DOE JOINT GENOME INSTITUTE

US DEPARTMENT OF ENERGY

OFFICE OF SCIENCE

LBNL-58994

Introduction to the DOE-Joint Genome Institute

Martha Lucia Posada-Buitrago, Ph.D.
Molecular Biologist

Production Genomic Facility

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Opened in 1999
~240 UC Employees
60,000 sf
~$66M Annual Budget

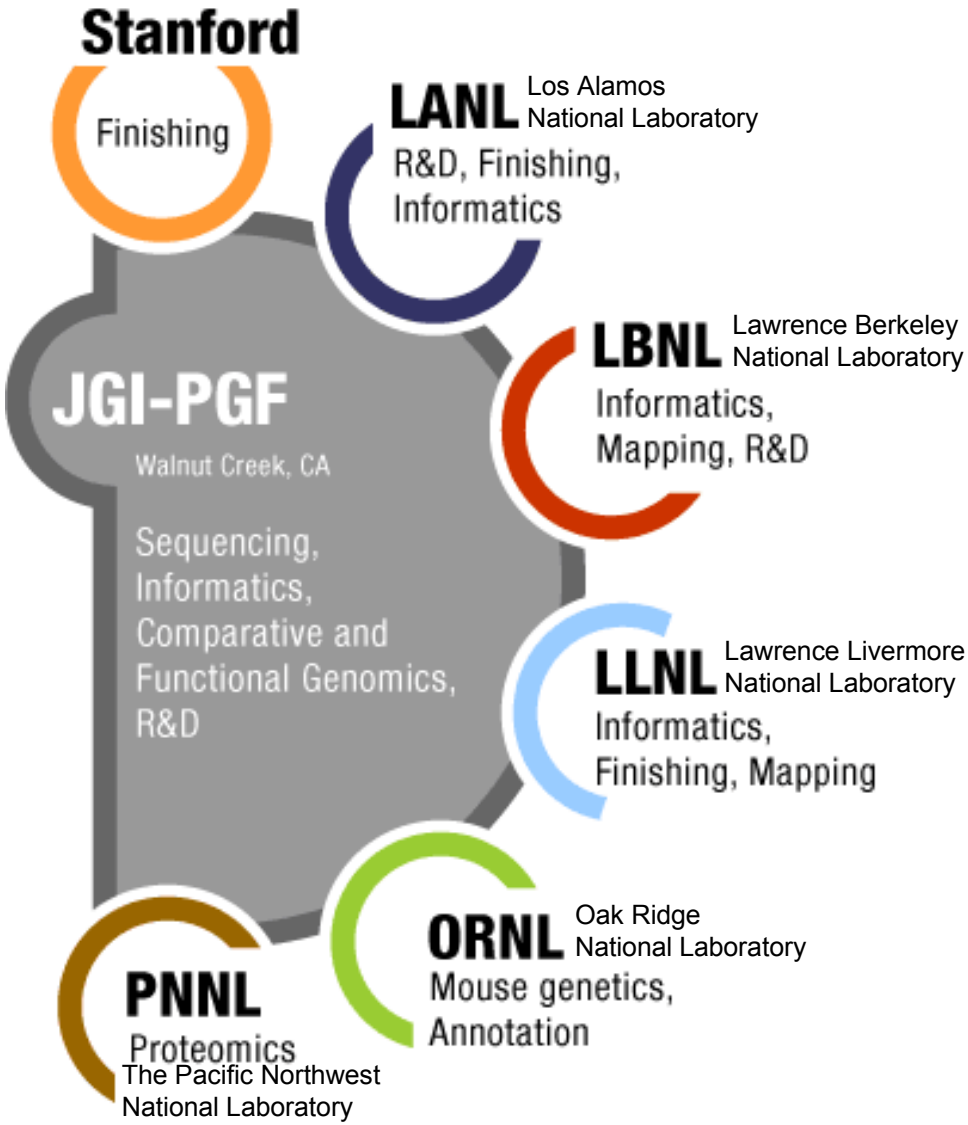
Mission:

To develop and exploit new sequencing and other high-throughput, genome-scale and computational technologies as a means for discovering and characterizing the basic principles and relationships underlying the organization, function, and evolution of living systems.

JGI Partnerships

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE



DNA Sequencing Production at the JGI

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

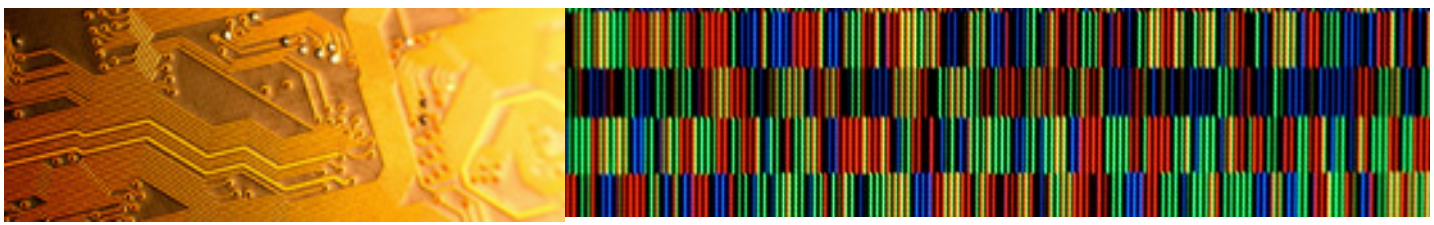
U.S. D.O.E. JOINT GENOME INSTITUTE

- April 2002: 1 gb/month
- January 2004: 2 gb/month
- July 2004: 2.5 gb/month
- March 2005: 3.1 gb/month

