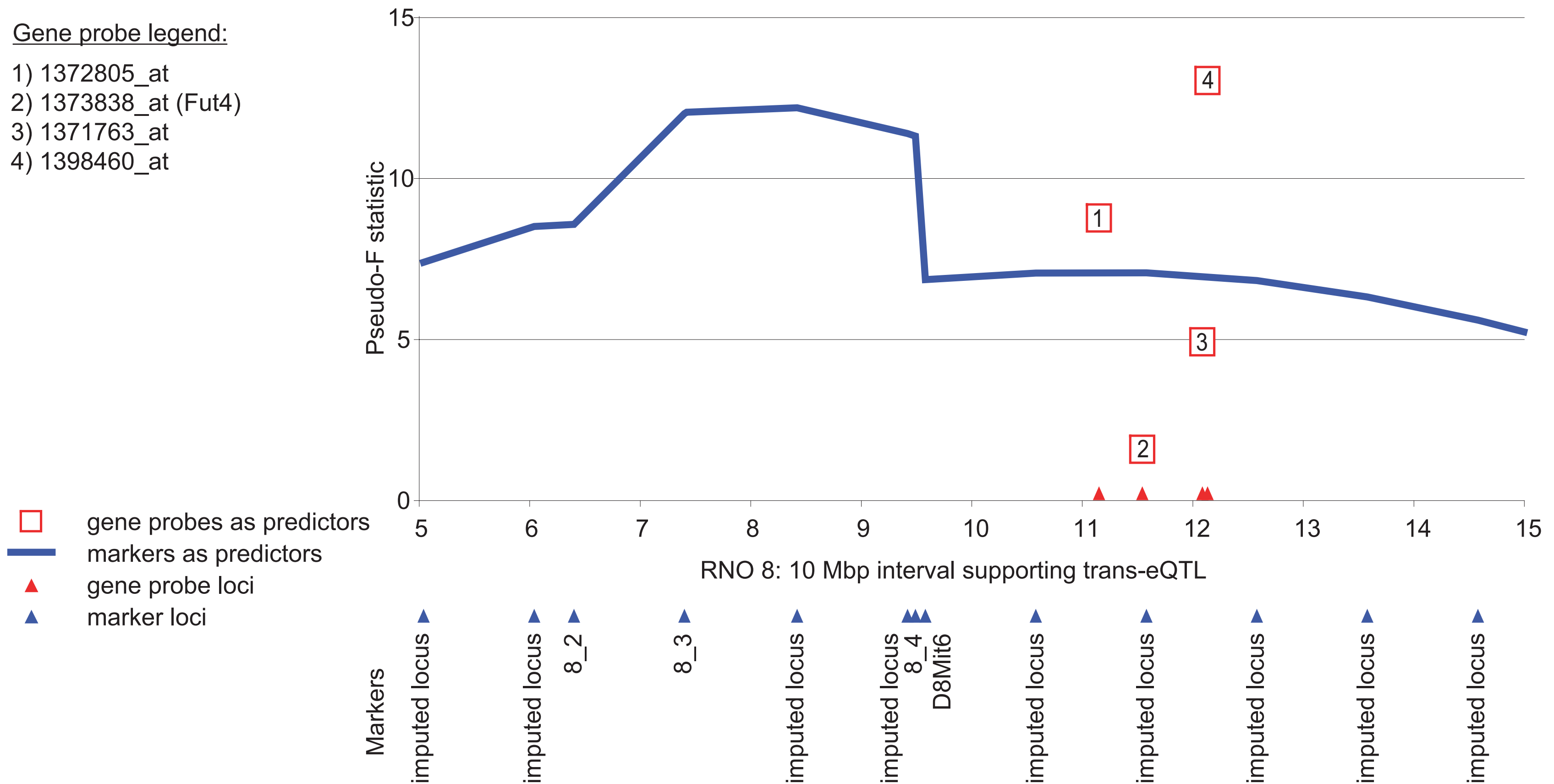
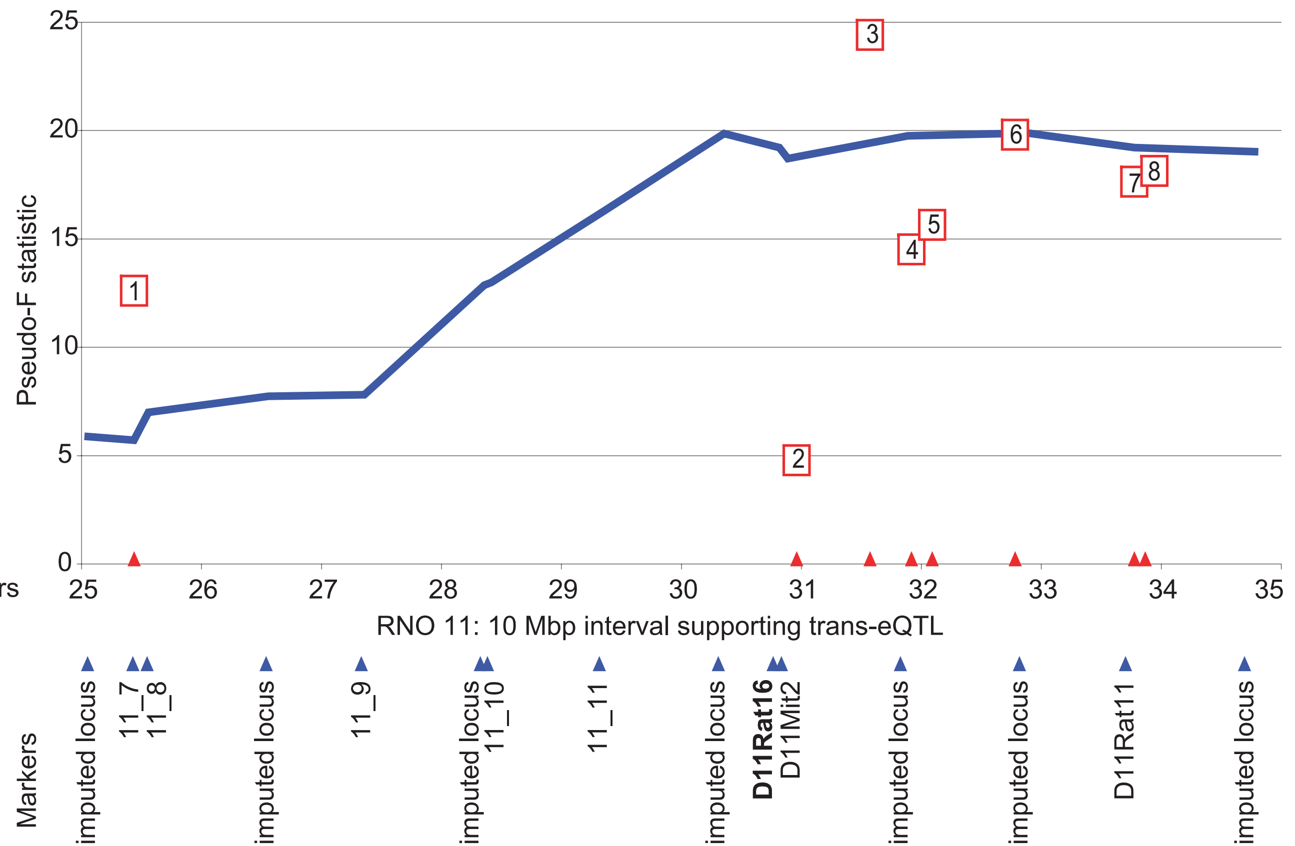


Figure 4-7 MDMR analysis of the *trans*-eQTL cluster #3 on RNO 8. 17 *trans*-eQTL genes, which mapped to the vicinity of the SNP 8_4 at a genome-wide *P*-value = 0.05 were included in the construction of a similarity matrix. Both genetic markers and *cis*-eQTL genes were then used as predictors of the variance captured by the matrix. The ordinate shows the pseudo-F statistic and the abscissa represents the 10 Mbp interval supporting the *trans*-eQTL cluster. Gene probes are numbered and detailed in the legend.

Gene probe legend:

1) 1368223_at (Adamts1)
2) 1388586_at (Synj1)
3) 1371347_at (Tmem50b)
4) 1370276_at (Atp5o)
5) 1389110_at (Slc5a3)
6) 1390364_at
7) 1370947_at
8) 1368037_at (Cbr1)



gene probes as predictors
markers as predictors
gene probe loci
marker loci

Figure 4-8 MDMR analysis of the *trans*-eQTL cluster #4 on RNO 11. 16 *trans*-eQTL genes, which mapped to the vicinity of marker D11Rat16 at a genome-wide *P*-value = 0.05 were included in the construction of a similarity matrix. Both genetic markers and *cis*-eQTL genes were then used as predictors of the variance captured by the matrix. The ordinate shows the pseudo-F statistic and the abscissa represents the 10 Mbp interval supporting the *trans*-eQTL cluster. Gene probes are numbered and detailed in the legend.

Gene probe legend:

1) 1373200_at (Eef1e1)
2) 1388884_at
3) 1376010_at (Prpf4b)
4) 1372686_at
5) 1388617_at (Bphl)
6) 1374959_at (Nqo2)
7) 1373672_at

gene probes as predictors
markers as predictors
gene probe loci
marker loci

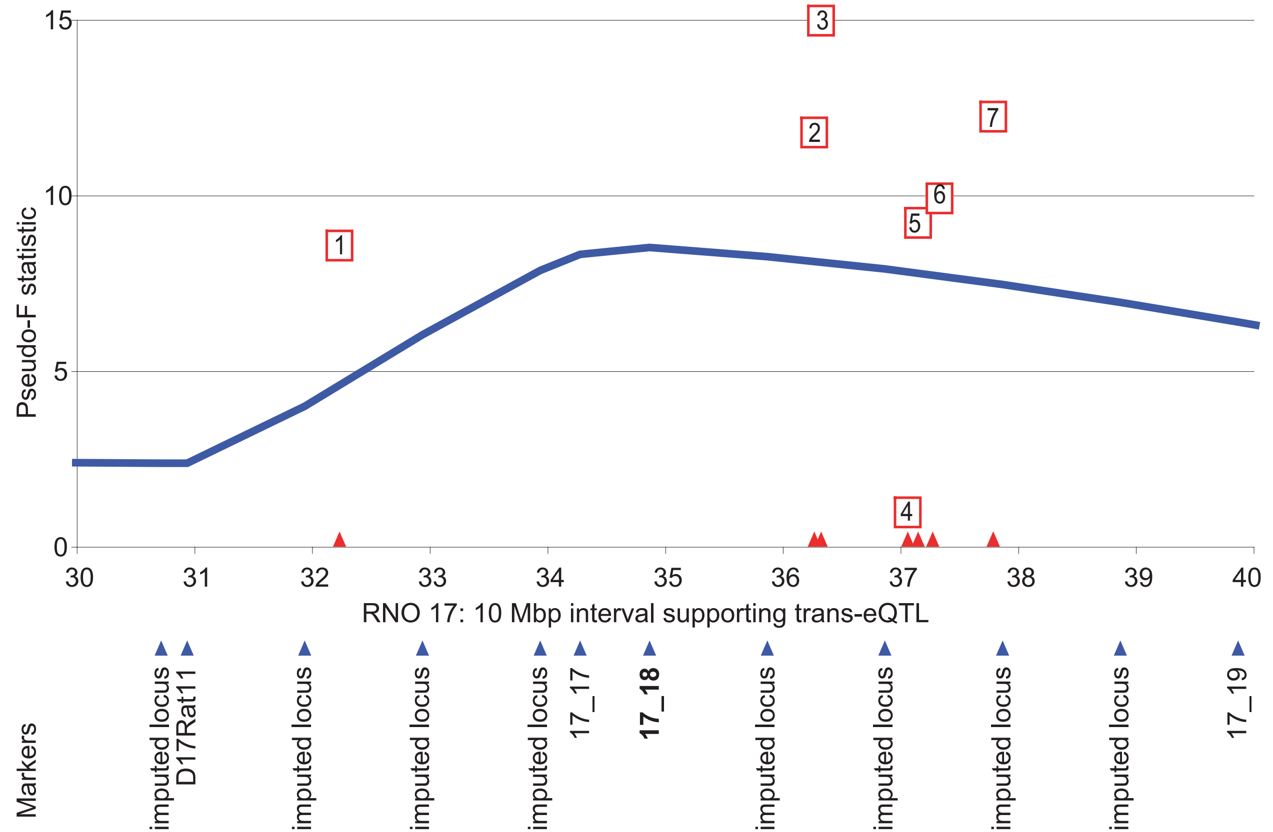


Figure 4-9 MDMR analysis of the *trans*-eQTL cluster #5 on RNO 17. 21 *trans*-eQTL genes, which mapped to the vicinity of the SNP 17_18 at a genome-wide *P*-value = 0.05 were included in the construction of similarity matrix. Both genetic markers and *cis*-eQTL genes were then used as predictors of the variance captured by the matrix. The ordinate shows the pseudo-F statistic and the abscissa represents the 10 Mbp interval supporting the *trans*-eQTL cluster. Gene probes are numbered and detailed in the legend.

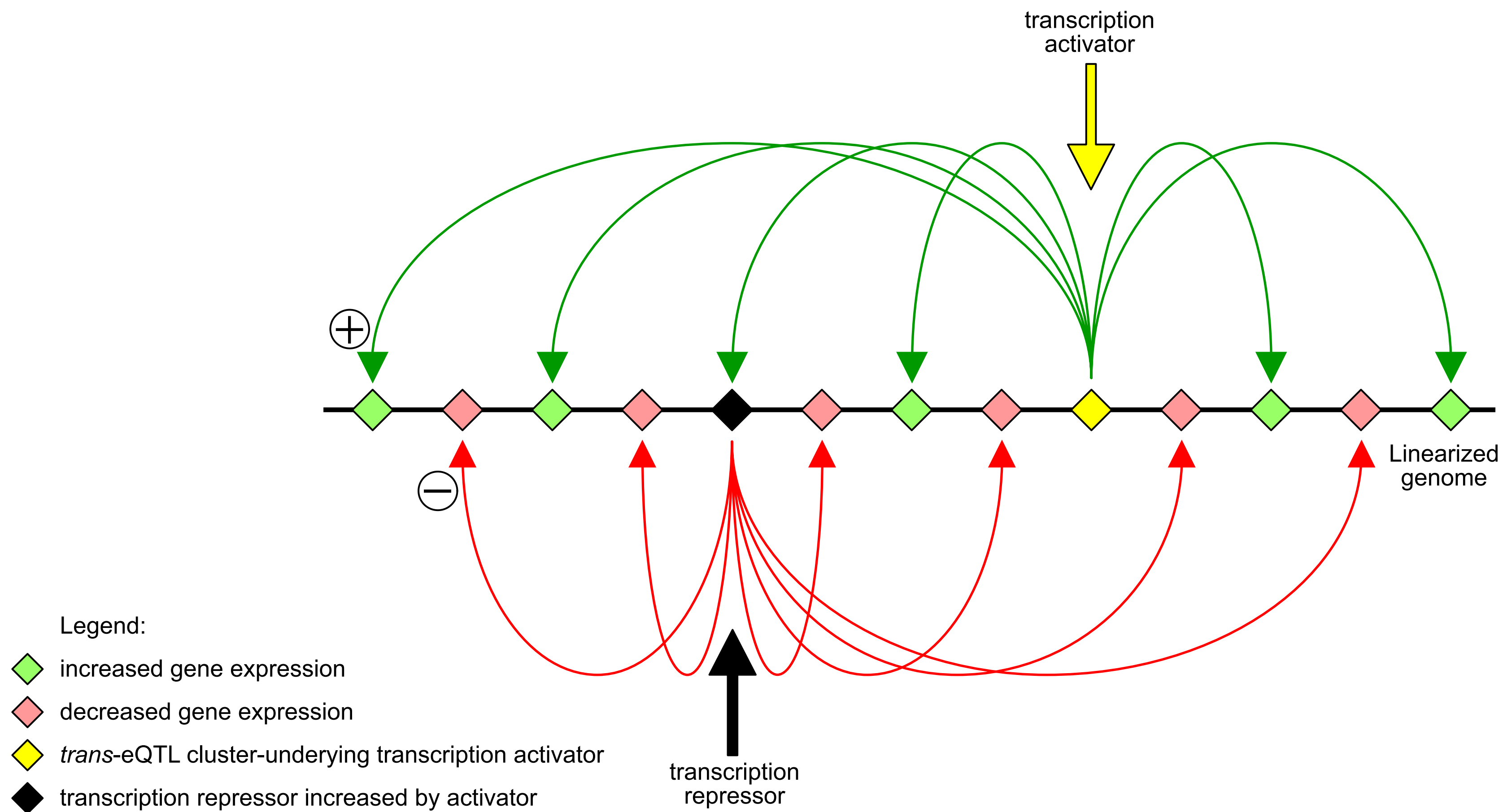


Figure 4-10 A *trans*-eQTL cluster model. A *trans*-acting gene can cause an overexpression of some genes and underexpression of others. This manifests itself as an absence of just one allelic effect within a cluster, i.e. the SHR allele is associated with an increased transcript in some, and a decreased transcript in other eQTLs. We can only speculate about the exact molecular mechanism involved. Here, a model is proposed where the main trans-acting cluster-underlying gene causes overexpression of a repressor gene, which is in turn responsible for a decreased expression of a subgroup of genes in the eQTL cluster.

Table 4-1 Number of eQTLs detected in the HXB/BXH adrenal tissue dataset. For each genome-wide *P*-value, the number of *cis*- and *trans*-eQTLs, as well as the percentages of the total they represent, and the median heritability (H^2_{median}) of the gene expression traits in each group is shown. The total number (regardless of the *P*-value) of *trans*-eQTLs in the dataset was 9044, compared to 887 *cis*-eQTLs.

Threshold	Detected eQTLs			% of total eQTLs		H^2_{median} of the eQTL genes		
	total	*cis*	*trans*	*cis*	*trans*	total	*cis*	*trans*
$P \leq 0.05$	1085	492	593	45.35%	54.65%	0.29	0.45	0.18
$P \leq 0.01$	500	372	128	74.40%	25.60%	0.42	0.48	0.21
$P \leq 0.001$	273	248	25	90.84%	9.16%	0.53	0.54	0.30
$P \leq 0.0001$	167	158	9	94.61%	5.39%	0.59	0.59	0.38
$P \leq 0.00001$	100	95	5	95.00%	5.00%	0.63	0.63	0.62

Table 4-2 RNO 1 t*rans*-eQTL cluster genes. Table contains details on the 16 gene transcripts, whose eQTLs map within the 37 - 47 Mbp region of chromosome 1 (TC1). These genes formed the input for TC1 similarity matrix calculation.

Gene					QTL					
Probe ID	Title	Symbol	Chr	Mbp	Chr	Locus	Mbp	*P*-val	LOD	Add
1374390_at	Transcribed locus	---	1	211.71	1	D1Rat15	37.8	0.02	3.8	-0.09
1371412_a_at	Neuronal regeneration related protein	Nrep	18	25.86	1	D1Rat15	37.8	0.02	4.6	0.25
1383080_at	Lysosomal membrane glycoprotein 2	Lamp2	X	6.93	1	1_4	39.2	0.00	5.2	0.09
1388558_at	Adenylate kinase 3	Ak3	1	232.67	1	1_4	39.2	0.01	4.9	-0.11
1377123_at	Solute carrier family 27 (fatty acid transporter), member 4	Slc27a4	3	8.80	1	1_4	39.2	0.03	3.6	-0.10
1371961_at	Phospholipase D family, member 3 MGC109410	Pld3	1	82.69	1	1_4	39.2	0.05	5.1	-0.19
1367679_at	CD74 antigen (MHC II antigen-associated)	Cd74	18	56.76	1	1_5	39.2	0.00	6.8	0.20
1370383_s_at	RT1 class II, locus Db1, RT1-D beta, RT1-Db, RT1-Db1n	RT1-Db1	20	4.68	1	1_5	39.2	0.01	5.9	0.16
1374053_at	Minichromosome maintenance deficient 3 assoc protein	Mcm3ap	20	12.56	1	1_5	39.2	0.01	4.4	0.06
1382339_a_at	Chromodomain helicase DNA binding protein 1	Chd1	1	54.60	1	1_5	39.2	0.03	4.4	-0.18
1370883_at	RT1 class II, locus Da, RT1-Daa, RT1-Dab, RT1-Dac, RT1-Dad	RT1-Da	20	4.64	1	1_5	39.2	0.03	3.7	0.14
1389332_at	Transcribed locus	---	7	55.74	1	1_5	39.2	0.04	4.2	0.08
1394012_at	Zinc finger, SWIM domain containing 6 Ab2-064	Zswim6	2	39.02	1	1_6	39.8	0.00	4.9	0.11
1372635_at	Transcribed locus	---	10	40.93	1	1_6	39.8	0.02	4.5	0.11
1389347_at	Phosphatidylinositol transfer protein, membrane-associated	Pitpnm1	1	206.69	1	D1Rat18	43.8	0.02	5.4	0.09
1368710_at	Serine/threonine kinase	Mark2	1	209.99	1	D1Mit9	43.8	0.05	2.7	0.04

Legend:
LOD = LOD score
Add = Additive effect

Table 4-3 RNO 3 t*rans*-eQTL cluster genes. Table contains details on the 21 gene transcripts, whose eQTLs map within the 6 - 16 Mbp region of chromosome 3 (TC2). These genes formed the input for TC2 similarity matrix calculation.

Gene					QTL					
Probe ID	Title	Symbol	Chr	Mbp	Chr	Locus	Mbp	*P*-val	LOD	Add
1376585_at	Mitochondrial ribosomal protein L50	Mrpl50	5	66.26	3	3_6	6.7	0.00	5.6	-0.07
1369352_at	Homeodomain interacting protein kinase 3	Hipk3	3	89.78	3	Dbh	6.1	0.00	6.4	-0.25
1393593_at	Transcribed locus	---	2	83.58	3	D3Rat194	10.2	0.00	6.2	-0.15
1376137_at	Pleckstrin homology domain containing, family B	Plekhb2	9	33.60	3	D3Rat194	10.2	0.01	8.9	-0.21
1369912_at	V-crk sarcoma virus CT10 oncogene homolog	Crk	10	63.03	3	3_11	14.4	0.01	4.6	-0.13
1387641_at	RAB5A, member RAS oncogene family	Rab5a	9	1.50	3	D3Rat194	10.2	0.01	6.2	-0.09
1393467_at	Proprotein convertase subtilisin/kexin type 5	Pcsk5	1	221.12	3	3_11	14.4	0.02	5.2	-0.19
1371108_a_at	Atpase, Na+/K+ transporting, alpha 1 polypeptide	Atp1a1	2	196.67	3	3_11	14.4	0.02	5.8	-0.18
1388072_at	SH3-binding kinase 1 Sbk	Sbk1	1	185.35	3	D3Rat194	10.2	0.02	6.7	-0.47
1367506_at	Mitochondrial ribosomal protein L11	mrpl11	1	207.58	3	3_11	14.4	0.02	3.9	0.05
1374964_at	Dipeptidylpeptidase 8	Dpp8	8	69.30	3	3_9	11.8	0.02	4.1	-0.05
1371192_at	Neurofibromatosis 2	Nf2	14	85.47	3	3_11	14.4	0.02	6.2	-0.27
1369779_at	Myosin IC	Myo1c	10	68.86	3	3_8	10.6	0.03	6.3	-0.33
1387368_at	Muscle and microspikes RAS	Mras	8	104.26	3	3_11	14.4	0.03	3.6	-0.07
1375233_at	Transcribed locus	---	5	156.11	3	D3Rat194	10.2	0.03	3.7	0.11
1385889_at	Transcribed locus	---	9	36.37	3	D3Rat194	10.2	0.03	4.8	-0.16
1370527_a_at	Casein kinase 1, delta	Csnk1d	10	110.18	3	3_11	14.4	0.03	4.6	-0.12
1367910_at	MAD homolog 4 (Drosophila)	Madh4	18	70.45	3	3_11	14.4	0.04	4.1	-0.09
1376990_at	Transcribed locus	---	15	34.04	3	3_11	14.4	0.04	4.1	0.07
1387786_at	Myotrophin	Mtpn	4	62.91	3	3_11	14.4	0.04	5.0	-0.11
1370488_a_at	Protein tyrosine phosphatase, receptor type, D	Ptprd	9	0.63	3	3_9	11.8	0.04	4.8	-0.16

Legend:
LOD = LOD score
Add = Additive effect

Table 4-4 RNO 8 t*rans*-eQTL cluster genes. Table contains details on the 17 gene transcripts, whose eQTLs map within the 5 - 15 Mbp region of chromosome 8 (TC3). These genes formed the input for TC3 similarity matrix calculation.

Gene					QTL					
Probe ID	Title	Symbol	Chr	Mbp	Chr	Locus	Mbp	*P*-val	LOD	Add
1389102_at	Similar to dedicator of cyto-kinesis 2	---	1	194.63	8	8_3	7.4	0.00	5.3	0.08
1372050_at	Glycosyltransferase 25 domain containing 1	Glt25d1	16	18.82	8	D8Mit6	9.6	0.01	4.6	-0.05
1392746_x_at	Transcribed locus	---	10	43.65	8	8_3	7.4	0.02	6.6	-0.10
1388543_at	Transcribed locus	---	1	188.58	8	8_4	9.5	0.02	3.6	-0.07
1383331_at	Transcribed locus	---	20	30.06	8	D8Mit6	9.6	0.02	4.1	0.12
1383884_at	Transcribed locus	---	15	2.57	8	8_4	9.5	0.02	4.5	0.16
1374326_at	Peter pan homolog	Ppan	8	19.91	8	8_3	7.4	0.03	5.0	-0.11
1371626_at	Signal recognition particle 68	Srp68	10	106.37	8	8_3	7.4	0.03	5.6	-0.05
1398864_at	Ubiquitin-conjugating enzyme E2G 1	Ube2g1	10	59.54	8	8_3	7.4	0.03	4.3	0.06
1388951_at	Transcribed locus	---	1	85.63	8	8_2	6.4	0.04	3.9	-0.05
1389086_at	Transcribed locus	---	13	98.89	8	8_4	9.5	0.04	4.2	-0.10
1368282_at	Dipeptidase 1 (renal)	Dpep1	19	53.51	8	8_4	9.5	0.04	3.6	-0.14
1398849_at	H3 histone, family 3B	RGD:621095	10	106.13	8	8_3	7.4	0.04	3.6	-0.06
1375692_at	Mitogen activated protein kinase 1	Mapk1	11	86.00	8	8_4	9.5	0.04	4.8	0.09
1374178_at	ADP-ribosylation factor-like 5	Arl5	3	33.92	8	D8Mit6	9.6	0.04	5.4	0.10
1370426_a_at	ATPase, Ca++ transporting, cardiac muscle	Atp2a2	12	35.29	8	D8Mit6	9.6	0.05	3.6	0.04
1398808_at	Inositol (myo)-1(or 4)-monophosphatase 1	Impa1	2	93.43	8	8_2	6.4	0.05	4.0	0.08

Legend:
LOD = LOD score
Add = Additive effect

Table 4-5 RNO 11 *trans*-eQTL cluster genes. Table contains details on the 16 gene transcripts, whose eQTLs map within the 25 - 35 Mbp region of chromosome 11 (TC4). These genes formed the input for TC4 similarity matrix calculation.

Gene					QTL					
Probe ID	Title	Symbol	Chr	Mbp	Chr	Locus	Mbp	*P*-val	LOD	Add
1370408_at	Putative small membrane protein NID67	Nid67	18	56.44	11	11_9	27.4	0.02	4.7	-0.13
1370384_a_at	Prolactin receptor RATPRLR	Prlr	2	59.52	11	11_11	29.4	0.01	5.3	0.16
1367680_at	Acyl-Coenzyme A oxidase 1, palmitoyl	Acox1	10	106.29	11	D11Rat16	30.8	0.00	5.0	-0.10
1373135_at	Transcribed locus	---	10	90.02	11	D11Rat16	30.8	0.00	6.0	-0.10
1370964_at	Arginosuccinate synthetase	Ass	3	10.60	11	D11Rat16	30.8	0.00	5.7	-0.47
1389903_at	Pituitary tumor-transforming 1 interacting protein	Pttg1ip	20	11.42	11	D11Rat16	30.8	0.02	4.6	-0.08
1369917_at	Neurotrophin receptor associated death domain PLAIDD	Nradd	8	115.00	11	D11Rat16	30.8	0.02	4.6	0.07
1377266_at	Transcribed locus	---	X	86.20	11	D11Rat16	30.8	0.02	4.4	0.09
1398450_at	Transcribed locus	---	3	59.76	11	D11Rat16	30.8	0.03	4.3	-0.15
1374725_at	Transcribed locus	---	2	200.09	11	D11Rat16	30.8	0.03	3.8	0.10
1368082_at	Solute carrier family 4, member 2	Slc4a2	4	6.11	11	D11Rat16	30.8	0.03	4.9	0.19
1372900_at	Phosphatidylinositol glycan, class T	RGD:1307156	3	155.52	11	D11Rat16	30.8	0.03	4.6	0.07
1371409_at	Transcribed locus	---	18	71.87	11	D11Mit2	30.9	0.00	5.8	-0.06
1375266_at	Transcribed locus	---	4	163.77	11	D11Mit2	30.9	0.02	4.3	-0.17

Legend:
LOD = LOD score
Add = Additive effect

Table 4-6 RNO 17 t*rans*-eQTL cluster genes. Table contains details on the 21 gene transcripts, whose eQTLs map within the 30 - 40 Mbp region of chromosome 17 (TC5). These genes formed the input for TC5 similarity matrix calculation.

Gene					QTL					
Probe ID	Title	Symbol	Chr	Mbp	Chr	Locus	Mbp	*P*-val	LOD	Add
1368657_at	Matrix metallopeptidase 3	Mmp3	8	4.32	17	D17Rat11	30.9	0.05	4.7	0.10
1372815_at	Mago-nashi homolog, proliferation-associated	Magoh	5	128.99	17	17_17	34.3	0.01	6.3	-0.09
1389227_at	Ras homolog gene family, member g arhg	Rhog	1	159.80	17	17_17	34.3	0.01	4.7	0.09
1370899_at	Splicing factor proline/glutamine rich	Sfpq	5	146.48	17	17_17	34.3	0.02	5.6	-0.09
1373969_at	Sh3 domain protein 4	Sorbs3	15	50.60	17	17_17	34.3	0.02	4.0	0.07
1387780_at	Dnaj (hsp40) homolog, subfamily a, member 2	Dnaja2	19	22.86	17	17_17	34.3	0.04	4.3	-0.06
1372242_at	Dead (asp-glu-ala-asp) box polypeptide 3, x-linked	Ddx3x	X	21.49	17	17_17	34.3	0.04	3.5	-0.06
1373045_at	Potassium channel tetramerisation domain containing 13	Kctd13	1	186.11	17	17_18	34.9	0.00	4.4	0.11
1375674_at	Transcribed locus	---	10	10.90	17	17_18	34.9	0.01	6.0	0.17
1377391_at	Calcium binding protein 39-like	Cab39l	15	38.63	17	17_18	34.9	0.01	3.5	0.08
1373821_at	Slc30a5_predicted: solute carrier family 30, member 5	Slc30a5	2	31.62	17	17_18	34.9	0.01	4.5	-0.07
1369974_at	Vesicle-associated membrane protein 2	Vamp2	10	55.85	17	17_18	34.9	0.02	6.0	0.12
1370188_at	Splicing factor, arginine/serine-rich 10	Sfrs10	11	81.04	17	17_18	34.9	0.02	4.5	-0.05
1374870_at	Collagen, type xxvii, alpha 1	Col27a1	5	80.26	17	17_18	34.9	0.02	5.0	0.13
1392604_at	Nad(p) dependent steroid dehydrogenase-like	Nsdhl	X	158.94	17	17_18	34.9	0.03	4.1	-0.15
1374945_at	Transcribed locus	---	6	136.46	17	17_18	34.9	0.04	4.0	-0.13
1368029_at	Guanine nucleotide binding protein, alpha inhibiting 3	Gnai3	2	203.69	17	17_18	34.9	0.04	4.0	-0.10
1388333_at	Ring-box 1	Rbx1	7	119.83	17	17_18	34.9	0.04	4.1	-0.07
1389721_at	Transcribed locus	---	1	77.72	17	17_18	34.9	0.04	4.5	0.17
1372143_at	Ubiquitin-conjugating enzyme e2 variant 2	Ube2v2	11	87.24	17	17_18	34.9	0.04	4.2	-0.09
1373531_at	Cleavage and polyadenylation specific factor 1, 160kda	Cpsf1	7	114.65	17	17_19	40.0	0.02	5.0	0.08

Legend:
LOD = LOD score
Add = Additive effect

Table 4-7 RNO 20 *cis*-eQTL cluster genes. Table contains details on the 23 gene transcripts, whose eQTLs map within the 0 - 10 Mbp region of chromosome 20.

Gene					QTL					
Probe ID	Title	Symbol	Chr	Mbp	Chr	Locus	Mbp	*P*-val	LOD	Add
1371171_at	RT1 class Ib, locus Aw2	RT1-Aw2	20	0.06	20	20_2	2.9	0.00	16.6	0.57
1390562_s_at	RT1 class Ib, locus Aw2	RT1-Aw2	20	0.08	20	20_2	2.9	0.00	13.0	-0.94
1388236_x_at	RT1 class I, CE12	RT1-CE12	20	0.15	20	20_2	2.9	0.00	13.4	-0.37
1389734_x_at	RT1 class Ib, locus Aw2	RT1-Aw2	20	2.84	20	20_2	2.9	0.00	6.8	0.29
1388694_at	RT1 class Ib, locus Aw2	RT1-Aw2	20	2.87	20	20_2	2.9	0.00	6.7	-0.44
1374927_at	Transcribed locus	---	20	3.04	20	20_2	2.9	0.00	8.6	0.27
1370290_at	Tubulin, beta 5	Tubb5	20	3.06	20	20_2	2.9	0.00	12.6	-0.31
1388255_x_at	RT1 class I, CE5	RT1-CE5	20	3.51	20	20_2	2.9	0.00	12.1	0.63
1370972_x_at	RT1 class Ib, locus Aw2	RT1-Aw2	20	3.53	20	20_2	2.9	0.00	8.2	-0.18
1371213_at	RT1 class I, A3	RT1-A3	20	3.60	20	20_2	2.9	0.00	15.8	-0.39
1372956_at	RT1 class Ib, locus Aw2	RT1-Aw2	20	3.76	20	20_2	2.9	0.01	5.2	-0.07
1371985_a_at	HLA-B associated transcript 5	Bat5	20	3.79	20	20_2	2.9	0.00	8.2	-0.25
1377091_at	NG5 protein	Ng5	20	4.22	20	20_2	2.9	0.00	6.8	0.20
1371033_at	RT1 class II, locus Bb	RT1-Bb	20	4.73	20	20_2	2.9	0.00	13.2	0.62
1370428_x_at	RT1 class I, A3	RT1-A3	20	5.02	20	20_2	2.9	0.00	17.1	0.72
1394386_s_at	Vacuolar protein sorting 52	Vps52	20	5.08	20	20_2	2.9	0.00	12.9	-0.46
1369726_at	TAP binding protein	Tapbp	20	5.11	20	20_2	2.9	0.00	6.2	-0.15
1383225_at	Transcribed locus	---	20	6.41	20	20_2	2.9	0.01	5.8	0.16
1377136_at	Mitogen activated protein kinase 14	Mapk14	20	6.97	20	20_3	6.8	0.00	6.0	-0.17
1374429_at	Transcribed locus	---	20	7.82	20	20_3	6.8	0.00	17.6	-0.73
1389481_at	Transcribed locus	---	20	7.60	20	20_4	8.0	0.00	6.7	-0.10
1399098_at	Glyoxylase 1	Glo1	20	8.91	20	20_6	8.3	0.00	11.8	0.12
1389369_at	Transcribed locus	---	20	5.07	20	20_7	9.1	0.00	7.7	-0.13

Legend:
LOD = LOD score
Add = Additive effect

Table 4-8 ***Cis*-eQTL genes located within the *trans*-eQTL cluster regions used as predictors in the MDMR analysis.** Horizontal lines separate the five clusters. The results are given in the last theree columns of the table as pseudo-F statistic, *P*-value (based on 100 000 permutations) and amount of variance (%) in the similarity matrix that can be explained by the predictor. Gene position, gene transcript abundance heritability in the RI strains, t-test *P*-value for differences between progenitors, as well as *cis*-eQTL peak position and *P*-value for each respective gene are also detailed in the table.

(Continued.)

Genes used as predictors in MDMR				Gene transcript info			*cis*-eQTL details			MDMR results		
Probeset ID	Chr	Position (Mbp)	Gene symbol	*t*-test *P*-value	H^2 (%) in RI strains	NR	Chr	Position (Mbp)	eQTL *P*-value	pseudo-F statistic	MDMR *P*-value	% variance explained
1369896_s_at	1	38.1	Rbm16	$9x10^{-1}$	25	1.03	1	37.1	$5x10^{-2}$	11.62	$3x10^{-4}$	29
1386882_at	1	41.1	Tctex1	$6x10^{-3}$	53	1.64	1	37.1	$5x10^{-1}$	2.37	$1x10^{-1}$	8
1370564_at	3	6.1	Dbh	$2x10^{-2}$	64	-2.35	3	6.0	$4x10^{-4}$	5.04	$7x10^{-3}$	15
1372323_at	3	6.1	Sardh	$1x10^{-2}$	44	-1.52	3	6.0	$2x10^{-5}$	6.53	$1x10^{-3}$	18
1372775_at	3	6.4	Brd3	$4x10^{-1}$	18	1.35	3	11.8	$6x10^{-1}$	9.59	$1x10^{-4}$	25
1389816_at	3	9.2	Endog	$2x10^{-1}$	26	-2.14	3	6.0	$2x10^{-4}$	2.93	$4x10^{-2}$	9
1373537_at	3	10.1	Fnbp1	$1x10^{-2}$	49	1.25	3	10.2	$9x10^{-4}$	6.48	$2x10^{-3}$	18
1389713_at	3	11.3	---	$2x10^{-1}$	56	1.19	3	10.6	$4x10^{-6}$	4.08	$1x10^{-2}$	12
1368267_at	3	11.3	Pomt1	$6x10^{-3}$	69	-1.59	3	10.6	$3x10^{-3}$	3.06	$4x10^{-2}$	10
1373100_at	3	11.4	---	$1x10^{-1}$	3	1.31	3	15.4	$3x10^{-1}$	5.65	$3x10^{-3}$	16
1390214_a_at	3	11.5	Ciz1	$2x10^{-1}$	14	-1.20	3	10.6	$2x10^{-1}$	0.40	$8x10^{-1}$	1
1388624_at	3	11.5	---	$4x10^{-1}$	39	1.68	3	10.6	$8x10^{-1}$	8.93	$3x10^{-4}$	24
1372759_at	3	11.7	Cdk9	$2x10^{-1}$	7	1.17	3	11.8	$1x10^{0}$	1.13	$3x10^{-1}$	4
1373331_at	3	11.9	Lrsam1	$5x10^{-2}$	36	-1.72	3	11.8	$3x10^{-4}$	2.66	$6x10^{-2}$	8
1375181_at	3	12.0	---	$2x10^{-1}$	43	1.27	3	11.8	$8x10^{-1}$	2.46	$7x10^{-2}$	8
1372267_at	3	13.9	Psmd5	$4x10^{-2}$	26	1.28	3	11.8	$1x10^{-3}$	2.15	$9x10^{-2}$	7
1375687_at	3	14.2	Rab14	$8x10^{-1}$	30	-1.07	3	14.4	$1x10^{-2}$	10.61	$5x10^{-5}$	27
1370335_at	3	14.8	Dab2ip	$5x10^{-1}$	25	-1.21	3	14.4	$6x10^{-3}$	1.74	$1x10^{-1}$	6

Table 4-8 C*is*-eQTL genes located within the *trans*-eQTL cluster regions used as predictors in the MDMR analysis. (Continued.)