(equivalent to 1 human genome/month)

Total (3/99-4/05) 82.893 gb

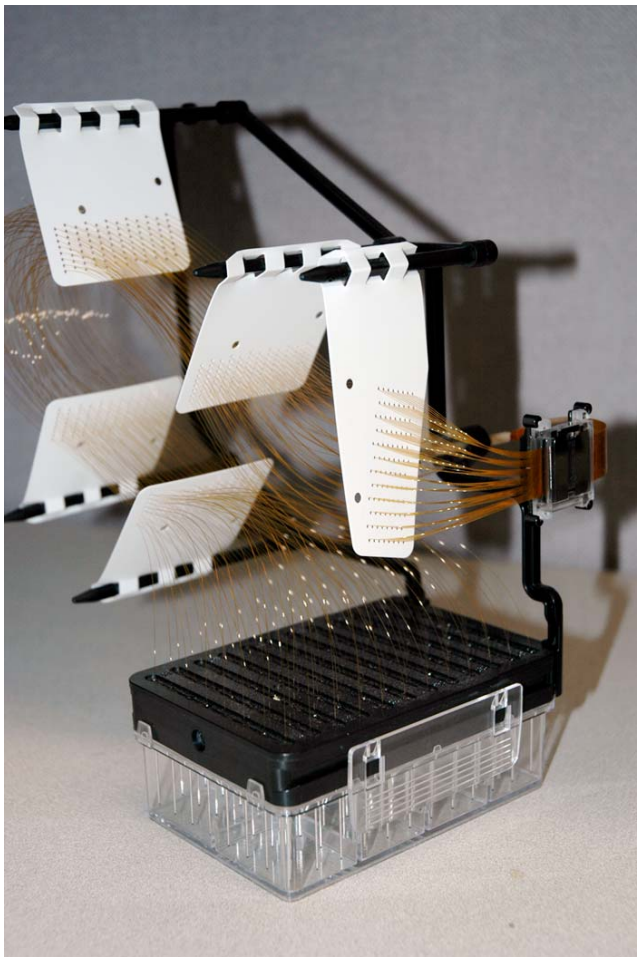
(equivalent of sequencing 27 human genomes)



Automated DNA Sequencing

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

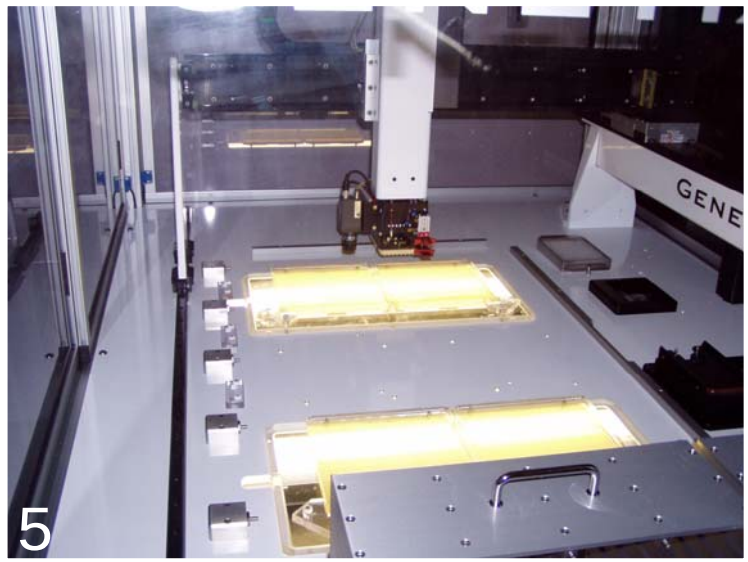
U.S. D.O.E. JOINT GENOME INSTITUTE

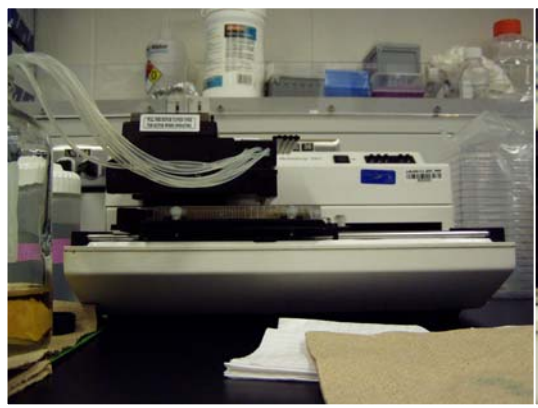


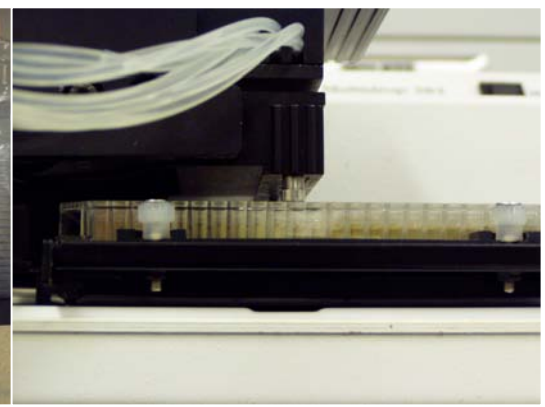
DNA sequencing process
Library Construction Group