Genes used as predictors in MDMR				Gene transcript info			*cis*-eQTL details			MDMR results		
Probeset ID	Chr	Position (Mbp)	Gene symbol	t-test *P*-value	H^2 (%) in RI strains	NR	Chr	Position (Mbp)	eQTL *P*-value	pseudo-F statistic	MDMR *P*-value	% variance explained
1372805_at	8	11.2	---	$3x10^{-3}$	64	-1.56	8	9.5	$1x10^{-4}$	8.77	$2x10^{-4}$	23
1373838_at	8	11.5	Fut4	$4x10^{-1}$	28	-1.20	8	7.4	$1x10^{-1}$	1.60	$2x10^{-1}$	5
1371763_at	8	12.1	---	$8x10^{-1}$	2	-1.04	8	6.4	$7x10^{-1}$	4.93	$4x10^{-3}$	15
1398460_at	8	12.1	---	$1x10^{-1}$	61	1.43	8	9.5	$1x10^{-5}$	13.04	$1x10^{-5}$	31
1368223_at	11	25.4	Adamts1	$5x10^{-1}$	26	1.23	11	28.4	$3x10^{-1}$	12.61	$1x10^{-5}$	30
1388586_at	11	31.0	Synj1	$6x10^{-1}$	21	-1.09	11	34.8	$1x10^{0}$	4.79	$1x10^{-2}$	14
1371347_at	11	31.6	Tmem50b	$3x10^{-1}$	46	-1.34	11	30.9	$2x10^{-5}$	24.41	$1x10^{-5}$	46
1370276_at	11	31.9	Atp5o	$2x10^{-1}$	49	1.26	11	30.8	$8x10^{-2}$	14.52	$4x10^{-5}$	33
1389110_at	11	32.1	Slc5a3	$8x10^{-2}$	61	1.14	11	34.8	$2x10^{-2}$	15.64	$1x10^{-5}$	35
1390364_at	11	32.8	---	$5x10^{-2}$	17	-1.49	11	30.8	$7x10^{-4}$	19.80	$1x10^{-5}$	41
1370947_at	11	33.8	---	$3x10^{-1}$	56	1.07	11	29.4	$8x10^{-3}$	17.64	$2x10^{-5}$	38
1368037_at	11	33.8	Cbr1	$1x10^{-2}$	78	1.70	11	29.4	$1x10^{-5}$	17.77	$1x10^{-5}$	38
1373200_at	17	32.2	Eef1e1	$1x10^{-1}$	34	1.92	17	34.3	$3x10^{-3}$	8.58	$5x10^{-5}$	23
1388884_at	17	36.3	---	$3x10^{-1}$	42	1.26	17	34.9	$9x10^{-2}$	11.80	$1x10^{-5}$	29
1376010_at	17	36.3	Prpf4b	$1x10^{-1}$	49	1.26	17	34.9	$5x10^{-2}$	14.99	$1x10^{-5}$	34
1372686_at	17	37.1	---	$3x10^{-1}$	14	-1.29	17	34.3	$2x10^{-1}$	0.99	$4x10^{-1}$	3
1388617_at	17	37.1	Bphl	$4x10^{-3}$	77	2.65	17	34.9	$1x10^{-5}$	9.23	$7x10^{-5}$	24
1374959_at	17	37.3	Nqo2	$1x10^{-3}$	88	-2.20	17	34.9	$1x10^{-5}$	9.77	$3x10^{-5}$	25
1373672_at	17	37.8	---	$3x10^{-1}$	66	1.22	17	34.9	$1x10^{-5}$	12.15	$1x10^{-5}$	30

Chapter 5

Conclusions and Perspectives:

Significance of the Presented Results and Future Directions

SIGNIFICANCE OF THE PRESENTED RESULTS

The construction of a high resolution linkage map and the genetic characterization of the HXB/BXH RI strain panel (described in Chapter 2), which is one of only two rat RI population (the other being the LEXF/FXLE RI set[166]), will allow researchers to maximize the potential of these strains for mapping genes contributing to the expression of complex phenotypes that are measurable in the HXB/BXH model population. Furthermore, the analysis presented in this dissertation revealed important limitations in terms of power to detect QTLs of small phenotypic effect, largely attributable to the relatively low number of strains in the HXB/BXH RI population. Another important conclusion emerging from this analysis is that the recently undertaken extensive SNP genotyping in the RI strains[51] provides only a limited increment in mapping resolution over the current microsatellite map, again most likely attributable to the number of strains in the RI panel.

The delineation of a regulatory genetic network controlling catecholamine biosynthesis in the SHR (described in Chapter 3) represents a significant advance on previously published work on several accounts. Firstly, we have solved the molecular basis of dysregulated catecholamine synthesis and secretion in the SHR. This abnormality was described more than 30 years ago in two Nature papers[89,90], and scores of papers have been written in the intervening decades trying to understand the underlying mechanism. The findings presented in this dissertation conclude this work, by identifying the molecular basis of these traits which are central to the hypertensive phenotype in the SHR. Secondly, we have described the molecular basis of these

phenotypes comprehensively, with causative changes found at the level of genomic sequence, transcript, enzyme activity and catecholamine content. In addition, the discovery of molecular mechanism for a physiological QTL is significant by itself, since only less than 20 of the more than 2,000 QTLs so far identified in mice and rats were characterized at a molecular level[44]. Thirdly, these observations of the basis of catecholamine dysregulation in the SHR have potentially profound physiological and evolutionary consequences on the basis of correlations between biochemical changes and both blood pressure and heart rate.

The analysis of the adrenal transcriptome (described in Chapter 4) resulted in the characterization of the natural variation in gene expression levels. The expression level of most genes is a heritable trait, regulated by genetic variation, either in the genic region itself (*cis*) or from more distant loci (*trans*). Identification of *trans*-eQTL clusters implies that a group of genes are jointly regulated from a single locus. Such grouping is conceivable either on the basis of functional relatedness of the co-regulated genes that could elicit a regulatory (compensatory) response to one another, or on the basis of genes exhibiting pleiotropic effects due to their involvement in vital cellular processes, such as energy metabolism, transcription, and translation. In our analysis we established that the latter was the case for the HXB/BXH adrenal transcriptome. Using a novel multivariate distance-matrix regression analysis (MDMR) to ascertain similarities among the co-regulated transcripts (and suppress the effect of noise) we identified two genes involved in pre-mRNA processing – a process that is extremely relevant to the examined phenotype, i.e. transcript abundance. An

additional finding is the *cis*-eQTL clustering in the MHC complex genomic region, indicating that MHC haplotypes confer substantial variation in MCH gene expression. This is noteworthy especially because of the known MHC association with numerous diseases.

THE FUTURE OF COMPLEX TRAIT ANALYSIS

Understanding the genetic architecture of complex traits continues to be of great interest to the biomedical community. The majority of common chronic human diseases, including cancer, cardiovascular disease, obesity, diabetes, neurodegenerative diseases, and behavioral disorders, are a result of complex pathologies that implicate many molecular and physiologic processes. Disease susceptibility and progression are influenced by numerous genetic, developmental and environmental factors. The contributing genetic factors are presumed to be a combination of common sequence variants of small effect and rare sequence variants of large effect, as proposed by R.A. Fisher[20]. This concept was recently shown to be consistent with the genetic basis of type 1 diabetes (T1D) susceptibility, as a recent analysis yielded a characteristic L-shaped distribution of allelic effect sizes for the ten loci so far confirmed in T1D[167].

The recent explosion of genome-wide association studies (GWAS), made possible chiefly by the availability of the high-throughput genotyping technologies, has resulted in the identification of a large number of risk alleles for common human diseases. Almost all of the newly identified risk alleles, however, have small effect sizes (odds ratio < 2). A complementary strategy to the GWAS is deep population re-

sequencing of candidate genes[168]. This strategy consists of use of knowledge of disease pathophysiology to select plausible candidates for re-sequencing in many diseased and healthy individuals, with the hope that mutations will be discovered that can be further subjected to testing. A great advantage of this strategy is the ability to detect alleles of large phenotypic effect, which would be missed in GWAS because of their likely low population frequency.

Linkage studies have the ability to detect the causative genomic regions likely to harbor important DNA sequence variations, without *a priori* knowledge of the location of any specific disease-causing allele. A gene can be affected by many different mutations in various individuals, which still can lead to the same disease due the same genic function being disrupted (e.g. cystic fibrosis). In such situations, linkage analysis will have advantage over association study because the heterogeneity of the disease-causing alleles would plague association analyses involving specific variations, but not in the identification of the gross genomic region harboring these variations via linkage analysis.

Despite the progress of genetic analysis in humans, model organisms will likely continue to contribute important pieces to genetic puzzles surrounding complex disease expression. This is particularly true of the large-scale effort to create "The Collaborative Cross", a community resource for the genetic analysis of complex traits, which will consist of a thousand recombinant inbred strains of the mouse originating from eight inbred progenitors[169], thus segregating eight potential alleles at each locus, compared to the two customary alleles in crosses like the HXB/BXH. Such model

population will better represent the genetic structure of human populations and will allow more accurate modeling of gene-gene and gene-environment interactions[170].

It can be safely predicted that the future of complex trait analysis lies with a combination of all four above mentioned approaches. As the cost of re-sequencing continues to fall, deep re-sequencing will be carried out in large number of individuals and across a wide range of disease. Information on rare disease causing mutations will continue to grow and will eventually be combined with information from GWAS to construct multifactorial models of disease in an attempt to quantify individual risk and predict disease outcome. This will potentially lead to timely identification of individuals at risk, improved prevention, and treatment tailored to the genetic profile, as is the stated goal of Personalized Medicine. A catalog of human variation in heath and disease is already being compiled via the “Human Variome Project”[171]. Such resources, completed with the results of longitudinal studies of the carriers of specific genetic variants, will further help increase our understanding of the genetic structure of complex traits.

Appendices

Appendix 1: Marker strain distribution patterns

Legend: Markers are identified by chromosome number and name. Marker position (Haldane cM) and strain distribution pattern (rows) for each strain (columns) are given. Strain distribution patterns are listed with the strains in the following order: 1HXB, 2HXB, 3HXB, 4HXB, 5HXB, 7HXB, 10HXB, 15HXB, 17HXB, 18HXB, 20HXB, 21HXB, 22HXB, 23HXB, 24HXB, 25HXB, 27HXB, 29HXB, 31HXB, 2BXH, 3BXH, 5BXH, 6BXH, 8BXH, 9BXH, 10BXH, 11BXH, 12BXH and 13BXH. Genotype coding: 'H' = SHR, 'B' = BN.*Lx*, '-' = unknown.

Chr	Marker	cM	Strain distribution patterns
Chr1	D1Rat327	0.0	BHBHHBHBBBBBHBHHBBBBHHHHBBHHB
Chr1	D1Rat7	3.2	BHBHHBHBBBBBBHHHHBBBHHHHBBHHB
Chr1	D1Rat186	4.1	HHBHHBHBBBBBBHHHHBBBHHHHBBHHB
Chr1	D1Mgh2	21.2	HHBHHBHHHBHHBBBHBBBBHHHBBBBHB
Chr1	D1Rat252	25.8	BHBHHBHHHBBHHBBHBBBBHHHBBBBBB
Chr1	D1Utr6	27.7	HHHHHBHHHBBHHBBHBBBBHHHBBBBBB
Chr1	Slc9a3	32.7	HHBHHBH-HBBBHBBHB-BBHHBHBBBBB
Chr1	D1Rat15	37.7	HBBHHBHHHBBHBBBHBBBBHHHHBBBBB
Chr1	D1Cebr68s1	42.3	HBHHHHHHHBBHBBBHHBHBHHHHBBBBB
Chr1	D1Rat18	45.4	HBBHHHHBHHBHBBBHHBHBHHHHBBBBB
Chr1	D1Mit9	47.4	HBBHHHBBHBBHBBBHHBHBHHHHBBBBB
Chr1	D1Rat20	49.4	HHBHHHHBHBBHBBBHHBHBHHHHBBBBB
Chr1	D1Rat256	52.5	HHHHHHHHHHBHBBBHHBHBHHHHBBBBB
Chr1	D1Rat24	63.0	BHHHHHHHBHBHBBHHBBBHHHHHBBHBB
Chr1	Cyp2b2	66.3	HHHHHHBHBHBHBHHHBBBHHHHHBBHB-
Chr1	D1Cebrp131s2	68.4	HHHHHHHHBHBHBHHHBBHHHHHHB-HBH
Chr1	D1Rat212	71.7	HHHHHHHHBHBHBHHHBBBHHHHHHHHBB
Chr1	D1Rat27	77.9	HHHHHHBHBBBHBHHHBBHBHBHHHHHBB
Chr1	D1Rat266	79.9	HHHHHHBHHHBHBHHHBBHBHBHHHHHBB
Chr1	Klk1	84.4	HHHHHBBHBHBHHHHHBBHBHBHBHHHBB
Chr1	D1Rat30	92.6	BHHHHHBHHHBHHHHHHBHBHBHBHBHHB
Chr1	D1Rat268	98.7	BHBHHHHHHHBHHHHHBHHBBBHBHBHHB
Chr1	D1Cebr31s2	101.9	HHHHHHHHHHBHHBHHBHHBBBHBHBHHB
Chr1	D1Arb11	104.5	-H-HHHHHH-BH-HHH-HHBBBBBH-HHB
Chr1	D1Cebr72s1	107.0	HHBHHHHHHBBHHBHHBHHBBBHBHBHHB
Chr1	D1Cebr100s2	109.0	HHHHHHHHHHBHHBHHBHHBBBHBHBHHB
Chr1	D1Rat35	113.5	BHHHHHHHHHBHHBHBBHHBBBBBHBHBB
Chr1	D1Rat270	118.1	BHHHHHHHBBBHHBBBBHHBBBBBBBHBB
Chr1	D1Rat42	124.3	BHBHHHHBBBBHBBBBBBBBBBBBBBHBB
Chr1	D1Cebr16s1	127.4	HHBHHBHBBBBHBBBBBBBBBBBHBBHBB
Chr1	D1Arb15	128.3	HHBHHBHBBBBHBBBBBBBBBBBBBBHBB
Chr1	D1Utr9	131.5	HHBHHBHBBBBHBBBBBHHBBBBBBHHBB
Chr1	D1Arb16	136.5	HHBHHBHBBHBH-HBBBBHBHBBBBHHB-
Chr1	D1Rat47	137.5	HHBHHBHBBBBHBHBBBBHBHBBBBHHBB
Chr1	D1Rat277	138.4	HHBHHBHBBBBHBHBBBBBBHBBBBHHBB
Chr1	D1Cebr21s2	139.9	HHBHHBBBBBBHBHBBBBB----------
Chr1	D1Cebr7s3	141.3	HHBHHBBBBBBHBHBBBHBBHBBBBHHBB
Chr1	D1Arb17	142.2	HHBHHBBBBBBHBHBBBBBBHBBBBHHBB
Chr1	D1Rat287	146.8	HHBHHBBBHBBHBHBBBHBBHBBBHBHBB

Chr	Marker	cM	Strain distribution patterns
Chr1	Mt1pa	147.7	HHBHHBBBHBBHBBBBBHBBHBBBHBHBB
Chr1	Lsn	149.7	HHBHHBBBHBBHBBBBBHBBHHBHHBHBB
Chr1	Scnn1g	153.0	HHBHHBBBHBBHB-BBBHBBHBBBHHHBB
Chr1	D1Cebr10s3	161.5	HHHHHBBBHHBHHHBHBHBBHBBHHBHBB
Chr1	Igf2	163.5	HHHHHBBBHBBHHBBHBHBBHBBHHBHBB
Chr1	D1Rat292	165.5	HHHHHBBBHHBHHBBHBHHBHBBHHBHBB
Chr1	D1Rat293	173.6	HHBHHBBBHHBHBBBHBHBHHBHHHBHHB
Chr1	D1Rat71	178.1	BBBHHBBBHHBHBHHHBHBHHBHHHBHHB
Chr1	D1Rat296	188.6	HHBHHHBBHHHHBHHBBHBHBBBHHBHHB
Chr1	D1Mit34	196.8	BHHHHHBBHBHHBBHBBHBHBBBHHBHBH
Chr1	Jak2	201.2	BHH-H-B-BBBHBB-B--BHBBBHH-HHH
Chr1	D1Rat77	207.9	BHBHHHBBBBHHBBHBBHBHBBBBBBHHH
Chr1	D1Rat304	216.0	BHHHBHBBBBHHBBHHBHHBBBBBBBHHB
Chr1	D1Utr5	217.0	BHHHBHBBHBHHBBHHBHHBBBBBBBHHB
Chr1	D1Cebrp29s6	217.9	BHBHBHBBHBHHBBHHBHHBBBBBBBHHB
Chr1	D1Utr7	221.0	BHHHBHBBHHHHBHHHBHHBBBBBBBHHB
Chr1	D1Rat235	224.2	BHHHBHBBBHBHBBHHBHHBBBBBBBHHB
Chr1	D1Rat81	226.2	HHHHBHBBHHBHBBHHBHHBBBBBBBHHB
Chr1	D1Rat225	232.3	HHHHBHBHHHBHBBHHBHHHHHBBHBHHB
Chr1	D1Mit14	233.3	HHHHBHHHHHBHBBHHBHHHHHBBHB-HB
Chr1	Adrb1	234.3	HHHHBHBHHHBHBBH-BHHHHHBBH-HHB
Chr1	D1Cebr19s2	236.4	HHHHBHHHHHBHBBHHBHHHHHBBHBBHB
Chr1	D1Utr4	237.4	HHHHBH-HHHBHBBHHBHHHHHBBHBHHB
Chr1	D1Utr10-U7	241.2	-HHHBHHHHHBHBBHHBHHHHBBHBBH--
Chr2	D2Rat189	0.0	BBHBHHBHBHHBBBHHBBHHHHBBHHHBH
Chr2	D2Rat124	8.1	BBBBHBBHBHBBBBHBBBHHHBBBBHHBH
Chr2	D2Rat94	14.3	HHBHHBHHBHBHBBHBBBHHHBBBBHHBH
Chr2	D2Rat116	15.2	HHBHHHHHBHBHBBHBBBHHHBBBBHHBH
Chr2	D2Rat11	18.4	HBHHHHHHBBBHBBHBBBHHHBBBBHHBH
Chr2	D2Rat201	26.5	BHBBHHHHHBBHBHHBBBHHHBBBBHHBH
Chr2	D2Rat202	28.5	BBHBHHHHHBBHBHHBBBHHHBBBBHHBH
Chr2	D2Mgh14	33.0	HBHBHHHHHBBHHHHBBBHHHBBBBBHBB
Chr2	D2Rat197	36.3	BBH-HHHHBBBHHHHBBBHHHBBBBBHBH
Chr2	D2Mit4	41.1	BBBBHBHHHBBHHHHBBBHHHBBBBHHBH
Chr2	D2Mit5	43.1	BBBBHBHHHBBHHHH-BBHHHBBHBBHBH
Chr2	D2Ucsf2	44.1	BBBBHBHHHBBHHHHBBBHHHBBHBBHBB
Chr2	D2Rat75	46.0	BBBBBBHHHBBHHHHBBBHHHBBHBBHBH
Chr2	D2Utr8	47.0	BBBBBBHHHBBHHHHBBBHHBBBHBBHBH
Chr2	D2Utr1	50.3	BBBBHBHHHBBHH-BBBBHHHBBHBBHBH
Chr2	D2Utr10	51.2	BBBBHBHHHBBHHHHBBBHHHBBHBBHBH
Chr2	D2Rat320	54.4	BHHBHBHHHBBHHHHBBBHHHBBHBHHBH
Chr2	D2Mit6	60.5	BBBBBBHHHBHHHHHBBBHHHBBHBBHBH
Chr2	D2Rat95	68.7	BHBBBHHHHBBHHHHBBBHHBHHHBBHBH
Chr2	D2Cebr11042	73.2	BBHBBHHBHBBHHHHBBBHHBBHHBBHBH
Chr2	D2Cebr11041	74.1	BBBBBHHBHBBHHHHBBBHHBBHHBBHBH
Chr2	Cpb	75.3	BBBHBHH-H-BHH-HBBBHHBBHHBBH--
Chr2	D2Mit7	79.5	HBBH-HHBHBBHBHHBBBHHBBHHBHHHH
Chr2	D2Mit18	84.5	BBBHBHHHHBBHB-HBBBHHBBHHBBHBH
Chr2	D2Rat24	87.8	BHBBBHHHHBBHHBHBBBHHBBHHBBHBH
Chr2	D2Rat147	98.3	BHBHBHHHHBBHHBBBBBHHHBHBHHHHH
Chr2	D2Rat115	100.2	BHBHBHHHHHBHHBHBBBHHHBHBHHHHH
Chr2	D2Rat221	103.4	HHBHBHHHHBBHHBHBBBHHBBHBHHHHH
Chr2	D2Rat34	105.4	BHBBBHHHHBBHHBHBBBHHBBHBHHHHH

Chr	Marker	cM	Strain distribution patterns
Chr2	D2Rat222	107.5	HHBHBHH-HBBHHBHBBBHHBBHBHHHH-
Chr2	D2Utr2	109.7	HBBHBHHH-BBHHBHBBBHHBBHBHHBHH
Chr2	D2Utr7	111.8	HBBHBBHHHBBH-BHBBBHHHBHBHHBHH
Chr2	D2Mit9	114.0	-BBHBHHHHBBHHBHBBBHHBBHBHHBHH
Chr2	D2Rat38	114.9	HBHHBHHHHBBHHBHBBBHHBBHBHHBHH
Chr2	D2Rat152	116.9	HBHHBBHHHBBHHBHBBBBHBBHBHHBHH
Chr2	D2Rat228	127.4	BBHHBBHBHBBHBBHHBHBHBHBBHHBHH
Chr2	Fgg	129.3	BBHHBBHBHBBHBBHHBHBHBBHBHHBHH
Chr2	D2Utr11	130.2	BBBHBBHBHBBHBBHHBHBHBBHBHHBHH
Chr2	D2CebrP476s2	134.8	BBBHBBHBHBBHBBHBBHBHBBBBBHHHH
Chr2	D2Cebr11s2	139.3	BBBHBBHHHBBHBBHBBHBHBBHBHHBHH
Chr2	D2Utr9	141.4	BBBHBBHBHBBHBB-HBHBHBBHBHHBHH
Chr2	Fga	142.4	BBHHBBHBHBBHBBHHBHBHBBHBHHB-H
Chr2	D2Cebr28s4	143.3	BBHHBBHHHBBHBBHHBHBHBBHBHHBHH
Chr2	R802	146.6	BBHHBHH-HBBHBBHHBHBHBHBBHHBHH
Chr2	D2Rat42	147.7	BBHHBBHBHBBHBBHHBHBHBHBBHH--H
Chr2	P9ka	151.1	BBHHBHHHHBBBBBHHBHBHBHBBHHBHH
Chr2	Npr1	154.3	BBHHBBHBHBBHBBHHBHBHBHBBHHBHH
Chr2	Atp1a1	156.2	BBHHHBHHHBBHBBHHBHBHBHBBHHBHH
Chr2	D2Cebr10s2	159.7	BBHHHBHHHBHHBBHHBHBHBBBB-HBB-
Chr2	D2Mit14	161.8	BBHHHBHHHBHHBBHHBHBHBHBBHHBHH
Chr2	D2Cebr204s17	163.9	BBHHHBHHHBHHBBHHBHBH-HHHHH-HH
Chr2	D2Cebr42s3	166.1	BBHHHBHHHBHHBBHHBHBHBHBBHHHHH
Chr2	D2Utr3	167.0	BBBHHBHHHBHHBBHHBHBHBHBBHHHHH
Chr2	D2Rat57	170.3	BBBHHHHBH-HHBHHHBHBHBHBBHHHHH
Chr2	D2Cebr4s8	172.3	BHBBHHHBHBHHBHHHBHBHBHBBHHHHH
Chr2	D2Ucsf1	174.0	----HHHH--HHB-HHBHBHB-B-H---H
Chr2	D2N91	177.7	BBHBHHHBHBHHBHHHBHBHHHBBHHHHH
Chr2	D2Rat62	183.8	BHHBHBHBHBBBBHHHBBBHHHBBHHHHH
Chr2	D2N35	184.8	BHHBHBHBHBBBBHHHBBBBHHBBHHHHH
Chr2	D2Rat61	185.7	-HHBHBHBHBBBBHHHBBBHHHBBHHHHH
Chr2	D2CebrP133s9	186.7	BHBBHBHBHBBBB-HHBBBHHHBBHHHHH
Chr2	D2Rat247	190.0	BHBBHBHBBBBBBHHHBHBHHHBBHHHHB
Chr2	D2Rat66	196.2	HBBHBBHBBBHBBHHHBHBHHHBBHHHHB
Chr2	D2Rat67	200.7	HHBHBBHBBBHHBHHHBHBHHHBBHHBBB
Chr2	D2Rat69	205.3	HHBHHBHBBHHHBHHHBHHHHHBHHHBBB
Chr2	D2Rat70	211.5	HBBHHBHBBHHHBBBBBHHHHBBHHHBBB
Chr2	D2Mit16	212.4	HBBBHBHBBHHHBBBBBHHHHB-HHHBB-
Chr3	D3Cebrp207s7	0.0	BBHBHHHHBHBBBBBBBHHBHBHHBHBHB
Chr3	D3Cebr204s4	0.9	BBHHHHHHBHBBBBBBBHHBHBHHBHBHB
Chr3	D3Ucsf1	1.9	BBBHHHHHBHBBBBBBBHHBHBHHBHB--
Chr3	D3Cebr26s1	4.0	BBHHHHHHHHBBBBBBBHHBHBHHBHBHB
Chr3	Dbh	5.0	BBHHHHHHBHBBBBBBBHHBHBHHBHBHB
Chr3	D3Rat194	6.9	HBHHHHHHHHBBBBBBBHHBHBHHBHBHB
Chr3	D3Rat53	15.0	BBBBHHHBHHBBHBBBBBHBHBHHBHBHB
Chr3	D3Utr7	19.6	HBBBHHHBHHBBHBBBBBHBHHBHBHBBB
Chr3	D3Utr5	22.9	HHBHHHHBHHBBH-BBBBHBHBBHBHBBB
Chr3	D3Mit9	23.9	HHBBHHHBHHBBHBBBBBHBH-BHBHBBB
Chr3	D3Rat82	27.2	HBBBHBHBHHBBHBBBBBHBHHBHHHBBB
Chr3	D3Rat188	37.7	HHHHHBHHHHHBHBBBBBHBHHBBHBBBB
Chr3	D3Rat185	39.6	HHHHHBHHHHHBHBBBBHHBHBBBHBBBB
Chr3	Scn2a	41.6	HHHHHHHHHHHBHBBBBHHBHBBBHHBBB
Chr3	D3Utr8	43.6	HHHBHHHHHHHBHBBBHH-BHBBBHHBBB

Chr	Marker	cM	Strain distribution patterns
Chr3	D3Rat183	46.9	BHHBHHHHHHHBHBBBBHHBHHBBHHBBB
Chr3	D3Rat180	57.4	HHHBBBHHBHHBBBBBBHHBHHHBHHHBB
Chr3	D3Rat35	62.0	HHHBHBHHBBHBBBBBBHBBHHHBHBHBB
Chr3	D3Utr4	63.9	HHHBHBHHBBHBBHBBBHBBHHHBBBHBB
Chr3	D3Rat173	67.1	HHHBHBBHBBHBBBBBBHBBHHHBBBBBB
Chr3	D3CebrP97s12	75.2	HHHBHBBHBHHBHBHBBHBBHHHHBHBBH
Chr3	Cat	79.8	HHHBHBBHBHHBBBHBBHBBHHHBHHBBB
Chr3	D3Mit16	83.0	HHHBHBBHBHHBBBHBBHBB-HBBBBBBB
Chr3	D3Cebr2s4	85.1	HHHBHBBHBHHBBBBBBHBBHHHBBBBBB
Chr3	D3Cebr9s1	86.0	HHHBHBBHBHHBBBBBBHBBHHBBBBBBB
Chr3	D3Mit15	87.1	HHHBHBBHBH-BBBBBB--B--BBBHBBB
Chr3	D3Mit6	88.3	HHHBHBBHBHHBBBBBBHHBBHBBBBBBB
Chr3	D3Ucsf3	90.2	HHHBHBBBBHHBBBBBBHHBBHBBBBBBH
Chr3	D3Mit17	92.2	HHHBHBBBBHHBBBBHBHHBBHBBBBBBB
Chr3	D3Rat166	93.1	HHHBHBBBBHHBBBBBBHHBBHBBBBBBB
Chr3	D3Rat20	97.6	HHBBHBBBBHHBBBHHBHHBBHBBBHBBB
Chr3	Slc12a1	99.9	HH-BHBBBBBBHBBBH-BHBBBHBBB-BBB
Chr3	D3Mit14	103.5	HHHBBBBBHHHBBBHHBHBBBHBBBHBBB
Chr3	D3Rat159	106.6	HHBBBBBBHHHBBBHHBHBBHHHBBHBBB
Chr3	D3Cebr4s2	107.6	HHBBBBBBHHHBBBHHBHBBHHHHBHBBB
Chr3	Gox1	110.7	BHHBBBBHHHHBBBHHBHBBHHHHBHBBB
Chr3	D3Mit4	116.3	BHBBBBB-HHHB-BHHBH-HHHHHB-BHH
Chr3	D3Mit2	118.6	BHBBBBBHHHHBHBBHBHBBHHHHBHBHH
Chr3	D3Rat6	123.2	BHHBBBHHHHHBHHBHBHHBHHHHBHBHH
Chr3	D3Cebr45s8	131.3	BHHBHBHBHHHBHBBBBHBBHHHHBHBHB
Chr3	Svp1	136.0	BBHBHBBBHHBBHBBBBBBBHHHHBHBH-
Chr3	D3Cebr80s2	139.3	BBHBHBHBHHBBHBBHBHBBHHHHBHBHB
Chr3	D3Cebr80s1	141.3	BBBBHBBBHHBBHBBHBHBBHHHHBHBHB
Chr3	D3Rat143	144.5	BHHBHBBBHHBBHBBHBHHBHHHHBHBHB
Chr3	D3Rat132	150.6	BHHBHHBBBHBBHBBHBHHBHHBHBHHHH
Chr3	D3Rat1	152.6	BBHBHHBBBHBHHBBHBHHBHHBHBHHHH
Chr3	Edn3	153.5	BBHBHHBBBHBBHBBHBHHBHHBHBHHHH
Chr4	D4Ucsf1	0.0	HHBHHBHBHB--HBBHHBHHHHHBHBHHH
Chr4	D4Cebr88s1	1.0	HHBHHBHBHBBHHHBHHBHHHHHBHBHHH
Chr4	R133	3.0	HHBHHBHBHBBHHHBHHBHHHHBBHBHBH
Chr4	Il6	4.9	HHBHHBHBHBBHHHBHHBHHHHHBHBHHH
Chr4	D4Utr2	5.8	HHBHHBHBHBBHHHBHBBHHHHHBHBHHH
Chr4	Cd36	7.8	HHBHHBHBHBBHHHBHBHHHBHHBHBHHH
Chr4	D4Cebr6s16	10.9	HHBHHBHBHBBHHHBHHBHHHHHBHBHHH
Chr4	D4Rat4	11.9	HHBHHBHBHHBHHHBHHBHHHHHBHBHHH
Chr4	D4Rat7	13.8	HHBHHBHBHHBHHHBHBHHHHHHBHBHHH
Chr4	D4Rat10	24.3	HBBHBBHBHHHBHBBHBHHHBHHBBBHHH
Chr4	D4Rat151	32.4	BBBHHBHBHHHHHBHHHHHHBHHBBBHBH
Chr4	D4Utr3	34.4	BBBHHBHBHHHHBBHHHHHHBHHBBBHHH
Chr4	D4Rat16	39.4	BBBH-HHBHHHHBHHH-HBBBHHBBBHHH
Chr4	D4Cebrp149s8	46.3	HHBHHHBBHHHBBBHHBHBBBHHBBBHHH
Chr4	D4Cebr46s5	47.3	B-BHHHB-HHHB-BHHBHBBBHHBBBHHH
Chr4	D4Mit9	50.9	HBHHHHHBHHHBBBHHBHBBBHHBBBHHH
Chr4	D4Utr1	53.0	HBBHHHHBHHHHBBHHBHBBB-HBB-HHH
Chr4	D4Rat153	58.0	BHBHHHHBHHHHBHHHBHBHBHHBBBHHH
Chr4	D4Rat102	66.6	BHBHBHBBHHH-BBHBBHBBBBHBBBHHH
Chr4	Try1	68.8	BHHHBHBBHHHBBBBBBHBBBBHB-BHHH
Chr4	Klk1rs	70.8	BHHHHHBBHHHBBBBBBHBBBHHBHBHHH

Chr	Marker	cM	Strain distribution patterns
Chr4	D4Cebrp1016s14	74.4	BHBHHBB-HHHBB-BBB-BBBHHBBBHHH
Chr4	Hoxa11	76.6	BHHHHBBBHHHBBHBHBHBBBHHBBBHHH
Chr4	Npy	78.6	BHHHHBBBHHHBBBBHBHBBBHHBBBHBH
Chr4	D4Mit5	81.8	BHHHBBBBHHHBBBBHBHBBBBHBBBHHH
Chr4	D4Rat168	86.3	BHBHBBBBHHHBBBBHBHBHBHHBHBHHH
Chr4	D4Cebrp215s9	89.8	BHBHBBBBHHHBBHBHBHBBB--BBBHHH
Chr4	D4Rat235	91.9	BHBHHBBBHHBBBHBHBHBBBHHBBBHHH
Chr4	D4Rat37	98.1	BHBBHBBBHHBBBHHHHHBHBHBBBBHHH
Chr4	D4Rat176	106.2	BHBBHBBBHHBHBBHHHBBBHHHBBBHHH
Chr4	Spr	109.4	BHHBHBBBHHBHBHHHHBBBHHHBBBHBH
Chr4	D4Rat44	113.9	BHBBHBBBHHBHBHBHHBBBHHHHBBHHH
Chr4	D4Rat58	127.3	HHBHHBBBHHHHBHHHHBHBHHHHHBBBH
Chr4	D4Rat240	135.9	BHBHHHBBH-BBBHHHHHHBHHHHHBHBH
Chr4	Cacna1s	138.0	HHBHHHBBHBBBBHHHHHHBHHHHHBHHH
Chr4	D4Cebr7s17	138.9	HHHHHHBBHBBBBHHHHHHBHH-HHBHHH
Chr4	Eno2	142.4	HHHHH-BBHBBBBHHHHHHHHHHBHBHHB
Chr4	D4Cebr7s7	145.7	HHBHHBBBHHBBBHHHHHHBHHHBHBHHB
Chr4	A2m	148.8	HHHHHBBBHBBBBHHHHHHBHHHHHBHHB
Chr4	D4Mit19	150.8	HHHHHBBBHBBBBHHHHHHBHHHHHBHBH
Chr4	Pparg	152.0	HHBHH-BBH-BBB----HHBHHHHH-HBH
Chr4	D4Rat198	153.2	HHBHHHBBHHBBBHHHHHHBHHHHHBBBH
Chr4	D4Rat202	159.4	HHBHHBBBHHBBBHHHHHHBHHHBHBHHB
Chr4	D4Rat68	165.6	HHBHHHBBHBHBBHBHHHHBHHHBHBBHB
Chr5	D5Mgh17	0.0	HBHBBHBHBBBBHBHBBHHHBHBHHBBHB
Chr5	D5Rat188	6.2	HBHBBBBHHHBBHBHBBHHHBHBHHHHHB
Chr5	D5Rat218	12.4	HBHBBHBHHHHBBHHBBHHHBHBHBHHHB
Chr5	D5Rat6	18.5	HBHHBHBHHHBBHHHHBHHHBBBHBHHHB
Chr5	D5Mit10	20.5	HBHHBHBHBHBBHHBHBHHHBBBHBHHHB
Chr5	D5Cebr63s1	21.4	BBHHBHBHBHBBHHBHBHHHBBBHBHHHB
Chr5	D5Cebr63s2	23.4	BBHBBHBHBHBBHHBHBHBHBBBHBHHHB
Chr5	D5Utr1	26.5	BBHBBHBHBHBBHHBHBHHHHBHHBHHHB
Chr5	D5Mit9	28.6	BBHHBHBHHHBBHHBHBHHHHB-HBHHHB
Chr5	D5Rat135	30.6	BBHHBHBHBHBBHHBHBHHHBBBHBHHHB
Chr5	D5Rat228	41.1	BBHBBHBHBHBHBHBHBHHHHHHHBBHHB
Chr5	D5Cebrp312s4	43.1	BBHBBHBHBHBBBHBHBBHHHHHHBBHHB
Chr5	D5Mit2	46.4	BBHBBHBHBHBBBHBHHHHHHBHHB-HHB
Chr5	D5Rat144	51.1	BBHBBHBHHHBHBHBHBBHHHBHHBBHHB
Chr5	D5Rat147	55.7	BHHHBHBHHHBBBHBHBBHHBBHHBBHHB
Chr5	D5Utr2	58.8	BHHHBHBHHHBBHHHHBBHHHBHHBBHHB
Chr5	D5Utr5	60.8	BHHHBHHHHHBBHHBHBBHHHBHHBBHHB
Chr5	D5Utr4	61.7	BHHHBHHHHHHBHHBHBBHHHBHHBBHHB
Chr5	D5Mit4	63.7	BHHHBHHHHHBBHHBHBBHHBBHHBBHHB
Chr5	D5Utr3	65.6	BHHHBHHBHHBBHHBHBBHHBBHHBBBHB
Chr5	D5Mit5	72.1	HHHHBHHHHHB-HHHHBBHBBBHHBBHHB
Chr5	Jun	73.1	HHHHBHHHHHBBHHHHBBHBHBHHBBHHB
Chr5	Pgm1	75.4	HHHBB-HH--BBHHHHBBHBHBHHB-HBB
Chr5	D5Rat158	77.7	HHHBBHHHBHBBHHHHBBHBHHBHBHHBB
Chr5	Slc2a1	82.3	HHHBBBHHBHBBHHHHHBHBHHBHBBBBB
Chr5	D5Rat169	84.3	HHHBHBHHBHBBHHHHHBHBHHBHBHBBB
Chr5	D5Rat34	90.4	HHHBHBHHHHBBHHHBHHHHHHBHBHBBH
Chr5	D5Rat63	100.9	HHHBHBHHBHBBHHHHHHHBHBHBBBBBH
Chr5	D5Rat93	111.4	BBHBHBBHBBBBHBHHHHHBHBHBBBHHH
Chr5	D5Rat45	117.6	BBHHHBBHBBHBHBHHHHHBHHHBHBHBH

Chr	Marker	cM	Strain distribution patterns
Chr5	D5Rat245	128.1	BBHHBBHHHBHHHBHHHBBHHHHBHBHBH
Chr5	D5Mgh15	129.0	BBHHBBHHH-HHHBHHHBBHHHHBHHHBH
Chr5	Clcnkb	132.8	BBHHHB-HHHH-H-HHHHHHHHHBHHHBH
Chr5	D5Cebr2s2	138.1	BBHHHBHHBBHBHBHHHHHBHHHBHBHBH
Chr5	D5Cebr2s1	139.0	BBHHHBBHBBHBHBHHHHHBHHHBHBHBH
Chr6	D6Cep8	0.0	BBBBHHBBHBBBHBBHBBHHHHBBHHBB-
Chr6	D6Cebrp424s2	1.0	BBBBHHBBHBBBHBBHBBHHHHBBBHBBB
Chr6	D6Cebr204s20	4.6	-BHBHHBHHBB-B-BHBBHHHHBBBHBBB
Chr6	D6Cebrp40s27	5.6	BBHBHHBHHBBBHB-HBBHHHHBBBHBBB
Chr6	D6Mit5	10.4	BBBBBHHBHBBBHBBHBBHHHHBBBHBBB
Chr6	D6Rat167	11.6	----BBHBHBBBHBBHBBHH-HBBB-BB-
Chr6	D6Rat46	12.9	BHBBBHHBHBBBHBBHBBHHHHBBBHBBB
Chr6	D6Rat80	19.1	BBBBHHHBBBHBHBBHBBHHHHBBHHBBB
Chr6	D6Rat147	23.6	HBBHBHHBBBHBHBBHBHHHHHBBHHBBB
Chr6	D6Rat171	31.7	HBBHBHHBBBBHBBHHBHHHHBBBHBBBB
Chr6	D6Rat84	37.9	HBBHBHHBBBBBBHHBBHHHHBBBHBHHB
Chr6	D6Mit9	40.1	HBHH--HB-BBBBHHBBBHHHBBBHBHHB
Chr6	D6Rat36	43.9	HB-HBHHBBBBHBBHBBHHHHBBBHBHHB
Chr6	D6Rat29	44.9	HB-HBHHBBBBHBBHBBHHHHBBB-BHBB
Chr6	D6Rat37	45.9	HBBHBHHBBBBHBBHBBHHHHBBBHBHHB
Chr6	D6Rat28	52.1	HBBHBHBHBBBHBBHBBHHBHBBBBBHBB
Chr6	D6Rat132	65.5	BBBHBHBHBBBHHHHBBBHBHHBHBHHHB
Chr6	D6Rat165	78.9	BHBHBBBBBBBHHHHBHBHHHBHHHHHHB
Chr6	D6Mit2	83.5	BBHHBBBBBBBHHHHBHBHHHBHBBHHHB
Chr6	D6Mit8	86.6	BHHHBBBBBBBHHHHBHBHHHBHHHHHHB
Chr6	D6Cebr36s1	87.5	BHBHBBBBBBBHHHHBHBHHHBHHHHHHB
Chr6	D6Rat87	92.1	BBBHBBHBBBBHHHHHHBHHBBHHHHHHB
Chr6	D6Rat88	96.6	HHBHBBBHBBBHHHHHHBHHBBHHHHHHB
Chr6	D6Rat117	107.1	HHBHBBBBBBBHHHHBHHHHHHHHBHBHB
Chr6	D6Rat11	115.3	HHBHBHBBBBBHHHHHHHBHHBHHBHBBH
Chr6	D6Utr3	120.3	HBB-BBBBBBB-HHHHHBBHHBHHBHBHH
Chr6	D6Rat184	121.3	HHBHBBBBBBBBHHHHHBBHHBHHBHBHH
Chr6	D6Cebrp165s2	125.8	HHBBBBHBBBBBHHHHHBBBHHHHBHBHH
Chr6	D6Cebr2s3	130.3	HHBBBBHBBBBBHHBHHBBBHHBHBBHHH
Chr6	D6Cebrp91s1	133.5	HHBBBBHBBBBBHHHHHBBBHHHHBBBHH
Chr6	D6Rat111	134.4	HHBBBBHBBBBBHHHHHBBBHHHHBHBHH
Chr6	Chga	135.3	BHBBBBHBBBBBHHHHHBBBHHHHBHBHH
Chr6	D6Rat101	146.5	-HBBHBBBBBHBHHBHHBBBHHHHHBHHH
Chr6	D6Rat1	155.1	HHBBHBBBHBHBHHBBHBBBBBBHBBHHH
Chr6	D6Rat3	161.3	HHBBHBBBHBBBHHHBHBBBHHHHBBHHH
Chr6	D6Utr2	166.0	HHBBHBBBBBHB-BBBHBBBHHHHBBHHH
Chr6	D6Utr6	171.0	HHBBHHBBBBHBHHBHHBBBHHBHB-HHH
Chr6	D6Utr5	174.3	HHBBHBBBBBHBHHBHHBBBHHHHHBHHH
Chr6	D6Cebr82s1	175.3	HHBBHBBBBBHBHHBHHBBBHHBHHBHHH
Chr6	Ighe	176.2	HHHBHBBBBBHBHHBHHBBBHHBHHBHHH
Chr6	Igh@	179.8	HBHBHBBBBBHBHHBH-BBH-HBHB-HHH
Chr6	D6Mit10	185.1	HHHBHBBBBBHBHHBHHBBBHHHHHHHHH
Chr6	Ckb	188.5	BBBBHBBBBBHBHHBHHBBBHHHHH-HH-
Chr7	D7Cebrp179s6	0.0	BBBHHBBBBHHBHBBBHBHBBHHHHBHBB
Chr7	D7Utr5	3.2	BBBBBBBBHHHBHBBBHBHBBHHHHBHBB
Chr7	D7Cebr10s1	6.5	BBBHHBBBBHHBHBBBHBHBBHHHHB-BB
Chr7	D7Cebr205s3	8.9	BBHHHBB-BHBBHBBBHBHB-HHHHBH--
Chr7	D7Rat35	11.2	BBHHHBBHBHBBHBBBHHHBHHHBHBHBB

Chr	Marker	cM	Strain distribution patterns
Chr7	D7Rat32	13.2	BBHHBBBHBBBBHBBBHHHBHHHBHBHBB
Chr7	D7Rat152	21.3	BBHHBBHHBBBBHBHBBBHBBHHHHBHBB
Chr7	D7Mit17	29.4	BBHHHBBHBHBBHBBBHBHBHHHHHBHBB
Chr7	D7Utr1	39.9	BBHHBBHHBHHHHBBBBBHBHBHBHBHBB
Chr7	D7Mit7	41.9	BBBHBBHHBHHHHBBBBBHHHBHBHBHBB
Chr7	D7Rat181	46.6	H-BHBHHBBHHHHBBBBBHBHBHBHBHBB
Chr7	D7Rat103	51.4	HBHHBHHHBHHHHHHBBBHBHBHBHBHBB
Chr7	D7Rat25	61.9	HBBHHHBHBHHHBHHBBBBBHBBHHBHBB
Chr7	D7Mit6	65.1	HBBBHHBBBHHHBHBBBBBBHBBHHBHBB
Chr7	D7Cebr77s3	67.2	HBBBBHBBBHHHBHHBBBBBHBBHHBH--
Chr7	D7Cebr204s12	72.2	HBHBBHBHBHHHBHBBBHBBHBBHHBHBB
Chr7	D7Cebr204s11	73.1	HBHBBHBHBHBHBHBBBHBBHBBHHBHBB
Chr7	D7Rat110	75.1	HBHBBHBHBHHHBHBBBBBBHBBHHBHBB
Chr7	D7Mit5	79.6	BBHBBHBHBHHHHHBBHBBBHBBBHBHBB
Chr7	D7Rat112	87.7	BHHHBBBHBBHHHHBBHHBHHBBBHBHBB
Chr7	D7Rat19	98.2	BHHHBBBHBHHHBHBBBHHHHHBBHHHHB
Chr7	D7Rat133	108.7	BHBHBHBHHHHHBHBHHHHBHBBBHHHHB
Chr7	D7Mit3	112.2	BBBHBHBH-HHHBHBHBHHBHHBBHHHH-
Chr7	D7Mit2	116.0	BBBHBHB-BBHHBH-HBHHHHHBBHHBHH
Chr7	Bzrp	117.0	BBBHBHBHBBHHBHBHBHHBHHBBHHBHH
Chr7	D7Mit13	117.9	BBBHBHBHBHHHBHBHBHHBHHBBHHBHH
Chr7	D7Mit14	119.9	BBBHBHBHBHHHBH-HHHHBHHBBHHBHB
Chr7	Cyp11b1	120.9	BBBHBHBHB-HHBH-HHHHB-HBBHHBBB
Chr7	Cyp11b2	122.0	BBBHBHBHBHHHBH-HHHHBHHBBHHBHB
Chr7	D7Cebr77s1	124.0	BBHHBHBHBHHHBHBHBHHBHHBBHHBHB
Chr7	D7Mit4	126.1	BBHHB-BHBBHHBHBHBHHBHHBBHHHHB
Chr7	D7Rat102	128.2	H-HHBHBHBBHHBHBHBHHBHHBBHHBHB
Chr7	D7Rat196	137.3	-BHHBHBHBBHBBHHHBHHBBHHBHHHHH
Chr7	D7Mit8	139.4	HBBHBHBHHBHBBHHHB-HBBHHBHHHHH
Chr7	D7Mit10	144.2	BBBHBHBHBBHBBHHHBHHBBBBBHHHHH
Chr7	D7Utr3	148.7	BBHHBHBHBBHBBHBHBHHBBHHBHHHHH
Chr7	D7Cebr74s1	150.8	BBHHBHBHHBHBBB-HBHHBBHHBHHHHH
Chr7	D7Rat4	154.1	BBHHBBBHHHHBBBHHBHHBBHHHHHHHH
Chr7	D7Rat2	158.6	BHHHBBBHHHHHBBBHBHHHBHHHHHHHH
Chr7	D7Ntr11	160.6	BBHHBBBHHBHHBBBHBHHHBHHHHHHHH
Chr7	D7Ucsf2	162.6	BBBHBBBHHBHHBBBHBHHBBHHHHHHHH
Chr7	Prph	164.7	BBHHBBBHHBHHBBBHBHHHBH-HHHHH-
Chr7	D7Cebr24s2	166.8	BBHHHBBHHBHHBBBHBHHHBHHHHHHBH
Chr7	D7Cebr205s1	168.9	BBHHHBBHHBHHBBBHBBBHBHHHHHH--
Chr7	D7Cebr6s5	169.9	BBHHHBBHHBHHBBBHBBBBBHHHHHH-H
Chr7	D7Cebr6s13	176.8	HBHHHHBHHBHHBBBHBBBHHH-HHHB-H
Chr7	D7Cebr69s5	179.2	BBHHHHBHHBHHBBBHBBBH-HHH-HH--
Chr7	D7Ucsf1	180.4	BBHHHBBHHBHHB--HBBBHBHHHHHHH-
Chr7	D7Cebr46s1	183.1	B-HHBBB-B-HH-B-HBBBHBHHHHHHHH
Chr8	D8Utr4	0.0	BBBHHHBBHHHBHBHBBHBBBHBBHHBBH
Chr8	D8Utr3	4.5	BBBBHHBBHHHBHHHBBBBBBHBBHHHBH
Chr8	D8Rat58	10.7	BBHBHHBHBBHBHHHBBHBBBHBBHHHBH
Chr8	D8Rat56	16.9	BBBBHHBBHHHBHHHBBHBBBHBBHHHHH
Chr8	D8Rat68	27.4	HBBBBHBBHHBBHHHBBBBHBHBBHHBHB
Chr8	D8Mit6	39.3	BBHB-HBBHHHBHHHBBBBBBHBB-HHBH
Chr8	D8Mgh9	64.9	HHHHB-BBHHBBHHHHBBHBB-B-BBB--
Chr8	D8Utr2	66.0	HH-HBHBBHHBBHHHHBBBBBBBBBBBBB
Chr8	D8Cebrp97s13	69.5	HHBHBBBBHHBBHHHHBBHBBHBB-BBBB

Chr	Marker	cM	Strain distribution patterns
Chr8	Es6	71.5	HHHHBBBBHHBBHHHHBBHBBBBBBBBBB
Chr8	D8Cebr81s4	72.4	HHBHBBBBHHBBHHHHBBHBBBBBBBBBB
Chr8	D8Cebr81s1	73.4	HHBHBBBBHHBBHHHHBBHBBHBBBBBBB
Chr8	D8Mit3	79.5	HHHBBBBBHHBHHHHHBBHBHBBBBBBBB
Chr8	Thy1	80.5	BHHBBBBBHHBHHHHHBBHBHBBBBBBB-
Chr8	Apoc3	82.5	HHHBBBBBHHBHHHHHBBHBBBBBBBBBB
Chr8	Kcnj1	85.7	HHHHBBBBHHBBHHHHBBHBBHBBBBBBB
Chr8	D8Rat150	93.8	HHHBBBHHHHBBHHHBBBHBBBBBBBBBH
Chr8	D8Rat213	94.7	HHHBBBHHHHBBHHHBBBHBBBBBBBBBB
Chr8	*Lx*	95.7	HHHBBBBHHHBBHHHBBBHBBBBBBBBBB
Chr8	D8Mit12	96.6	HHHBBBHHHHBBHHHBBBHBBBBBBBBBB
Chr8	Tpm1	99.9	BHHBB-HHBHBBHBHBBBHBBBBBBBBBB
Chr8	D8Utr5	101.9	BHHBBBHHHHBBHBBBBBHBBBBBBBBBB
Chr8	D8Rat21	102.8	BHHBBBHHHHBBHBBBBBHBBBBBBHBBB
Chr8	D8Cebr49s2	106.0	BHBBBBHHHHBBHBHBBBHHBBBBBHBBB
Chr8	D8Cebr92s1	106.9	BHHBBBHHHHBBHBHBBBHHBBBBBHBBB
Chr8	D8Rat130	110.2	BBB-BBHBHHBBHBHBBBHHBBBBBHBBB
Chr8	D8Rat123	116.7	BBBBHBHBHHBBHBHHBBHHBBHHBHBBH
Chr8	Rbp2	119.9	BBBBBBHBHHBBHBHHBBHHBBHHBBBBB
Chr8	D8Cebr10s5	123.0	BBBBHBHBHHBBHBHHBBHHBBHHBHBBH
Chr8	Mylc1v	126.3	BHBHHBHBHHBBHBHHBBHHBBB-BHBBH
Chr8	Apeh	128.4	BHBHHBHBHHBBHBHHBBHHBBHHHHBBH
Chr8	D8Rat7	136.5	BBBHHBHBHHBBHBHHBBHBBBBHBBBBB
Chr8	Acaa	137.4	BBBHHBHBHHBBHBHHBHHBBBBHBBBBB
Chr8	D8Cebr103s2	140.6	BBBHHHHBHHBBBBHHBHHBBBBHBHBBB
Chr9	D9Cebr16s3	0.0	HHHHHBHHHHHBHBHHBHBHHBBBBHBBH
Chr9	D9Cebr25s1	0.9	HHHHHBHHHHHBHBHHBHBHHBHBBHBBH
Chr9	D9Mit6	1.8	HHHHHBHHHBHBHBHHBHBHHBHBBHBBH
Chr9	D9Cebr65s1	3.8	HHHHHBHHBBHBHBHBBHBHHBHBBHBBH
Chr9	D9Cebr65s2	4.7	HHHHHBHHHBHBHBHBBHBHHBHBBHBBH
Chr9	D9Rat88	5.6	HHHHHBHHHBHBHBHHBHBHHBHBBHBBH
Chr9	D9Rat131	16.1	BHHBHBHHHHBBHBHHBHBBHBHBHHBHH
Chr9	D9Rat158	29.5	HBHBBBHHHBBBHBHHBHBBHBBHHHHBH
Chr9	D9Rat180	32.7	HBHBBBHHHBHBHBHHBHBBHHBHHHBBH
Chr9	D9Rat104	49.8	BHHBBBHHHHBBHBHHHHBHHBBHHBHBH
Chr9	D9Rat60	67.0	BHHBBBBHHBHBHBHHBBBHBBHBHBHBB
Chr9	D9Rat19	73.2	BHBBBBHBHBBBHBHHBBHHBBHBHBHBB
Chr9	D9Utr2	79.3	BHBBBBHBBBBBBBHHHBHHBBBBHHHBB
Chr9	D9Rat12	80.3	BBBBBBHBBBBBBBHHHBHHBBBBHHHBB
Chr9	D9Rat171	86.4	BBBHHBHBHBBBBHHHHBHBBBBBHHHBB
Chr9	D9Utr3	91.0	BHBBHBHBHBBBBHHHHBBBBBBBHHHHB
Chr9	Inha	93.0	BHBBHBHBBBBBBHHHHBBHBBBBHHHHB
Chr9	Alp1	99.5	BBBBHBHBHBBBBHHHHBBBBBB-HHBHH
Chr9	D9Utr1	100.4	BBBBHBHBHBBBBHHHHBBBBHBBHHBHH
Chr9	D9Rat153	117.6	BHHBBBHHHHBHBHHHHBHBHHBBHBBHH
Chr9	D9Cebr204s1	120.7	HHHBBHHHHHBBBHHHHBHBHHBBHBBHH
Chr9	D9Mit1	122.8	HHBBBHHHHHHBBHHHHBHBHHBBH-BHH
Chr9	D9Cebr16C27s1	123.7	HHBBBHHHHHBBBHHHHBHBHH-BHBBHH
Chr9	D9Cebr16C27s2	124.7	HHHBBHHHHHBBBHHHHBHBHHBBHBBHH
Chr9	D9Rat108	127.9	HHHHBHHBHHBHBHHHHBHBHHBBHBBHH
Chr9	D9Rat1	138.3	HHBHBHHBBBBHBHBHBBHBHHBBHHBBH
Chr10	D10Rat95	0.0	HBHHHHHBHHBBHHBBBHHBBBBBBBHHB
Chr10	D10Utr3	3.2	HHBHHHBBHHBBHHBBBHHBBBBBBBHHB

Chr	Marker	cM	Strain distribution patterns
Chr10	D10Cebr27s2	5.1	HHBHHHBBHHBBHHBBBHHBBBBBBBBHH
Chr10	D10Rat218	8.3	HBBHHHBBHHBBHHBBBHHBBBBBBBHHB
Chr10	D10Mit6	12.8	HHBHHHBBHHBBHHBBBHHBBBHBBBBHH
Chr10	D10Rat121	17.4	HBHHHHBHHHBBHHBBBHHBBBHBBHBHH
Chr10	D10Rat45	21.9	BBBHHHBBHHBBHHBBBHHBBBHBBHBHB
Chr10	D10Cebrp312s3	26.5	BHBHHHBBHHBBHHBBBHHBBHHBHHBHH
Chr10	D10Mit5	28.4	BHBHHHBBHHBBHHBBBHHBBBHBBHBHH
Chr10	D10Utr5	31.7	BHBHHHBBHHBBHHHBBHHHBBBBBHB-H
Chr10	D10Rat72	38.2	BBBHHHBBBHBBHHHHBHBHBBBBBBBBH
Chr10	D10Mit4	46.4	BBBHHHBBBBBBHHHHHHHHBBBHBBBHB
Chr10	D10Cebrp1016s2	47.4	BBBHHHBBBB-BHHHH---HBBBHBBBBB
Chr10	D10Cebr4s7	48.6	BBBHHHBBBBBBHBHHHBB-BBBHBBBBB
Chr10	D10Cebr4s9	49.5	BBBHHHBBBBBBHBHHHHBHBBBHBBBBB
Chr10	D10Rat166	51.5	BBBHHHBBBBBBHBBHHHBHBBHHBBBBB
Chr10	Srebp1	55.1	BBBHHHBBBBBB-HHHHHB-BBHHBB-BH
Chr10	D10Cebrp97s5	57.4	BBBHHHBBBBBBBHHBHBBHBB-HBBBBH
Chr10	Myh3	62.2	BBBHHHBBBBBBBBHHHBBHBBHHBBHHH
Chr10	D10Rat59	64.1	HBBHHHBBBBBBBBHHHBBBBBHHBBHHH
Chr10	Syb2	65.1	HBBHHHBBBBBHBBHHHBBBBBHHBBHHH
Chr10	D10Wox14	66.0	HBBHHHBBBBBHBHHHHBBBBBHHBBHHH
Chr10	D10Mgh7	66.9	HBBHHHBBBBBHBBHHHBBBBBHHBBHHH
Chr10	D10Rat116	71.4	BBBHHHHHBBBBBBHHHBBBBBHHBBHHH
Chr10	D10Rat102	72.4	BBBHHHBHBBBBBBHHHBBBBBHHBBHHH
Chr10	Abpa	75.5	HBBHHHBBBBBHBBHHHBBBBBHHBBHHH
Chr10	D10Rat160	76.4	BBBHHHBBBBBHBBHHHBBBBBHHBBHHH
Chr10	D10Rat80	77.4	BBBHHHBBBBBHBBHHHHBBBBHHBBHHH
Chr10	D10Mit2	78.3	BBBHHHBBBBBHBBHHHHBBBBBHBBHHH
Chr10	D10Cebrp1016s5	81.4	BBBHHHBBBBBHHBHHHHBBHBBHHBHHH
Chr10	D10Rat28	83.4	BBBHHHBBBBBHHBHHHBBBHBBHBBHHH
Chr10	D10Rat240	84.3	BBBHHHBBBBBHHBHHHHBBHBBHBBHHH
Chr10	D10Ntr44S7	85.2	BBHHHHBBBBBHHBHHHHBBHBBHBBHHH
Chr10	D10Utr1	89.8	BBHHHHBBBBBHBBHHHBBHHBBHHBHHH
Chr10	D10Mit7	96.0	HBHHHBBBBBBHBHHHHBBBHBHHHBHHH
Chr10	Pnmt	96.9	HBBHHBBBBBBHBHHHHBBBHB-HHBHHH
Chr10	D10Utr2	103.4	HBHHHBBBBBBHBHBBHBBBHBHHBBHBH
Chr10	D10Rat267	109.6	HHHHHBBHBBHHBHHBHBBBHBHHBHHBH
Chr10	D10Rat228	120.1	HHBBHBBBBHHBBHHBHBBHHBHHBHHHH
Chr10	D10Rat7	133.5	HHHBHHHHBHHBHHHBHHBBHBHHBHBHH
Chr10	D10Cebr39s1	134.4	HHHBHHHHBBHBHHHBHHBBHBHHBHBHH
Chr10	D10Cebr39s2	136.4	HHHHHHHHBHHBHHHBHHBBHBHHBHBHH
Chr10	D10Rat226	142.5	HHHBHHHHHHHBHHHHHHBBBBHHBBBHH
Chr11	D11Cebr77s6	0.0	HHHBBHBHBBBBBBHBBHHHHBBB-BH-B
Chr11	D11Rat28	2.1	HHHHBHBHBBBBBBHBBHHHHBBBBHHHB
Chr11	D11Cebr204s16	4.2	HHHBBHBHBBB-BHHBBHHHHBBBBHHHB
Chr11	D11Cebr77s5	5.2	HHHBBHBHBBBHBBHBBHHHHBBB-HH-B
Chr11	D11Mit4	7.3	HHHBBHBHBBHHHBHBBHHHHBBBBHHHB
Chr11	D11Utr1	8.2	HHHBBHBHHBHHHBHBBHHHHBBBBHHHB
Chr11	D11Rat20	10.2	HHHBBHBHBBHBHBHBBHHHHBBBBHHHB
Chr11	D11Rat16	20.7	HHHHBBHHBBBHHBHBBHHHHBBBBHBBB
Chr11	D11Cebr87s1	23.9	BHHBBBHHBBBBHBHBBHHHHBBBBHBBB
Chr11	D11Mit2	25.8	HHHBBBHHBBBHHBHBBHHHHBBBBHBBB
Chr11	D11Cebr15s1	26.7	BHHBBBHHBBBHHBHBBHHHHBBBBHBBB
Chr11	D11Mit1	29.0	HHHBBBHHBBBBHBH-BHHHHBBBB-BB-

Chr	Marker	cM	Strain distribution patterns
Chr11	D11Rat7	30.0	HBHBBBHHBBBBHBHBBHHHHBB-BBBBH
Chr11	D11Rat65	41.2	HBBBHBHBBHBBBBHBBBBHHBBBBBBBH
Chr11	D11Rat94	63.3	BBBHBBHBBBBBHBBBBBBHHBHBHHHBH
Chr11	D11Rat47	67.8	BBHHBBHBBBBBHBBHBBBHBBHBHBHBH
Chr11	Sst	72.4	HBHBBBHBBHBBHBBHBBHHBBHBHBHBH
Chr11	D11Cebr105s1	76.9	HBHBBBHBBBBBHHBHBBHBBBHBHHHBH
Chr11	D11Rat1	85.0	HHBHBBBBBBBHHHBBBBHBBBHBHHHBH
Chr12	D12Cebrp454s1	0.0	BHBHHBBBHBBHBHHBHHBBHHBBBHHHB
Chr12	D12Cebr4s3	0.9	BHBHHBBBHBBHBHHBHHBBHHHBBHHHB
Chr12	D12Cebrp97s4	2.9	BHHHHBBBHBBHBHHHHHBBHHHBBHHHB
Chr12	D12Ntr2	4.8	BHBHHBBBHBBHBHHHHHBBHBHBBHHHB
Chr12	D12Rat40	5.8	BHBHHBBBHHBHBHHHHHBBHBHBBHHHB
Chr12	D12Rat61	10.3	BHHHHBBBHHBHBHHHBHBBBHHBBHHHB
Chr12	D12Rat10	20.8	BHBHHBBBBBBBBHBHBHBBHHHBBHHHH
Chr12	D12Rat42	27.0	HHHBBBBBBBBBBHBHBHBBHHHHBHHHH
Chr12	D12Rat14	28.9	HHHHBBBBBBBBBHBHBHBBHHHBBHHHH
Chr12	Eln	30.9	BHHHHBBBBBBBBHBHBHBBHHHBBHHHH
Chr12	D12Mit7	31.8	BBHHHBBBBBBBBHBHBHBBHHHBBHHHH
Chr12	Pai1	32.8	BBHHHBBB-BBBBHHHBHBBHHHBBHHHH
Chr12	Mdh2	33.8	BBHHHBBBBBBBBHBHBHBBHHHBB-HHH
Chr12	Lsn2	34.7	BBHHHBBBBBBBBHBHBHBBHHHHBHHHH
Chr12	Hsp27	35.6	BBBHHBBBBBBBBHBHBHBBHHHHBHHHH
Chr12	D12Mit1	38.8	BBHHHBBBBBBBBHBHBHBBBHHBBHHHH
Chr12	D12Mit5	43.3	BHHHHBBBHBBHBHHHBHBBBHHBBHHHH
Chr12	D12Mit3	47.9	BHHHHBBBBBBBBHBHBHBBHHHBBHHHH
Chr12	D12Rat16	49.8	BHBHBBBBBBBBBHBHBHBBHHHBBHHHH
Chr12	D12Rat36	58.0	HHHBHBBBBBBBBHBBBHBBHHHHBHHHH
Chr12	D12Cebr1s1	61.1	HHHHHBBBBBBBBHBHBHBBHHHBBHHHH
Chr12	D12Cebr6s4	65.9	B-HHBBBBBBBBBBBHBHBBBHHBBHHHH
Chr13	D13Mit1	0.0	-BHHBHHHBHHHBBBHHHHHBHBBBBHBH
Chr13	D13Rat113	14.4	HHHHBBHHHBHHBHBBBHBHBHBBBBHBH
Chr13	D13Cebr9s3	15.3	HHHHBBHHHBHHBHBBBHHHBHBBBBHBH
Chr13	Bcl2	16.3	HHHHBBHHHBHHBH-HBHHHBHBBBBHBH
Chr13	D13Utr5	17.3	HHHHBBH-HHHHBHBH-HHHBHBBBBHBH
Chr13	D13Utr6	18.3	HHHHBBHHHHHHBHBHBHBHBHBBBBHBH
Chr13	D13Cebr9s2	20.2	HHHHBBHHHHHHBHBBBHHHBHBBBBHBH
Chr13	D13Cebr28s8	22.2	HHHHBBHHHHHHBHBBBHHHBBBHBBHBH
Chr13	D13Rat88	24.2	HHHHBBHHHHHHBHBHBHHHBHBHBBHBH
Chr13	Ren	27.3	HHHHBBHHHHHHHHBBBHHHBHBHBBHHH
Chr13	Lca	28.2	HHHHBBHHHHHHHHBBBHHHBBBHBBHHH
Chr13	D13Cebr5s4	30.2	HHHHBBHHHHHHHHBBBHHHBHBHBBHBH
Chr13	D13Cebr5s3	31.1	HHHHBBHHHBHHHHBBBHHHBHBHBBHBH
Chr13	D13Cebr2s5	35.9	HHHHBBH-HBHHHHBBBHHHHBBHBHHBB
Chr13	D13N2	36.8	BHHHBBHHHBHHHHBBBHHHHBBHBHHBB
Chr13	D13Mit2	40.0	HHHHBHHHHBHHHHBBBHHHHBBHBHHHB
Chr13	D13Utr8	47.2	HHHHBH-HHHHH-HBBBHHHHHBHHBH-H
Chr13	D13Rat126	48.3	HHHHBHHHHBHHHHBBBHHHHHBHHBHBH
Chr13	D13Rat131	58.8	BHHHBHHHHBHHBBHBBHBHBHBHHBHBB
Chr13	D13Mit5	61.9	BHHHBBHHHBHHBBHBBHBBBBBHHBHBB
Chr13	D13Mit3	63.9	BHHHBBHHHBHHBBHBBHBHBHBHHBHBB
Chr13	D13Utr7	64.8	BHHHBBHHHHHHB-HBBHBHBHBHHBHBB
Chr13	Trneglr	69.8	BHHHBHHHHHHHBBHBBBBH-HBHHHHBH
Chr13	D13Rat152	73.1	BHHHBBHHHHBHBBHBBBBHHHBHHBHBH

Chr	Marker	cM	Strain distribution patterns
Chr13	D13Utr1	75.3	HHHHHBHHHHBHBBHBBBBHH-BHH-HBH
Chr13	Fh	80.3	HHHHBBHHHHHHBBHBBHHHHHBHHBHBH
Chr14	D14Cebr85s1	0.0	HHBHBHHBBBBHHHHBHBBHHBHHHBBBB
Chr14	D14Mit5	3.3	H-HHHHHBBBBBHHHBHBBHHBHHHBBBB
Chr14	D14Mit1	10.6	HHHHHHHHBBBBHHHBBBB--BBHBBBBH
Chr14	D14Utr5	14.0	HHHHHBBHBBBBHHHBBBBBHHBHBBBBH
Chr14	Alb	14.9	HHHHHBBHBBBBHHHBBBBBHHBHBBBBB
Chr14	D14Rat77	15.8	HHHHHBBHBBBBHHHBBBBBHHBHBHBBB
Chr14	D14Rat8	24.0	BHHHHBBHBBHBHHHHBBHBHBBHBHBBH
Chr14	D14Utr2	27.1	BHHBHHBHBBHBHHHHBBHBHBBHBHHBH
Chr14	D14Mit3	30.3	BBHBHBBHBBHBHHHHBBHBHBBHBHBBH
Chr14	D14Mit8	32.2	BBHBHHBHBBHBHHHHBBHBHBBHBHHBH
Chr14	D14Mit9	34.3	BBBBHHBHBHHB-HHHBBHBHBBHBHHBH
Chr14	D14Cebr7s14	36.3	BBHBHHBHBBHBHHHHBBHBHBBHBHHBH
Chr14	D14Rat64	38.3	BBHHHHBHBBHBHHHHBBHBHHBHBHHBH
Chr14	D14Utr1	41.7	BBHHHHHHBBHBHBHBBBHBH-BHB-HBH
Chr14	D14Rat37	42.7	BBHHHHHHBBBBHBHBBBHBHHBHBHHBH
Chr14	D14Rat94	47.3	BBHHHHHHBBHBHBHBBBHBHHHHBBBBH
Chr14	D14Rat38	57.8	BHHHHHHHHBBBBHHBBBHBHHHBBBBBB
Chr15	D15Rat1	0.0	HBBHHBHBBBBHHBHHBHBBBHBBHBHHH
Chr15	D15Mit3	22.1	BBBHHBBBBBHHBBHHBBBHHHBBBBBHB
Chr15	D15Utr1	29.4	HBBHHBBBBBHHBHHBBBBH--BBB-BBH
Chr15	D15Rat6	30.4	HBBHHBBBBBHHBHHBBBBBBHBBBBBBH
Chr15	D15Utr2	36.6	HBBHHBBBBHBHBBBBHBBBBHBBBBBBH
Chr15	D15Rat123	47.0	HBHBHBBHHBHHBBBBHBBBBHBBBBBBB
Chr15	D15Cebr7s13	51.8	HBHHHHBBBBHHBBBBHBBBBHBBB-BBB
Chr15	D15Utr3	53.9	HBBHHHBBBBHHBHBBHBBBBHBBBBBBB
Chr15	D15Rat68	55.8	HBHHHHBBBBHHBBBBHBBBBHBBBBBBB
Chr15	D15Cebr204s39	62.0	HBHHHBHBBHBHBBBBHBBBBHBBBBBBH
Chr15	D15Rat21	62.9	HHHHHBHBBHBHBBBBHBBBBHBBBBBBH
Chr15	Ednrb	69.4	B-HHHBHBHHBHBBBBHBBHHHBBBBHBH
Chr15	D15Rat101	78.0	BHBHHBHBBHBHBHBHHBBBBHBBBBHBH
Chr15	D15Rat107	91.4	BBBHHBBBBHBHHHBHBHBBHHBBHBBBH
Chr16	D16Cebr204s13	0.0	HHHBBHHHHHHBHBBBBBHBHHBBBBBBH
Chr16	Mbpa	3.2	BHHBBHBHHHHBHBBBBBHBHHBHBBBBH
Chr16	D16	4.1	H-HBBHBHHHHBHBBBBBHBHHBHBBBBH
Chr16	D16Rat6	5.2	HHHBBHHHHH-BHBBBBBHBHHBHB-BB-
Chr16	D16Utr1	6.3	H-HBBHBHHHHBHBBBBBHBHHBHBBBBH
Chr16	D16Cebr10s10	8.3	HHHBBHHHHHHBHBBBBBHBHHBBBBBBH
Chr16	D16Cebrp1038s2	9.2	HHHBBHHHHHHBHBBBBBHBHHBBBBBHH
Chr16	D16Mit2	12.4	BHHBBHHHHHHBHBBBBBHBHBBBBBBBH
Chr16	D16Mit1	16.9	BHHBBBHHHHHBHBBBBBBBHHBHBBBBH
Chr16	D16Rat41	20.4	HHHBBHHHHH-BHBBBBBHBHHBHBBBB-
Chr16	D16Mit5	22.5	BHHBBHBHHHHBHBBBBBHBHHBHBBBBH
Chr16	D16Rat67	35.9	HHHHBBHHHHHBBHBBBBBBBHBHBBBBH
Chr16	D16Rat72	64.9	HBHHHHHBHBHHBHBHBHBBBBBHHHBBH
Chr16	D16Rat66	67.1	HBHHHHHHHB-HBHBHBHBBHBBHH-BB-
Chr16	D16Rat60	72.4	HBHHHHHHHB-HBHHHBHBBBBHBH-BB-
Chr16	CT8-3	74.7	HBHHHHHHH-HHBHHHBHBBHBBBHHBBH
Chr16	P205_2_6	78.0	HBHHHHHHHBBHBBHHHHBBHBBBHHBBH
Chr16	D16Rat53	82.6	HBHHBHHHHBHHBHHHBHBBHBBBHHBBH
Chr16	D16Rat34	93.1	BHHHHHHHHBBHBHHHHBBBHBBBHHHBH
Chr16	D16Rat14	99.2	BHHHHBBHHHBHBHHHHBBBBHBBHHHBH

Chr	Marker	cM	Strain distribution patterns
Chr16	D16Rat15	107.4	BBBHBBHBHHBHBHHHHBBHBHBBHHHBH
Chr16	D16Cebr48s1	108.3	BBBHBBHBHHBHBBHHHBBHBHBBHHHBH
Chr17	D17Rat11	0.0	BHBHBHBHBBHHHHBBHHHBHHBHBHBHB
Chr17	D17Rat144	8.1	BHBHBBBBBBHHHHBBBHHBHBBBBBBHB
Chr17	D17Cebrp203s2	10.1	HHBHBBBBBBHBHHBBBHHBHBBBBBBHB
Chr17	Prl	14.6	BHBBBBBB----HHBBBHHBBBBBBB---
Chr17	D17Rat20	17.3	BBBBBBBBBBHBHBBBBHHBBBBBBBBHB
Chr17	D17Mit3	24.1	BHBBBBBBBBHBHBBBB-BHBBBBHHB-B
Chr17	D17Rat151	36.1	HHBHBBBHBBHHHBHBHHBHBBBBHBBBB
Chr17	D17Mit6	44.6	B-BHBBBHHHHHHHHBHBBHBHBBHBBBB
Chr17	D17Rat50	53.2	BHBHBHHBHHBHHHHBHBBHBBBBHBHBB
Chr18	D18Mit7	0.0	BBHHHBHHHBHHBBBHBBBHHHHHHBHHB
Chr18	Ttr	4.8	HBHHHBHHH-HHBBBHHBHHHBHHHBHHB
Chr18	D18Rat112	6.8	HHHHHBHHHHHHBBBHHBHHHBHHHBHBB
Chr18	D18Cebrp97s6	8.8	BHHHHBHHHHHHBBBHBBHHHBHHHBHBB
Chr18	D18Rat29	10.7	BBHHHBHHHHHHBBBHBBBHHBHHHBHBB
Chr18	D18Rat47	16.9	BBHHHHBHHHHHBBBHHBHHHBHHBBHBB
Chr18	D18Mit2	21.5	BBHHHHHHHBHHBBBHBBHHHBHHBBHHB
Chr18	D18Mit3	24.8	BBHHHHHBHHHHBBBHHBHHHBHH-BHHB
Chr18	D18Rat103	26.8	BBBHHHHBHHHHBBBHHBHHHBHHBBHBB
Chr18	D18Rat99	37.3	BBBHHHHBHHHBBHBHBBBHBBHHHBHHB
Chr18	D18Cebr19s1	40.4	BBBHHHHBHHHBBHBHHBBHBBHBBBHHB
Chr18	Grl	43.6	BBHHHHHBHHHBBHBHHBBHBBHHHBHHB
Chr18	D18Cebrp60s11	49.8	BBBHBHHBHHHBHHBHBBBHBBHHHBHBB
Chr18	D18Rat19	54.3	BBBHBHHBHBHHBHBHBBBHBBHHHBHHB
Chr18	D18Rat55	64.8	BBBHHHHBHBHHBHBHHHBHHHHBHBHHH
Chr18	Adrb2	71.0	BBHHHHHBHBBHBHBHHHBHHHHHHBBBH
Chr18	D18Utr3	75.5	BBHHHHHBBBBHBHBBHBBBHHHHHBBBH
Chr18	D18Mit10	80.5	BBHHH-HBHBHHBH-HHHBBHHHHHBBBH
Chr18	D18Rat89	82.7	BBBHHHHBHBBHBHBHHHBBHHHHHBBBH
Chr18	D18Rat9	90.8	BHBBHHHBHBBHHHBHHBHBHBHHHBBBH
Chr18	D18Rat5	97.0	HBBBHHBBHBBHHHBHHBHBHHBHHBBBH
Chr18	D18Cebr51s2	98.9	HBBBHHBBBBBHHHBHHBHBHHBHHHBBH
Chr18	D18Cebr51s1	99.9	HBBBHHBBHBBHHHBHHBHBHHBHHHBBH
Chr18	D18Cebrp187s6	100.8	HBBBHHBBHBBHHHBHHBHBHHBHHBBBH
Chr18	D18Mit9	101.7	HBHBHHBBHBBHHHBHHBHBHHBHHBBBH
Chr18	D18Ucsf2	102.7	HBHBHHBB--BHHHBHHBHBHHBBHB-BH
Chr18	D18Utr2	103.8	HBBBHHBBHBBHHHBHHBHBHH-BHBBBH
Chr19	Hmox	0.0	HHHHBHBBBBHHHBBHHBHBBBHHBBHBB
Chr19	D19Utr3	2.0	HHHHBHBBBBHHHBBHHBBBBB-HHBHBB
Chr19	D19Utr4	4.1	HHHHBHBBHBHHHBBHHBHBBBHHHBHBB
Chr19	D19Utr5	7.4	HHHHB-BBHBHHHBBHHBBHBBHHHBHBH
Chr19	D19Rat56	10.7	HHHHBBBHHBHHHBBHHBBBBBHHHBHBB
Chr19	D19Cebrp97s10	15.2	HHBHBBBBBBHBHBBHHBBBBBHHHBHBB
Chr19	D19Mit2	19.8	HHHHBHBBBBHHHBBHHBHBBBHHHBHBB
Chr19	D19Rat52	22.0	HHHHBHBBH-HHHBBHHBH-BBHHHBBB-
Chr19	Es8	24.2	HHHHBBBBHBHHHBBHHBHHBBHHBBBBB
Chr19	Es3	25.1	HHHHBBBBHBHHHBBHHBHBBBHHBBBBB
Chr19	D19Ucsf2	31.6	HHHHBHHBHH-HHBBBHBHBBBHHHBBBB
Chr19	Ednra	34.0	HHHHBHHBH-HHHB-BHBHB-BBBHBBBB
Chr19	D19Utr6	39.5	HHHBBHBBHB-HHBBBHBHBBBHHHBBBB
Chr19	D19Utr1	43.3	HBBBB-BBHBHHHBBBHBHHB-HHH-BBB
Chr19	Es4	50.6	HHHHBBBBHBHHHBBHHBHHBBHHBBBBB

Chr	Marker	cM	Strain distribution patterns
Chr19	Rt2	55.6	HHH-BBBBHH-HHBBBHBHBBBHHHBBBB
Chr19	D19Ucsf1	60.9	HHHHBHHBB--HHBBBHBHHBBHHHBBBB
Chr19	D19Cebr204s23	63.0	HHHHBHHBHBBHHBBBHBHBBBHHHBBBB
Chr19	D19Cebr204s27	66.3	HHBBBBHBHBBHHBBBHBHB-BHHHBBBB
Chr19	D19Rat48	68.3	HHBHBHHBHBBHHBBBHBHBBBHHHBBBB
Chr19	D19Mit7	82.7	HHHHHHHBHB-HBHBBHBBBBBBHHBHHB
Chr19	Tat	87.5	HHHBHHHBHBBBBHBBHBBBBBBBHBHBB
Chr19	D19Rat71	89.4	HHHBHHHBHBBBBHBBBBBBBBBBHBHHB
Chr19	D19Rat64	97.6	HHHHHBHBHBBBBHBBBBHBBBBHBBHBB
Chr19	D19Rat61	103.7	HBHHBHHBHBHBBHBBBHHBBBBHBBHBB
Chr19	D19Rat5	114.9	-HHBBHBHHHHBBHBBBHHBBHBHBBBBB
Chr19	D19Cebrp150s1	116.9	HHHHBHBHHBHBBHBBBHHBBHBHBBBBB
Chr19	D19Rat1	117.9	-HHHBHBHHHHBBHBBBHHBBH-HBBBBB
Chr20	D20Cebr32s3	0.0	BHHBHBBHHHBHBBBBBBHHBHHBBBHHB
Chr20	Rt1a	0.9	BHHBHBBHHBBHBBBBBBHHBHHBBBHHB
Chr20	D20Utr3	3.0	BHHBHBBHHBHHBBBBBBHBBH-BB-HHB
Chr20	D20Rat41	5.2	BHHBHBBHHBBHBBBBBBHHBHHBBBHHB
Chr20	D20Mgh5	8.3	BBHBBBBHHBBHBBBBBBHHBHHBBHHHB
Chr20	D20Rat4	21.7	BHHHBBBHBHBHBBBBBBHHHHBBHHHHH
Chr20	D20Rat75	36.1	HHHHBBBHH-BBBBBHHBBHBHHBHHHHH
Chr20	D20Rat23	42.6	HBHHBBBHHHBBBBBHHBBHBHHBHBBBB
Chr20	D20Rat9	48.8	BBBHBBBHHHHBBHBHHBHHBHHBHBBBB
Chr20	D20Rat10	53.4	BBBHBBBHHHHBBHBBHBHBBBBBHBBBB
Chr20	D20Rat55	61.5	BHBHBBBHBHHBBBBBHHHBBBHBBBBBB
Chr20	D20Mit1	67.7	BBBHBBBBBHHBBBHHHBHBBBHBBBBBB
Chr20	D20Utr4	70.8	BBBHBBBHBHHBBHHHHBHBHBHBBBBBB

Appendix 2: RT-PCR primers

Legend: Each primer name consists of: gene symbol, NCBI reference sequence (in parentheses), primer position in the reference sequence, primer direction/function (F = forward, R = reverse, T = tagged)