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE







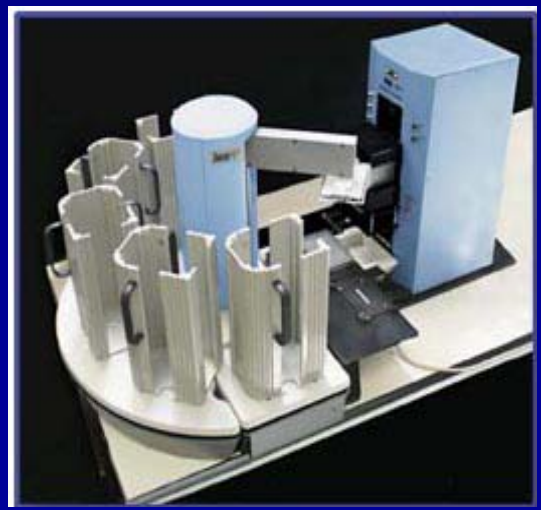
1. Shear DNA
2. Ligate into pUC18
3. Transform
4. Plate
5. Pick colonies
6. Grow overnight

DNA sequencing process
Rolling circle amplification of plasmid clones for sequencing template

1. PlateMate adds lysis buffer to small amount of culture

2. Cells are heat-lysed

3. Hydra 384's with Twister arms add RCA reagents.



4. ON incubation

JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

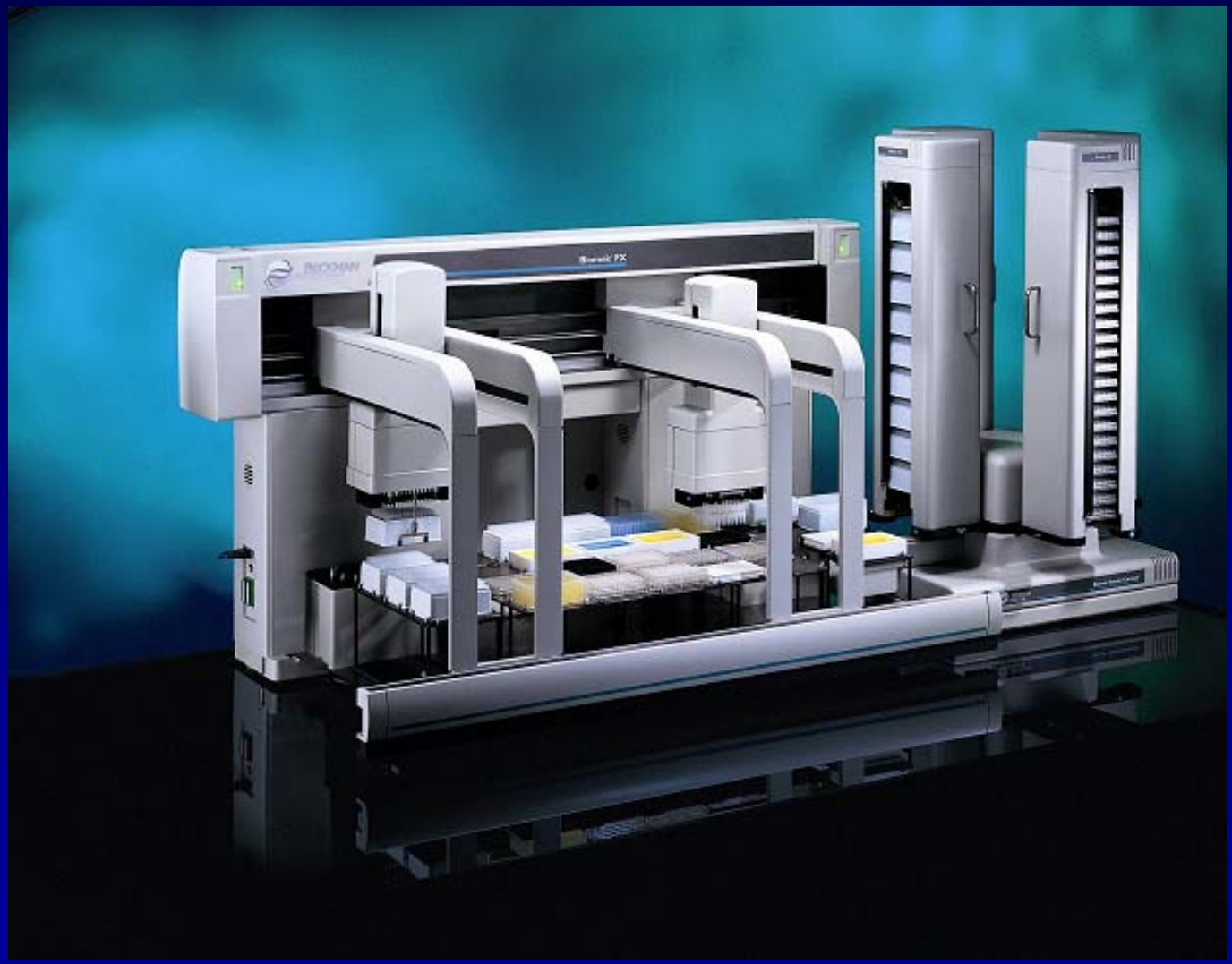
DNA sequencing process
Sequencing Chemistry