Th-(NM_013158)-1725F	5'-AGCGCCCATTCTCTGTGAAG-3'
Th-(NM_013158)-17292R	5'-GGTGTGAGGGCTGTCCAGTAC-3'
Th-(NM_013158)-1746T	5'-TTGACCCGTACACACTGGCCATTGA-3'
Ddc-(NM_012545)-799F	5'-CCCTCGCTTTGAAATTTGCA-3'
Ddc-(NM_012545)-867R	5'-AACTGGTTGGAGCCCTTTAGC-3'
Ddc-(NM_012545)-820T	5'-TCATCCTCGGGTTGGTCTGCTTC-3'C
Dbh-(NM_013158)-1730F	5'-GCTTCCCGGGTAACTGGAA-3'
Dbh-(NM_013158)-1800R	5'-GCGTGGGTCGGGTTCTT-3'
Dbh-(NM_013158)-1750T	5'-CTGCAGCCTCTGCCTAATATCACTTCCG-3'
Pnmt-(X14211)-340F	5'-GAGTCCTGGCAGGAGAAAGAAC-3'
Pnmt-(X14211)-407R	5'-TGCACATCAATGGGCAAGAC-3'
Pnmt-(X14211)-363T	5'-CCAGCTCCGAGCGAGGGTGAAG-3'
Chga-(NM_021655)-765F	5'-AGAAGGCTGGGCCTAAAGAAGT-3'
Chga-(NM_021655)-838R	5'-CTGGATCTTCTTGTAGCCTGAATAGA-3'
Chga-(NM_021655)-788T	5'-CCCACGGCAGCATCCAGTTCTCA-3'
Chgb-(NM_012526)-1877F	5'-TGGACCAGCTCCTTCACTACAG-3'
Chgb-(NM_012526)-1948R	5'-CCATCTGCTCCTCTGAATCGTA-3'
Chgb-(NM_012526)-1900T	5'-AAGAAGGCAGCCGAATTTCCCGACT-3'
Scg2-(NM_022669)-1668F	5'-GCAGGCCATCAAGGAGCAT-3'
Scg2-(NM_022669)-1735R	5'-TGCTCACCTTGGCCAGTTTC-3'
Scg2-(NM_022669)-1688T	5'-TGGGTCAAGGAAGCTCCCAGGAAATG-3'

Appendix 3: Primers for re-sequencing in rat *Dbh*

<u>Legend:</u> Each primer name consists of: gene symbol, amplicon number in sequential order from 5'-end and primer direction (F = forward, R = reverse)

Dbh_1F	5'-GCCTCAACATCTGGTTTTTCTT-3'
Dbh_1R	5'-ACAGGCTCTGAGTAGGGTATGG-3'
Dbh_2F	5'-AGCACAACTGTTGCTCTCACC-3'
Dbh_2R	5'-CACACCTGTTCCCATCTCACTA-3'
Dbh_3F	5'-GCTGAGCAATAGGAAGGTCAA-3'
Dbh_3R	5'-AAACACTTTGCTCCGATCCTC-3'
Dbh_4F	5'-GTGTCCTTGGGACCTATGTCTG-3'
Dbh_4R	5'-ACACAGAAGCAGTAGGCGACTT-3'
Dbh_5F	5'-ACCTCGTCATGCTCTGGACT-3'
Dbh_5R	5'-AAGTAGGAGACTTCCCCTTTGC-3'
Dbh_6F	5'-GGGTGCTAGAAAGCTTGGTTTA-3'
Dbh_6R	5'-GAATACATCCCAGGGCAAGAG-3'
Dbh_7F	5'-AGCTCCCTGTCAAACTCAGAAT-3'
Dbh_7R	5'-AGCACCTCAGATTCTTGCAACT-3'
Dbh_8F	5'-TCAGGCCTACATACGGGACTAC-3'
Dbh_8R	5'-TCCTTGTGTGTATGGGTGACTC-3'
Dbh_9F	5'-CACTAGTCCCCTATGCTTCCTG-3'
Dbh_9R	5'-GTGGCGACAGTAGTTGAGTCTG-3'
Dbh_10F	5'-CCCCAGTTCAGGGTAGTCTGT-3'
Dbh_10R	5'-GGACATTTCAGGATGTGGTTTA-3'
Dbh_11F	5'-ATTGCACCATCCCCTCCT-3'
Dbh_11R	5'-TGTGGTAATGAACTTCCAGTCG-3'
Dbh_12F	5'-CGATAGGACCTTTTGTGTGTGA-3'
Dbh_12R	5'-AAAGAACCTGTCCAGAGCAGTC-3'
Dbh_13F	5'-AAACACCCCATAGAGGTGACAG-3'
Dbh_13R	5'-CTCAGGGACACTGGGTACTGTG-3'
Dbh_14F	5'-CAGGTTTACCATGGGGATTAGA-3'
Dbh_14R	5'-GTCTCTTTTGGGCACAGTTCTC-3'
Dbh_15F	5'-GTTGAAGCAGAACTGACAATGC-3'
Dbh_15R	5'-GGAAAAGCCAGTGTGACTTACC-3'
Dbh_16F	5'-GGATCTGACATGGCTACAAGC-3'
Dbh_16R	5'-TGCTGAAGTTTCTGTGACCCTA-3'

Dbh_17F	5'-GTCACAAGAAAGCCACAGACAG-3'
Dbh_17R	5'-CCTTGAGCATATCACGATTGAA-3'
Dbh_18F	5'-CAGAGAATACAACGGCTTAGGG-3'
Dbh_18R	5'-GAGCCACTCTGCAAAGGACTAT-3'
Dbh_19F	5'-CCCCTTCCATAGAATAGTGCTG-3'
Dbh_19R	5'-TATAGAGCCCAGAAAGGGTCAG-3'
Dbh_20F	5'-AAACCCCAACATGTAGTTCCAC-3'
Dbh_20R	5'-CAGGCAGTACAGATGACAGAGG-3'
Dbh_21F	5'-TTGGAAAGGAGTAGGAGTCTGG-3'
Dbh_21R	5'-TTTAGGTAGTTGGTGGGGACCT-3'
Dbh_22F	5'-GAGAGTACGAGAAATGCTCACG-3'
Dbh_22R	5'-AGGCCCTCTCAACACTGATCTA-3'

Appendix 4: Primers for re-sequencing in rat *Pnmt*.

Legend: Each primer name consists of: gene symbol, amplicon number in sequential order from 5'-end and primer direction (F = forward, R = reverse)

Pnmt_1F	5'-CAGGCACCTAGAGCACCAAG-3'
Pnmt_1R	5'-ACCTTTATGGGCTCTTGATCG-3'
Pnmt_2F	5'-AAGGAGGATAGAGACGGGGTAG-3'
Pnmt_2R	5'-GTAGTTGTTGCGGAGGTAGGC-3'
Pnmt_3F	5'-GGAGGGGACCCAGTAGTAGA-3'
Pnmt_3R	5'-CTCCCAGGTCCCATCTGTCT-3'
Pnmt_4F	5'-CAGGAGCGGACGATAGTCTT-3'
Pnmt_4R	5'-GTCCTCGATGAGGCAGACAT-3'
Pnmt_5F	5'-TGATATCCCCTCCTCTGCTC-3'
Pnmt_5R	5'-GCAAGGTACCACGACTCCTC-3'
Pnmt_6F	5'-GAGCCTCTGGGGTAGTCTGA-3'
Pnmt_6R	5'-CCTTCTAAGCCTCGGAGACC-3'