F and R reactions are separated with hydra 384's with twister arms

Sequencing reagents are added with Cavro Dispense System

. . . more sequencing chemistry

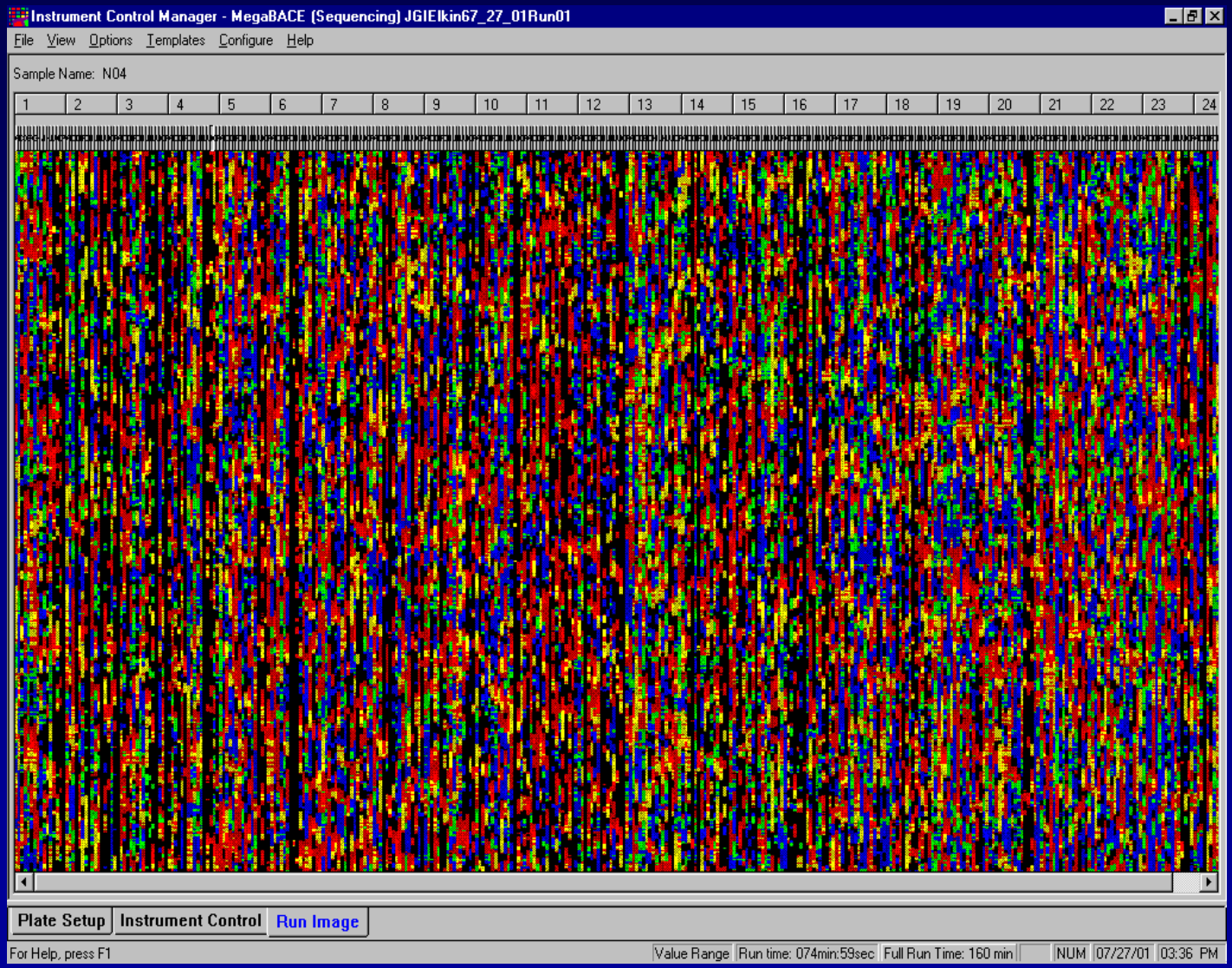
Sequence reactions with Quad-head PCR machines, then clean-up using BioMek robots and SPRI



DNA sequencing process
Capillary Group

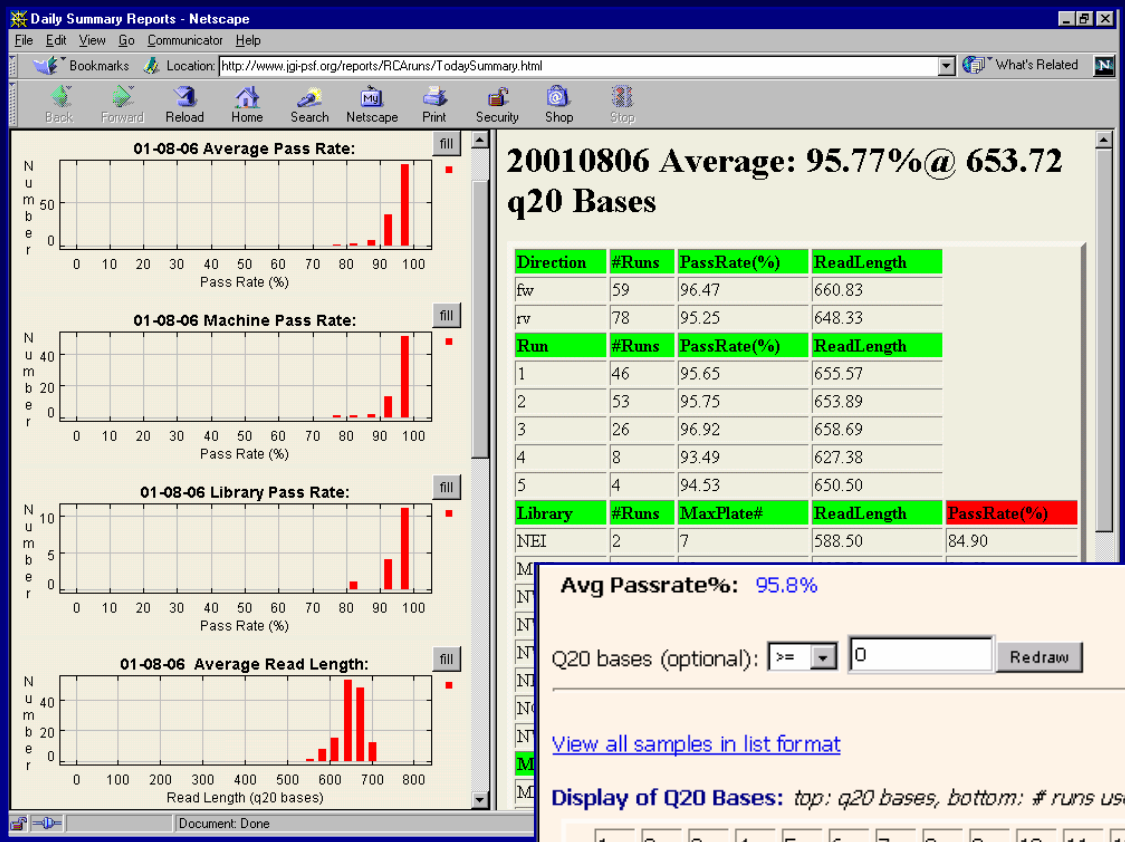
35 MegaBACE 4000
60 ABI 3730

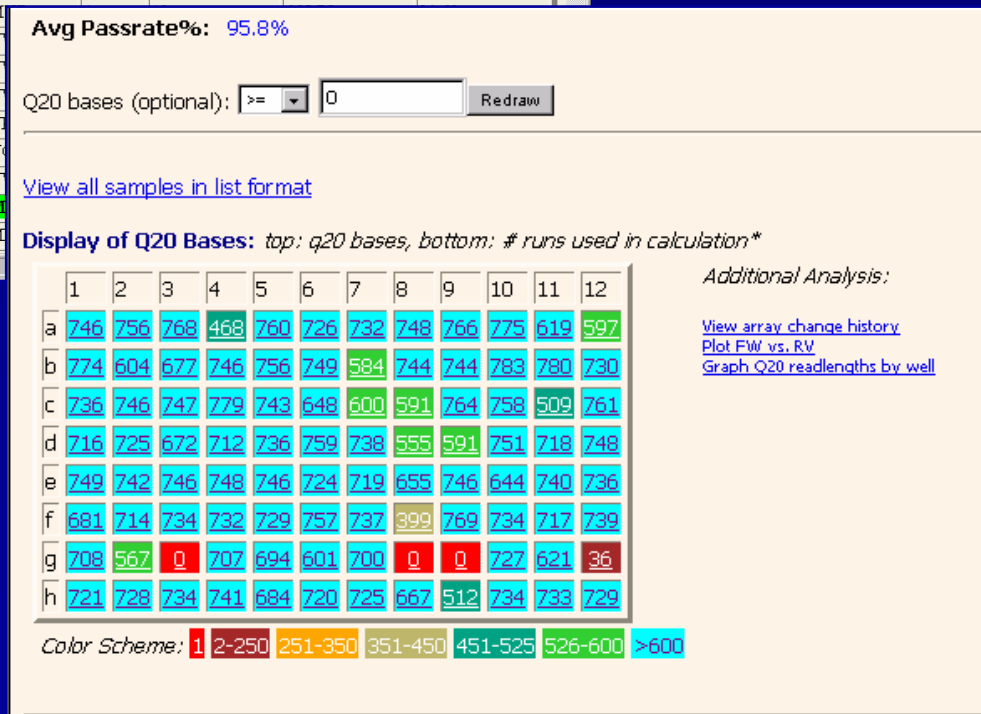
Q20 / month = 3.1 Gb



Online tracking of progress

LIMS uses bar code readers at every step and allows real time tracking of all reagents, personnel, and processes