Appendix 5: Polymorphism discovery (re-sequencing) in rat *Dbh*

```
               -1800     -1790     -1780     -1770     -1760
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TTT
DBH NM_013158  tcagttctgttcagcacatctacatgagtcctgtgagatttttctgattt
SHR            ~~~~~~~~GTTCAGCACATCTACATGAGTCCTGTGAGATTTTTCTGATTT
EXONS

               -1750     -1740     -1730     -1720     -1710
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          TGGNTGCGTACTCCTACCCCTTATGTTGCCAAGGGAACCCAGTTTTGGGT
DBH NM_013158  tggctgtgtactcctaccccttatgttgccaagggaacccagttttgggt
SHR            TGGCTGTGTACTCCTACCCCTTATGTTGCCAAGGGAACCCAGTTTTGGGT
EXONS

               -1700     -1690     -1680     -1670     -1660
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          TCCTGAATGCTGTCCTGCCTATGCCCTACCCTGCTCTGCCCTGCTACTGA
DBH NM_013158  tcctgaatgctgtcctgcctatgccctaccctgctctgccctgctactga
SHR            TCCTGAATGCTGTCCTGCCTATGCCCTACCCTGCTCTGCCCTGCTACTGA
EXONS

               -1650     -1640     -1630     -1620     -1610
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          TGGCTGTGTGGCCCTGGGTACCTTCCCCTCTATCCCCTTGTGTGTCAGAT
DBH NM_013158  tggctgtgtggccctgggtaccttcccctctatccccttgtgtgtcagat
SHR            TGGCTGTGTGGCCCTGGGTACCTTCCCCTCTATCCCCTTGTGTGTCAGAT
EXONS

               -1600     -1590     -1580     -1570     -1560
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          GGGGACAAACACAGGCTGGTCTCTCAAAGCCAGCAAAGCAAGCAGGCCTG
DBH NM_013158  ggggacaaacacaggctggtctctcaaagccagcaaagcaagcaggcctg
SHR            GGGGACAAACACAGGCTGGTCTCTCAAAGCCAGCAAAGCAAGCAGGCCTG
EXONS

               -1550     -1540     -1530     -1520     -1510
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          CTTCCCTCTCCTGGTAACTACCAAGACTGGGTAATTGTTACTTCTGAAGT
DBH NM_013158  cttccctctcctggtaactaccaagactgggtaattgttacttctgaagt
SHR            CTTCCCTCTCCTGGTAACTACCAAGACTGGGTAATTGTTACTTCTGAAGT
EXONS

               -1500     -1490     -1480     -1470     -1460
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          CGGCTGAATCACTTCTGCCAACGTTCAGCCTTGTTCCTAGCTGCAGAACG
DBH NM_013158  cggctgaatcacttctgccaacgttcagccttgttcctagctgcagaacg
SHR            CGGCTGAATCACTTCTGCCAACGTTCAGCCTTGTTCCTAGCTGCAGAACG
EXONS

               -1450     -1440     -1430     -1420     -1410
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          GGAGTGCTTTTTGAATTTGTTAAAAATAAACGTGTGTGTGTGTGTGTGTG
DBH NM_013158  ggagtgctttttgaatttgttaaaaataaacgtgtgtgtgtgtgtgtgtg
SHR            GGAGTGCTTTTTGAATTTGTTAAAAATAAACGTGTGTGTGTGTGTGTGTG
EXONS

               -1400     -1390     -1380     -1370     -1360
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          TGTGTGTGTGTGTGTGTGTGTGTGTGTGTCACTCTGCTGTTTACTAAGAA
DBH NM_013158  tgtgtgtgtgtgtgtgtgtgtgtgtgtgtcactctgctgtttactaagaa
SHR            TGTGTGTGTGTGTGTGTGTGTGTGTGTGTCACTCTGCTGTTTACTAAGAA
EXONS

               -1350     -1340     -1330     -1320     -1310
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx          CTGTGCTTCTCCTCCTCTAAGACCTGANCACAACTGTTGCTCTCACCCTG
DBH NM_013158  ctgtgcttctcctcctctaagacctgagcacaactgttgctctcaccctg
SHR            CTGTGCTTCTCCTCCTCTAAGACCTGATCACAACTGTTGCTCTCACCCTG
EXONS
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                -1300     -1290     -1280     -1270     -1260
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GATCCAGGAAAGAGCTGCTGCTGTCAGGCTCCTGCCTGGCACCCCTACCC
DBH NM_013158     gatccaagaaagagctgctgctgtcaggctcctgcctggcacccctaccc
SHR               GATCCAGGAAAGAGCTGCTGCTGTCAGGCTCCTGCCTGGCACCCCTACCC
EXONS                   --------------------------------------------

                -1250     -1240     -1230     -1220     -1210
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TTCTTAGGAGAGAACCTGGAGCTTCACTGCCCCAGGTGGTCCTAAGGCCT
DBH NM_013158     ttcttaggagagaacctggagcttcactgccccaggtgggactaaggcct
SHR               TTCTTAGGAGAGAACCTGGAGCTTCACTGCCCCAGGTGGTCCTAAGGCCT
EXONS             --------------------------------------------------

                -1200     -1190     -1180     -1170     -1160
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CACTGAAAACCAGGCTGTGGCTAGCTGAGCCATACCCTACTCAGAGCCTG
DBH NM_013158     cactgaaaaccaggctgtgggtagctgagccataccctactcagagcctg
SHR               CACTGAAAACCAGGCTGTGGCTAGCTGAGCCATACCCTACTCAGAGCCTG
EXONS             --------------------------------------------------

                -1150     -1140     -1130     -1120     -1110
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGACTCTTGTCTTGACTTGTGTAGAACAATGTGCCCATTAACCCTGGACA
DBH NM_013158     tgactcttgtcttgacttgtgtagaccaatgtgcccattaaccctggaca
SHR               TGACTCTTGTCTTGACTTGTGTAGAACAATGTGCCCATTAACCCTGGACA
EXONS             --------------------------------------------------

                -1100     -1090     -1080     -1070     -1060
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TTCTGCCTAGTCCCAGGGCAAAGCTGGGACTATCTGGAGACATCACCTGC
DBH NM_013158     ttctgcctagtcccagggcaaagctgggactatctggagacatcacctgc
SHR               TTCTGCCTAGTCCCAGGGCAAAGCTGGGACTATCTGGAGACATCACCTGC
EXONS             --------------------------------------------------

                -1050     -1040     -1030     -1020     -1010
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GTGCAAGACTGAGGTCCCTTTTCCTCTGTGGAACAAAGCTTAGTGAAGAA
DBH NM_013158     gtgcaagactgaggtcccttttcctctgtggaacaaagcttagtgaagaa
SHR               GTGCAAGACTGAGGTCCCTTTTCCTCTGTGGAACAAAGCTTAGTGAAGAA
EXONS             --------------------------------------------------

                -1000     -990      -980      -970      -960
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TCAGCCTTGCTGGCCAGAGGCTCAGGCTGGGCTACTGTGTTTCTGAGAAG
DBH NM_013158     tcagccttgctggccagaggctcaggctgggctactgtgtttctgagaag
SHR               TCAGCCTTGCTGGCCAGAGGCTCAGGCTGGGCTACTGTGTTTCTGAGAAG
EXONS             --------------------------------------------------

                -950      -940      -930      -920      -910
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GAAGCACAAGGAAAGCAAGGAAGGAAAAAGAGGGAGAGAAGGACTCACAA
DBH NM_013158     gaagcacaaggaaagcaaggaaggaaaaagagggagagaaggactcacaa
SHR               GAAGCACAAGGAAAGCAAGGAAGGAAAAAGAGGGAGAGAAGGACTCACAA
EXONS             --------------------------------------------------

                -900      -890      -880      -870      -860
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CTAGGTTGTAGGGACTTATAGTCACAGTGAGGTACAGAGGTGGATGGCTT
DBH NM_013158     ctaggttgtagggacttatagtcacagtgaggtacagaggtggatggctt
SHR               CTAGGTTGGAGGGACCTATAGTCACAGTGAGGTACAGAGGTGGATGGCTT
EXONS             --------------------------------------------------

                -850      -840      -830      -820      -810
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGGATACAAGTAGAATGTTCTGGTGTTGGGGTACGGACAGCACCGGTCCT
DBH NM_013158     tggatacaagtagaatgttctggtgttggggtacggacagcaccggtcct
SHR               TGGATACAAGTAGAATGTTCTGGTGTTGGGGTACGGACAGCACCGGTCCT
EXONS             --------------------------------------------------

                -800      -790      -780      -770      -760
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CATCATGAAACAGACAGGGCTGAGCAATAGGAAGGTCAAAACTTCGGACT
DBH NM_013158     catcatgaaacagacagggctgagcaataggaaggtcaaaacttcggact
SHR               CATCATGAAACAGACAGGGCTGAGCAATAGGAAGGTCAAAACTTCGGACT
EXONS             --------------------------------------------------
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              -750      -740      -730      -720      -710
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GGTGGTCAGCCTGTGAAGAGGCCCTGTGGTAGGGATGGGAGAAATAGTGC
DBH NM_013158   ggtggtcagcctgtgaagaggccctgtggtagggatgggagaaatagtgc
SHR             GGTGGTCAGCCTGTGAAGAGGCCCTGTGGTAGGGATGGGAGAAATAGTGC
EXONS           --------------------------------------------------

              -700      -690      -680      -670      -660
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TGGGGGTGGCTGGCCCGAGAAGCAAGAGGAAGTCCTGGCTTTGAGAGCTT
DBH NM_013158   tgggggtggctggcccgagaagcaagaggaagtcctggctttgagagctt
SHR             TGGGGGTGGCTGGCCCGAGAAGCAAGAGGAAGTCCTGGCTTTGAGAGCTT
EXONS           --------------------------------------------------

              -650      -640      -630      -620      -610
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TAGTGAGATGGGAACAGGTGTGTCCCCATAGCCACGAGTGCCCATTCACC
DBH NM_013158   tagtgagatgggaacaggtgtgtccccatagccacgagtgcccattcacc
SHR             TAGTGAGATGGGAACAGGTGTGTCCCCATAGCCACGAGTGCCCATTCACC
EXONS           ------------------------------

              -600      -590      -580      -570      -560
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           CCCTTCCTGCCTGCCCTGTGCTGTCTCTGCACCCACTCCTTCTGTAGCAT
DBH NM_013158   cccttcctgcctgccctgtgctgtctctgcacccactccttctgtagcat
SHR             CCCTTCCTGCCTGCCCTGTGCTGTCTCTGCACCCACTCCTTCTGTAGCAG
EXONS

              -550      -540      -530      -520      -510
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           CCGTGGCGCCAGCTGGAGCCCGTGGGGAAACAGGAGAAAAAGTGGGAACA
DBH NM_013158   ccgtggcgccagctggagcccgtggggaaacaggagaaaaagtgggaaca
SHR             CCGTGGCGCCAGCTGGAGCCCGTGGGGAAACAGGAGAAAAAGTGGGAACA
EXONS

              -500      -490      -480      -470      -460
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GCAGGCCTCGGAGAGAGTGTCTAAACACGGACTATTTGGGGACTGTCTTT
DBH NM_013158   gcaggcctcggagagagtgtctaaacacggactatttggggactgtcttt
SHR             GCAGGCCTCGGAGAGAGTGTCTAAACACGGACTATTTGGGGACTGTCTTT
EXONS

              -450      -440      -430      -420      -410
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TATTTTTAGTTTGAAACTTATCCATCCTCCAGCATTGTAAAAAAAAAAAA
DBH NM_013158   tatttttagtttgaaacttatccatcctccagcattgtaaaaaaaaaaaa
SHR             TATTTTTAGTTTGAAACTTATCCATCCTCCAGCATTGTAAAAAAAAAAAA
EXONS

              -400      -390      -380      -370      -360
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           AAAACAGGATCCAGGAACCCACCACTCACTGTCACTCAGGAAGGAGCCCC
DBH NM_013158   aaaacaggatccaggaacccaccactcactgtcactcaggaaggagcccc
SHR             AAAACAGGATCCAGGAACCCACCACTCACTGTCACTCAGGAAGGAGCCCC
EXONS

              -350      -340      -330      -320      -310
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TTGAACCTCAGTTGGAGCAGGTAGAGAGGCCCTGACTGCCTATGAGGCAT
DBH NM_013158   ttgaacctcagttggagcaggtagagaggccctgactgcctatgaggcat
SHR             TTGAACCTCAGTTGGAGCAGGTAGAGAGGCCCTGACTGCCTATGAGGCAT
EXONS

              -300      -290      -280      -270      -260
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TCAGCAGGGCCTGGCTGGAGGTGTCCTTGGGACCTATGTCTGCAGAGAGT
DBH NM_013158   tcagcagggcctggctggaggtgtccttgggacctatgtctgcagagagt
SHR             TCAGCAGGGCCTGGCTGGAGGTGTCCTTGGGACCTATGTCTGCAGAGAGT
EXONS

              -250      -240      -230      -220      -210
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           AGCTGTTTCCAACAGGCGTCAGAGATCCATTGGAGGACATGGCCATTCTG
DBH NM_013158   agctgtttccaacaggcgtcagagatccattggaggacatggccattctg
SHR             AGCTGTTTCCAACAGGCGTCAGAGATCCATTGGAGGACATGGCCATTCTG
EXONS
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                 -200      -190      -180      -170      -160
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            CTTCGATTCTCTTGATGATGTCCATGCGTCATTAGTGTCAATTAGGGGAG
DBH_NM_013158    cttcgattctcttgatgatgtccatgcgtcattagtgtcaattaggggag
SHR              CTTCGATTCTCTTGATGATGTCCATGCGTCATTAGTGTCAATTAGGGGAG
EXONS

                 -150      -140      -130      -120      -110
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            GATCGGAGCAAAGTGTTTGCCCCAGGGCATGGGCTGGTGGGAGAGCCACC
DBH_NM_013158    gatcggagcaaagtgtttgccccagggcatgggctggtgggagagccacc
SHR              GATCGGAGCAAAGTGTTTGCCCCAGGGCATGGGCTGGTGGGAGAGCCACC
EXONS

                 -100      -90       -80       -70       -60
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            AGGACAATTGAATTCCCCACCAGACAAATGTGATTAGGTACAGCCTGGCC
DBH_NM_013158    aggacaattgaattccccaccagacaaatgtgattaggtacagcctggcc
SHR              AGGACAATTGAATTCCCCACCAGACAAATGTGATTAGGTACAGCCTGGCC
EXONS

                  -50       -40       -30       -20       -10      -1
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            CAACCCCACCGAACAGACATAAATGGCCCAGTGGGGCTGGGGTGCTCATC
DBH_NM_013158    caaccccaccgaacagacataaatggcccagtggggctggggtgctcatc
SHR              CAACCCCACCGAACAGACATAAATGGCCCAGTGGGGCTGGGGTGCTCATC
EXONS

                    1        10        20        30        40
                    ....|....|....|....|....|....|....|....|....|....
BN.Lx            _CCAGCCATGCAGCCTCACCTCAGCCACCAGCCTTGCTGGAGCCTCCCCA
DBH_NM_013158    _CCAGCCATGCAGCCTCACCTCAGCCACCAGCCTTGCTGGAGCCTCCCCA
SHR              _CCAGCCATGCAGCCTCACCTCAGCCACCAGCCTTGCTGGAGCCTCCCCA
EXONS            _5'UTR-EXON_1_XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                  50        60        70        80        90
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            GCCCCAGCGTCCGTGAGGCGGCTTCCATGTATGGCACTGCTGTGGCCATC
DBH_NM_013158    GCCCCAGCGTCCGTGAGGCGGCTTCCATGTATGGCACTGCTGTGGCCATC
SHR              GCCCCAGCGTCCGTGAGGCGGCTTCCATGTATGGCACTGCTGTGGCCATC
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                  100       110       120       130       140
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            TTCCTGGTCATCCTGGTGGCTGCACTGCAGGGCTCGGAGCCTCCGGAGAG
DBH_NM_013158    TTCCTGGTCATCCTGGTGGCTGCACTGCAGGGCTCGGAGCCTCCGGAGAG
SHR              TTCCTGGTCATCCTGGTGGCTGCACTGCAGGGCTCGGAGCCTCCGGAGAG
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                  150       160       170       180       190
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            CCCCTTCCCTTACCACATCCCCCTGGACCCTGAAGGGACTTTAGAGCTCT
DBH_NM_013158    CCCCTTCCCTTACCACATCCCCCTGGACCCTGAAGGGACTTTAGAGCTCT
SHR              CCCCTTCCCTTACCACATCCCCCTGGACCCTGAAGGGACTTTAGAGCTCT
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                  200       210       220       230       240
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            CGTGGAACGTCAGCTATGACCAGGAGATCATCCACTTCCAGCTCCAGGTG
DBH_NM_013158    CGTGGAACGTCAGCTATGACCAGGAGATCATCCACTTCCAGCTCCAGGTG
SHR              CGTGGAACGTCAGCTATGACCAGGAGATCATCCACTTCCAGCTCCAGGTG
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                  250       260       270       280       290
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            CAAGGGCCGAGGGCTGGGGTCCTGTTCGGAATGTCGGATCGAGGTGAGAT
DBH_NM_013158    CAAGGGCCGAGGGCTGGGGTCCTGTTCGGAATGTCGGATCGAGGTGAGAT
SHR              CAAGGGCCGAGGGCTGGGGTCCTGTTCGGAATGTCGGATCGAGGTGAGAT
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                  300       310       320       330       340
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            GGAGAACGCAGACCTCGTCATGCTCTGGACTGACGGGGACAGGACCTACT
DBH_NM_013158    GGAGAACGCAGACCTCGTCATGCTCTGGACTGACGGGGACAGGACCTACT
SHR              GGAGAACGCAGACCTCGTCATGCTCTGGACTGACGGGGACAGGACCTACT
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
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               350       360       370       380       390
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          TTGCGGTGAGTCCTCTGCCATCTTTCTGTCTTGTCCTCACGCCCATACCC
DBH NM_013158  TTGCGgtgagtcctctgccatctttctgtcttgtcctcacgcccataccc
SHR            TTGCGGTGAGTCCTCTGCCATCTTTCTGTCTTGTCCTCACGCCCATACCC
EXONS          XXXXX

               400       410       420       430       440
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          CGAGCTGGCTATCTTCATACAGCCAGGCCCTACGAGCAGCAAAGCAAAGT
DBH NM_013158  cgagctggctatcttcatacagccaggccctacgagcagcaaagcaaagt
SHR            CGAGCTGGCTATCTTCATACAGCCAGGCCCTACGAGCAGCAAAGCAAAGT
EXONS

               450       460       470       480       490
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          CGCCTACTGCTTCTGTGTTTGATGTCACTAATCTGAATCAAGCCCTTCAG
DBH NM_013158  cgcctactgcttctgtgtttgatgtcactaatctgaatcaagcccttcag
SHR            CGCCTACTGCTTCTGTGTTTGATGTCACTAATCTGAATCAAGCCCTTCAG
EXONS

               500       510       520       530       540
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          CATCACAAGGACACTCACTAGAGGATGCGACAGTAAGTGGACTCTTCCTG
DBH NM_013158  catcacaaggacactcactagaggatgcgacagtaagtggactcttcctg
SHR            CATCACAAGGACACTCACTAGAGGATGCGACAGTAAGTGGACTCTTCCTG
EXONS

               550       560       570       580       590
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          GAGTCCTTGACTGCACCCCCAAATACTGGTTACAGCAATCCCAAGGACGG
DBH NM_013158  gagtccttgactgcacccccaaatactggttacagcaatcccaaggacgg
SHR            GAGTCCTTGACTGCACCCCCAAATACTGGTTACAGCAATCCCAAGGACGG
EXONS

               600       610       620       630       640
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          TAAGAGCATAGGAAGGACCCATATGCCATGCAGCCCCCCTTCCCACCACG
DBH NM_013158  taagagcataggaaggacccatatgccatgcagccccccttcccaccacg
SHR            TAAGAGCATAGGAAGGACCCATATGCCATGCAGCCCCCCTTCCCACCACG
EXONS

               650       660       670       680       690
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          TGAGTACAGACCGTGCATATCCTCCAGCGTTACAAATGACAGCTCATACC
DBH NM_013158  tgagtacagaccgtgcatatcctccagcgttacaaatgacagctcatacc
SHR            TGAGTACAGACCGTGCATATCCTCCAGCGTTACAAATGACAGCTCATACC
EXONS

               700       710       720       730       740
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          ACACTGCCAGCTCATGGGCACCTGTTGTGGGCCAGGTGCACTGACTTAGT
DBH NM_013158  acactgccagctcatgggcacctgttgtgggccaggtgcactgacttagt
SHR            ACACTGCCAGCTCATGGGCACCTGTTGTGGGCCAGGTGCACTGACTTAGT
EXONS

               750       760       770       780       790
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          AGCACAGGTGGCACGCACACCAGCCTGGAACACAGCACACACAGGCCTTC
DBH NM_013158  agcacaggtggcacgcacaccagcctggaacacagcacacacaggccttc
SHR            AGCACAGGTGGCACGCACACCAGCCTGGAACACAGCACACACAGGCCTTC
EXONS

               800       810       820       830       840
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          TGTGCTATTCCCGATGATGGTTGCAGATAGCATCGCAACCCAGGTAAGGG
DBH NM_013158  tgtgctattcccgatgatggttgcagatagcatcgcaacccaggtaaggg
SHR            TGTGCTATTCCCGATGATGGTTGCAGATAGCATCGCAACCCAGGTAAGGG
EXONS

               850       860       870       880       890
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          ATAACCCCACCCTCCTCACAAGAGATGAGCCCAAGACGACAGAATAGCTC
DBH NM_013158  ataaccccaccctcctcacaagagatgagcccaagacgacagaatagctc
SHR            ATAACCCCACCCTCCTCACAAGAGATGAGCCCAAGACGACAGAATAGCTC
EXONS
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                  900       910       920       930       940
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGCTTCCTTCCGTTCAAAAATCTTCCTGAACACCCTATATGCTAGAGGAG
DBH NM_013158     tgcttccttccgttcaaaaatcttcctgaacaccctatatgctagaggag
SHR               TGCTTCCTTCCGTTCAAAAATCTTCCTGAACACCCTATATGCTAGAGGAG
EXONS

                  950       960       970       980       990
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CAAAGGGGAAGT--------------------------------------
DBH NM_013158     caaaggggaagtctcctacttagtggagcatccttcaggggcctctacaa
SHR               CAAAGGGGAAGT---~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
EXONS

                  1000      1010      1020      1030      1040
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gctacagggatcagtagaggagggggaaacacccacatatatatcctcgt
SHR               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
EXONS

                  1050      1060      1070      1080      1090
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     agctctactcttcctctgcagagggcttctttgggatagactcttactct
SHR               ~~~~~~~~~~~~--------------------------------------
EXONS

                  1100      1110      1120      1130      1140
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ctgcacctacaagagaagaggtggtcaaagattttccgtatcaaatcaga
SHR               --------------------------------------------------
EXONS

                  1150      1160      1170      1180      1190
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     aagtactttgtgttaatgcctctgcctcagcttccccagtgctgggatca
SHR               --------------------------------------------------
EXONS

                  1200      1210      1220      1230      1240
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     caggtgtgagccactaaacctgactaggagatgctttctcgagccgagca
SHR               --------------------------------------------------
EXONS

                  1250      1260      1270      1280      1290
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tggcagccaccccccttccccagccatttgctcagaactctcaggcgggg
SHR               --------------------------------------------------
EXONS

                  1300      1310      1320      1330      1340
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tgacagagcagggctggctgtacagtggctttggatcagtccatctgagg
SHR               --------------------------------------------------
EXONS

                  1350      1360      1370      1380      1390
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tgtgtgggagacagaagagccttgttgggccccagaggggttgccattcc
SHR               --------------------------------------------------
EXONS

                  1400      1410      1420      1430      1440
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tttcgccttcctggcatagcttttccgtgcagcagctcccaccagagagg
SHR               --------------------------------------------------
EXONS
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                    1450      1460      1470      1480      1490
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       cctgttctacccctgccccatttgtttgcacaatgaatgtctgtgaacac
SHR                 --------------------------------------------------
EXONS

                    1500      1510      1520      1530      1540
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       agacatcagaaatggagcccccgtgggcagcaggatgctcaggtatgcac
SHR                 --------------------------------------------------
EXONS

                    1550      1560      1570      1580      1590
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       tgtagagtcctggcagcaatgccagctgcctggctggtcctgctctaagt
SHR                 --------------------------------------------------
EXONS

                    1600      1610      1620      1630      1640
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       caacagggtccccagcaggagcttcaccatgaggctgtccccaggggaaa
SHR                 --------------------------------------------------
EXONS

                    1650      1660      1670      1680      1690
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ttttgctaatttctttcaggctttgctttcctaagccctggaggtaggac
SHR                 --------------------------------------------------
EXONS

                    1700      1710      1720      1730      1740
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       taaacagaaacagccccagcagtcctcacagcagaggcctgtgacgttca
SHR                 --------------------------------------------------
EXONS

                    1750      1760      1770      1780      1790
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       tgaattcagaaggaggcccacttgctggatgctaccccacagcctgcttc
SHR                 --------------------------------------------------
EXONS

                    1800      1810      1820      1830      1840
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ccttacagagaaggcagggagacctggacccgtgatctaggttccaggag
SHR                 --------------------------------------------------
EXONS

                    1850      1860      1870      1880      1890
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ctattctggggaaatgctcactcttgctgccaatgcagtctccccagcc
SHR                 --------------------------------------------------
EXONS

                    1900      1910      1920      1930      1940
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       cagacctcagtttactccctccaggcaatgctagccctagagtgagaggt
SHR                 --------------------------------------------------
EXONS

                    1950      1960      1970      1980      1990
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       tcccatggtacagtagttaaggcagcttttgctctcagcgttatgcaagc
SHR                 --------------------------------------------------
EXONS
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              2000      2010      2020      2030      2040
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 atggagcgcacacctgggtgagcaagtgccaccttaaatgctgccctgcc
SHR           --------------------------------------------------
EXONS

              2050      2060      2070      2080      2090
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 ttttcccattctggctccatcatcatctgccttgatccaagactctagtc
SHR           --------------------------------------------------
EXONS

              2100      2110      2120      2130      2140
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 tcagatgtctaatcctggggtcagtcctgccagaagccatggtctctaag
SHR           --------------------------------------------------
EXONS

              2150      2160      2170      2180      2190
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 ccctgagaatctgtatggttaactgtctctgggagtttcaggtaccccac
SHR           --------------------------------------------------
EXONS

              2200      2210      2220      2230      2240
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 ttacatgtggcaagttgaaagcaagctgggtgagggtacaactttaggca
SHR           --------------------------------------------------
EXONS

              2250      2260      2270      2280      2290
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         -------------------------GGGTGCTAGAAAGCTTGGTTTAAGG
DBH NM_013158 ggacgggtaacagaatgtgatatgggggtgctagaaagcttggtttaagg
SHR           -------------------------GGGTGCTAGAAAGCTTGGTTTAAGG
EXONS

              2300      2310      2320      2330      2340
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GAGGCCTGATCCCTTTACGTACACTGGACACTGGGTTTGATCCCAACATT
DBH NM_013158 gaggcctgatccctttacgtacactggacactgggtttgatcccaacatt
SHR           GAGGCCTGATCCCTTTACGTACACTGGACACTGGGTTTGATCCCAACATT
EXONS

              2350      2360      2370      2380      2390
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         AAAAGGAATGAGACAGATAAAAATCCAACAGGGGCGTCATCGATCAGCAG
DBH NM_013158 aaaaggaatgagacagataaaaatccaacaggggcgtcatcgatcagcag
SHR           AAAAAGAATGAGACAGATAAAAATCCAACAGGGGCGTCATCGATCAGCAG
EXONS

              2400      2410      2420      2430      2440
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCTCTTGGCCTCTCTGGGTGTCCCTGTGAAGCTGGCGACTCACTCAGTTA
DBH NM_013158 cctcttggcctctctgggtgtccctgtgaagctggcgactcactcagtta
SHR           CCTCTTGGCCTCTCTGGGTGTCCCTGTGAAGCTGGCGACTCACTCAGTTA
EXONS

              2450      2460      2470      2480      2490
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         ATGGACGTGATCTCTAATAGAGAAGTGCGCTCTACTGTGGGTCGGGGCTC
DBH NM_013158 atggacgtgatctctaatagagaagtgcgctctactgtgggtcggggctc
SHR           ATGGACGTGATCTCTAATAGAGAAGTGCGCTCTACTGTGGGTCGGGGCTC
EXONS

              2500      2510      2520      2530      2540
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TTTCCTGCTCAGATTCTGAGCCTGTCCCTGCAGGACGCCTGGAGTGACCA
DBH NM_013158 tttcctgctcagattctgagcctgtccctgcagGACGCCTGGAGTGACCA
SHR           TTTCCTGCTCAGAGTCTGAGCCTGTCCCTGCAGGACGCCTGGAGTGACCA
EXONS                                          EXON_2_XXXXXXXXXX
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               2550      2560      2570      2580      2590
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GAAAGGGCAGATCCATCTGGATACCCATCAGGACTACCAGCTGCTCCAGG
DBH NM_013158   GAAAGGGCAGATCCATCTGGATACCCATCAGGACTACCAGCTGCTCCAGG
SHR             GAAAGGGCAGATCCATCTGGATACCCATCAGGACTACCAGCTGCTCCAGG
EXONS           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

               2600      2610      2620      2630      2640
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           CACAGAGGGTGTCAAACAGCCTATCCCTGCTCTTCAAGAGGCCCTTTGTC
DBH NM_013158   CACAGAGGGTGTCAAACAGCCTATCCCTGCTCTTCAAGAGGCCCTTTGTC
SHR             CACAGAGGGTGTCAAACAGCCTATCCCTGCTCTTCAAGAGGCCCTTTGTC
EXONS           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

               2650      2660      2670      2680      2690
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           ACCTGCGACCCCAAGGATTATGTCATTGAGGTAGGCCACCCAGGGACTTT
DBH NM_013158   ACCTGCGACCCCAAGGATTATGTCATTGAGgtaggccacccagggacttt
SHR             ACCTGCGACCCCAAGGATTATGTCATTGAGGTAGGCCACCCAGGGACTTT
EXONS           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

               2700      2710      2720      2730      2740
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           CCTGAACCATCCCGGCATCCTAAGGTGTCAGGGAAGCCTGAGGTAGAGTT
DBH NM_013158   cctgaaccatcccggcatcctaaggtgtcagggaagcctgaggtagagtt
SHR             CCTGAACCATCCCGGCATCCTAAGGTGTCAGGGAAGCCTGAGGTAGAGTT
EXONS

               2750      2760      2770      2780      2790
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           CACACGTGAGAGAGAGCCCCTTTGGCAGATGGAAGCTGTTTTGAGAGAGG
DBH NM_013158   cacacgtgagagagagcccctttggcagatggaagctgttttgagagagg
SHR             CACACGTGAGAGAGAGCCCCTTTGGCAGATGGAAGCTGTTTTGAGAGAGG
EXONS

               2800      2810      2820      2830      2840
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GCCTCTAGCTTTCTATCCAGCAGCGAGGGGTTCCAAAAAAGCTCCCTGCA
DBH NM_013158   gcctctagctttctatccagcagcgaggggttccaaaaaagctccctgca
SHR             GCCTCTAGCTTTCTATCCAGCAGCGAGGGGTTCCAAAAAAGCTCCCTGCA
EXONS

               2850      2860      2870      2880      2890
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GACATGTATCCAGTGTTCTTTCCAGATGTGACTGTTGTTTGGGGCCCTCT
DBH NM_013158   gacatgtatccagtgttctttccagatgtgactgttgtttggggccctct
SHR             GACATGTATCCAGTGTTCTTTCCAGATGTGACTGTTGTTTGGGGCCCTCT
EXONS

               2900      2910      2920      2930      2940
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TGCC----------------------------------------------
DBH NM_013158   tgccctgggatgtattcatcagcctgtcatgtagaactctgtatgaagac
SHR             TGCC----------------------------------------------
EXONS

               2950      2960      2970      2980      2990
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   taacatacccgactttctaccctgctcagtcgctgatctgctttgaggct
SHR             --------------------------------------------------
EXONS

               3000      3010      3020      3030      3040
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   caggactggtccctttctcctccaaatctcagtctactcgtctgtaaggc
SHR             --------------------------------------------------
EXONS

               3050      3060      3070      3080      3090
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   taggagctgctggattcccaataaggaaggtctgcagtgtacttcatggc
SHR             --------------------------------------------------
EXONS
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                  3100      3110      3120      3130      3140
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tgccacatagttccaaagcacctgagcttcttagccactcccgtcaggaa
SHR               --------------------------------------------------
EXONS

                  3150      3160      3170      3180      3190
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     aagccagtctgtcttgcccctgatggaaaccttacgttcttgtttcctta
SHR               --------------------------------------------------
EXONS

                  3200      3210      3220      3230      3240
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gttaatctttcctgatggaacggacaggagctggaagtttgtggtggtgc
SHR               --------------------------------------------------
EXONS

                  3250      3260      3270      3280      3290
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tgagagacagagtagagatgctggtgccacgggaagaaactcatttactc
SHR               --------------------------------------------------
EXONS

                  3300      3310      3320      3330      3340
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gcagtcccccgggaagggccaccagcatgtaggaagccccaggctcagtc
SHR               --------------------------------------------------
EXONS

                  3350      3360      3370      3380      3390
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     actaggtggaatagagaaaagaaccttggcaataagctccactgggctta
SHR               --------------------------------------------------
EXONS

                  3400      3410      3420      3430      3440
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     taagggcaaaagctaggaagggtctggaaagcagcaggagggataattct
SHR               --------------------------------------------------
EXONS

                  3450      3460      3470      3480      3490
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gactcttcagggctttggggtgctctctgcttaccagttctggttctgag
SHR               --------------------------------------------------
EXONS

                  3500      3510      3520      3530      3540
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gcaatttatggcaggggagatggtggcttggagtagaaaagttgttcaaa
SHR               --------------------------------------------------
EXONS

                  3550      3560      3570      3580      3590
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     aaggtgggtggtgatttgggtccagccagactgcaggattcggaaggcgg
SHR               --------------------------------------------------
EXONS

                  3600      3610      3620      3630      3640
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gttgtactcctcctaagaatgggtctcgagaaaggataaacacatttggc
SHR               --------------------------------------------------
EXONS
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              3650      3660      3670      3680      3690
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 catttgcttagttctgtgattgattaatggatgccaaacagataactcta
SHR           --------------------------------------------------
EXONS

              3700      3710      3720      3730      3740
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 gattccaagaaaacaaatgaggaattttggccaaagaggggatagaataa
SHR           --------------------------------------------------
EXONS

              3750      3760      3770      3780      3790
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 cccaggagctcgtgacacacagaggctagggcaaggccattggaaatggg
SHR           --------------------------------------------------
EXONS

              3800      3810      3820      3830      3840
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 tcatttaagtccccgccctcctgtagacaaggcaagagcagtcagagaac
SHR           --------------------------------------------------
EXONS

              3850      3860      3870      3880      3890
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 cttccagaatgcaccacagggcaggccaatgtttatatgttctggggcca
SHR           --------------------------------------------------
EXONS

              3900      3910      3920      3930      3940
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 gagacttctatgtagaacatgcaagatgtctttcccaagagcagcccttg
SHR           --------------------------------------------------
EXONS

              3950      3960      3970      3980      3990
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 ggactcctaccaaccagagcacactgactgtgcatccttgggtgcccggg
SHR           --------------------------------------------------
EXONS

              4000      4010      4020      4030      4040
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 aaaagggctgttggcatttccagcaaagcatccacccagagctgccagcc
SHR           --------------------------------------------------
EXONS

              4050      4060      4070      4080      4090
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 tctgtccatagcaacctgtgaggaaaccaacccatatctgtggtctgttt
SHR           --------------------------------------------------
EXONS

              4100      4110      4120      4130      4140
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 gggctctccatcacagcaatgacctgtagactttacatgtccagcttct
SHR           --------------------------------------------------
EXONS

              4150      4160      4170      4180      4190
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         ----------------------------------TCAGAATTCCCCAGAT
DBH NM_013158 ggggaaaactcaatcacacagctccctgtcaaactcagaattccccagat
SHR           ----------------------------------TCAGAATTCCCCAGAT
EXONS
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              4200      4210      4220      4230      4240
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TGGTTTTGTTTTTGTTTTTGTTTTTGTTTTTTGTTTTTGTTTTTTGTTTT
DBH NM_013158 tggttttgtttttgtttttgtttttgttttttgtttttgttttttgtttt
SHR           TGGTTTTGTTTTTGTTTTTGTTTTTGTTTTTTGTTTTTGTTTTTTGTTTT
EXONS

              4250      4260      4270      4280      4290
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TTGTTTTTGTTTTTTTTTTGCTGCCTAGATGGTCACTGACTACTCAGTTG
DBH NM_013158 ttgtttttgttttttttttgctgcctagatggtcactgactactcagttg
SHR           TTGTTTTTTTTTTTTTTTTGCTGCCTAGATGGTCACTGACTACTCAGTTG
EXONS

              4300      4310      4320      4330      4340
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TGACCAATACCTTTCCCCTGCCCCCAGTGACCTTGGGGTTCATAGAAGAA
DBH NM_013158 tgaccaatacctttcccctgcccccagtgaccttggggttcatagaagaa
SHR           TGACCAATACCTTTCCCCTGCCCCCAGTGACCTTGGGGTTCATAGAAGAA
EXONS

              4350      4360      4370      4380      4390
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GTAAGAGTCCCAGCCTGCCTTATGGGTTGTAAACATGAATGGATGCCAAG
DBH NM_013158 gtaagagtcccagcctgccttatgggttgtaaacatgaatggatgccaag
SHR           GTAAGAGTCCCAGCCTGCCTTATGGGTTGTAAACATGAATGGATGCCAAG
EXONS

              4400      4410      4420      4430      4440
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CAAATGAAGAGTGTGTGAGAAAGAAAAAAGTGTTTCACAGGTGGGCACAG
DBH NM_013158 caaatgaagagtgtgtgagaaagaaaaaagtgtttcacaggtgggcacag
SHR           CAAATGAAGAGTGTGTGAGAAAGAAAAAAGTGTTTCACAGGTGGGCACAG
EXONS

              4450      4460      4470      4480      4490
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GATTTCAGAAACAGCCTGGGGCCAGAGCATTTGCTGGCTCTGAGTGGGCA
DBH NM_013158 gatttcagaaacagcctggggccagagcatttgctggctctgagtgggca
SHR           GATTTCAGAAACAGCCTGGGGCCAGAGCATTTGCTGGCTCTGAGTGGGCA
EXONS

              4500      4510      4520      4530      4540
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         ATCAACTGGTTCTGTCTGGGCTACAGGATGACACTGTCCATCTAGTGTAT
DBH NM_013158 atcaactggttctgtctgggctacagGATGACACTGTCCATCTAGTGTAT
SHR           ATCAACTGGTTCTGTCTGGGCTACAGGATGACACTGTCCATCTAGTGTAT
EXONS                                   EXON_3_XXXXXXXXXXXXXXXXX

              4550      4560      4570      4580      4590
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GGGATCCTGGAGGAGCCATTCCAGTCCCTGGAGGCCATCAACACCTCAGG
DBH NM_013158 GGGATCCTGGAGGAGCCATTCCAGTCCCTGGAGGCCATCAACACCTCAGG
SHR           GGGATCCTGGAGGAGCCATTCCAGTCCCTGGAGGCCATCAACACCTCAGG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              4600      4610      4620      4630      4640
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCTACATACGGGACTACAGCAGGTGCAGCTTCTGAAGCCTGAGGTCTCCA
DBH NM_013158 CCTACATACGGGACTACAGCAGGTGCAGCTTCTGAAGCCTGAGGTCTCCA
SHR           CCTACATACGGGACTACAGCAGGTGCAGCTTCTGAAGCCTGAGGTCTCCA
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              4650      4660      4670      4680      4690
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CTCCAGCCATGCCTGCGGATGTACAAACCATGGATATCCGGGCTCCTGAC
DBH NM_013158 CTCCAGCCATGCCTGCGGATGTACAAACCATGGATATCCGGGCTCCTGAC
SHR           CTCCAGCCATGCCTGCGGATGTACAAACCATGGATATCCGGGCTCCTGAC
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              4700      4710      4720      4730      4740
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GTCCTCATCCCCAGCACTGAGACCACATACTGGTGCTATATCACTGAGCT
DBH NM_013158 GTCCTCATCCCCAGCACTGAGACCACATACTGGTGCTATATCACTGAGCT
SHR           GTCCTCATCCCCAGCACTGAGACCACATACTGGTGCTATATCACTGAGCT
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
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              4750      4760      4770      4780      4790
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         ACCCCTACACTTCCCCCGACACCACATCATCATGGTAAATGGGGAACAGG
DBH NM_013158 ACCCCTACACTTCCCCCGACACCACATCATCATGgtaaatggggaacagg
SHR           ACCCCTACACTTCCCCCGACACCACATCATCATGGTAAATGGGGAACAGG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              4800      4810      4820      4830      4840
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GTGGGGGTGTCCAGCTCTACTCCCCCCCCGGGGGCCCAGAGTTGCAAGAA
DBH NM_013158 gtgggggtgtccagctctactccccccccgggggcccagagttgcaagaa
SHR           GTGGGGGTGTCCAGCTCTACTCCCCCCCCGGGGGCCCAGAGTTGCAAGAA
EXONS

              4850      4860      4870      4880      4890
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TCTGAGGTGCTAGCCCAACTCCCTTAGAATAACACATATGCACTAGTCCC
DBH NM_013158 tctgaggtgctagcccaactcccttagaataacacatatgcactagtccc
SHR           TCTGAGGTGCTAGCCCAACTCCCTTAGAATAACACATATGCACTAGTCCC
EXONS

              4900      4910      4920      4930      4940
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CTATGCTTCCTGTCCCCACTCACCATCCGCCTCGGGGTCCCTGAGATGAA
DBH NM_013158 ctatgcttcctgtccccactcaccatccgcctcggggtccctgagatgaa
SHR           CTATGCTTCCTGTCCCCACTCACCATCCGCCTCGGGGTCCCTGAGATGAA
EXONS

              4950      4960      4970      4980      4990
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TATTTTCTGTCTTACCTGCCTGGAGCAAAGCAATAATTTCTTTACTTCTG
DBH NM_013158 tattttctgtcttacctgcctggagcaaagcaataatttctttacttctg
SHR           TATTTTCTGTCTTACCTGCCTGGAGCAAAGCAATAATTTCTTTACTTCTG
EXONS

              5000      5010      5020      5030      5040
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CAATTGCCCTTGAGGTTAGAACTGCTGTCAATCTTTCTGGCCACCTCCTC
DBH NM_013158 caattgcccttgaggttagaactgctgtcaatctttctggccacctcctc
SHR           CAATTGCCCTTGAGGTTAGAACTGCTGTCAATCTTTCTGGCCACCTCCTC
EXONS

              5050      5060      5070      5080      5090
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TCATGTAAAAGTAGCTAAATTCAGTTGCTCCAAAGCTGCAGCTAGGGACT
DBH NM_013158 tcatgtaaaagtagctaaattcagttgctccaaagctgcagctagggact
SHR           TCATGTAAAAGTAGCTAAATTCAGTTGCTCCAAAGCTGCAGCTAGGGACT
EXONS

              5100      5110      5120      5130      5140
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TTGTCACAGAAGTCCCAAAGGCTGCCCCTGTTCCAGAAGACGAAGCATAA
DBH NM_013158 ttgtcacagaagtcccaaaggctgcccctgttccagaagacgaagcataa
SHR           TTGTCACAGAAGTCCCAAAGGCTGCCCCTGTTCCAGAAGACGAAGCATAA
EXONS

              5150      5160      5170      5180      5190
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCCAGAAAGGTCATGTGAATGCCCAGGGTCACACAGCAAACAGTATAGCT
DBH NM_013158 cccagaaaggtcatgtgaatgcccagggtcacacagcaaacagtatagct
SHR           CCCAGAAAGGTCATGTGAATGCCCAGGGTCACACAGCAAACAGTATAGCT
EXONS

              5200      5210      5220      5230      5240
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GTCCTTGAGGCCCAGGCTCTAGCCTGGAGTCCATTCTGTCTCCTAGTGGA
DBH NM_013158 gtccttgaggcccaggctctagcctggagtccattctgtctcctagtgga
SHR           GTCCTTGAGGCCCAGGCTCTAGCCTGGAGTCCATTCTGTCTCCTAGTGGA
EXONS

              5250      5260      5270      5280      5290
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GCTTCATGGGCCCAGAGTCACCCATACACACAAGGACAGTTGGCTTCCTA
DBH NM_013158 gcttcatgggcccagagtcacccatacacacaaggacagttggcttccta
SHR           GCTTCATGGGCCCAGAGTCACCCATACACACAAGGACAGTTGGCTTCCTA
EXONS
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                 5300      5310      5320      5330      5340
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GTTCCATCAGGAATAGTCAGTGTAGGTCTCTACTTTTTTTCTGGAGCTCT
DBH NM_013158    gttccatcaggaatagtcagtgtaggtctctactttttttctggagctct
SHR              GTTCCATCAGGAATAGTCAGTGTAGGTCTCTACTTTTTTTCTGGAGCTCT
EXONS

                 5350      5360      5370      5380      5390
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GTGGGTTCTCTTACCTCCTGGCTCTGAACTGGAAACCCTCAAGTACCCGA
DBH NM_013158    gtgggttctcttacctcctggctctgaactggaaaccctcaagtacccga
SHR              GTGGGTTCTCTTACCTCCTGGCTCTGAACTGGAAACCCTCAAGTACCCGA
EXONS

                 5400      5410      5420      5430      5440
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            AGTTGCCTTCTCATTTATAGCTGCTCCAGCCAAGTGGGAGGGGTAGGCAG
DBH NM_013158    agttgccttctcatttatagctgctccagccaagtgggaggggtaggcag
SHR              AGTTGCCTTCTCATTTATAGCTGCTCCAGCCAAGTGGGAGGGGTAGGCAG
EXONS

                 5450      5460      5470      5480      5490
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GTACTGGACCCCCAGTTCAGGGTAGTCTGTCCCTCTGCAGTATGAGGCCA
DBH NM_013158    gtactggacccccagttcagggtagtctgtccctctgcagTATGAGGCCA
SHR              GTACTGGACCCCCAGTTCAGGGTAGTCTGTCCCTCTGCAGTATGAGGCCA
EXONS                                                    EXON_4_XXX

                 5500      5510      5520      5530      5540
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            TTGTCACGGAGGGCAATGAGGCCCTGGTGCACCACATGGAGGTCTTCCAA
DBH NM_013158    TTGTCACGGAGGGCAATGAGGCCCTGGTGCACCACATGGAGGTCTTCCAA
SHR              TTGTCACGGAGGGCAATGAGGCCCTGGTGCACCACATGGAGGTCTTCCAA
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 5550      5560      5570      5580      5590
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            TGTACAAATGAGTCTGAGGCCTTCCCCATGTTCAACGGACCCTGCGACTC
DBH NM_013158    TGTACAAATGAGTCTGAGGCCTTCCCCATGTTCAACGGACCCTGCGACTC
SHR              TGTACAAATGAGTCTGAGGCCTTCCCCATGTTCAACGGACCCTGCGACTC
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 5600      5610      5620      5630      5640
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            CAAGATGAAACCTGACAGACTCAACTACTGTCGCCACGTGCTGGCGGCAT
DBH NM_013158    CAAGATGAAACCTGACAGACTCAACTACTGTCGCCACGTGCTGGCGGCAT
SHR              CAAGATGAAACCTGACAGACTCAACTACTGTCGCCACGTGCTGGCGGCAT
EXONS            XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 5650      5660      5670      5680      5690
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GGGCCCTGGGCGCCAAGGTATGTACATATGGCTGAACGCCTCTTCTAGTT
DBH NM_013158    GGGCCCTGGGCGCCAAGgtatgtacatatggctgaacgcctcttctagtt
SHR              GGGCCCTGGGCGCCAAGGTATGTACATATGGCTGAACGCCTCTTCTAGTT
EXONS            XXXXXXXXXXXXXXXXX

                 5700      5710      5720      5730      5740
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            TGTTTTCTGTTGTTGTGATATACCATGGCCAAAATCAACTTGGGGGAAGA
DBH NM_013158    tgttttctgttgttgtgatataccatggccaaaatcaacttgggggaaga
SHR              TGTTTTCTGTTGTTGTGATATACCATGGCCAAAATCAACTTGGGGGAAGA
EXONS

                 5750      5760      5770      5780      5790
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            AAGGATTTACTTGGCAAACATTTTCAGGTCATAGTTAGTCCTTCATGGAG
DBH NM_013158    aaggatttacttggcaaacattttcaggtcatagttagtccttcatggag
SHR              AAGGATTTACTTGGCAAACATTTTCAGGTCATAGTTAGTCCTTCATGGAG
EXONS

                 5800      5810      5820      5830      5840
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GGAAGTCGAGGCAAGAATCAAGGCTTGAGGTACCACATCACCTCTGACCA
DBH NM_013158    ggaagtcgaggcaagaatcaaggcttgaggtaccacatcacctctgacca
SHR              GGAAGTCGAGGCAAGAATCAAGGCTTGAGGTACCACATCACCTCTGACCA
EXONS
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             5850      5860      5870      5880      5890
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        AGGAACGCATAGCCAAAGAAGAACGGCAGGAATCACGGGGGATGCTGGCA
DBH NM_013158 aggaacgcatagccaaagaagaacggcaggaatcacgggggatgctggca
SHR          AGGAACGCATAGCCAAAGAAGAACGGCAGGAATCACGGGGGATGCTGGCA
EXONS

             5900      5910      5920      5930      5940
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        CTGGCTGGTCTTCAGGCCATCTAAGGACTCAGATAGTGTCCTTACATAGC
DBH NM_013158 ctggctggtcttcaggccatctaaggactcagatagtgtccttacatagc
SHR          CTGGCTGGTCTTCAGGCCATCTAAGGACTCAGATAGTGTCCTTACATAGC
EXONS

             5950      5960      5970      5980      5990
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        CCAGAGGATGGCGCCGCACACAGTCGCCTTGGCCTTCCTAAGTCAGTGAA
DBH NM_013158 ccagaggatggcgccgcacacagtcgccttggccttcctaagtcagtgaa
SHR          CCAGAGGATGGCGCCGCACACAGTCGCCTTGGCCTTCCTAAGTCAGTGAA
EXONS

             6000      6010      6020      6030      6040
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        GAATCAAAACAAACCTCATAGATGAGCCTCCAGCCAATCTGACAGAGGTA
DBH NM_013158 gaatcaaaacaaacctcatagatgagcctccagccaatctgacagaggta
SHR          GAATCAAAACAAACCTCATAGATGAGCCTCCAGCCAATCTGACAGAGGTA
EXONS

             6050      6060      6070      6080      6090
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        ACGCCATAGCTGAGGCTGTCTCTGGTGAGTCTAGGCTTTGCCAGCTTGGC
DBH NM_013158 acgccatagctgaggctgtctctggtgagtctaggctttgccagcttggc
SHR          ACGCCATAGCTGAGGCTGTCTCTGGTGAGTCTAGGCTTTGCCAGCTTGGC
EXONS

             6100      6110      6120      6130      6140
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        AGGTTAAAGTTAGTTAGGACACAATTGCACCATCCCCTCCTTGGGAGTCC
DBH NM_013158 aggttaaagttagttaggacacaattgcaccatcccctccttgggagtcc
SHR          AGGTTAAAGTTAGTTAGGACACAATTGCACCATCCCCTCCTTGGGAGTCC
EXONS

             6150      6160      6170      6180      6190
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        TCGGGATTTTAACTCACTGCACCCGTGTCCTAAACCACATCCTGAAATGT
DBH NM_013158 tcgggattttaactcactgcacccgtgtcctaaaccacatcctgaaatgt
SHR          TCGGGATTTTAACTCACTGCACCCGTGTCCTAAACCACATCCTGAAATGT
EXONS

             6200      6210      6220      6230      6240
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        CCCAGGAGACAGATGGGAAGGAGGCACACCAGGCGCCGGCAGAGTGTAGT
DBH NM_013158 cccaggagacagatgggaaggaggcacaccaggcgccggcagagtgtagt
SHR          CCCAGGAGACAGATGGGAAGGAGGCACACCAGGCGCCGGCAGAGTGTAGT
EXONS

             6250      6260      6270      6280      6290
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        TCACCTGCAGGATTCTCCTGCTTTTGTTCTTTGGGGGTCACGAAAGCTCC
DBH NM_013158 tcacctgcaggattctcctgcttttgttctttgggggtcacgaaagctcc
SHR          TCACCTGCAGGATTCTCCTGCTTTTGTTCTTTGGGGGTCACGAAAGCTCC
EXONS

             6300      6310      6320      6330      6340
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        TTGCATCCTTCTATGAAAATGCACACGACCATGGGTATGCACCTCTCATC
DBH NM_013158 ttgcatccttctatgaaaatgcacacgaccatgggtatgcacctctcatc
SHR          TTGCATCCTTCTATGAAAATGCACACGACCATGGGTATGCACCTCTCATC
EXONS

             6350      6360      6370      6380      6390
             |....|....|....|....|....|....|....|....|....|....
BN.Lx        CTGGATTAGCACTTACAGGGCTCTGGGTTCTGTGCCCAGCATGATCCCAG
DBH NM_013158 ctggattagcacttacagggctctgggttctgtgcccagcatgatcccag
SHR          CTGGATTAGCACTTACAGGGCTCTGGGTTCTGTGCCCAGCATGATCCCAG
EXONS
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                6400      6410      6420      6430      6440
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GGCTGCCTGTGGCTGGGCAAGCTGTCAACAGACCATGCCCGAGATTCTTA
DBH NM_013158   ggctgcctgtggctgggcaagctgtcaacagaccatgcccgagattctta
SHR             GGCTGCCTGTGGCTGGGCAAGCTGTCAACAGACCATGCCCGAGATTCTTA
EXONS

                6450      6460      6470      6480      6490
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TCAAGCCCCTCTTCGGTCCCCAGGCCAGAGGATGACAAGGGCAGGACTGG
DBH NM_013158   tcaagcccctcttcggtccccaggccagaggatgacaagggcaggactgg
SHR             TCAAGCCCCTCTTCGGTCCCCAGGCCAGAGGATGACAAGGGCAGGACTGG
EXONS

                6500      6510      6520      6530      6540
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           AAGGCCTGGGAGAGAGGCTCAGCTCAGGTTCAGTGCCCTCACGATAGGAC
DBH NM_013158   aaggcctgggagagaggctcagctcaggttcagtgccctcacgataggac
SHR             AAGGCCTGGGAGAGAGGCTCAGCTCAGGTTCAGTGCCCTCACGATAGGAC
EXONS

                6550      6560      6570      6580      6590
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           CTTTTGTGTGTGACAGCAAGGTCTACTTAGCCCCAGGAGAGAATCCTTTT
DBH NM_013158   cttttgtgtgtgacagcaaggtctacttagccccaggagagaatcctttt
SHR             CTTTTGTGTGTGACAGCAAGGTCTACTTAGCCCCAGGAGAGAATCCTTTT
EXONS

                6600      6610      6620      6630      6640
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           AACCCAGCCACTTGCCGAGCTTCTGTTCGGGCCCCTATTGCTGGCCAGGC
DBH NM_013158   aacccagccacttgccgagcttctgttcgggcccctattgctggccaggc
SHR             AACCCAGCCACTTGCCGAGCTTCTGTTCGGGCCCCTATTGCTGGCCAGGC
EXONS

                6650      6660      6670      6680      6690
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TTGTCCCTGAAGGACACTTGTCTAATACCCCTGTCCCACACAGGCATTTT
DBH NM_013158   ttgtccctgaaggacacttgtctaatacccctgtcccacacagGCATTTT
SHR             TTGTCCCTGAAGGACACTTGTCTAATACCCCTGTCCCACACAGGCATTTT
EXONS                                                      EXON_5_

                6700      6710      6720      6730      6740
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           ACTACCCAGAAGAAGCCGGTGTCCCCTTTGGGGGCTCAGGATCCTCCCGG
DBH NM_013158   ACTACCCAGAAGAAGCCGGTGTCCCCTTTGGGGGCTCAGGATCCTCCCGG
SHR             ACTACCCAGAAGAAGCCGGTGTCCCCTTTGGGGGCTCAGGATCCTCCCGG
EXONS           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                6750      6760      6770      6780      6790
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           TTTCTCCGACTGGAAGTTCATTACCACAATCCACGGAATATACAAGGTAC
DBH NM_013158   TTTCTCCGACTGGAAGTTCATTACCACAATCCACGGAATATACAAGgtac
SHR             TTTCTCCGACTGGAAGTTCATTACCACAATCCACGGAATATACAAGGTAC
EXONS           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                6800      6810      6820      6830      6840
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GAGTGCCGAGAGCACATCTCCCGACCCTGCTTCAGTTTCTCAGGTGCTCG
DBH NM_013158   gagtgccgagagcacatctcccgaccctgcttcagtttctcaggtgctcg
SHR             GAGTGCCGAGAGCACATCTCCCGACCCTGCTTCAGTTTCTCAGGTGCTCG
EXONS

                6850      6860      6870      6880      6890
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GGAGCAGCACAGAGGAAACAGGATGTACGTGTACGTCGGAAATAACACAG
DBH NM_013158   ggagcagcacagaggaaacaggatgtacgtgtacgtcggaaataacacag
SHR             GGAGCAGCACAGAGGAAACAGGATGTACGTGTACGTCGGAAATAACACAG
EXONS

                6900      6910      6920      6930      6940
                |....|....|....|....|....|....|....|....|....|....
BN.Lx           GCCTTTGCTCCTTGTCTACTTTATTGTAGTCAATACCCTAATGAGTTCCC
DBH NM_013158   gcctttgctccttgtctactttattgtagtcaataccctaatgagttccc
SHR             GCCTTTGCTCCTTGTCTACTTTATTGTAGTCAATACCCTAATGAGTTCCC
EXONS
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               6950      6960      6970      6980      6990
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          AGCCTAGGTTCTAGGTGACTTCCTCAAGCCGGTGCTCTAATTGATTAGTG
DBH NM_013158  agcctaggttctaggtgacttcctcaagccggtgctctaattgattagtg
SHR            AGCCTAGGTTCTAGGTGACTTCCTCAAGCCGGTGCTCTAATTGATTAGTG
EXONS

               7000      7010      7020      7030      7040
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          ATGTCTGCCATGGACCCTGGGGCAGGGTTTGGTACCATGCTGTTGAGCAT
DBH NM_013158  atgtctgccatggaccctggggcagggtttggtaccatgctgttgagcat
SHR            ATGTCTGCCATGGACCCTGGGGCAGGGTTTGGTACCATGCTGTTGAGCAT
EXONS

               7050      7060      7070      7080      7090
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          CTGAACACTCAGTGAAAAGAGAGCCTCACAGGTTCCACAGAGTAGGCACG
DBH NM_013158  ctgaacactcagtgaaaagagagcctcacaggttccacagagtaggcacg
SHR            CTGAACACTCAGTGAAAAGAGAGCCTCACAGGTTCCACAGAGTAGGCACG
EXONS

               7100      7110      7120      7130      7140
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          ATGTCGTTTACCTCCTTGCAGGGCATGACAGCAGGCACTGACTGCTCTG-
DBH NM_013158  atgtcgtttacctccttgcagggcatgacagcaggcactgactgctctgg
SHR            ATGTCGTTTACCTCCTTGCAGGGCATGACAGCAGGCACTGACTGCTCTG-
EXONS

               7150      7160      7170      7180      7190
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          --------------------------------------------------
DBH NM_013158  acaggttctttgtccagcttgtgtcatgtgtttagatggcccaagatgtc
SHR            --------------------------------------------------
EXONS

               7200      7210      7220      7230      7240
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          --------------------------------------------------
DBH NM_013158  cagttaactcagaaatgcccagggcagtgccacacccgtccccctgcttc
SHR            --------------------------------------------------
EXONS

               7250      7260      7270      7280      7290
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          --------------------------------------------------
DBH NM_013158  tgttccccaacctggcacggagctggaaaaaagtcgtggacggatggatg
SHR            --------------------------------------------------
EXONS

               7300      7310      7320      7330      7340
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          --------------------------------------------------
DBH NM_013158  ggtctgagaataccgtattaggccaagggatctcaaggaagataaggcac
SHR            --------------------------------------------------
EXONS

               7350      7360      7370      7380      7390
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          --------------------------------------------------
DBH NM_013158  atccatgctgcaaaaagcttctagaagagagacaaggggtgtactgtgtg
SHR            --------------------------------------------------
EXONS

               7400      7410      7420      7430      7440
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          --------------------------------------------------
DBH NM_013158  cctgtgtgtttgcgtgcctgtgtgtgcgtgtgtgtgtgtgaaagagagag
SHR            --------------------------------------------------
EXONS

               7450      7460      7470      7480      7490
               |....|....|....|....|....|....|....|....|....|....
BN.Lx          --------------------------------------------------
DBH NM_013158  acacacacatgcacacacacacacacagagagagagagagagagagacag
SHR            --------------------------------------------------
EXONS
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                   7500      7510      7520      7530      7540
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       agacagagacagagacagagacagagagagagacagagaggaattatggt
SHR                 --------------------------------------------------
EXONS

                   7550      7560      7570      7580      7590
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       gccatttagtacaggtattgcaggcttagcacaatctcagtcactatcat
SHR                 --------------------------------------------------
EXONS

                   7600      7610      7620      7630      7640
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ggctggatcaccccgtgcctgaagctgaacggcttgagtgggtcattaag
SHR                 --------------------------------------------------
EXONS

                   7650      7660      7670      7680      7690
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       gggtctgatagaggacaccacatcagacactaaatatttccttgctcttg
SHR                 --------------------------------------------------
EXONS

                   7700      7710      7720      7730      7740
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       tgccccaccttccatcaaatgtctcccctttcctttctctccctggcctc
SHR                 --------------------------------------------------
EXONS

                   7750      7760      7770      7780      7790
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ctctctctccccctcccccgccctttgaatgacacttatcctcgtccatg
SHR                 --------------------------------------------------
EXONS

                   7800      7810      7820      7830      7840
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       caaacgaggagtagaaagaccctgagggacatggaggcgtgtccacagtg
SHR                 --------------------------------------------------
EXONS

                   7850      7860      7870      7880      7890
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       agactggagcagctactgcagccagccctcagctggacgtggaactggca
SHR                 --------------------------------------------------
EXONS

                   7900      7910      7920      7930      7940
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       gtgcaaacttaatgtcacgcgggacttccccccttggctgtggcaatgcct
SHR                 --------------------------------------------------
EXONS

                   7950      7960      7970      7980      7990
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ccaccgtgggaattatctatctactgcacgtgggggagcggcctgagcac
SHR                 --------------------------------------------------
EXONS

                   8000      8010      8020      8030      8040
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ccacaggggatttattgctcatttaaaacccgagagcaaaggatttttga
SHR                 --------------------------------------------------
EXONS
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                8050      8060      8070      8080      8090
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   aaactccttgggcgtcagggaagtctgtcttccttgatcatccgcgtgta
SHR             --------------------------------------------------
EXONS

                8100      8110      8120      8130      8140
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   tttatggatcttttgccaaatataagggtgctcctgtcagttgatgagtt
SHR             --------------------------------------------------
EXONS

                8150      8160      8170      8180      8190
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   ttgagacaggctgcatttctattaataccgaaatccgtgattatctcatt
SHR             --------------------------------------------------
EXONS

                8200      8210      8220      8230      8240
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   atttaatccccacctaaattttaaccaattgcttcttctttttattattt
SHR             --------------------------------------------------
EXONS

                8250      8260      8270      8280      8290
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   gcctctgatgtcatcagcctagagacacaagggctgcttgagtttgaatg
SHR             --------------------------------------------------
EXONS

                8300      8310      8320      8330      8340
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   tcgttggcctggggttggggaggtagagacaacacaaaaggaggtactct
SHR             --------------------------------------------------
EXONS

                8350      8360      8370      8380      8390
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   caactgatgagcaggagagagcctcggcctgccagcagcatcctgcatcc
SHR             --------------------------------------------------
EXONS

                8400      8410      8420      8430      8440
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   cagcctgccggtcctgggtttgaagccaagttcagatgcttcccaaacct
SHR             --------------------------------------------------
EXONS

                8450      8460      8470      8480      8490
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   ttggtcttaggcagagtcttccgttcagccttccacctcagttgctccct
SHR             --------------------------------------------------
EXONS

                8500      8510      8520      8530      8540
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------------------------------
DBH NM_013158   ctgtaaatggggacagtggtccttaccaagaaaacaccccatagaggtga
SHR             --------------------------------------------------
EXONS

                8550      8560      8570      8580      8590
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx           --------------------------TCCATTCTTTCTTGCTGTTTTCAG
DBH NM_013158   caggtgcgctaagagtggcttctgactccattctttcttgctgttttcag
SHR             --------------------------TCCATTCTTTCTTGCTGTTTTCAG
EXONS
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              8600      8610      8620      8630      8640
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TCAGAAAGGGTGACCAAGCACACTTTCTCAGGGGTATCTCAGGGGCTGCT
DBH NM_013158 tcagaaagggtgaccaagcacactttctcaggggtatctcaggggctgct
SHR           TCAGAAAGGGTGACCAAGCACACTTTCTCAGGGGTATCTCAGGGGCTGCT
EXONS

              8650      8660      8670      8680      8690
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GCTTATCACACGCCTCTCCCTCAGGCCGGCGCGACTCCTCTGGCATCCGT
DBH NM_013158 gcttatcacacgcctctccctcagGCCGGCGCGACTCCTCTGGCATCCGT
SHR           GCTTATCACACGCCTCTCCCTCAGGCCGGCGCGACTCCTCTGGCATCCGT
EXONS                                 EXON_6_XXXXXXXXXXXXXXXXXXX

              8700      8710      8720      8730      8740
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CTACACTACACAGCTAGTCTCCGACCCAATGAGGCAGGCATCATGGAGCT
DBH NM_013158 CTACACTACACAGCTAGTCTCCGACCCAATGAGGCAGGCATCATGGAGCT
SHR           CTACACTACACAGCTAGTCTCCGACCCAATGAGGCAGGCATCATGGAGCT
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              8750      8760      8770      8780      8790
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TGGACTGGTGTACACGCCCTTGATGGCCATCCCCCCTCAGGAGACCACCT
DBH NM_013158 TGGACTGGTGTACACGCCCTTGATGGCCATCCCCCCTCAGGAGACCACCT
SHR           TGGACTGGTGTACACGCCCTTGATGGCCATCCCCCCTCAGGAGACCACCT
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              8800      8810      8820      8830      8840
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TTGTTTTGACTGGCTACTGCACAGACAGGTGCACCCAGATGGTGAGTGGA
DBH NM_013158 TTGTTTTGACTGGCTACTGCACAGACAGGTGCACCCAGATGGtgagtgga
SHR           TTGTTTTGACTGGCTACTGCACAGACAGGTGCACCCAGATGGTGAGTGGA
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              8850      8860      8870      8880      8890
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         AAGGGCCTTACACGCAGCCCACTGTTTGCAATGACCAGGGGAAGCAGCAA
DBH NM_013158 aagggccttacacgcagcccactgtttgcaatgaccaggggaagcagcaa
SHR           AAGGGCCTTACACGCAGCCCACTGTTTGCAATGACCAGGGGAAGCAGCAA
EXONS

              8900      8910      8920      8930      8940
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GAGGACTCTGGGCCCCTGACCCCGGAATCAAGGTTGTCTGAGTCTCTGAC
DBH NM_013158 gaggactctgggcccctgaccccggaatcaaggttgtctgagtctctgac
SHR           GAGGACTCTGGGCCCCTGACCCCGGAATCAAGGTTGTCTGAGTCTCTGAC
EXONS

              8950      8960      8970      8980      8990
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCCCTGTCTCCAGCAAGAATTGGACAGTGCCATGCACCCACCCTTCTGAA
DBH NM_013158 cccctgtctccagcaagaattggacagtgccatgcacccacccttctgaa
SHR           CCCCTGTCTCCAGCAAGAATTGGACAGTGCCATGCACCCACCCTTCTGAA
EXONS

              9000      9010      9020      9030      9040
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         ATATGTCACTTTAAAAATATTTATTGATGTGTTTGATTATATGCTCCTCT
DBH NM_013158 atatgtcactttaaaaatatttattgatgtgtttgattatatgctcctct
SHR           ATATGTCACTTTAAAAATATTTATTGATGTGTTTGATTATATGCTCCTCT
EXONS

              9050      9060      9070      9080      9090
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCTCTCTCCCCCTTCTGTCTCTGTCTCCCCACCCCTACCCCTCTCTGTTT
DBH NM_013158 cctctctcccccttctgtctctgtctccccacccctacccctctctgttt
SHR           CCTCTCTCCCCCTTCTGTCTCTGTCTCCCCACCCCTACCCCTCTCTGTTT
EXONS

              9100      9110      9120      9130      9140
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CTCTGTCTCTGTCTCTGTCTCTCTCTGTCTCTGTCTCTCTCTTTCTCTGT
DBH NM_013158 ctctgtctctgtctctgtctctctctgtctctgtctctctctttctctgt
SHR           CTCTGTCTCTGTCTCTGTCTCTCTCTGTCTCTGTCTCTCTCTTTCTCTGT
EXONS
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                 9150      9160      9170      9180      9190
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GTGTATGTGTATGTGTGTGTGCGTGCGTGCGTGTGTGTGTGTGTGTGTGT
DBH NM_013158     gtgtatgtgtatgtgtgtgtgcgtgcgtgcgtgtgtgtgtgtgtgtgtgt
SHR               GTGTATGTGTATGTGTGTGTGCGTGCGTGCGTGTGTGTGTGTGTGTGTGT
EXONS

                 9200      9210      9220      9230      9240
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GTGTGTGTGTGTGTGTTCCAGCTAGGAAGTAGGGGAGGCAGCAC------
DBH NM_013158     gtgtgtgtgtgtgtgttccagctaggaagtaggggaggcagcacagtacc
SHR               GTGTGTGTGTGTGTGTTCCAGCTAGGAAGTAGGGGAGGCAGCAC------
EXONS

                 9250      9260      9270      9280      9290
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     cagtgtccctgaggctgtgcaggcgcgaggtgacacagcaggggccttgg
SHR               --------------------------------------------------
EXONS

                 9300      9310      9320      9330      9340
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     cttctgtagcaggtggacagtggctcctagcctgttccaggctgccctgg
SHR               --------------------------------------------------
EXONS

                 9350      9360      9370      9380      9390
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ctctcgactggaccactgacttagctgggctcacctcccatctggcatga
SHR               --------------------------------------------------
EXONS

                 9400      9410      9420      9430      9440
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     aggtctagaggagggcactgctcagtaggggtgggggacagtcaccaatg
SHR               --------------------------------------------------
EXONS

                 9450      9460      9470      9480      9490
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gggatctcacagagccctctctagactctgtgatggcttcccactcacac
SHR               --------------------------------------------------
EXONS

                 9500      9510      9520      9530      9540
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tatggccagttgtgacagttccctgtagtttgggaggcgggcaagtgcac
SHR               --------------------------------------------------
EXONS

                 9550      9560      9570      9580      9590
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     aggcctggagctctcagttatccgtagcctctgttcactcctgttaatgt
SHR               --------------------------------------------------
EXONS

                 9600      9610      9620      9630      9640
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ctgttctcaccctaagcgagtttctctggctgcctgccatcagtcttact
SHR               --------------------------------------------------
EXONS

                 9650      9660      9670      9680      9690
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gtctggagctcttttgatatcacagatgtgacttgattgtctgctgtgtg
SHR               --------------------------------------------------
EXONS
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```
                 9700      9710      9720      9730      9740
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tcctgtgttctcgctggcggcctccctgcttcttgcagtgacctgtcacg
SHR               --------------------------------------------------
EXONS

                 9750      9760      9770      9780      9790
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gaatggtacatattcctggatcgcagacagggagacccaggcctgccgag
SHR               --------------------------------------------------
EXONS

                 9800      9810      9820      9830      9840
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     agaggacacatggctcaaagctgcaccatggagcaggtctctttgtccca
SHR               --------------------------------------------------
EXONS

                 9850      9860      9870      9880      9890
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gagctcatcgtaaatgctcctttccagtgtactcatgggatgacgcctgt
SHR               --------------------------------------------------
EXONS

                 9900      9910      9920      9930      9940
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tctcaagagtcctctgtacctgagcacctggtctcggaagtgttagcgag
SHR               --------------------------------------------------
EXONS

                 9950      9960      9970      9980      9990
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gcctaaagttctcaggttgagacactggacacatctgttctttttttttt
SHR               --------------------------------------------------
EXONS

                10000     10010     10020     10030     10040
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tttttttttggttctttttttcggagctggggaccgaacccagggccttg
SHR               --------------------------------------------------
EXONS

                10050     10060     10070     10080     10090
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     cacttcctaggtaagtgctctaccactgagctaaatccccagccccgaca
SHR               --------------------------------------------------
EXONS

                10100     10110     10120     10130     10140
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     catctgttcttatacacaatcaaaacctttagcctgggggcaggatgaga
SHR               --------------------------------------------------
EXONS

                10150     10160     10170     10180     10190
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     aatcaactttcctcccaagtccaaacagacaggttaggaggggtcatgct
SHR               --------------------------------------------------
EXONS

                10200     10210     10220     10230     10240
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ggcttcttagacttgtgtccacagatcacgttctgccacacgcctgaatc
SHR               --------------------------------------------------
EXONS
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                 10250     10260     10270     10280     10290
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      cctggatcatagcaagggcccaggcccggcatatacagccacagtttagt
SHR                --------------------------------------------------
EXONS

                 10300     10310     10320     10330     10340
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      caggaagaccctggtttttgttgcaagggggataaagatgtctgcaatgt
SHR                --------------------------------------------------
EXONS

                 10350     10360     10370     10380     10390
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      gaatggcagggacagtagctgcccagagcaagacggacggctcagaagaa
SHR                --------------------------------------------------
EXONS

                 10400     10410     10420     10430     10440
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      aaggcagatgagtcaaagagagcacagcagtccggagccactgaaggtta
SHR                --------------------------------------------------
EXONS

                 10450     10460     10470     10480     10490
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      gcggccagcaccaggatcttcagctctggccggcagcacgggggagggga
SHR                --------------------------------------------------
EXONS

                 10500     10510     10520     10530     10540
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      ggaccaagcagggcagtgggatggtcacaggtatctaaaggcataaaccc
SHR                --------------------------------------------------
EXONS

                 10550     10560     10570     10580     10590
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      agatgagggaaacaactgttccttggaactgccctaattcctttaagagg
SHR                --------------------------------------------------
EXONS

                 10600     10610     10620     10630     10640
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      gagggggccttcctacattagggctggctatctttctgccactggctttg
SHR                --------------------------------------------------
EXONS

                 10650     10660     10670     10680     10690
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      tggtcaacatcagtgctgttgatcagggtcagggtgccctcagggggaat
SHR                --------------------------------------------------
EXONS

                 10700     10710     10720     10730     10740
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      ctggccttttgtgcacagatccctgcctgggaccctgcatgtggcccgaa
SHR                --------------------------------------------------
EXONS

                 10750     10760     10770     10780     10790
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      catcaattgacagggagcccacagagaaggaaggtcctatcctgccaatg
SHR                --------------------------------------------------
EXONS
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              10800     10810     10820     10830     10840
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 tgggaagggcttcaaagatctgctggttgaaaaggaggagaactcagctt
SHR           --------------------------------------------------
EXONS

              10850     10860     10870     10880     10890
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 ttgggcacccccaaggagaccaaaggacacgggtgtcatctgccctaaac
SHR           --------------------------------------------------
EXONS

              10900     10910     10920     10930     10940
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
DBH NM_013158 gtgttctagggtctggccgccggttggggtttcaaagtcctgggcttctg
SHR           --------------------------------------------------
EXONS

              10950     10960     10970     10980     10990
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         ------------------------------------------------GG
DBH NM_013158 gccaccgcacaatctctaacaagccgttctctggcaggtttaccatgggg
SHR           --------------------------------------------------
EXONS

              11000     11010     11020     11030     11040
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         ATTAGAGAGCATCATCTAGAAAAAGCGAATGTTCTGGTAAGGCAGGGGAT
DBH NM_013158 attagagagcatcatctagaaaaagcgaatgttctggtaaggcaggggat
SHR           --------------------------------------------------
EXONS

              11050     11060     11070     11080     11090
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         GCTGCCTCTGCTCTGGGTCCTTGTCACCCCCTCTGGGGTCTGATTCTCAT
DBH NM_013158 gctgcctctgctctgggtccttgtcacccccctctggggtctgattctcat
SHR           ------------------CCTTGTCNCCCCNANTGGGGTCTGATTCTCAT
EXONS

              11100     11110     11120     11130     11140
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCTCCCACAGGCACTGCCGAAATCTGGAATCCGCATCTTTGCCTCACAGC
DBH NM_013158 cctcccacagGCACTGCCGAAATCTGGAATCCGCATCTTTGCCTCACAGC
SHR           CCTCCCACAGGCACTGCCNAAATCTGGAATCCNCATCTTTGCCTCACAGC
EXONS                   EXON_7_XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              11150     11160     11170     11180     11190
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         TCCACACGCACCTGACCGGCAGGAAGGTGATTACTGTGCTCGCCAGGGAT
DBH NM_013158 TCCACACGCACCTGACCGGCAGGAAGGTGATTACTGTGCTCGCCAGGGAT
SHR           TCCNCACNCACCTGACCGGGAGGAAGGGGATTACTGTGCTCNCCAGGGAT
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              11200     11210     11220     11230     11240
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         GGCCAACAGAGGGAAGTGGTGAACAGAGACAACCACTACAGCCCCCACTT
DBH NM_013158 GGCCAACAGAGGGAAGTGGTGAACAGAGACAACCACTACAGCCCCCACTT
SHR           GGCCAACAGAGGGAAGTGGTGAACAGAGACAACCACTACAGCCCCCACTT
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              11250     11260     11270     11280     11290
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         TCAGGTGGGTGTCGGCCTCCCAGGCCTTCCGCATCCTAGGGCTGACCTCT
DBH NM_013158 TCAGgtgggtgtcggcctcccaggccttccgcatcctagggctgacctct
SHR           TCAGGTGGGTGTCGGCCTCCCAGGCCTTCCGCATCCTAGGGCTGACCTCT
EXONS         XXXX

              11300     11310     11320     11330     11340
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx         GACCTTCGGTGGTGTACCCTGACCCGGGCCCGTGGGTGCCAACAATAGCT
DBH NM_013158 gaccttcggtggtgtaccctgacccgggcccgtgggtgccaacaatagct
SHR           GACCTTCNGTGGTGTNTNNTGACCCGGGCCCGTGGGTGCCAACAATAGCT
EXONS
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                11350     11360     11370     11380     11390
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           TGGTGACAACTTGATTGACCTTATCTTCCCCTGACTGAAGCCCATACTTA
DBH NM_013158   tggtgacaacttgattgaccttatcttcccctgactgaagcccatactta
SHR             TGGTGACAACTTGATTGACCTTATCTTCCCCTGANTGAAGCCCATACTTA
EXONS
                11400     11410     11420     11430     11440
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GTCAGCCCTGACTTCCGCCCAGAAAGGTTGAAGCAGAACTGACAATGCAG
DBH NM_013158   gtcagccctgacttccgcccagaaaggttgaagcagaactgacaatgcag
SHR             GTCAGCCCTGACTTCCGCCCAGAAAGGTTGAAGCAGAACTGACAATGCAG
EXONS
                11450     11460     11470     11480     11490
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GGGACTTTGGTAGGGAAGAACAAGGTGGCTGGATCTGGTCCCAGAGCTCT
DBH NM_013158   gggactttggtagggaagaacaaggtggctggatctggtcccagagctct
SHR             GGGACTTTGGTAGGGAAGAACAAGGTGGCTGGATCTGGTCCCAGAGCTCT
EXONS
                11500     11510     11520     11530     11540
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           TTTTCACTCCTCTCTTCCCAACCCCAGGAGATCAGAATGCTGAAGAATGC
DBH NM_013158   ttttcactcctctcttcccaaccccagGAGATCAGAATGCTGAAGAATGC
SHR             TTTTCACTCCTCTCTTCCCAACCCCAGGAGATCAGAATGCTGAAGAATGC
EXONS                                      EXON_8_XXXXXXXXXXXXXXXX
                11550     11560     11570     11580     11590
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           TGTGACTGTCCACCAGGTGAGTGCCCGGCAGGGACAGATGGCTGGGGCAC
DBH NM_013158   TGTGACTGTCCACCAGgtgagtgcccggcagggacagatggctggggcac
SHR             TGTGACTGTCCACCAGGTGAGTGCCCGGCAGGGACAGATGGCTGGGGCAC
EXONS           XXXXXXXXXXXXXXXX
                11600     11610     11620     11630     11640
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GAGATATTGGGAGAGAACTGTGCCCAAAAGAGACAGAAAGCAGAGGAACA
DBH NM_013158   gagatattgggagagaactgtgcccaaaagagacagaaagcagaggaaca
SHR             GAGATATTGGGAGAGAACTGTGCCCAAAAGAGACAGAAAGCAGAGGAACA
EXONS
                11650     11660     11670     11680     11690
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GGAGAGACAGGGCGGGCACAGCAGGGCTGGGTAGGGTACATGAGGACTTC
DBH NM_013158   ggagagacagggcgggcacagcagggctgggtagggtacatgaggacttc
SHR             GGAGAGACAGGGCGGGCACAGCAGGGCTGGGTAGGGTACATGAGGACTTC
EXONS
                11700     11710     11720     11730     11740
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           ATGGCACAGAGGGGACATTGTCTTGTATCCTCAGCTTAATCACAACCCCA
DBH NM_013158   atggcacagaggggacattgtcttgtatcctcagcttaatcacaacccca
SHR             ATGGCACAGAGGGGACATTGTCTTGTATCCTCAGCTTAATCACAACCCCA
EXONS
                11750     11760     11770     11780     11790
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           AAGAATTTCATTCCCTGACAACACTGGGAAGAGATGGGGTTACCAAGATG
DBH NM_013158   aagaatttcattccctgacaacactgggaagagatggggttaccaagatg
SHR             AAGAATTTCATTCCCTGACAACACTGGGAAGAGATGGGGTTACCAAGATG
EXONS
                11800     11810     11820     11830     11840
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           TAGTCTTTGCCTGTGGGGATCTGACATGGCTACAAGCCCGGGGATGCTTA
DBH NM_013158   tagtctttgcctgtggggatctgacatggctacaagcccggggatgctta
SHR             TAGTCTTTGCCTGTGGGGATCTGACATGGCTACAAGCCCGGGGATGCTTA
EXONS
                11850     11860     11870     11880     11890
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GATTTCGAACCTCATTGGAGTGGGCGCTGGGCTCCTCTTGGGAGATCACA
DBH NM_013158   gatttcgaacctcattggagtgggcgctgggctcctcttgggagatcaca
SHR             GATTTCGAACCTCATTGGAGTGGGCGCTGGGCTCCTCTTGGGAGATCACA
EXONS
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               11900     11910     11920     11930     11940
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          CATCACAGTGGCTTGGTCTGAGCACCCCGTCTGCTGCCTCTCTCTTCAGG
DBH NM_013158  catcacagtggcttggtctgagcaccccgtctgctgcctctctcttcagG
SHR            CATCACAGTGGCTTGGTCTGAGCACCCCGTCTGCTGCCTCTCTCTTCAGG
EXONS                                                           E

               11950     11960     11970     11980     11990
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          GGGATGTCCTCATCACTTCGTGCACATACAACACAGAAAACAGGACAATG
DBH NM_013158  GGGATGTCCTCATCACTTCGTGCACATACAACACAGAAAACAGGACAATG
SHR            GGGATGTCCTCATCACTTCGTGCACATACAACACAGAAAACAGGACAATG
EXONS          XON_9_XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

               12000     12010     12020     12030     12040
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          GCCACAGTGGTAAGTCACACTGGCTTTTCCAGCCACCCACACAGAAAAGC
DBH NM_013158  GCCACAGTGgtaagtcacactggcttttccagccacccacacagaaaagc
SHR            GCCACAGTGGTAAGTCACACTGGCTTTTCCAGCCACCCACACAGAAAAGC
EXONS          XXXXXXXXX

               12050     12060     12070     12080     12090
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          GAGCAGCCCAGAGTCTGCTTAAAGATTTCTAAGATTCTAACCATTCCCAT
DBH NM_013158  gagcagcccagagtctgcttaaagatttctaagattctaaccattcccat
SHR            GAGCAGCCCAGAGTCTGCTTAAAGATTTCTAAGATTCTAACCATTCCCAT
EXONS

               12100     12110     12120     12130     12140
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          GGTTGAATGTAGGTTGCCAGAGGCAGGACTGCCCCTCCTACATTCCCCAG
DBH NM_013158  ggttgaatgtaggttgccagaggcaggactgcccctcctacattccccag
SHR            GGTTGAATGTAGGTTGCCAGAGGCAGGACTGCCCCTCCTACATTCCCCAG
EXONS

               12150     12160     12170     12180     12190
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          ATGGCCTGACCACTCTCGGGGGATGTTTCTGAACCCCTATCTCTAGCAAC
DBH NM_013158  atggcctgaccactctcgggggatgtttctgaacccctatctctagcaac
SHR            ATGGCCTGACCACTCTCGGGGGATGTTTCTGAACCCCTATCTCTAGCAAC
EXONS

               12200     12210     12220     12230     12240
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          CCTTCTCCCCTAGGCCCCTAGTCACGCAGGCAGCATGCTAGATTCTGCAC
DBH NM_013158  ccttctcccctaggcccctagtcacgcaggcagcatgctagattctgcac
SHR            CCTTCTCCCCTAGGCCCCTAGTCACGCAGGCAGCATGCTAGATTCTGCAC
EXONS

               12250     12260     12270     12280     12290
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          TTGCTGCACTGAGTCCAGCATTGAGGTAACACTGTCAATACAGCCTCAGG
DBH NM_013158  ttgctgcactgagtccagcattgaggtaacactgtcaatacagcctcagg
SHR            TTGCTGCACTGAGTCCAGCATTGAGGTAACACTGTCAATACAGCCTCAGG
EXONS

               12300     12310     12320     12330     12340
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          ACCTCAGCTAAGAACCACTAAGGTGGGTTCTCATTGGTACCCGCGTGCTC
DBH NM_013158  acctcagctaagaaccactaaggtgggttctcattggtacccgcgtgctc
SHR            ACCTCAGCTAAGAACCACTAAGGTGGGTTCTCATTGGTACCCGCGTGCTC
EXONS

               12350     12360     12370     12380     12390
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          AGTGCTTCTCTGGCACGGGAGGGAGCAGGATATACTTCGGAATTCAAAAG
DBH NM_013158  agtgcttctctggcacgggagggagcaggatatacttcggaattcaaaag
SHR            AGTGCTTCTCTGGCACGGGAGGGAGCAGGATATACTTCGGAATTCAAAAG
EXONS

               12400     12410     12420     12430     12440
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx          TCCAGCTGGAGAGATCTCTGCTACAGGAGATATCCAAAATGATAGGGTCA
DBH NM_013158  tccagctggagagatctctgctacaggagatatccaaaatgatagggtca
SHR            TCCAGCTGGAGAGATCTCTGCTACAGGAGATATCCAAAATGATAGGGTCA
EXONS
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                12450     12460     12470     12480     12490
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           CAGAAACTTCAGCAGACATTTCCCAAGAGGTGACAGGGAGGATGCATGCG
DBH NM_013158   cagaaacttcagcagacatttcccaagaggtgacagggaggatgcatgcg
SHR             CAGAAACTTCAGCAGACATTTCCCAAGAGGTGACAGGGAGGATGCATGCG
EXONS

                12500     12510     12520     12530     12540
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           AGAGTCGGGGAGGGATAGAAAAAAGCTTTGGCTGGGGTGTGTGCCAAAAG
DBH NM_013158   agagtcggggagggatagaaaaaagctttggctggggtgtgtgccaaaag
SHR             AGAGTCGGGGAGGGATAGAAAAAAGCTTTGGCTGGGGTGTGTGCCAAAAG
EXONS

                12550     12560     12570     12580     12590
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           TCTCAGAGGACCAAAGTTGTCCGTAGGATGAGGAAATGTTCAGCTGTAAG
DBH NM_013158   tctcagaggaccaaagttgtccgtaggatgaggaaatgttcagctgtaag
SHR             TCTCAGAGGACCAAAGTTGTCCGTAGGATGAGGAAATGTTCAGCTGTAAG
EXONS

                12600     12610     12620     12630     12640
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GGAGTGTGGAGATGTGGCTACACCAGACAAGTAGATGTGGTCAGCCACTC
DBH NM_013158   ggagtgtggagatgtggctacaccagacaagtagatgtggtcagccactc
SHR             GGAGTGTGGAGATGTGGCTACACCAGACAAGTAGATGTGGTCAGCCACTC
EXONS

                12650     12660     12670     12680     12690
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           CAGGGAAGTAACAGGGGTCTTGTTTTGGGCTGGATTGTGGATAGTGCAGG
DBH NM_013158   cagggaagtaacaggggtcttgttttgggctggattgtggatagtgcagg
SHR             CAGGGAAGTAACAGGGGTCTTGTTTTGGGCTGGATTGTGGATAGTGCAGG
EXONS

                12700     12710     12720     12730     12740
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GAGGAGGAGTACCTCGTGGGAGGACTATTTTCAAGGTGAGGTATGCAGGA
DBH NM_013158   gaggaggagtacctcgtgggaggactattttcaaggtgaggtatgcagga
SHR             GAGGAGGAGTACCTCGTGGGAGGACTATTTTCAAGGTGAGGTATGCAGGA
EXONS

                12750     12760     12770     12780     12790
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           AATGTGTCATCCGGCTGGAGCCCGTTGGGCAGGAAGAGGGATGTTTACTG
DBH NM_013158   aatgtgtcatccggctggagcccgttgggcaggaagagggatgtttactg
SHR             AATGTGTCATCCGGCTGGAGCCCGTTGGGCAGGAAGAGGGATGTTTACTG
EXONS

                12800     12810     12820     12830     12840
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GTTTCCTGGATCCACACTATTCCCAGTCACCAATCCCACTGGCCTTCTAA
DBH NM_013158   gtttcctggatccacactattcccagtcaccaatcccactggccttctaa
SHR             GTTTCCTGGATCCACACTATTCCCAGTCACCAATCCCACTGGCCTTCTAA
EXONS

                12850     12860     12870     12880     12890
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           TTGATATTTGTGCAATCAGTAACAAACACATCAACTGTGAGGTTCAGAGT
DBH NM_013158   ttgatatttgtgcaatcagtaacaaacacatcaactgtgaggttcagagt
SHR             TTGATATTTGTGCAATCAGTAACAAACACATCAACTGTGAGGTTCAGAGT
EXONS

                12900     12910     12920     12930     12940
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           GGGCCAGACCTGGGCTATACCCAACCCTGGCACCCCTGAACCCTAGGAAC
DBH NM_013158   gggccagacctgggctatacccaaccctggcacccctgaaccctaggaac
SHR             GGGCCAGACCTGGGCTATACCCAACCCTGGCACCCCTGAACCCTAGGAAC
EXONS

                12950     12960     12970     12980     12990
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx           TCCTACCAGTACTGCAGCCCTGTTCTCTGCACAATGTCTCGTGCCTTCAG
DBH NM_013158   tcctaccagtactgcagccctgttctctgcacaatgtctcgtgccttcag
SHR             TCCTACCAGTACTGCAGCCCTGTTCTCTGCACAATGTCTCGTGCCTTCAG
EXONS
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               13000     13010     13020     13030     13040
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            CCATGGTCCAGGGTTCCCCACTCTGTCTCACGTGTGTTCTTTAAATAGCC
DBH NM_013158    ccatggtccagggttccccactctgtctcacgtgtgttctttaaatagcc
SHR              CCATGGTCCAGGGTTCCCCACTCTGTCTCACGTGTGTTCTTTAAATAGCC
EXONS

               13050     13060     13070     13080     13090
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            TTCTTGTGAGATGATTCACATACCAAACAGCTGAAGTGTGTGAAGTGTGC
DBH NM_013158    ttcttgtgagatgattcacataccaaacagctgaagtgtgtgaagtgtgc
SHR              TTCTTGTGAGATGATTCACATACCAAACAGCTGAAGTGTGTGAAGTGTGC
EXONS

               13100     13110     13120     13130     13140
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GGCTTGTTGCCCGGAAGCCTGCTCACAAATCCATGCACACAGTCAAGCCA
DBH NM_013158    ggcttgttgcccggaagcctgctcacaaatccatgcacacagtcaagcca
SHR              GGCTTGTTGCCCGGAAGCCTGCTCACAAATCCATGCACACAGTCAAGCCA
EXONS

               13150     13160     13170     13180     13190
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            CGGCTAGTGCCTCCCGGTCCCCTCACCCAGCCCAACGCAGATCCACTTGC
DBH NM_013158    cggctagtgcctcccggtcccctcacccagcccaacgcagatccacttgc
SHR              CGGCTAGTGCCTCCCGGTCCCCTCACCCAGCCCAACGCAGATCCACTTGC
EXONS

               13200     13210     13220     13230     13240
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            TCTAGATGTTTCTGTCCTGAACACTTCTCATCACTGGATTTACATGTTAC
DBH NM_013158    tctagatgtttctgtcctgaacacttctcatcactggatttacatgttac
SHR              TCTAGATGTTTCTGTCCTGAACACTTCTCATCACTGGATTTACATGTTAC
EXONS

               13250     13260     13270     13280     13290
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GCAGCCTCTGTGGCTGGATTCTTTCCCATACATTTAATCCATCTTAATCT
DBH NM_013158    gcagcctctgtggctggattctttcccatacatttaatccatcttaatct
SHR              GCAGCCTCTGTGGCTGGATTCTTTCCCATACATTTAATCCATCTTAATCT
EXONS

               13300     13310     13320     13330     13340
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            CCTGCCAGTCTCCAAGTCAAGCATTTCCCCCAGACACTTCCACAGGCATA
DBH NM_013158    cctgccagtctccaagtcaagcatttcccccagacacttccacaggcata
SHR              CCTGCCAGTCTCCAAGTCAAGCATTTCCCCCAGACACTTCCACAGGCATA
EXONS

               13350     13360     13370     13380     13390
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GTCTCCTTTCTCCAGGTGCTGTGGGTGGGGGCAGGGAGGCCCTGCCAGTC
DBH NM_013158    gtctcctttctccaggtgctgtgggtgggggcagggaggccctgccagtc
SHR              GTCTCCTTTCTCCAGGTGCTGTGGGTGGGGGCAGGGAGGCCCTGCCAGTC
EXONS

               13400     13410     13420     13430     13440
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            ATTGGTGGAAGGGAGCCCCTAGGCCTGCTCTGCTAGAGGCGCTTCTGTCA
DBH NM_013158    attggtggaagggagcccctaggcctgctctgctagaggcgcttctgtca
SHR              ATTGGTGGAAGGGAGCCCCTAGGCCTGCTCTGCTAGAGGCGCTTCTGTCA
EXONS

               13450     13460     13470     13480     13490
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            GGGCTGTATTCCTGCCTTGTCAGATCCGTGGCCAATAAATAGTGCCTTAC
DBH NM_013158    gggctgtattcctgccttgtcagatccgtggccaataaatagtgccttac
SHR              GGGCTGTATTCCTGCCTTGTCAGATCCGTGGCCAATAAATAGTGCCTTAC
EXONS

               13500     13510     13520     13530     13540
                 |....|....|....|....|....|....|....|....|....|....
BN.Lx            CTGTCTTCAGATGTTGATGGCCACTGGTCTAGCAAGCAACTAAAATATCA
DBH NM_013158    ctgtcttcagatgttgatggccactggtctagcaagcaactaaaatatca
SHR              CTGTCTTCAGATGTTGATGGCCACTGGTCTAGCAAGCAACTAAAATATCA
EXONS
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                13550     13560     13570     13580     13590
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               GATCCTAGGTCCTGAAGTCCAAGTCTCAAGGACAA---------------
DBH NM_013158       gatcctaggtcctgaagtccaagtctcaaggacaagatacctccttggct
SHR                 GATCCTAGGTCCTGAAGTCCAAGTCTCAAGGACAA---------------
EXONS
                13600     13610     13620     13630     13640
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       atttaggactcgtgaattcttggtggtggtggtggtggtgggaaggttca
SHR                 --------------------------------------------------
EXONS
                13650     13660     13670     13680     13690
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       cctgcgatcagaggaaatgggaacttctgggagcttccagaaggcagatc
SHR                 --------------------------------------------------
EXONS
                13700     13710     13720     13730     13740
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       tgagcttcctgcctctgacaacctgtggatctaaggagacagtggccttt
SHR                 --------------------------------------------------
EXONS
                13750     13760     13770     13780     13790
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       gaagtgcagcttccttcccaaagtgagagagagctctgtcccctgtcctc
SHR                 --------------------------------------------------
EXONS
                13800     13810     13820     13830     13840
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       agtcttcacctctgaggggacagagcaggggggtgtgatgtggggaggag
SHR                 --------------------------------------------------
EXONS
                13850     13860     13870     13880     13890
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       gggttgtcccatgctcattgagatctatgaatgctgaaaaataagagctt
SHR                 --------------------------------------------------
EXONS
                13900     13910     13920     13930     13940
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       gggacaggcagaccctgcaggaagacccatctagcacacaggaagcaggc
SHR                 --------------------------------------------------
EXONS
                13950     13960     13970     13980     13990
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       caacttcttatgatgaacttcctctcccactcctccatagagacacccca
SHR                 --------------------------------------------------
EXONS
                14000     14010     14020     14030     14040
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       ggccccaaccattcgaccagggaccaactgagctttgcatcaccctgtgg
SHR                 --------------------------------------------------
EXONS
                14050     14060     14070     14080     14090
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               --------------------------------------------------
DBH NM_013158       tctgtcatgaattaatgaaggcctttctccctttaccagtnnnnnnnnnn
SHR                 --------------------------------------------------
EXONS
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                 14100     14110     14120     14130     14140
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
SHR              --------------------------------------------------
EXONS
                 14150     14160     14170     14180     14190
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
SHR              --------------------------------------------------
EXONS
                 14200     14210     14220     14230     14240
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
SHR              --------------------------------------------------
EXONS
                 14250     14260     14270     14280     14290
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
SHR              --------------------------------------------------
EXONS
                 14300     14310     14320     14330     14340
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
SHR              --------------------------------------------------
EXONS
                 14350     14360     14370     14380     14390
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnntagaagaatgtgttccaca
SHR              --------------------------------------------------
EXONS
                 14400     14410     14420     14430     14440
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    ggtcagggcacagcttgagggacagccccagctccagttgttgggggatc
SHR              --------------------------------------------------
EXONS
                 14450     14460     14470     14480     14490
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    caatgaagactgagctgcacgtctgctacaggtgtgcatgtgtgtgcttg
SHR              --------------------------------------------------
EXONS
                 14500     14510     14520     14530     14540
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    tgtggccttgctgaggaagtacatgattagaagtcagagaacaccctcag
SHR              --------------------------------------------------
EXONS
                 14550     14560     14570     14580     14590
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    gtgtcaatccccaacgtctaccttgcttgagacagggcctctttgctgtt
SHR              --------------------------------------------------
EXONS
                 14600     14610     14620     14630     14640
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx            --------------------------------------------------
DBH NM_013158    ggatgctccgtacaccaggctgtctggtctgtgagcttccggggtttctc
SHR              --------------------------------------------------
EXONS
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                 14650     14660     14670     14680     14690
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      ggtttctatctcatagaggcttgctgtagggatgctaagttgcagactgg
SHR                --------------------------------------------------
EXONS

                 14700     14710     14720     14730     14740
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      cactaccacattcagctttagagtgagtcctggggatctgagttcagggc
SHR                --------------------------------------------------
EXONS

                 14750     14760     14770     14780     14790
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      cagcaagcacgctgcctaccaggctacctccttagccctttaagaatagt
SHR                --------------------------------------------------
EXONS

                 14800     14810     14820     14830     14840
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              ------AAAGCCACAGACAGGCAGGGTGCTGAGAGCGATGTGTTGTGGGC
DBH NM_013158      cacaagaaagccacagacaggcagggtgctgagagcgatgtgttgtgggc
SHR                ----------CCACAGACAGGCAGGGTGCTGAGAGCGATGTGTTGTGGGC
EXONS

                 14850     14860     14870     14880     14890
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              TGATGGCTTCTTTTCTCTCCTGCAGGGGGGGTTTGGAATCTTGGAGGAGA
DBH NM_013158      tgatggcttcttttctctcctgcagGGGGGGTTTGGAATCTTGGAGGAGA
SHR                TGATGGCTTCTTTTCTCTCCTGCAGGGGGGGTTTGGAATCTTGGAGGAGA
EXONS                                        EXON_10_XXXXXXXXXXXXXXXX

                 14900     14910     14920     14930     14940
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              TGTGCGTCAACTACGTCCACTACTACCCCAAAACAGAGCTGGAGCTCTGC
DBH NM_013158      TGTGCGTCAACTACGTCCACTACTACCCCAAAACAGAGCTGGAGCTCTGC
SHR                TGTGCGTCAACTACGTCCACTACTACCCCAAAACAGAGCTGGAGCTCTGC
EXONS              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 14950     14960     14970     14980     14990
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              AAGAGTGCCGTGGATGATGGCTTCCTGCAGAAATACTTCCACATAGTAAA
DBH NM_013158      AAGAGTGCCGTGGATGATGGCTTCCTGCAGAAATACTTCCACATAGTAAA
SHR                AAGAGTGCCGTGGATGATGGCTTCCTGCAGAAATACTTCCACATAGTAAA
EXONS              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 15000     15010     15020     15030     15040
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              CCGGTGAAGCACTTCTTGCTTTCTGCCCCCGGGGAACCCAGCATAGACAG
DBH NM_013158      CCGgtgaagcacttcttgctttctgcccccggggaacccagcatagacag
SHR                CCGGTGAAGCACTTCTTGCTTTCTGCCCCCGGGGAACCCAGCATAGACAG
EXONS              XXX

                 15050     15060     15070     15080     15090
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              CCTCCATACCCTACACCATGCTCTCTGACCCACATCCTCTCCTGTTAGGA
DBH NM_013158      cctccataccctacaccatgctctctgacccacatcctctcctgttagga
SHR                CCTCCATACCCTACACCATGCTCTCTGACCCACATCCTCTCCTGTTAGGA
EXONS

                 15100     15110     15120     15130     15140
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              GGTTGGGTGGCCAAATGCAAACTTTGAGCTAAGTTCTTGGAGCAAAGTTA
DBH NM_013158      ggttgggtggccaaatgcaaactttgagctaagttcttggagcaaagtta
SHR                GGTTGGGTGGCCAAATGCAAACTTTGAGCTAAGTTCTTGGAGCAAAGTTA
EXONS

                 15150     15160     15170     15180     15190
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              GCATGCATGCCAGCAGACCTTCTGTGTCTTTGGAAGTGACTGTGGCAGGC
DBH NM_013158      gcatgcatgccagcagaccttctgtgtctttggaagtgactgtggcaggc
SHR                GCATGCATGCCAGCAGACCTTCTGTGTCTTTGGAGGTGACTGTGGCAGGC
EXONS
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                 15200     15210     15220     15230     15240
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              ATTGCTAATCGATCCCTCAACTTACAAGTTACTGCCAGAGAGACCACTTT
DBH NM_013158      attgctaatcgatccctcaacttacaagttactgccagagagaccacttt
SHR                ATTGCTAATCGATCCCTCAACTTACAAGTTACTGCCAGAGAGACCACTTT
EXONS

                 15250     15260     15270     15280     15290
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              CAACAAGCAAATATAATATAAAAGACGGAGGCAGGCGGTGGCTAAAAGAA
DBH NM_013158      caacaagcaaatataatataaaagacggaggcaggcggtggctaaaagaa
SHR                CAACAAGCAAATATAATATAAAAGACGGAGGCAGGCGGTGGCTAAAAGAA
EXONS

                 15300     15310     15320     15330     15340
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              GCAAGGCTAGGGAAGAGGTGAAGGGTTACTGTTGGGAGCCATGCTGCCTC
DBH NM_013158      gcaaggctagggaagaggtgaagggttactgttgggagccatgctgcctc
SHR                GCAAGGCTAGGGAAGAGGTGAAGGGTTACTGTTGGGAGCCATGCTGCCTC
EXONS

                 15350     15360     15370     15380     15390
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              TCCATCCCTCAGTTATGCCTGTAAATTGAGTTTTTCTCTTTCCTTCCAGG
DBH NM_013158      tccatccctcagttatgcctgtaaattgagtttttctctttccttccagG
SHR                TCCATCCCTCAGTTATGCCTGTAAATTGAGTTTTTCTCTTTCCTTCCAGG
EXONS                                                                E

                 15400     15410     15420     15430     15440
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              TTCGGCAATGAGGAGGTCTGCACCTGCCCTCAGGCCTCTGTCCCCCAGCA
DBH NM_013158      TTCGGCAATGAGGAGGTCTGCACCTGCCCTCAGGCCTCTGTCCCCCAGCA
SHR                TTCGGCAATGAGGAGGTCTGCACCTGCCCTCAGGCCTCTGTCCCCCAGCA
EXONS              XON_11_XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 15450     15460     15470     15480     15490
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              GTTCGCCTCTGTGCCCTGGAACTCTTTCAATCGTGATATGCTCAAGG---
DBH NM_013158      GTTCGCCTCTGTGCCCTGGAACTCTTTCAATCGTGATATGCTCAAGGCTT
SHR                GTTCGCCTCTGTGCCCTGGAACTCTTTCAATCGTGATATGCTCAAGG---
EXONS              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 15500     15510     15520     15530     15540
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      TGTATAACTATGCCCCTATCTCTGTGCACTGTAACAAGACCTCTGCCGTC
SHR                --------------------------------------------------
EXONS              XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 15550     15560     15570     15580     15590
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      CGCTTCCCGgtatgacagcatgaggttgagtgacattcaacagtagctct
SHR                --------------------------------------------------
EXONS              XXXXXXXXX

                 15600     15610     15620     15630     15640
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      ccccatccaataccaataaaccccactgctgcctgattcctccaatcccg
SHR                --------------------------------------------------
EXONS

                 15650     15660     15670     15680     15690
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      ggtctgttcaaatcttgaggagaaccccaggtaggctgaggaagggcacc
SHR                --------------------------------------------------
EXONS

                 15700     15710     15720     15730     15740
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx              --------------------------------------------------
DBH NM_013158      ctggacttctcaacaagcaatgctcacatgagactgagaaaatggccact
SHR                --------------------------------------------------
EXONS
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              15750     15760     15770     15780     15790
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tagtgatagaaacaggcgtgcagagagatagacgataagggaaccagcca
SHR               --------------------------------------------------
EXONS

              15800     15810     15820     15830     15840
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gggatactgccgggaaggtcagggaggccacttctcctgggcacagtaca
SHR               --------------------------------------------------
EXONS

              15850     15860     15870     15880     15890
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     acagaggactagcctcggccctgtctgggtggtgtgtgactgagtgtcag
SHR               --------------------------------------------------
EXONS

              15900     15910     15920     15930     15940
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     caccctcctcagggaataaaaatagagctatgggcagaggaggcaagaaa
SHR               --------------------------------------------------
EXONS

              15950     15960     15970     15980     15990
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ccagcagtctgtgacaagagccccacagcacagcttctccctccttatct
SHR               --------------------------------------------------
EXONS

              16000     16010     16020     16030     16040
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gaggcctgctgggattcagaggcacctaatttatttcccagcagagccag
SHR               --------------------------------------------------
EXONS

              16050     16060     16070     16080     16090
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ccagcagcagcggtaccagcccagagtgctggtcagccaacccagtctgc
SHR               --------------------------------------------------
EXONS

              16100     16110     16120     16130     16140
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     tctgttaggctggggcgggggtttgggggagaggaggcactgcagcagga
SHR               --------------------------------------------------
EXONS

              16150     16160     16170     16180     16190
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ggtgaacaaacctttcctggcagcatctgggcactgactggattaattgc
SHR               --------------------------------------------------
EXONS

              16200     16210     16220     16230     16240
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     taacccaaacacccacgccatggggaggcttgcttacctcagagcagttt
SHR               --------------------------------------------------
EXONS

              16250     16260     16270     16280     16290
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ggggcactgccggttatggctagatcttggcttttacagaagccttgttg
SHR               --------------------------------------------------
EXONS
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                 16300     16310     16320     16330     16340
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gacagtggctgaagccatagccagcctccaaagactcagtcctttcctag
SHR               --------------------------------------------------
EXONS

                 16350     16360     16370     16380     16390
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gggtatacaattgagtgaaggagcagacatggaggtggagaagcctagag
SHR               --------------------------------------------------
EXONS

                 16400     16410     16420     16430     16440
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             -----------------CAGAGAATACAACGGCTTAGGGCAGGGACATGG
DBH NM_013158     ggttccaggtttctggccagagaatacaacggcttagggcagggacatgg
SHR               ----------------CCAGAGAATACAACGGCTTAGGGCAGGGACATGG
EXONS

                 16450     16460     16470     16480     16490
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             GTCCTCTAGTGGTGACCCTCAAAGGCTGACTTGCAAGAGGTGGGGAGTGT
DBH NM_013158     gtcctctagtggtgaccctcaaaggctgacttgcaagaggtggggagtgt
SHR               GTCCTCTAGTGGTGACCCTCAAAGGCTGACTTGCAAGAGGTGGGGAGTGT
EXONS

                 16500     16510     16520     16530     16540
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             TCAGCACGGGATAAATGAATTTAAGGACAGGAATCCAACCAGCTAGGGAG
DBH NM_013158     tcagcacgggataaatgaatttaaggacaggaatccaaccagctagggag
SHR               TCAGCACGGGATAAATGAATTTAAGGACAGGAATCCAACCAGCTAGGGAG
EXONS

                 16550     16560     16570     16580     16590
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             ATAGCACCTTGCTTGGTCCTTCCAGCCTCAGTTTACCTCCTGGTTCCTTC
DBH NM_013158     atagcaccttgcttggtccttccagcctcagtttacctcctggttccttc
SHR               ATAGCACCTTGCTTGGTCCTTCCAGCCTCAGTTTACCTCCTGGTTCCTTC
EXONS

                 16600     16610     16620     16630     16640
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             CTTACAGGGTAACTGGAACCTGCAGCCTCTGCCTAAGATCACTTCCGCAG
DBH NM_013158     cttacagGGTAACTGGAACCTGCAGCCTCTGCCTAAGATCACTTCCGCAG
SHR               CTTACAGGGTAACTGGAACCTGCAGCCTCTGCCTAAGATCACTTCCGCAG
EXONS                    EXON_12_XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                 16650     16660     16670     16680     16690
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGGAAGAACCCGACCCACGCTGCCCCATCCGACAGACTCGGGGACCCGCC
DBH NM_013158     TGGAAGAACCCGACCCACGCTGCCCCATCCGACAGACTCGGGGACCCGCC
SHR               TGGAAGAACCCGACCCACGCTGCCCCATCCGACAGACTCGGGGACCCGCC
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                 16700     16710     16720     16730     16740
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             GGCCCCTTCGTTGTCATCACCACGGAGGCAGACACTGAGTAATTGTTCTT
DBH NM_013158     GGCCCCTTCGTTGTCATCACCACGGAGGCAGACACTGAGTAATTGTTCTT
SHR               GGCCCCTTCGTTGTCATCACCACGGAGGCAGACACTGAGTAATTGTTCTT
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX---3'UTR-XXXXXX
                 16750     16760     16770     16780     16790
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             CAGCCTCTCCTCGTTTTGTCCCTACTGGGCTCACTCCAGCTCTGCGCACC
DBH NM_013158     CAGCCTCTCCTCGTTTTGTCCCTACTGGGCTCACTCCAGCTCTGCGCACC
SHR               CAGCCTCTCCTCGTTTTGTCCCTACTGGGCTCACTCCAGCTCTGCGCACC
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
                 16800     16810     16820     16830     16840
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx             CCACATGAAGACCCCCTTCCATAGAATAGTGCTGTTCACCTAGGAGGAAG
DBH NM_013158     CCACATGAAGACCCCCTTCCATAGAATAGTGCTGTTCACCTAGGAGGAAG
SHR               CCACATGAAGACCCCCTTCCATAGAATAGTGCTGTTCACCTAGGAGGAAG
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
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                16850     16860     16870     16880     16890
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               GGGGTATCACCTTGGAGACACCTGCACGGCTGCATCCCATGAGGTCTGAC
DBH NM_013158       GGGGTATCACCTTGGAGACACCTGCACGGCTGCATCCCATGAGGTCTGAC
SHR                 GGGGTATCACCTTGGAGACACCTGCACGGCTGCATCCCATGAGGTCTGAC
EXONS               XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                16900     16910     16920     16930     16940
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               TGGACAGAGCAGCTCTGGACATCATCACTGCTGGCTCCACAGAGGGACAA
DBH NM_013158       TGGACAGAGCAGCTCTGGACATCATCACTGCTGGCTCCACAGAGGGACAA
SHR                 TGGACAGAGCAGCTCTGGACATCATCACTGCTGGCTCCACAGAGGGACAA
EXONS               XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                16950     16960     16970     16980     16990
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               CTCAATGTGGGAGTCCAGATTCAACTCCACAGGACCTCCCTTGCCTCCAG
DBH NM_013158       CTCAATGTGGGAGTCCAGATTCAACTCCACAGGACCTCCCTTGCCTCCAG
SHR                 CTCAATGTGGGAGTCCAGATTCAACTCCACAGGACCTCCCTTGCCTCCAG
EXONS               XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                17000     17010     17020     17030     17040
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               AACAGCCTTACCAGGCTGGGTGCAGACTTCCCAAGCCTCACAGTCCTGAC
DBH NM_013158       AACAGCCTTACCAGGCTGGGTGCAGACTTCCCAAGCCTCACAGTCCTGAC
SHR                 AACAGCCTTACCAGGCTGGGTGCAGACTTCCCAAGCCTCACAGTCCTGAC
EXONS               XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                17050     17060     17070     17080     17090
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               CCTGTTGGCCCTCTGGTGGTAACTTGTGTTGGTGTATGCCATGACAACAC
DBH NM_013158       CCTGTTGGCCCTCTGGTGGTAACTTGTGTTGGTGTATGCCATGACAACAC
SHR                 CCTGTTGGCCCTCTGGTGGTAACTTGTGTTGGTGTATGCCATGACAACAC
EXONS               XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                17100     17110     17120     17130     17140
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               TGTTTAAATAGTCCTTTGCAGAGTGGCTCATGTTTCCCAGTGGGCGTCCT
DBH NM_013158       TGTTTAAATAGTCCTTTGCAGAGTGgctcatgtttcccagtgggcgtcct
SHR                 TGTTTAAATAGTCCTTTGCAGAGTGGCTCATGTTTCCCAGTGGGCGTCCT
EXONS               XXXXXXXXXXXXXXXXXXXXXXXXX

                17150     17160     17170     17180     17190
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               CCTTGCAACAAGACAGGACAAGTCATTTAGCTAGTTAGAGACTAGCCAGG
DBH NM_013158       ccttgcaacaagacaggacaagtcatttagctagttagagactagccagg
SHR                 CCTTGCAACAAGACAGGACAAGTCATTTAGCTAGTTAGAGACTAGCCAGG
EXONS

                17200     17210     17220     17230     17240
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               GGAACTCGCTTCGTGGCAGAGTCAATAGATATTTTCGCCCACCTAGAGGG
DBH NM_013158       ggaactcgcttcgtggcagagtcaatagatattttcgcccacctagaggg
SHR                 GGAACTCGCTTCGTGGCAGAGTCAATAGATATTTTCGCCCACCTAGAGGG
EXONS

                17250     17260     17270     17280     17290
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               AAACCCCAACATGTAGTTCCACCATGGAGAGCCAAGATGGCTAGAGCCAG
DBH NM_013158       aaaccccaacatgtagttccaccatggagagccaagatggctagagccag
SHR                 AAACCCCAACATGTAGTTCCACCATGGAGAGCCAAGATGGCTAGAGCCAG
EXONS

                17300     17310     17320     17330     17340
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               GCTCTGTAGGGGAATGGAATGATCAGAACCAGTTGCTTCTCCTGCTGGTG
DBH NM_013158       gctctgtaggggaatggaatgatcagaaccagttgcttctcctgctggtg
SHR                 GCTCTGTAGGGGAATGGAATGATCAGAACCAGTTGCTTCTCCTGCTGGTG
EXONS

                17350     17360     17370     17380     17390
                    |....|....|....|....|....|....|....|....|....|....
BN.Lx               ACAGGCCGGGCTCTGAAATAGGTAGGTGAAGAGCGGGCTTGGGCCCTTAA
DBH NM_013158       acaggccgggctctgaaataggtaggtgaagagcgggcttgggcccttaa
SHR                 ACAGGCCGGGCTCTGAAATAGGTAGGTGAAGAGCGGGCTTGGGCCCTTAA
EXONS
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              17400     17410     17420     17430     17440
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TAAAGGAGTGCCTAGGTCAAGCCTCTCAGCTGCCTACCTTCAGTCTCCTT
DBH NM_013158     taaaggagtgcctaggtcaagcctctcagctgcctaccttcagtctcctt
SHR               TAAAGGAGTGCCTAGGTCAAGCCTCTCAGCTGCCTACCTTCAGTCTCCTT
EXONS
              17450     17460     17470     17480     17490
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GTCTCTTAAGTGAGACTCGCCCTTACCTACACTAATTGTATGCACATATG
DBH NM_013158     gtctcttaagtgagactcgcccttacctacactaattgtatgcacatatg
SHR               GTCTCTTAAGTGAGACTCGCCCTTACCTACACTAATTGTATGCACATATG
EXONS
              17500     17510     17520     17530     17540
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CATGGCCAATATTCCTGCCTGTGCTGACCCTTTCTGGGCTCTATAGTATC
DBH NM_013158     catggccaatattcctgcctgtgctgaccctttctgggctctatagtatc
SHR               CATGGCCAATATTCCTGCCTGTGCTGACCCTTTCTGGGCTCTATAGTATC
EXONS
              17550     17560     17570     17580     17590
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             ACATATGTCCCATGTGAGTGAGGCCTGCTTCTACAGTTCTAGTCACCTCT
DBH NM_013158     acatatgtcccatgtgagtgaggcctgcttctacagttctagtcacctct
SHR               ACATATGTCCCATGTGAGTGAGGCCTGCTTCTACAGTTCTAGTCACCTCT
EXONS
              17600     17610     17620     17630     17640
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             ACCCTGTTTTCTCTTCCTCAGTCTCCTGCCGAAGGGCCAGCTCAGCTCTT
DBH NM_013158     accctgttttctcttcctcagtctcctgccgaagggccagctcagctctt
SHR               ACCCTGTTTTCTCTTCCTCAGTCTCCTGCCGAAGGGCCAGCTCAGCTCTT
EXONS
              17650     17660     17670     17680     17690
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGATGGCTTTGGAAAGGAGTAGGAGTCTGGGCCTTTTCAGCTATTTCAGC
DBH NM_013158     tgatggctttggaaaggagtaggagtctgggccttttcagctatttcagc
SHR               TGATGGCTTTGGAAAGGAGTAGGAGTCTGGGCCTTTTCAGCTATTTCAGC
EXONS
              17700     17710     17720     17730     17740
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             AAAATGCAGCCCAGACTTCTTCAGGCAGGGAGGGTGATGGATGAGCCTCA
DBH NM_013158     aaaatgcagcccagacttcttcaggcagggagggtgatggatgagcctca
SHR               AAAATGCAGCCCAGACTTCTTCAGGCAGGGAGGGTGATGGATGAGCCTCA
EXONS
              17750     17760     17770     17780     17790
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GGTCTCCTGCACCCCCACCCCACTGCAGACATCAGCTAAGCACTGCCTCC
DBH NM_013158     ggtctcctgcacccccaccccactgcagacatcagctaagcactgcctcc
SHR               GGTCTCCTGCACCCCCACCCCACTGCAGACATCAGCTAAGCACTGCCTCC
EXONS
              17800     17810     17820     17830     17840
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CCTTCAGCAAGGCGCTTTATTACTCGGGAACAAAAGTGCCTGGGCTCTCT
DBH NM_013158     ccttcagcaaggcgctttattactcgggaacaaaagtgcctgggctctct
SHR               CCTTCAGCAAGGCGCTTTATTACTCGGGAACAAAAGTGCCTGGGCTCTCT
EXONS
              17850     17860     17870     17880     17890
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GCGCCTTGTGGTATGAATCATGGCTCCAGGCACCCATAAGTCAAGGGCAT
DBH NM_013158     gcgccttgtggtatgaatcatggctccaggcacccataagtcaagggcat
SHR               GCGCCTTGTGGTATGAATCATGGCTCCAGGCACCCATAAGTCAAGGGCAT
EXONS
              17900     17910     17920     17930     17940
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             AATCGGTGCGAAATGCCAGGCCTGACGGCCCAGAGCAATGCTCTGCCCTC
DBH NM_013158     aatcggtgcgaaatgccaggcctgacggcccagagcaatgctctgccctc
SHR               AATCGGTGCGAAATGCCAGGCCTGACGGCCCAGAGCAATGCTCTGCCCTC
EXONS
```

```
                 17950     17960     17970     17980     17990
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            TGTCATCTGTACTGCCTGAGAGAGGGGGGAGGCAGGCAGTCCCCATAGCT
DBH NM_013158    tgtcatctgtactgcctgagagaggggggaggcaggcagtccccatagct
SHR              TGTCATCTGTACTGCCTGAGAGAGGGGGGAGGCAGGCAGTCCCCATAGCT
EXONS
                 18000     18010     18020     18030     18040
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            GAGCCTTCCCTCTATAGCCGCCTGCCTGAAGAGCCTAGAGTCAGAGAGTA
DBH NM_013158    gagccttccctctatagccgcctgcctgaagagcctagagtcagagagta
SHR              GAGCCTTCCCTCTATAGCCGCCTGCCTGAAGAGCCTAGAGTCAGAGAGTA
EXONS
                 18050     18060     18070     18080     18090
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            CGAGAAATGCTCACGGAGCTCTGAGGAACCGGACCTTCAACCCAGTTACT
DBH NM_013158    cgagaaatgctcacggagctctgaggaaccggaccttcaacccagttact
SHR              CGAGAAATGCTCACGGAGCTCTGAGGAACCGGACCTTCAACCCAGTTACT
EXONS
                 18100     18110     18120     18130     18140
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            CTACTTCTAGTGGTGTGCCAACTTACAGACAGGAAAACTGAGGCTCATGG
DBH NM_013158    ctacttctagtggtgtgccaacttacagacaggaaaactgaggctcatgg
SHR              CTACTTCTAGTGGTGTGCCAACTTACAGACAGGAAAACTGAGGCTCATGG
EXONS
                 18150     18160     18170     18180     18190
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            GTTAGCACTTTTCCAAAAGACCACAAAAGGACCTACCTGCTGCCCATGTG
DBH NM_013158    gttagcacttttccaaaagaccacaaaaggacctacctgctgcccatgtg
SHR              GTTAGCACTTTTCCAAAAGACCACAAAAGGACCTACCTGCTGCCCATGTG
EXONS
                 18200     18210     18220     18230     18240
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            ACATGTCCAGACCAACAGTCCCCTGCTCCCCATCTAGGCTGTTATGCTAC
DBH NM_013158    acatgtccagaccaacagtcccctgctccccatctaggctgttatgctac
SHR              ACATGTCCAGACCAACAGTCCCCTGCTCCCCATCTAGGCTGTTATGCTAC
EXONS
                 18250     18260     18270     18280     18290
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            TTCCCTGCCCTACATCTGTCACAACAGGCCTCACTGGGGTGACCAGGGTT
DBH NM_013158    ttccctgccctacatctgtcacaacaggcctcactggggtgaccagggtt
SHR              TTCCCTGCCCTACATCTGTCACAACAGGCCTCACTGGGGTGACCAGGGTT
EXONS
                 18300     18310     18320     18330     18340
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            GAGGCACATGAGGTCCCCACCAACTACCTAAACCTTTAAGGACTTGTTAT
DBH NM_013158    gaggcacatgaggtccccaccaactacctaaacctttaaggacttgttat
SHR              GAGGCACATGAGGTCCCCACCAACTACCTAAACCTTTAAGGACTTGTTAT
EXONS
                 18350     18360     18370     18380     18390
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            GTCTCTTCACTAGAAGAGGAGCCTGCAGCCCAGAGACCTGAGGTTGGATA
DBH NM_013158    gtctcttcactagaagaggagcctgcagcccagagacctgaggttggata
SHR              GTCTCTTCACTAGAAGAGGAGCCTGCAGCCCAGAGACCTGAGGTTGGATA
EXONS
                 18400     18410     18420     18430     18440
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            ACAAGGCCCCATAGTGAGCTGTAGAGCCCAGGTCTGTCTGACACCAGCAT
DBH NM_013158    acaaggccccatagtgagctgtagagcccaggtctgtctgacaccagcat
SHR              ACAAGGCCCCATAGTGAGCTGTAGAGCCCAGGTCTGTCTGACACCAGCAT
EXONS
                 18450     18460     18470     18480     18490
                     |....|....|....|....|....|....|....|....|....|....
BN.Lx            CTGGGCTCCCAGCTCCATAACCTTATTTTCCAGGCAGGAATCATGGGCGA
DBH NM_013158    ctgggctcccagctccataaccttattttccaggcaggaatcatgggcga
SHR              CTGGGCTCCCAGCTCCATAACCTTATTTTCCAGGCAGGAATCATGGGCGA
EXONS
```

```
                 18500     18510     18520     18530     18540
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             GCTTACAAGGTCTGATCCTGAGGATGTAAGGGTAACAGGCTCTTGAGGTC
DBH NM_013158     gcttacaaggtctgatcctgaggatgtaagggtaacaggctcttgaggtc
SHR               GCTTACAAGGTCTGATCCTGAGGATGTAAGAGTAACAGGCTCTTGAGGTC
EXONS

                 18550     18560     18570     18580     18590
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             ACATTCCTCCTATATGCTCTAAGCCAAAGCGGGTCTTCAGGCCTACCCTT
DBH NM_013158     acattcctcctatatgctctaagccaaagcgggtcttcaggcctaccctt
SHR               ACATTCCTCCTATATGCTCTAAGCCAAAGCGGGTCTTCAGGCCTACCCTT
EXONS

                 18600     18610     18620     18630     18640
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             CTTCTTCTTCCCTGAGGAAGAAAGAGCCTGGGGGTGAGGCACTCCCACGC
DBH NM_013158     cttcttcttccctgaggaagaaagagcctgggggtgaggcactcccacgc
SHR               CTTCTTCTTCCCTGAGGAAGAAAGAGCCTGGGGGTGAGGCACTCCCACGC
EXONS

                 18650     18660     18670     18680     18690
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             CTGCTGGGTGCATTGCTTTCCCTTGCTGGGCTTGGGTGTCCCAAGGAGCT
DBH NM_013158     ctgctgggtgcattgctttcccttgctgggcttgggtgtcccaaggagct
SHR               CTGCTGGGTGCATTGCTTTCCCTTGCTGGGCTTGGGTGTCCCAAGGAGCT
EXONS

                 18700     18710     18720     18730     18740
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             GAGGGGTTTCTAGCCTCCCAGAAGCCAGTGCCAAAGGACAGGGGCGGGAG
DBH NM_013158     gaggggtttctagcctcccagaagccagtgccaaaggacaggggcgggag
SHR               GAGGGGTTTCTAGCCTCCCAGAAGCCAGTGCCAAAGGACAGGGGCGGGAG
EXONS

                 18750     18760     18770     18780     18790
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGCTACTCTAGATCA-----------------------------------
DBH NM_013158     tgctactctagatcagtgttgagagggcctggggtacaaattgggtgtcc
SHR               TGCTACTCTAGATCA-----------------------------------
EXONS

                 18800     18810     18820     18830     18840
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     ccaggccacggataaggtttaaatgagctggccctaaactgctccagagc
SHR               --------------------------------------------------
EXONS

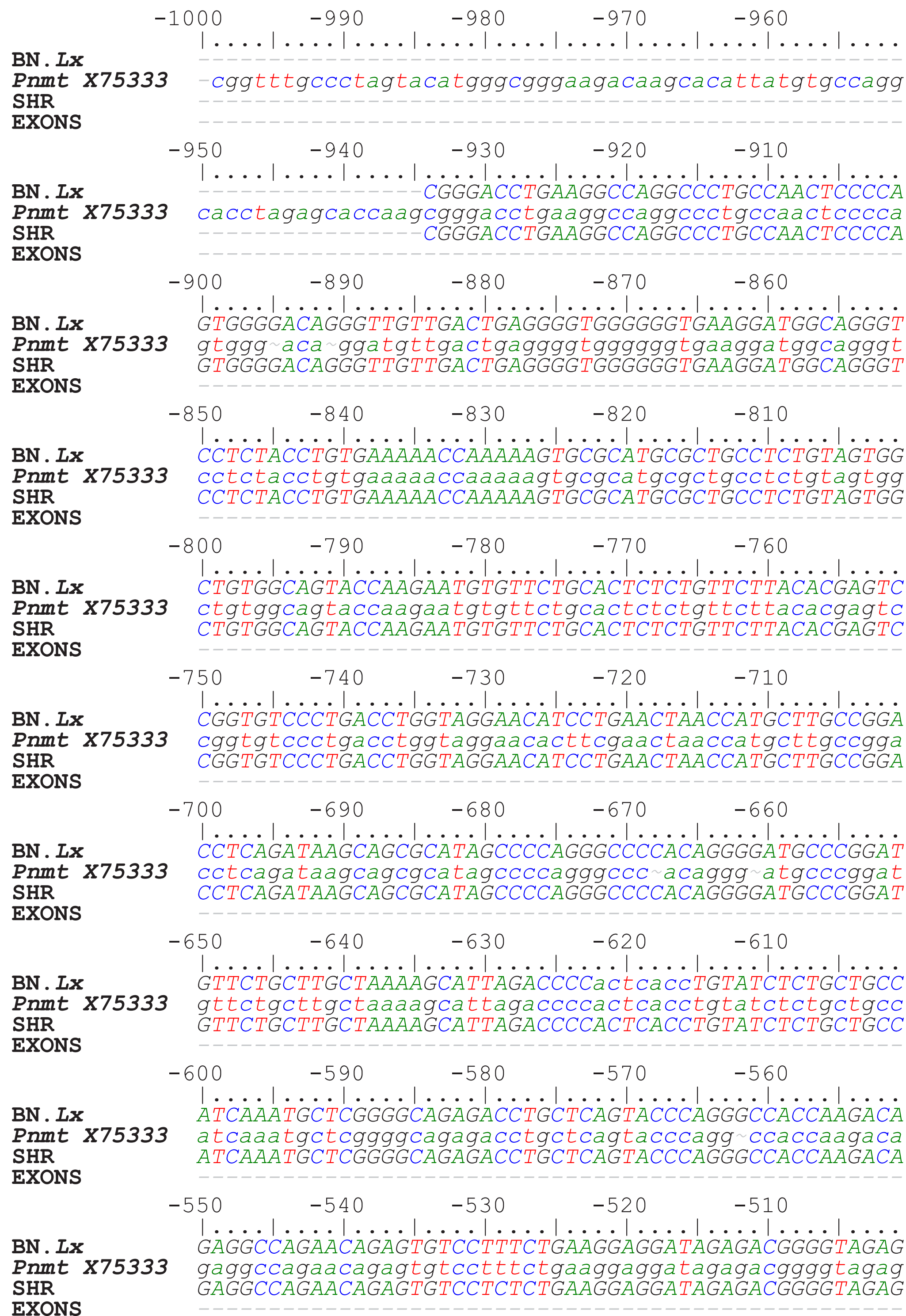
                 18850     18860     18870     18880     18890
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     agtgcccccagtctcagtggctatttcatttttatttagattaatttaag
SHR               --------------------------------------------------
EXONS

                 18900     18910     18920     18930     18940
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     caccgtggtgagggatgtggctcagttggtcgaacactttagcctaacaa
SHR               --------------------------------------------------
EXONS

                 18950     18960     18970     18980     18990
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gcacaaagccttctaaccccagatccacacaaaactggggacaggcacac
SHR               --------------------------------------------------
EXONS

                 19000     19010     19020     19030     19040
                   |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
DBH NM_013158     gagcacacccgccatcccatctcttgggaggtgtagacaggaggatcaga
SHR               --------------------------------------------------
EXONS
```