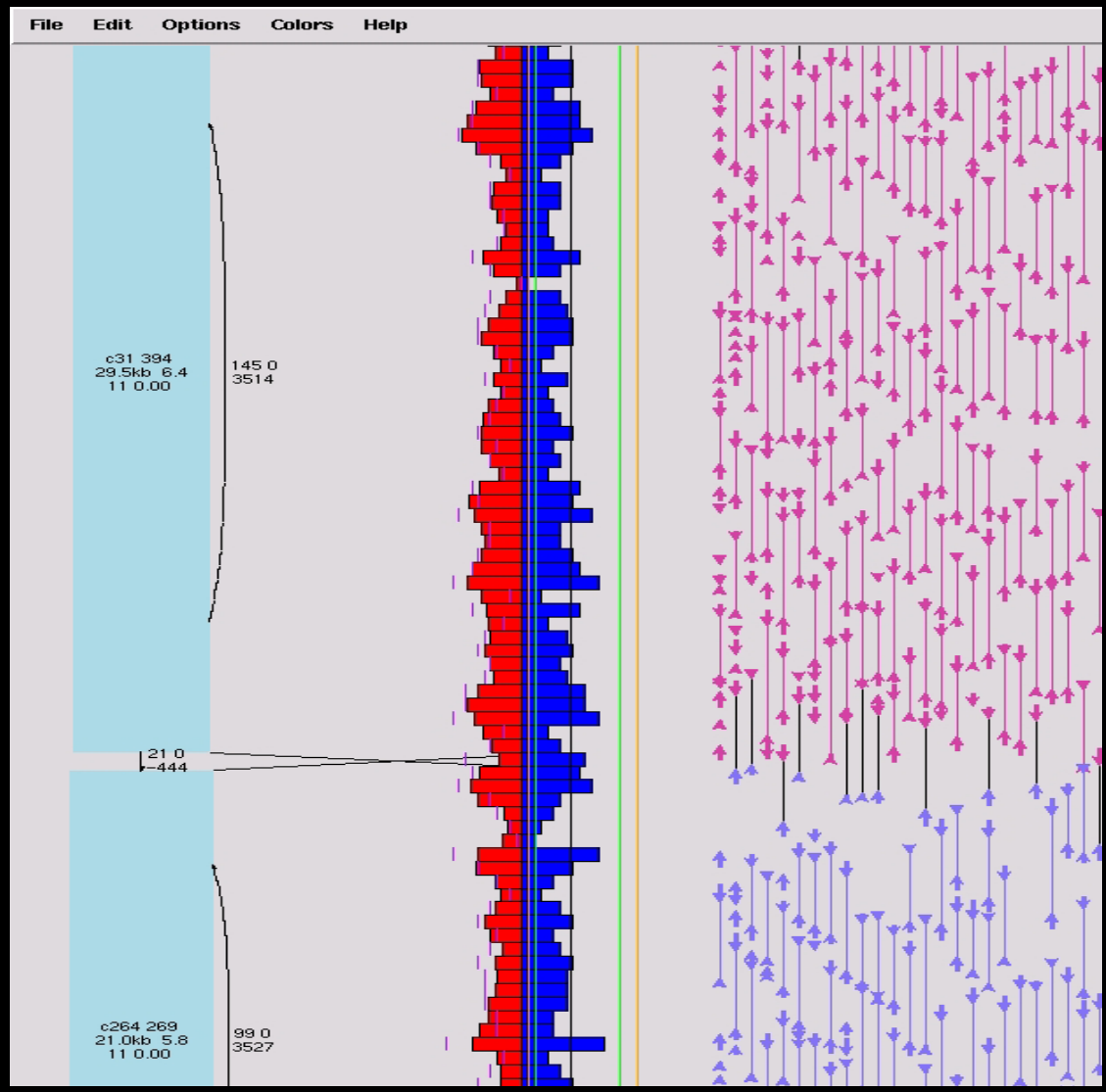


DNA Sequence Assembly

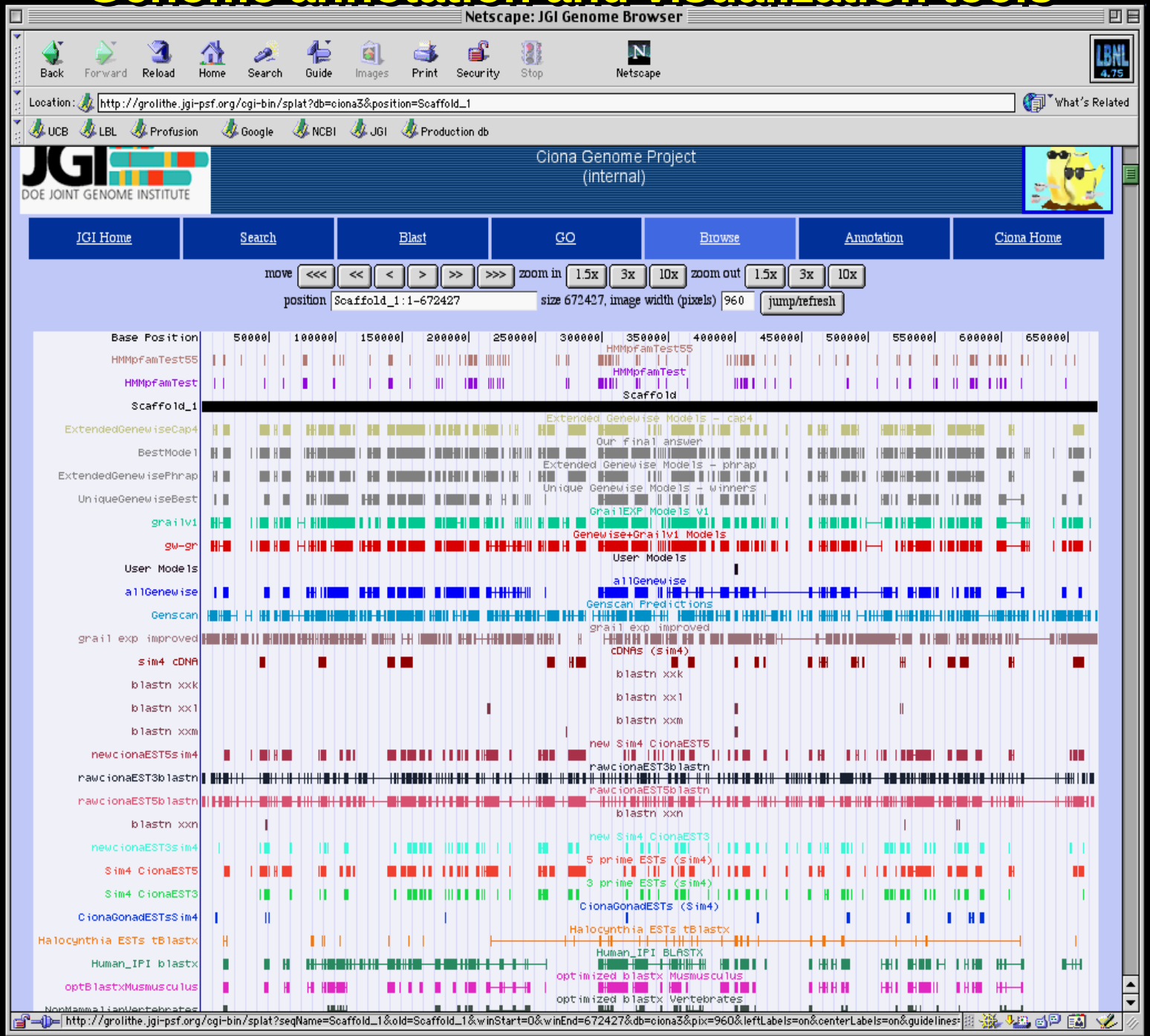
Informatics team assembles, verifies, annotates genomes

Best assemblies come from end sequences from a mixture of clone sizes.

Typically, the JGI makes 3 libraries:
3-4 Kb in plasmids
8-10 Kb in plasmids
40 Kb in fosmids



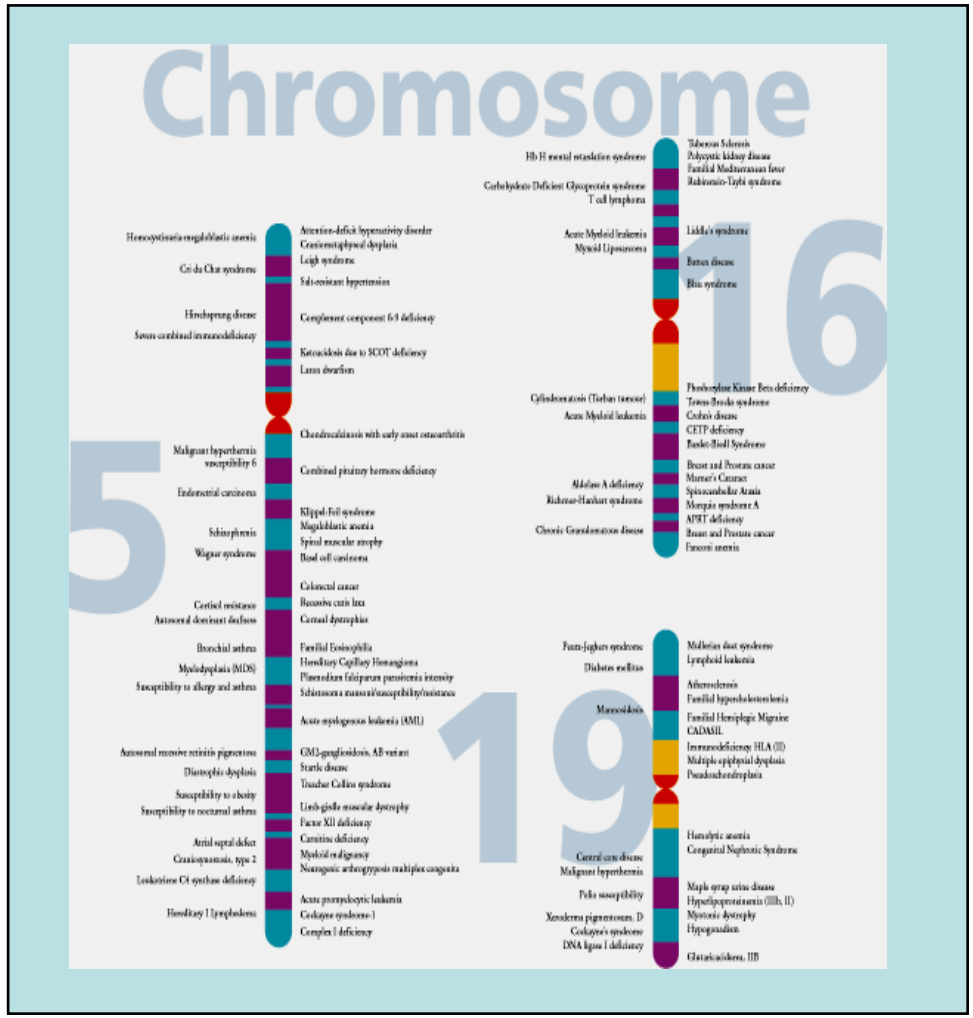
Genome annotation and visualization tools