Appendix 6: Polymorphism discovery (re-sequencing) in rat *Pnmt*



```
               -500      -490      -480      -470      -460
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          GTCTGGAGGTGGGGATGGCATGATAAAGAAGGGGAGTTTGTAAAGGGTAC
Pnmt X75333    gtctggaggtggggatggcatgataaagaaggggagtttgtaaagggtac
SHR            GTCTGGAGGTGGGGATGGCATGATAAAGAAGGGGAGTTTGTAA-GGGTAC
EXONS          --------------------------------------------------

               -450      -440      -430      -420      -410
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          CCCGAGAGAGGGGAGGAGGCCGGGAAGGATGCTGGGACTGGGAACATGAG
Pnmt X75333    cccgagag~~gggaggaggccgggaaggatgctgggatcgggaacatgag
SHR            CCCGAGAGAGGGGAGGAGGCCGGGAAGGATGCTGGGACTGGGAACACGAG
EXONS          --------------------------------------------------

               -400      -390      -380      -370      -360
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          CTAGCTCAGACCTT-GGGAAAGAGAAGGGATGGGATGCTGGACGCTGGAC
Pnmt X75333    ctagctcagacctttgggaaagagaagggatgggatgctggacgctggac
SHR            CTAGTTCAGACCTT-GGGAAAGAGAAGGGATGGGATGCTGGACGCTGGAT
EXONS          --------------------------------------------------

               -350      -340      -330      -320      -310
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          GGAGTCTGTGGGAGGCTGGAGAGGAGTCAGGGGGCCACTGGAACTGAGGC
Pnmt X75333    ggagtctgtgggaggctggagaggagtcagggggccactggaactgaggc
SHR            GGAGTCTGTGGGAGGCTGGAGAGGAGTCAGGGGGCCACTGGAACTGAGGC
EXONS          --------------------------------------------------

               -300      -290      -280      -270      -260
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          TGGGGGAGCAGAGCAGCACTGGAGCGATCAAGAGCCCATAAAGGTGGAGG
Pnmt X75333    tgggggagcagagcagcactggagcgatcaagagcccataaaggtggagg
SHR            TGGGGGAGCAGAGCAGCACTGGAGCGATCAAGAGCCCATAAAGGTGGAGG
EXONS          --------------------------------------------------

               -250      -240      -230      -220      -210
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          GTTGTCAAGCAAGAGTAAAGCAGGAGTGGGGCAGGAGCGATGTTCTAAAG
Pnmt X75333    gttgtcaagcaagagtaaagcaggagtggggcaggagcgatgttctaaag
SHR            GTTGTCAAGCAAGAGTAAAGCAGGAGTGGGGCAGGAGCGATGTTCTAAAG
EXONS          --------------------------------------------------

               -200      -190      -180      -170      -160
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          GGCGCCCTCCACATCTCCCCCCTCCCCGCCCCCGCGCGTCCGTCCGGCGC
Pnmt X75333    ggcgccctccacatctcccccctccccgcccccgcgcgtccgtccggcgc
SHR            GGCGCCCTCCACATCTCCCCCCTCCCCGCCCCCGCGCGTCCGTCCGGCGC
EXONS          --------------------------------------------------

               -150      -140      -130      -120      -110
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          TCAGACACTAACTGAGATGGATGGGGTGACAGAGATGTGGCGGCCTCGGG
Pnmt X75333    tcagacactaactgagatggatggggtgacagagatgtggcggcctcgg~
SHR            TCAGACACTAACTGAGATGGATGGGGTGACAGAGATGTGGCGGCCTCGGG
EXONS          --------------------------------------------------

               -100       -90       -80       -70       -60
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          CGCCTCATCCCTCAGCAGCCACCCACCCCTGTGATGGAGGGGTCTGGGCG
Pnmt X75333    cgcctcatccctcagcagccacccacccctgtgatggaggggtctgggcg
SHR            CGCCTCATCCCTCAGCAGCCACCCACCCCTGTGATGGAGGGGTCTGGGCG
EXONS          --------------------------------------------------

                -50       -40       -30       -20       -10
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          GGGGGGAGGGGACCCAGTAGTAGATAAAGGGATGGGGAGGTTAGCGGAGA
Pnmt X75333    ggggggaggggacccagtagtagataaagggatggggaggttagcggaga
SHR            GGGGGGAGGGGACCCAGTAGTAGATAAAGGGATGGGGAGGTTAGCGGAGA
EXONS          --------------------------------------------------

                  0         10        20        30        40
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx          _TAGGCGGCCTCAACAGGAGCATGGACCGTGGCTCAGACCCGAAGCACAC
Pnmt X75333    _taggcggcctcaacaggagcATGGACCGTGGCTCAGACCCGAAGCACAC
SHR            _TAGGCGGCCTCAACAGGAGCATGGACCGTGGCTCAGACCCGAAGCACAC
EXONS          _5'UTR--------------EXON_1_XXXXXXXXXXXXXXXXXXXXXXX
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```
                  50        60        70        80        90
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGCAGGGATGGACTCTGACTCCGACCCTGGCCAGGCAGAGGTCGCCTTGG
Pnmt X75333       TGCAGGGATGGACTCTGACTCCGACCCTGGCCAGGCAGAGGTCGCCTTGG
SHR               TGCAGGGATGGACTCTGACTCCGACCCTGGCCAGGCAGAGGTCGCCTTGG
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 100       110       120       130       140
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CTTACCAGCGCTTTGAGCCCCGTGCCTACCTCCGCAACAACTACGCGCCT
Pnmt X75333       CTTACCAGCGCTTTGAGCCCCGTGCCTACCTCCGCAACAACTACGCGCCT
SHR               CTTACCAGCGCTTTGAGCCCCGTGCCTACCTCCGCAACAACTACGCGCCT
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 150       160       170       180       190
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CCTCGTGGGGACCTGAGCAACCCTGATGGTGTGGGGCCTTGGAAGCTTCG
Pnmt X75333       CCTCGTGGGGACCTGAGCAACCCTGATGGTGTGGGGCCTTGGAAGCTTCG
SHR               CCTCGTGGGGACCTGAGCAACCCTGATGGTGTGGGGCCTTGGAAGCTTCG
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

                 200       210       220       230       240
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             CTGCATGGCACAAGTCTTTGCCACCGGTGAGCACTGGAAACAGAGGCACG
Pnmt X75333       CTGCATGGCACAAGTCTTTGCCACCGgtgagcactggaaacagaggcacg
SHR               CTGCATGGCGCAAGTCTTTGCCACCGGTGAGCACTGGAAACAGAGGCACG
EXONS             XXXXXXXXXXXXXXXXXXXXXXXXXX------------------------

                 250       260       270       280       290
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             AGAGAGCAGAAGTCATAGGGAAATGAAACGGGGAGAGAGAAACAAGAGTG
Pnmt X75333       agagagcagaagtcatagggaaatgaaacggggagagagaaacaagagtg
SHR               AGAGAGCAGAAGTCATAGGGAAATGAAACGGGGAGAGAGAAACAAGAGTG
EXONS             --------------------------------------------------

                 300       310       320       330       340
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TGCACCAAATGGGGAACCAAAAAGCAGAACTATAGGTGTGTAGCCGGCAC
Pnmt X75333       tgcaccaaatggggaaccaaaaagcagaactataggtgtgtagccggcac
SHR               TGCACCAAATGGGGAACCAAGAAGCAGAACTATAGGTGTGTAGCCGGCAC
EXONS             --------------------------------------------------

                 350       360       370       380       390
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             AAACAGGAGCGGACGATAGTCTTGGCTACTAGGGATGCGGAGGCTTGGAG
Pnmt X75333       aaacaggagcggacgatagtcttggctactagggatggggaggcttggag
SHR               AAACAGGAGCGGACGATAGTCTTGGCTACTAGGGATGCGGAGGCTTGGAG
EXONS             --------------------------------------------------

                 400       410       420       430       440
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GACAAGTAGTTGAGGCCCGGAATTGGAAGACTGACTAGACAACTTAAGGT
Pnmt X75333       gacaagtagttgaggcccggaattggaagactgactagacaacttaaggt
SHR               GACAAGTAGTTGAGGCCCGGAATTGGAAGACTGACTAGACAACTTAAGGT
EXONS             --------------------------------------------------

                 450       460       470       480       490
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             TCTTTTCTTTCCAGGGGAGGATGCTTAAAGTTGAGAGACAGATGGGACCT
Pnmt X75333       tcttttctttccaggggaggatgcttaaagttgagagacagatgggacct
SHR               TCTTTTCTTTCCAGGGGAGGATGCTTAAAGTTGAGAGACAGATGGGACCT
EXONS             --------------------------------------------------

                 500       510       520       530       540
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             GGGAGA--------------------------------------------
Pnmt X75333       gggagagcgcgggagggggatgggagttggagactgcaaagagagaggct
SHR               GGGAGA--------------------------------------------
EXONS             --------------------------------------------------

                 550       560       570       580       590
                  |....|....|....|....|....|....|....|....|....|....
BN.Lx             --------------------------------------------------
Pnmt X75333       ccatccttgatgtcaagggatctcccccactaaattgtaactagagctag
SHR               --------------------------------------------------
EXONS             --------------------------------------------------
```

```
              600       610       620       630       640
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
Pnmt X75333   tttctgagtgctggaacccgagtcagaggagccctgtcagcacaagtgct
SHR           --------------------------------------------------
EXONS         --------------------------------------------------

              650       660       670       680       690
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
Pnmt X75333   ggggaggacacgagggagaggggtggctgaggcaggaacccccctgatatc
SHR           --------------------------------------------------
EXONS         --------------------------------------------------

              700       710       720       730       740
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         -----ctctgctctaccagGTGAGGTGTCTGGACAGGTCCTCATTGACAT
Pnmt X75333   ccctcctctgctctaccagGTGAGGTGTCTGGACAGGTCCTCATTGACAT
SHR           ------tctgctctaccagGTGAGGTGTCTGGACAGGTCCTCATTGACAT
EXONS         --------------------EXON_2_XXXXXXXXXXXXXXXXXXXXXXX

              750       760       770       780       790
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CGGCTCAGGCCCCACCATATACCAGCTGCTCAGCGCCTGTGCCCACTTCG
Pnmt X75333   CGGCTCAGGCCCCACCATATACCAGCTGCTCAGCGCCTGTGCCCACTTCG
SHR           CGGCTCAGGCCCCACCATATACCAGCTGCTCAGCGCCTGTGCCCACTTCG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              800       810       820       830       840
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         AGGACATCACCATGACAGACTTCTTGGAGGTCAACCGGCAGGAGCTGGGA
Pnmt X75333   AGGACATCACCATGACAGACTTCTTGGAGGTCAACCGGCAGGAGCTGGGA
SHR           AGGACATCACCATGACAGACTTCTTGGAGGTCAACCGGCAGGAGCTGGGA
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              850       860       870       880       890
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CTCTGGCTGCGAGAGGAACCAGGAGCCTTCGACTGGAGTGTGTATAGCCA
Pnmt X75333   CTCTGGCTGCGAGAGGAACCAGGAGCCTTCGACTGGAGTGTGTATAGCCA
SHR           CTCTGGCTGCGAGAGGAACCAGGAGCCTTCGACTGGAGTGTGTATAGCCA
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              900       910       920       930       940
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GCATGTCTGCCTCATCGAGGACAAGGGGTGAGAACTGGGCTGGGAGCTTC
Pnmt X75333   GCATGTCTGCCTCATCGAGGACAAGGGgtgagaactgggctgggagcttc
SHR           GCATGTCTGCCTCATCGAGGACAAGGGGTGAGAACTGGGCTGGGAGCTTC
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXX-----------------------

              950       960       970       980       990
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         ACACGGAGTGGTGGGCGGGTGCTGGAGGGCGGAGGAGGACTGAGCCTCTG
Pnmt X75333   acacggagtg~tgggcgggtgctggagggcggaggaggactgagcctctg
SHR           ACACGGAGTGGTGGGCGGGTGCTGGAGGGCGGAGGAGGACTGAGCCTCTG
EXONS         --------------------------------------------------

              1000      1010      1020      1030      1040
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GGGTAGTCCTGAGCCCCGCCTTGTGCCCCCTGTACAGAGAGTCCTGGCAG
Pnmt X75333   gggtagtc~tgagccccgccttgtgcccccctgtacagAGAGTCCTGGCAG
SHR           GGGTAGTCCTGAGCCCCGCCTTGTGCCCCCTGTACAGAGAGTCCTGGCAG
EXONS         -------------------------------------EXON_3_XXXXXX

              1050      1060      1070      1080      1090
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GAGAAAGAACGCCAGCTCCGAGCGAGGGTGAAGCGAGTCTTGCCCATTGA
Pnmt X75333   GAGAAAGAACGCCAGCTCCGAGCGAGGGTGAAGCGAGTCTTGCCCATTGA
SHR           GAGAAAGAACGCCAGCTCCGAGCGAGGGTGAAGCGAGTCTTGCCCATTGA
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              1100      1110      1120      1130      1140
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TGTGCACAAGCCCCAGCCCCTGGGAGCTTCGGGCCTGGCACCCCTGCCTG
Pnmt X75333   TGTGCACAAGCCCCAGCCCCTGGGAGCTTCGGGCCTGGCACCCCTGCCTG
SHR           TGTGCACAAGCCCCAGCCCCTGGGAGCTTCGGGCCTGGCACCCCTGCCTG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
```

```
              1150      1160      1170      1180      1190
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CTGACGCCTTGGTCTCTGCCTTCTGCCTGGAGGCTGTGAGCCCGGATCTC
Pnmt X75333   CTGACGCCTTGGTCTCTGCCTTCTGCCTGGAGGCTGTGAGCCCGGATCTC
SHR           CTGACGCCTTGGTCTCTGCCTTCTGCCTGGAGGCTGTGAGCCCGGATCTC
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              1200      1210      1220      1230      1240
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCAAGCTTCCGGCAGGCTTTGTATCATATCACGACGCTGCTGAGGCCCGG
Pnmt X75333   CCAAGCTTCCGGCAGGCTTTGTATCATATCACGACGCTGCTGAGGCCCGG
SHR           CCAAGCTTCCGGCAGGCTTTGTATCATATCACGACGCTGCTGAGGCCCGG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              1250      1260      1270      1280      1290
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GGGTCATCTCCTTCTCATCGGGGCCCTGGAGGAGTCGTGGTACCTTGCTG
Pnmt X75333   GGGTAATCTCCTCTTCATCGGGGCCCTGGAGGAGTCGTGGTACCTTGCTG
SHR           GGGTCATCTCCTTCTCATCGGGGCCCTGGAGGAGTCGTGGTACCTTGCTG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              1300      1310      1320      1330      1340
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GGGAGGCCAGGCTATCTGTGGTTCCAGTGTCAGAGGAGGAGGTGAGGGAG
Pnmt X75333   GGGAGGCCAGGCTATCTGTGGTTCCAGTGTCAGAGGAGGAGGTGAGGGAG
SHR           GGGAGGCCAGGCTATCTGTGGTTCCAGTGTCAGAGGAGGAGGTGAGGGAG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              1350      1360      1370      1380      1390
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GCCCTGGTCTGTAGTGGTTATGAGGTCCGAGACCTTCGCACCTACATCAT
Pnmt X75333   GCCCTGGTCTGTAGTGGTTATGAGGTCCGAGACCTTCGCACCTACATCAT
SHR           GCCCTGGTCTGTAGTGGTTATGAGGTCCGAGACCTTCGCACCTACATCAT
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              1400      1410      1420      1430      1440
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         GCCTGCCCACCTCCGCACGGGTGTGGATGACGTCAAGGGTATCTTCTTTG
Pnmt X75333   GCCTGCCCACCTCCGCACGGGTGTGGATGACGTCAAGGGTATCTTCTTTG
SHR           GCCTGCCCACCTCCGCACGGGTGTGGATGACGTCAAGGGTATCTTCTTTG
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

              1450      1460      1470      1480      1490
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         CCTGGGCCCAGAAGATAGAGGTGCAGGTGTGAGGCTCCATTTGCCTCCCT
Pnmt X75333   CCTGGGCCCAGAAGATAGAGGTGCAGGTGTGAggctccatttgcctccct
SHR           CCTGGGCCCAGAAGATAGAGGTGCAGGCGTGAGGCTCCATTTGCCTCCCT
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXX---3'UTR-XXXXXXXXXXXX

              1500      1510      1520      1530      1540
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         AACTCCTTATCACCCGAAGTGGCACCTAATAAAGTAACAGTCCCCTGCTA
Pnmt X75333   aactccttatcacccgaagtggcacctaataaagtaacagtccccagcta
SHR           AACTCCTTATCACCCGAAGTGGCACCTAATAAAGTAACAGTCCCCTGCTA
EXONS         XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX-

              1550      1560      1570      1580      1590
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         TGTCTGTGCTGTTTGTGACTCTCCTAGACACAAGGATAGGAAAAGGTCTC
Pnmt X75333   tgtctgtgctgtttgtgactctcctagacacaaggataggaaaaggtctc
SHR           TGTCTGTGCTGTTTGTGACTCTCCTAGACACAAGGATAGGAAAAGGTCTC
EXONS         --------------------------------------------------

              1600      1610      1620      1630      1640
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         C-------------------------------------------------
Pnmt X75333   cgaggcttagaaggaaccaatcctagctttttctttttgccctagaagcc
SHR           C-------------------------------------------------
EXONS         --------------------------------------------------

              1650      1660      1670      1680      1690
              |....|....|....|....|....|....|....|....|....|....
BN.Lx         --------------------------------------------------
Pnmt X75333   ttcgtgaaggctgctttctgtgccacccattctcccctccccaggaaggc
SHR           --------------------------------------------------
EXONS         --------------------------------------------------
```

Appendix 7: Primers for SNP genotyping at the *Dbh* and *Pnmt* loci

Primers to genotype *Dbh* T-550G

F-5'-ATTCACCCCCTTCCTGCCT-3'
R-5'-biotin-GGCCTGCTGTTCCCACTTTTT-3'
Sequencing primer 5'-CACCCACTCCTTCTGTAG-3'

Primers to genotype *Pnmt* T-529C

F-5'-biotin-CCAGGGCCACCAAGACAG -3'
R-5'-TCCCCACCTCCAGACCTCTA -3'
Sequencing primer 5'-CCGTCTCTATCCTCCTT -3'

Appendix 8: Primers for the *Dbh* and *Pnmt* promoter/reporter constructs

Dbh - forward (5’-CCATGAGCTCTGGACATTCTGCCTAGTCC-3’)
Dbh - reverse (5’-GAGGCTCGAGGGCTGGGATGAGCA-3’)

Pnmt - forward (5’-GTGAGAGCTCGCAGGGTCCTCTACCTGTGA-3’)
Pnmt - reverse (5’-CACGCTCGAGGCTCCTGTTG-3’)

Glossary of terms

This glossary is designed to help the reader understand the complex terminology associated with contemporary molecular and statistical genetics, genomics and systems biology. It was compiled from various textbooks, journals and on-line resources. Especially helpful was the Glossary of terms published in the May 1, 2007 special issue of the Journal of Experimental Biology, which was devoted to '*The Genomic Revolution*' [172].

ANNOTATION – as applied to proteins, DNA sequences or genes. The storage of data describing these entities (protein/gene identities, DNA motifs, gene ontology categorisation, etc.) within a biological database. Active projects include FlyBase and WormBase. See Gene ontology.

ASSEMBLY – the process of aligning sequenced fragments of DNA into their correct positions within the chromosome or transcript.

ASSOCIATION STUDY – an examination of genetic variation and relationships between genetic variants (alleles) and phenotypes in a population of unrelated individuals

cDNA – complementary DNA. This is DNA synthesised from a mature mRNA template by the enzyme reverse transcriptase. cDNA is frequently used as an early part of gene cloning procedures, since it is more robust and less subject to degradation than the mRNA itself.

Chip – see Microarray.

***cis*-acting –** a molecule is described as *cis*-acting when it affects other genes that are physically adjacent, on the same chromosome, or are genetically linked or in close proximity (for mRNA expression, typically a promoter).

COMPLEX TRAIT – see Quantitative Trait.

CpG ISLANDS – regions that show high density of `C followed by G' dinucleotides and are generally associated with promoter elements; in particular, stretches of DNA of at least 200 bp with a C–G content of 50% and an observed CpG/expected CpG in excess of 0.6. The cytosine residues can be methylated, generally to repress transcription, while demethylated CpGs are a hallmark of transcription. CpG dinucleotides are under-represented outside regulatory regions, such as promoters, because methylated C mutates into T by deamination.

ENHANCER – a short segment of genomic DNA that may be located remotely and that, on binding particular proteins (*trans*-acting factors), increases the rate of transcription of a specific gene or gene cluster.

EPISTASIS – a phenomenon when the properties of one gene are modified by one or more genes at other loci. Otherwise known as a genetic interaction, but epistasis refers to the statistical properties of the phenomenon.

eQTL – the combination of conventional QTL analysis with gene expression profiling, typically using microarrays. eQTLs describe regulatory elements controlling the expression of genes involved in specific traits.

EST – expressed sequence tag. A short DNA sequence determined for a cloned cDNA representing portions of an expressed gene. The sequence is generally several hundred base pairs from one or both ends of the cloned insert.

EXON – any region of DNA that is transcribed to the final (spliced) mRNA molecule. Exons interleave with segments of non-coding DNA (introns) that are removed (spliced out) during processing after transcription.

GENE – a functional physical unit of heredity that can be passed from parent to child. All genes in humans are pieces of DNA. Most genes contain information for making a specific protein, or RNA.

GENE INTERACTION NETWORK – a network of functional interactions between genes. Functional interactions can be inferred from many different data types, including protein–protein interactions, genetic interactions, co-expression relationships, the co-inheritance of genes across genomes and the arrangement of genes in bacterial genomes. The interactions can be represented using network diagrams, with lines connecting the interacting elements, and can be modelled using differential equations.

GENE MAP – the position of gene loci on chromosomes; a physical map refers to the absolute position of the gene loci, expressed by the number of base pairs; a genetic map expresses distance in terms of their frequency of recombination

GENE ONTOLOGY (GO) – an ontology is a controlled vocabulary of terms that have logical relationships with each other and that are amenable to computerised manipulation. The Gene Ontology project has devised terms in three domains: biological process, molecular function and cell compartment. Each gene or DNA sequence can be associated with these annotation terms from each domain, and this enables analysis of microarray data on groups of genes based on descriptive terms so provided. See http://www.geneontology.org

GENE SET ENRICHMENT ANALYSIS – a computational method that determines whether a defined set of genes, usually based on their common involvement in a biological process, shows statistically significant differences in transcript expression between two biological states.

GENE SILENCING – the switching-off of a gene by an epigenetic mechanism at the transcriptional or post-transcriptional levels. Includes the mechanism of RNAi.

GENETIC INTERACTION (NETWORK) – a genetic interaction between two genes occurs when the phenotypic consequences of a mutation in one gene are modified by the mutational status at a second locus. Genetic interactions can be aggravating (enhancing) or alleviating (suppressing). To date, most high-throughput studies have focused on systematically identifying synthetic lethal or sick

(aggravating) interactions, which can then be visualized as a network of functional interactions (edges) between genes (nodes).

GENOME – a portmanteau of gene and chromosome, the entire hereditary information for an organism that is embedded in the DNA (or, for some viruses, in RNA). Includes protein-coding and non-coding sequences.

GENOTYPE – the specific allelic makeup of an individual.

HERITABILITY – phenotypic variation within a population is attributable to the genetic variation between individuals and to environmental factors. Heritability is the proportion due to genetic variation usually expressed as a percentage.

HYBRIDISATION – the process of joining (annealing) two complementary single-stranded DNAs into a single double-stranded molecule. In microarray analysis, the target RNA/DNA from the subject under investigation is denatured and hybridised to probes that are immobilised on a solid phase (i.e. glass microscope slide).

INDEL – insertion and deletion of DNA, referring to two types of genetic mutation. To be distinguished from a `point mutation', which refers to the substitution of a single base.

INTERACTOME – a more or less comprehensive set of interactions between elements within cells. Usually applied to genes or proteins as defined by transcriptomic, proteomic or protein–protein interaction data.

INTRON – see Exon.

KEGG – The Kyoto Encyclopedia of Genes and Genomes is a database of metabolic and other pathways collected from a variety of organisms. See http://www.genome.jp/kegg

LINKAGE ANALYSIS – study aimed at establishing relationship between genes or markers. Because DNA segments that lie near each other on a chromosome tend to be inherited together, markers are often used as tools for tracking the inheritance pattern of a gene that has not yet been identified but whose approximate location is known. Linkage analysis is one of the most important tools of quantitative genetics used to find genes that underlie complex traits.

LINKAGE DISEQUILIBRIUM (LD) – term refers to polymorphisms in close physical proximity on a chromosome, such that a specific allele at one locus has a significant predictive value for specific alleles at the other (closely linked) loci.

MAP DISTANCE – distance between gene loci, expressed either in physical terms or in terms of recombination frequency

MEIOSIS – the special division of a germ cell nucleus that leads to reduction of the chromosome complement from the diploid to the haploid

METABOLOMICS – the systematic qualitative and quantitative analysis of small chemical metabolite profiles. The metabolome represents the collection of metabolites within a biological sample.

METAGENOMICS – the application of genomic techniques to characterise complex communities of microbial organisms obtained directly from environmental samples. Typically, genomic tags are sequence characterised as markers of each species to inform on the range and abundance of species in the community.

MICROARRAY – an arrayed set of probes for detecting molecularly specific analytes or targets. Typically, the probes are composed of DNA segments that are immobilised onto the solid surface, each of which can hybridise with a specific DNA present in the target preparation. DNA microarrays are used for profiling of gene transcripts.

miRNA – a category of novel, very short, non-coding RNAs, generated by the cleavage of larger precursors (pri-miRNA). These short RNAs are included in the RNA-induced silencing complex (RISC) and pair to the 3' ends of target RNA, blocking its translation into proteins (in animals) or promoting RNA cleavage and degradation (in plants).

MODEL SPECIES – a species used to study particular biological phenomena, the outcome offering insights into the workings of other species. Usually, the selection is based on experimental tractability, particularly ease of genetic manipulation. For the geneticist, it is an organism with inbred lines where sibs will be >98% identical (i.e. *Drosophila*, *Caenorhabditis elegans* and mice). For genomic science, it refers to a species for which the genomic DNA has been sequenced.

mRNA – a protein-coding mRNA containing a protein-coding region (CDS), preceded by a 5' and followed by a 3' untranslated region (5' UTR and 3' UTR). The UTRs contain regulatory elements. A full-length cDNA contains the complete sequence of the original mRNA, including both UTRs. However, it is often difficult to assign the starting–termination positions for protein synthesis unambiguously. A cDNA containing the entire CDS is often considered acceptable for bioinformatic and experimental studies requiring full-length cDNAs.

PCR – polymerase chain reaction. A molecular biology technique for replicating DNA *in vitro*. The DNA is thus amplified, sometimes from very small amounts. PCR can be adapted to perform a wide variety of genetic manipulations.

PERMUTATION TEST – method that uses empirical distribution of test statistic acquired by permuting the original sample to establish what is the likelihood of obtaining a certain value merely by chance. Frequently used to assess the significance of QTL mapping results.

PHENOTYPE – any observed quality of an organism, such as its morphology, development, or behavior, as opposed to its Genotype (see above).

POLYADENYLATION – the covalent addition of multiple A bases to the 3' tail of an mRNA molecule. This occurs during the processing of transcripts to form the mature, spliced molecule and is important for regulation of turnover, trafficking and translation.

POST-TRANSLATIONAL MODIFICATION – the chemical modification of a protein after synthesis through translation. Some modifications, notably phosphorylation, affect the properties of the protein, offering a means of regulating function.

PRINCIPAL COMPONENT ANALYSIS (PCA) – a technique for simplifying complex, multi-dimensional datasets to a reduced number of dimensions, the principal components. This procedure retains those characteristics of the data that relate to its variance.

PROMOTER – a regulatory DNA sequence, generally lying upstream of an expressed gene, which in concert with other often distant regulatory elements directs the transcription of a given gene.

PROTEOME – the entire protein complement of an organism, tissue or cell culture at a given time.

qPCR – quantitative real-time PCR, sometimes called real-time PCR. A more quantitative form of RT-PCR in which the quantity of amplified product is estimated after each round of amplification.

QUANTITATIVE GENETICS – a branch of genetics research that employs statistical and probabilistic principles to identify the genetic and non-genetic components of a complex trait and to describe them in statistical terms.

QTL – quantitative trait loci. A region of DNA that contains those genes contributing to the trait under study.

QUANTITATIVE TRAIT – inheritance of a phenotypic property or characteristic that varies continuously between extreme states and can be attributed to interactions between multiple genes and their environment.

QUANTITATIVE TRAIT – trait that has value wit a continuous distribution in a population (Gaussian curve) as opposed to a **QUALITATIVE TRAIT**, which has a bimodal (categorical) distribution.

QUANTITATIVE TRAIT LOCUS (QTL) – a region of DNA statistically associated with a quantitative trait; QTLs are believed to be closely linked to the actual genes that underlie variation in a particular quantitative trait.

RECOMBINANT INBRED STRAINS – set of strains derived from brother-sister inbreeding of F2 progeny from a cross of two standard inbred strains.

RNAi – RNA interference or RNA-mediated interference. The process by which double-stranded RNA triggers the destruction of homologous mRNA in eukaryotic cells by the RISC.

RT-PCR – reverse transcription–polymerase chain reaction. A technique for amplifying a defined piece of RNA that has been converted to its complementary DNA form by the enzyme reverse transcriptase. See qPCR.

siRNA – small interfering RNA, or silencing RNA. A class of short (20–25 nt), double-stranded RNA molecules. It is involved in the RNA interference pathway, which alters RNA stability and thus affects RNA concentration and thereby suppresses the normal expression of specific genes. Widely used in biomedical research to ablate specific genes.

snoRNA – small nucleolar RNA. A sub-class of RNA molecules involved in guiding chemical modification of ribosomal RNA and other RNA genes as part of the regulation of gene expression.

SNP – single nucleotide polymorphism. A single base-pair mutation at a specific locus, usually consisting of two alleles. Because SNPs are conserved over evolution, they are frequently used in QTL analysis and in association studies in place of microsatellites, and in genetic fingerprinting analyses.

SSH – suppressive subtractive hybridisation. A powerful protocol for enriching cDNA libraries for genes that differ in representation between two or more conditions. It combines normalisation and subtraction in a single procedure and allows the detection of low-abundance, differentially expressed transcripts, such as those involved in signalling and signal transduction.

STATISTICAL GENOMICS – a research discipline that considers the evaluation of entire genomes in order to identify and characterize genetic factors that influence a particular trait.

STRUCTURAL RNAs – a class of non-coding RNA, long known to have a structural role (for instance, the ribosomal RNAs), transcribed by RNA polymerase I or III.

SYSTEMS BIOLOGY – treatment of biological entities as systems composed of defined elements interacting in defined ways to enable the observed function and behaviour of that system. The properties of the systems are embedded in a quantitative model that guides further tests of systems behaviour.

TATA-BOXES – sequences in promoter regions constituted by TATAAA, or similar variants, which were considered the hallmark of Promoters. Recent data show that they are present only in the minority of promoters, where they direct transcription at a single well-defined location some 30 bp downstream of this element.

***trans*-acting –** a factor or gene that acts on another unlinked gene, a gene on a separate chromosome or genetically unlinked usually through some diffusible protein product (for mRNA expression, typically a transcription factor).

TRANSCRIPT – an RNA product produced by the action of RNA polymerase reading the sequence of bases in the genomic DNA. Originally limited to protein-coding sequences with flanking UTRs but now known to include large numbers of products that do not code for a protein product.

TRANSCRIPTOME – the full set of mRNA molecules (transcripts) produced by the system under observation. Whilst the genome is fixed for a given organism, the

transcriptome varies with context (i.e. tissue source, ontogeny, external conditions or experimental treatment).

TRANSINDUCTION – generation of transcripts from intergenic regions. At least some such products do not relate to a definable promoter or transcriptional start site.

TRANSPOSON – sequences of DNA able to move to new positions within the genome of a single cell. This event might cause mutation at the site of insertion. Also called `mobile genetic elements' or `jumping genes'.

UTR – untranslated region. Regions of the mRNA that lie at either the 3' or 5' flanking ends of the molecule (i.e. 3' UTR and 5' UTR). They bracket the protein-coding region and contain signals and binding sites that are important for the regulation of both protein translation and RNA degradation.

References

1. Antonarakis SE, Beckmann JS. Mendelian disorders deserve more attention. *Nat Rev Genet*. 2006;7:277-82.

2. Hirschhorn JN. Genetic approaches to studying common diseases and complex traits. *Pediatr Res*. 2005;57:74R-77R.

3. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science*. 1994;265:2037-48.

4. Botstein D, Risch N. Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nat Genet*. 2003;33 Suppl:228-37.

5. Dean M. Approaches to identify genes for complex human diseases: lessons from Mendelian disorders. *Hum Mutat*. 2003;22:261-74.

6. Van Heyningen V, Yeyati PL. Mechanisms of non-Mendelian inheritance in genetic disease. *Hum Mol Genet*. 2004;13 Spec No 2:R225-33.

7. Badano JL, Katsanis N. Beyond Mendel: an evolving view of human genetic disease transmission. *Nat Rev Genet*. 2002;3:779-89.

8. Lynch M, Walsh B. *Genetics and Analysis of Quantitative Traits*: Sinauer Associates, Inc.; 1998.

9. Hartl DL, Clark AG. *Principles of Population Genetics*. Sunderland, MA: . Sinaur Associates, Inc.; 1997.

10. Liu B-H. *Statistical Genomics: Linkage, Mapping, and QTL Analysis*: CRC Press; 1998.

11. Camp NJ, Cox A, eds. *Quantitative trait loci: methods and protocols*. Totowa, New Jersey: Humana Press; 2002.

12. Paterson AH. *Molecular Dissection of Complex Traits*: CRC Press; 1998.

13. Darwin C. *On the origin of species by means of natural selection*. London: Murray; 1859.

14. Bateson W. *Mendel's Principles of Heredity*. London: Cambridge University Press; 1902.

15. Mendel G. Versuche über Pflanzenhybriden. *Verh. naturforsch. Ver. Brünn*. 1866;4:3–47.

16. Morgan TH. What are "factors" in Mendelian explanations? *American Breeders Association Reports*. 1909;5:365-369.

17. Morgan TH, Sturtevant AH, Muller HJ, Bridges CB. *The Mechanism of Mendelian Heredity*. New York: Henry Holt Company; 1915.

18. Sturtevant AH. The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. *Journal of Experimental Zoology*. 1913;14:43-59.

19. Johannsen W. *Elemente der exakten Erblichkeitslehre*. Jena: Fischer; 1909.

20. Fisher RA. The correlation between relatives on the supposition of Mendelian inheritance. *Trans. Roy. Soc. Edinb.* 1918;52:399-433.

21. Wright S. Evolution in Mendelian populations. *Genetics*. 1931;16:97-159.

22. Avery OT, MacLeod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. *J Exp Med*. 1944;79:137-158.

23. Watson JD, Crick FH. Genetical implications of the structure of deoxyribonucleic acid. *Nature*. 1953;171:964-7.

24. Crick FH, Barnett L, Brenner S, Watts-Tobin RJ. General nature of the genetic code for proteins. *Nature*. 1961;192:1227-32.

25. Jacob HJ, Brown DM, Bunker RK, Daly MJ, Dzau VJ, Goodman A, Koike G, Kren V, Kurtz T, Lernmark A, et al. A genetic linkage map of the laboratory rat, Rattus norvegicus. *Nat Genet*. 1995;9:63-9.

26. Lander ES, Weinberg RA. Genomics: journey to the center of biology. *Science*. 2000;287:1777-82.

27. Jacob HJ, Kwitek AE. Rat Genetics: Attaching Physiology and Pharmacology to the Genome. *Nature Reviews Genetics*. 2002;3:33-42.

28. Venter JC, et al. The sequence of the human genome. *Science*. 2001;291:1304-51.

29. Lander ES, et al. Initial sequencing and analysis of the human genome. *Nature*. 2001;409:860-921.

30. Gibbs RA, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature*. 2004;428:493-521.

31. Waterston RH, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature*. 2002;420:520-62.

32. Hubner N, Wallace CA, Zimdahl H, Petretto E, Schulz H, Maciver F, Mueller M, Hummel O, Monti J, Zidek V, Musilova A, Kren V, Causton H, Game L, Born G, Schmidt S, Muller A, Cook SA, Kurtz TW, Whittaker J, Pravenec M, Aitman TJ. Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. *Nat Genet*. 2005;37:243-53.

33. Wong CM, O'Connor DT, Martinez JA, Kailasam MT, Parmer RJ. Diminished renal kallikrein responses to mineralocorticoid stimulation in African Americans: determinants of an intermediate phenotype for hypertension. *Am J Hypertens*. 2003;16:281-9.

34. Barr MM. Super models. *Physiol Genomics*. 2003;13:15-24.

35. Stoll M, Cowley AW, Jr., Tonellato PJ, Greene AS, Kaldunski ML, Roman RJ, Dumas P, Schork NJ, Wang Z, Jacob HJ. A genomic-systems biology map for cardiovascular function. *Science*. 2001;294:1723-6.

36. Cox RD, Brown SD. Rodent models of genetic disease. *Curr Opin Genet Dev*. 2003;13:278-83.

37. Lerman LO, Chade AR, Sica V, Napoli C. Animal models of hypertension: an overview. *J Lab Clin Med*. 2005;146:160-73.

38. Silver LM. *Mouse Genetics: Concepts and Applications*. Oxford.: Oxford University Press; 1995.

39. Nadeau JH, Singer JB, Matin A, Lander ES. Analysing complex genetic traits with chromosome substitution strains. *Nat Genet*. 2000;24:221-5.

40. Darvasi A, Soller M. Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics*. 1995;141:1199-207.

41. Valdar W, Solberg LC, Gauguier D, Burnett S, Klenerman P, Cookson WO, Taylor MS, Rawlins JN, Mott R, Flint J. Genome-wide genetic association of complex traits in heterogeneous stock mice. *Nat Genet*. 2006;38:879-87.

42. Pravenec M, Kren V, Krenova D, Bila V, Zidek V, Simakova M, Musilova A, van Lith HA, van Zutphen LF. HXB/Ipcv and BXH/Cub recombinant inbred strains of the rat: strain distribution patterns of 632 alleles. *Folia Biol (Praha)*. 1999;45:203-15.

43. Farrall M. Quantitative genetic variation: a post-modern view. *Hum Mol Genet*. 2004;13 Spec No 1:R1-7.

44. Flint J, Valdar W, Shifman S, Mott R. Strategies for mapping and cloning quantitative trait genes in rodents. *Nat Rev Genet*. 2005;6:271-86.

45. Jansen RC, Nap JP. Genetical genomics: the added value from segregation. *Trends Genet*. 2001;17:388-91.

46. McBride MW, Charchar FJ, Graham D, Miller WH, Strahorn P, Carr FJ, Dominiczak AF. Functional genomics in rodent models of hypertension. *J Physiol*. 2004;554:56-63.

47. Jirout M, Krenova D, Kren V, Breen L, Pravenec M, Schork NJ, Printz MP. A new framework marker-based linkage map and SDPs for the rat HXB/BXH strain set. *Mamm Genome*. 2003;14:537-46.

48. Pravenec M, Kren V, Bila V, Kabra P, Krsiakova M, Simonet L, Kurtz TW. HXB and BXH sets of recombinant inbred strains: strain distribution patterns of some genetic markers. *Transplant Proc*. 1990;22:2557-8.

49. Pravenec M, Gauguier D, Schott JJ, Buard J, Kren V, Bila V, Szpirer C, Szpirer J, Wang JM, Huang H, St Lezin E, Spence MA, Flodman P, Printz M, Lathrop GM, Vergnaud G, Kurtz TW. A genetic linkage map of the rat derived from recombinant inbred strains. *Mamm Genome*. 1996;7:117-27.

50. Steen RG, Kwitek-Black AE, Glenn C, Gullings-Handley J, Van Etten W, Atkinson OS, Appel D, Twigger S, Muir M, Mull T, Granados M, Kissebah M, Russo K, Crane R, Popp M, Peden M, Matise T, Brown DM, Lu J, Kingsmore S, Tonellato PJ, Rozen S, Slonim D, Young P, Jacob HJ, et al. A high-density integrated genetic linkage and radiation hybrid map of the laboratory rat. *Genome Res*. 1999;9:AP1-8, insert.

51. The STAR consortium. SNP and haplotype mapping for genetic analysis in the rat. *(Submitted)*.

52. Pravenec M, Klir P, Kren V, Zicha J, Kunes J. An analysis of spontaneous hypertension in spontaneously hypertensive rats by means of new recombinant inbred strains. *J Hypertens*. 1989;7:217-21.

53. Manly KF, Cudmore RH, Jr., Meer JM. Map Manager QTX, cross-platform software for genetic mapping. *Mamm Genome*. 2001;12:930-2.

54. Wang S, Basten CJ, Zeng ZB. Windows QTL Cartographer 2.5. In: Department of Statistics, North Carolina State University, Raleigh, NC; 2006.

55. Schork NJ. Genome partitioning and whole-genome analysis. *Adv Genet*. 2001;42:299-322.

56. Belknap JK, Mitchell SR, O'Toole LA, Helms ML, Crabbe JC. Type I and type II error rates for quantitative trait loci (QTL) mapping studies using recombinant inbred mouse strains. *Behav Genet*. 1996;26:149-60.

57. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet*. 1995;11:241-7.

58. Lazar J, Moreno C, Jacob HJ, Kwitek AE. Impact of genomics on research in the rat. *Genome Res*. 2005;15:1717-28.

59. Pravenec M, Gauguier D, Schott JJ, Buard J, Kren V, Bila V, Szpirer C, Szpirer J, Wang JM, Huang H, et al. Mapping of quantitative trait loci for blood pressure and cardiac mass in the rat by genome scanning of recombinant inbred strains. *J Clin Invest*. 1995;96:1973-8.

60. Hamet P, Pausova Z, Dumas P, Sun YL, Tremblay J, Pravenec M, Kunes J, Krenova D, Kren V. Newborn and adult recombinant inbred strains: a tool to search for genetic determinants of target organ damage in hypertension. *Kidney Int*. 1998;53:1488-92.

61. Pravenec M, Zidek V, Musilova A, Vorlicek J, Kren V, St Lezin E, Kurtz TW. Genetic isolation of a blood pressure quantitative trait locus on chromosome 2 in the spontaneously hypertensive rat. *Journal of Hypertension*. 2001;19:1061-1064.

62. Jaworski RL, Jirout M, Closson S, Breen L, Flodman PL, Spence MA, Kren V, Krenova D, Pravenec M, Printz MP. Heart rate and blood pressure quantitative trait loci for the airpuff startle reaction. *Hypertension*. 2002;39:348-52.

63. Conti LH, Jirout M, Breen L, Vanella JJ, Schork NJ, Printz MP. Identification of quantitative trait Loci for anxiety and locomotion phenotypes in rat recombinant inbred strains. *Behav Genet*. 2004;34:93-103.

64. Printz MP, Jirout M, Jaworski R, Alemayehu A, Kren V. Genetic Models in Applied Physiology. HXB/BXH rat recombinant inbred strain platform: a newly enhanced tool for cardiovascular, behavioral, and developmental genetics and genomics. *J Appl Physiol*. 2003;94:2510-22.

65. Pravenec M, Zidek V, Musilova A, Simakova M, Kostka V, Mlejnek P, Kren V, Krenova D, Bila V, Mikova B, Jachymova M, Horky K, Kazdova L, St Lezin E, Kurtz TW. Genetic analysis of metabolic defects in the spontaneously hypertensive rat. *Mamm Genome*. 2002;13:253-8.

66. Bielavska E, Kren V, Musilova A, Zidek V, Pravenec M. Genome scanning of the HXB/BXH sets of recombinant inbred strains of the rat for quantitative

trait loci associated with conditioned taste aversion. *Behav Genet*. 2002;32:51-6.

67. Bila V, Kren V, Liska F. The influence of the genetic background on the interaction of retinoic acid with Lx mutation of the rat. *Folia Biol (Praha)*. 2000;46:264-72.

68. Buresova M, Zidek V, Musilova A, Simakova M, Fucikova A, Bila V, Kren V, Kazdova L, Di Nicolantonio R, Pravenec M. Genetic relationship between placental and fetal weights and markers of the metabolic syndrome in rat recombinant inbred strains. *Physiol Genomics*. 2006;26:226-31.

69. Williams RW, Gu J, Qi S, Lu L. The genetic structure of recombinant inbred mice: high-resolution consensus maps for complex trait analysis. *Genome Biol*. 2001;2:RESEARCH0046.

70. Gavras I, Manolis A, Gavras H. Genetic epidemiology of essential hypertension. *J Hum Hypertens*. 1999;13:225-9.

71. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature*. 2006;444:881-7.

72. Murray C, Lopez A. *The Global Burden of Disease*. Cambridge: Harvard University Press; 1996.

73. Reddy KS, Naik N, Prabhakaran D. Hypertension in the developing world: a consequence of progress. *Curr Cardiol Rep*. 2006;8:399-404.

74. Ward R. Familial aggregation and genetic epidemiology of blood pressure. In: Laragh JH, Brenner, B.M., ed. *Hypertension: Pathophysiology, Diagnosis and Management.* 2 ed. New York: Raven Press; 1995:67-88.

75. Hollenberg NK. Genes, hypertension, and intermediate phenotypes. *Curr Opin Cardiol*. 1996;11:457-63.

76. Cowley AW, Jr. The genetic dissection of essential hypertension. *Nat Rev Genet*. 2006;7:829-40.

77. Stoll M, Kwitek-Black AE, Cowley AW, Jr., Harris EL, Harrap SB, Krieger JE, Printz MP, Provoost AP, Sassard J, Jacob HJ. New target regions for human hypertension via comparative genomics. *Genome Res*. 2000;10:473-82.

78. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci*. 2006;7:335-46.

79. Takiyyuddin MA, Cervenka JH, Sullivan PA, Pandian MR, Parmer RJ, Barbosa JA, O'Connor DT. Is physiologic sympathoadrenal catecholamine release exocytotic in humans? *Circulation*. 1990;81:185-95.

80. Hagberg JM, Hickson RC, McLane JA, Ehsani AA, Winder WW. Disappearance of norepinephrine from the circulation following strenuous exercise. *J Appl Physiol*. 1979;47:1311-4.

81. DeQuattro V, Feng M. The sympathetic nervous system: the muse of primary hypertension. *J Hum Hypertens*. 2002;16 Suppl 1:S64-9.

82. Esler M. The sympathetic system and hypertension. *Am J Hypertens*. 2000;13:99S-105S.

83. Greifenkamp JD, DiPette DJ. Adrenal medulla. *Curr Hypertens Rep*. 1999;1:241-5.

84. Anderson EA, Sinkey CA, Lawton WJ, Mark AL. Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. *Hypertension*. 1989;14:177-83.

85. Mancia G, Grassi G, Giannattasio C, Seravalle G. Sympathetic Activation in the Pathogenesis of Hypertension and Progression of Organ Damage. *Hypertension*. 1999;34:724-728.

86. Cabassi A, Vinci S, Calzolari M, Bruschi G, Borghetti A. Regional sympathetic activity in pre-hypertensive phase of spontaneously hypertensive rats. *Life Sci*. 1998;62:1111-8.

87. Wenzel RR, Bruck H, Noll G, Schafers RF, Daul AE, Philipp T. Antihypertensive drugs and the sympathetic nervous system. *J Cardiovasc Pharmacol*. 2000;35:S43-52.

88. Rabbia F, Martini G, Cat Genova G, Milan A, Chiandussi L, Veglio F. Antihypertensive drugs and sympathetic nervous system. *Clin Exp Hypertens*. 2001;23:101-11.

89. Nagatsu T, Kato T, Numata Y, Keiko I, Umezawa H. Serum dopamine beta-hydroxylase activity in developing hypertensive rats. *Nature*. 1974;251:630-1.

90. Grobecker G, Roizen MF, Weise V, Saavedra JM, Kopin IJ. Sympathoadrenal medullary activity in young, spontaneously hypertensive rats. *Nature*. 1975;258:267-8.

91. Nagaoka A, Lovenberg W. Plasma norepinephrine and dopamine-beta-hydroxylase in genetic hypertensive rats. *Life Sci*. 1976;19:29-34.

92. Borkowski KR, Quinn P. Adrenaline and the development of spontaneous hypertension in rats. *J Auton Pharmacol*. 1985;5:89-100.

93. Borkowski KR. Effect of adrenal demedullation and adrenaline on hypertension development and vascular reactivity in young spontaneously hypertensive rats. *J Auton Pharmacol*. 1991;11:1-14.

94. Lee RM, Borkowski KR, Leenen FH, Tsoporis J, Coughlin M. Combined effect of neonatal sympathectomy and adrenal demedullation on blood pressure and vascular changes in spontaneously hypertensive rats. *Circ Res*. 1991;69:714-21.

95. Grobecker H, Saavedra JM, Weise VK. Biosynthetic enzyme activities and catecholamines in adrenal glands of genetic and experimental hypertensive rats. *Circ Res*. 1982;50:742-6.

96. Moura E, Pinho Costa PM, Moura D, Guimaraes S, Vieira-Coelho MA. Decreased tyrosine hydroxylase activity in the adrenals of spontaneously hypertensive rats. *Life Sci*. 2005;76:2953-64.

97. Nakamura K. Enhanced sympathetic activity in young spontaneously hypertensive rats is not the trigger mechanism for genetic hypertension. *Naunyn Schmiedebergs Arch Pharmacol*. 1977;299:143-8.

98. Teitelman G, Ross RA, Joh TH, Reis DJ. Differences in utero in activities of catecholamine biosynthetic enzymes in adrenals of spontaneously hypertensive rats. *Clin Sci (Lond)*. 1981;61 Suppl 7:227s-230s.

99. Kumai T, Tanaka M, Watanabe M, Kobayashi S. Elevated tyrosine hydroxylase mRNA levels in the adrenal medulla of spontaneously hypertensive rats. *Jpn J Pharmacol*. 1994;65:367-9.

100. Kumai T, Tanaka M, Tateishi T, Watanabe M, Nakura H, Asoh M, Kobayashi S. Effects of anti-androgen treatment on the catecholamine synthetic pathway in the adrenal medulla of spontaneously hypertensive rats. *Naunyn Schmiedebergs Arch Pharmacol*. 1998;357:620-4.

101. Yamabe H, De Jong W, Lovenberg W. Further studies on catecholamine synthesis in the spontaneously hypertensive rat: catecholamine synthesis in the central nervous system. *Eur J Pharmacol*. 1973;22:91-8.

102. Ciaranello RD, Hoffman HJ, Shire JG, Axelrod J. Genetic regulation of the catecholamine biosynthetic enzymes. II. Inheritance of tyrosine hydroxylase, dopamine-beta-hydroxylase, and phenylethanolamine N-methyltransferase. *J Biol Chem*. 1974;249:4528-36.

103. Petretto E, Mangion J, Dickens NJ, Cook SA, Kumaran MK, Lu H, Fischer J, Maatz H, Kren V, Pravenec M, Hubner N, Aitman TJ. Heritability and tissue specificity of expression quantitative trait loci. *PLoS Genet*. 2006;2:e172.

104. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*. 2003;4:249-64.

105. O'Connor DT, Frigon RP, Stone RA. Human pheochromocytoma dopamine-beta-hydroxylase: purification and molecular parameters of the tetramer. *Mol Pharmacol*. 1979;16:529-38.

106. Kennedy B, Bigby TD, Ziegler MG. Nonadrenal epinephrine-forming enzymes in humans. Characteristics, distribution, regulation, and relationship to epinephrine levels. *J Clin Invest*. 1995;95:2896-902.

107. Ziegler MG, Kennedy B, Elayan H. A sensitive radioenzymatic assay for epinephrine forming enzymes. *Life Sci*. 1988;43:2117-22.

108. Kennedy B, Ziegler MG. A more sensitive and specific radioenzymatic assay for catecholamines. *Life Sci*. 1990;47:2143-53.

109. Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biol*. 2003;4:R7.

110. Grubbs FE. Procedures for detecting outlying observations in samples. *Technometrics*. 1969;11:1-21.

111. Belknap JK. Effect of within-strain sample size on QTL detection and mapping using recombinant inbred mouse strains. *Behav Genet*. 1998;28:29-38.

112. Basten CJ, Weir BS, Zeng ZB. *QTL Cartographer: A Reference Manual and Tutorial for QTL Mapping.*: Department of Statistics, North Carolina State University, Raleigh, NC; 2002.

113. Manly KF, Wang J, Williams RW. QTL Reaper. In. Memphis, Tennessee: University of Tennessee Health Science Center; 2004.

114. Doerge RW, Churchill GA. Permutation tests for multiple loci affecting a quantitative character. *Genetics*. 1996;142:285-94.

115. Van Ooijen JW. Accuracy of mapping quantitative trait loci in autogamous species. *Theor Appl Genet.* 1992;84:803-11.

116. Visscher PM, Thompson R, Haley CS. Confidence intervals in QTL mapping by bootstrapping. *Genetics*. 1996;143:1013-20.

117. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol*. 2000;132:365-86.

118. Colella S, Shen L, Baggerly KA, Issa JP, Krahe R. Sensitive and quantitative universal Pyrosequencing methylation analysis of CpG sites. *Biotechniques*. 2003;35:146-50.

119. Esch E, Weber E. Investigation of crossover interference in barley (Hordeum vulgare L.) using the coefficient of coincidence. *Theor Appl Genet*. 2002;104:786-796.

120. Betts MJ, Russell RB. *Amino acid properties and consequences of subsitutions*: Wiley; 2003.

121. Ungermann C, Langosch D. Functions of SNAREs in intracellular membrane fusion and lipid bilayer mixing. *J Cell Sci*. 2005;118:3819-28.

122. Kuchel O, Racz K, Debinski W, Buu NT. A defective beta-hydroxylation of dopamine may precede the full development of hypertension in spontaneously hypertensive rats. *Can J Cardiol*. 1989;5:327-31.

123. Saavedra JM, Grobecker H, Axelrod J. Changes in central catecholaminergic neurons in the spontaneously (genetic) hypertensive rat. *Circ Res*. 1978;42:529-34.

124. Howes LG, Rowe PR, Summers RJ, Louis WJ. Age related changes of catecholamines and their metabolites in central nervous system regions of spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. *Clin Exp Hypertens A*. 1984;6:2263-77.

125. Cornish JL, Wilks DP, Van den Buuse M. A functional interaction between the mesolimbic dopamine system and vasopressin release in the regulation of blood pressure in conscious rats. *Neuroscience*. 1997;81:69-78.

126. van den Buuse M. Pressor responses to brain dopaminergic stimulation. *Clin Exp Pharmacol Physiol*. 1997;24:764-9.

127. Takami T, Ito H, Suzuki T. Decreased norepinephrine content in the medulla oblongata in severely hypertensive rats. *Clin Exp Pharmacol Physiol*. 1993;20:161-7.

128. Oparil S, Yang RH, Jin HK, Wyss JM, Chen YF. Central mechanisms of hypertension. *Am J Hypertens*. 1989;2:477-85.

129. Shigetomi S, Buu NT, Kuchel O. Dopaminergic abnormalities in borderline essential hypertensive patients. *Hypertension*. 1991;17:997-1002.

130. DeQuattro V, Campese V, Lurvey A, Yen G, Kypridakis G. Low response of serum dopamine beta-hydroxylase to stimuli in primary hypertension. Decreased DbetaH response in hypertension. *Biochem Med*. 1976;15:1-9.

131. Zabetian CP, Romero R, Robertson D, Sharma S, Padbury JF, Kuivaniemi H, Kim KS, Kim CH, Kohnke MD, Kranzler HR, Gelernter J, Cubells JF. A revised allele frequency estimate and haplotype analysis of the DBH deficiency mutation IVS1+2T --> C in African- and European-Americans. *Am J Med Genet A*. 2003;123:190-2.

132. Her S, Bell RA, Bloom AK, Siddall BJ, Wong DL. Phenylethanolamine N-methyltransferase gene expression. Sp1 and MAZ potential for tissue-specific expression. *J Biol Chem*. 1999;274:8698-707.

133. Cui J, Zhou X, Chazaro I, DeStefano AL, Manolis AJ, Baldwin CT, Gavras H. Association of polymorphisms in the promoter region of the PNMT gene with essential hypertension in African Americans but not in whites. *Am J Hypertens*. 2003;16:859-63.

134. Rana BK, Insel PA, Payne SH, Abel K, Beutler E, Ziegler MG, Schork NJ, O'Connor DT. Population-based sample reveals gene-gender interactions in blood pressure in White Americans. *Hypertension*. 2007;49:96-106.

135. Koike G, Jacob HJ, Krieger JE, Szpirer C, Hoehe MR, Horiuchi M, Dzau VJ. Investigation of the phenylethanolamine N-methyltransferase gene as a candidate gene for hypertension. *Hypertension*. 1995;26:595-601.

136. Wong DL. Epinephrine biosynthesis: hormonal and neural control during stress. *Cell Mol Neurobiol*. 2006;26:891-900.

137. Wurtman RJ. Stress and the adrenocortical control of epinephrine synthesis. *Metabolism*. 2002;51:11-4.

138. Bao X, Lu CM, Liu F, Gu Y, Dalton ND, Zhu BQ, Foster E, Chen J, Karliner JS, Ross J, Jr., Simpson PC, Ziegler MG. Epinephrine is required for normal cardiovascular responses to stress in the phenylethanolamine N-methyltransferase knockout mouse. *Circulation*. 2007;116:1024-31.

139. Taupenot L, Harper KL, O'Connor DT. The chromogranin-secretogranin family. *N Engl J Med*. 2003;348:1134-49.

140. Henry JP, Botton D, Sagne C, Isambert MF, Desnos C, Blanchard V, Raisman-Vozari R, Krejci E, Massoulie J, Gasnier B. Biochemistry and molecular

biology of the vesicular monoamine transporter from chromaffin granules. *J Exp Biol*. 1994;196:251-62.

141. Mahapatra NR, O'Connor DT, Vaingankar SM, Hikim AP, Mahata M, Ray S, Staite E, Wu H, Gu Y, Dalton N, Kennedy BP, Ziegler MG, Ross J, Mahata SK. Hypertension from targeted ablation of chromogranin A can be rescued by the human ortholog. *J Clin Invest*. 2005;115:1942-52.

142. Richards M, Iijima Y, Kondo H, Shizuno T, Hori H, Arima K, Saitoh O, Kunugi H. Association study of the vesicular monoamine transporter 1 (VMAT1) gene with schizophrenia in a Japanese population. *Behav Brain Funct*. 2006;2:39.

143. Lohoff FW, Dahl JP, Ferraro TN, Arnold SE, Gallinat J, Sander T, Berrettini WH. Variations in the vesicular monoamine transporter 1 gene (VMAT1/SLC18A1) are associated with bipolar i disorder. *Neuropsychopharmacology*. 2006;31:2739-47.

144. Doss S, Schadt EE, Drake TA, Lusis AJ. Cis-acting expression quantitative trait loci in mice. *Genome Res*. 2005;15:681-91.

145. McClintick JN, Edenberg HJ. Effects of filtering by Present call on analysis of microarray experiments. *BMC Bioinformatics*. 2006;7:49.

146. Birney E, Andrews D, Caccamo M, Chen Y, Clarke L, Coates G, Cox T, Cunningham F, Curwen V, Cutts T, Down T, Durbin R, Fernandez-Suarez XM, Flicek P, Graf S, Hammond M, Herrero J, Howe K, Iyer V, Jekosch K, Kahari A, Kasprzyk A, Keefe D, Kokocinski F, Kulesha E, London D, Longden I, Melsopp C, Meidl P, Overduin B, Parker A, Proctor G, Prlic A, Rae M, Rios D, Redmond S, Schuster M, Sealy I, Searle S, Severin J, Slater G, Smedley D, Smith J, Stabenau A, Stalker J, Trevanion S, Ureta-Vidal A, Vogel J, White S, Woodwark C, Hubbard TJ. Ensembl 2006. *Nucleic Acids Res*. 2006;34:D556-61.

147. Anderson M. DISTLM v.5. In: University of Auckland; 2004:Distance-based multivariate analysis for a linear model.

148. McArdle B, Anderson M. Fitting Multivariate Models to Community Data: A Comment on Distance-Based Redundancy Analysis. *Ecology*. 2001;82:290–297.

149. Zapala MA, Schork NJ. Multivariate regression analysis of distance matrices for testing associations between gene expression patterns and related variables. *Proc Natl Acad Sci U S A*. 2006;103:19430-5.

150. Zapala MA, Schork NJ. Statistical properties of multivariate distance matrix regression for high-dimensional data analysis. *Communications in Statistic: Simulation and Computation*. 2007;(submitted).

151. Gower JC. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika*. 1966;53:325-338.

152. Johnson R, Wichern D. *Applied multivariate statistical analysis*. 3 ed. Englewood Cliffs, New Jersey: Prentice-Hall; 1992.

153. Gunther E, Walter L. The major histocompatibility complex of the rat (Rattus norvegicus). *Immunogenetics*. 2001;53:520-42.

154. Yuryev A, Patturajan M, Litingtung Y, Joshi RV, Gentile C, Gebara M, Corden JL. The C-terminal domain of the largest subunit of RNA polymerase II interacts with a novel set of serine/arginine-rich proteins. *Proc Natl Acad Sci U S A*. 1996;93:6975-80.

155. Meinhart A, Cramer P. Recognition of RNA polymerase II carboxy-terminal domain by 3'-RNA-processing factors. *Nature*. 2004;430:223-6.

156. Berezney R, Mortillaro MJ, Ma H, Wei X, Samarabandu J. The nuclear matrix: a structural milieu for genomic function. *Int Rev Cytol*. 1995;162A:1-65.

157. Bottner CA, Schmidt H, Vogel S, Michele M, Kaufer NF. Multiple genetic and biochemical interactions of Brr2, Prp8, Prp31, Prp1 and Prp4 kinase suggest a function in the control of the activation of spliceosomes in Schizosaccharomyces pombe. *Curr Genet*. 2005;48:151-61.

158. Dellaire G, Makarov EM, Cowger JJ, Longman D, Sutherland HG, Luhrmann R, Torchia J, Bickmore WA. Mammalian PRP4 kinase copurifies and interacts with components of both the U5 snRNP and the N-CoR deacetylase complexes. *Mol Cell Biol*. 2002;22:5141-56.

159. Schwelnus W, Richert K, Opitz F, Gross T, Habara Y, Tani T, Kaufer NF. Fission yeast Prp4p kinase regulates pre-mRNA splicing by phosphorylating a non-SR-splicing factor. *EMBO Rep*. 2001;2:35-41.

160. Alahari SK, Schmidt H, Kaufer NF. The fission yeast prp4+ gene involved in pre-mRNA splicing codes for a predicted serine/threonine kinase and is essential for growth. *Nucleic Acids Res*. 1993;21:4079-83.

161. Stojdl DF, Bell JC. SR protein kinases: the splice of life. *Biochem Cell Biol*. 1999;77:293-8.

162. Tennyson CN, Klamut HJ, Worton RG. The human dystrophin gene requires 16 hours to be transcribed and is cotranscriptionally spliced. *Nat Genet*. 1995;9:184-90.

163. Fry CJ, Peterson CL. Chromatin remodeling enzymes: who's on first? *Curr Biol*. 2001;11:R185-97.

164. Ioannidu S, Walter L, Dressel R, Gunther E. Physical map and expression profile of genes of the telomeric class I gene region of the rat MHC. *J Immunol*. 2001;166:3957-65.

165. Dressel R, Walter L, Gunther E. Genomic and funtional aspects of the rat MHC, the RT1 complex. *Immunol Rev*. 2001;184:82-95.

166. Voigt B, Kuramoto T, Mashimo T, Tsurumi T, Sasaki Y, Hokao R, Serikawa T. Evaluation of LEXF/FXLE rat recombinant inbred strains for the genetic dissection of complex traits. *Physiol Genomics*. 2007.

167. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S, Field SF, Payne F, Lowe CE, Szeszko JS, Hafler JP, Zeitels L, Yang JH, Vella A, Nutland S, Stevens HE, Schuilenburg H, Coleman G, Maisuria M, Meadows W, Smink LJ, Healy B, Burren OS, Lam AA, Ovington NR, Allen J, Adlem E, Leung HT, Wallace C, Howson JM, Guja C, Ionescu-Tirgoviste C, Simmonds MJ, Heward JM, Gough SC, Dunger DB, Wicker LS, Clayton DG. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet*. 2007;39:857-64.

168. Topol EJ, Frazer KA. The resequencing imperative. *Nat Genet*. 2007;39:439-40.

169. Churchill GA, et al. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet*. 2004;36:1133-7.

170. Valdar W, Flint J, Mott R. Simulating the collaborative cross: power of quantitative trait loci detection and mapping resolution in large sets of recombinant inbred strains of mice. *Genetics*. 2006;172:1783-97.

171. Ring HZ, Kwok PY, Cotton RG. Human Variome Project: an international collaboration to catalogue human genetic variation. *Pharmacogenomics*. 2006;7:969-72.

172. Glossary of terms. *J Exp Biol*. 2007;210:1492-6.