11% of Human Genome Sequenced by JGI

JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

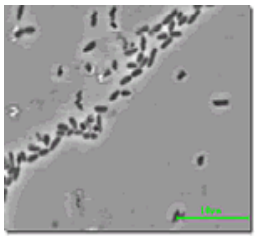
U.S. D.O.E. JOINT GENOME INSTITUTE



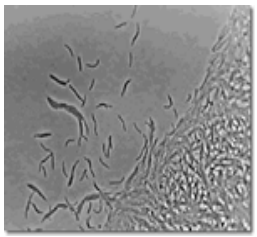
Selected JGI Microbes

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

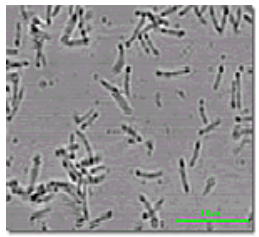
U.S. D.O.E. JOINT GENOME INSTITUTE



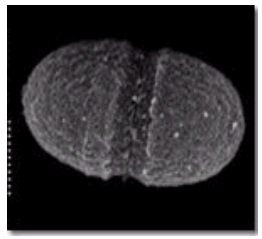
Burkholderia cepacia



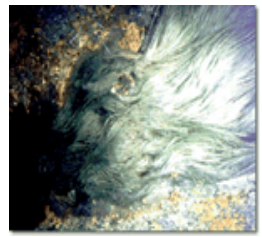
Cytophaga hutchinsonii



Desulfitobacterium halfniense

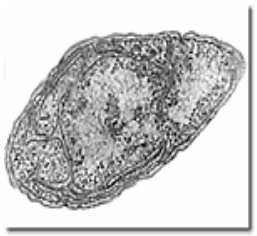


Enterococcus faecium



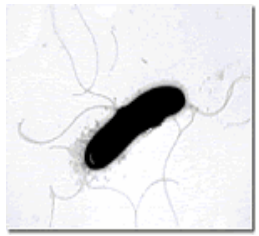
Ferroplasma acidarmanus

Magnetospirillum magnetotacticum

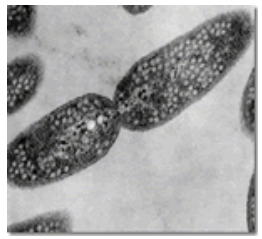


Nitrosomonas europaea

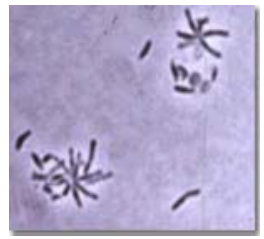
Prochlorococcus marinus



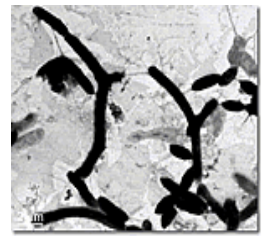
Pseudomonas fluorescens



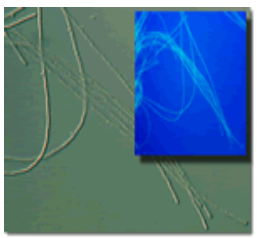
Rhodobacter sphaeroides



Rhodopseudomonas palustris



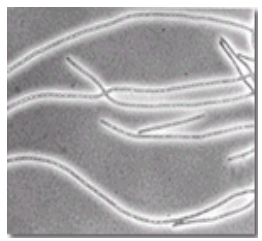
Sphingomonas aromaticivorans



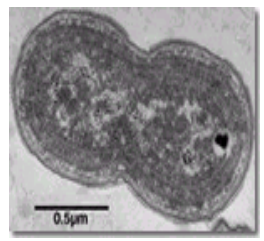
Thermomonospora fusca

Trichodesmium erythraeum

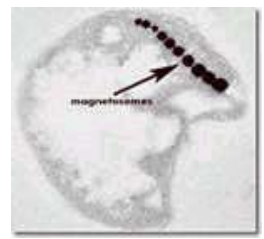
Xylella fastidiosa



Nostoc punctiforme



Marine synechococcusß

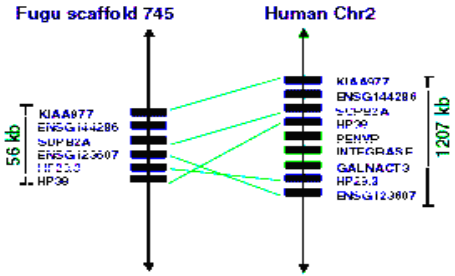


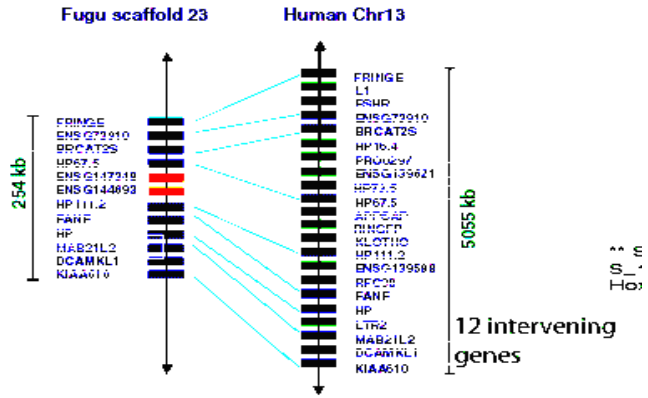
Magnetococcus MC-1

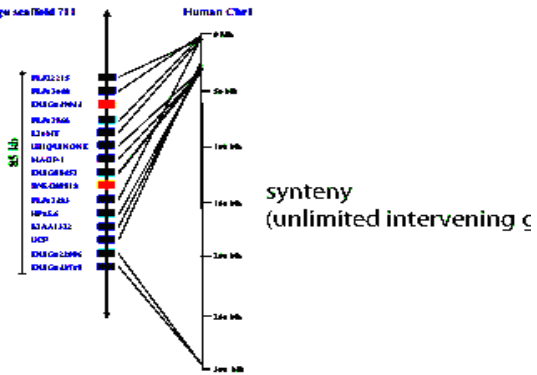
Pufferfish Genome

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE



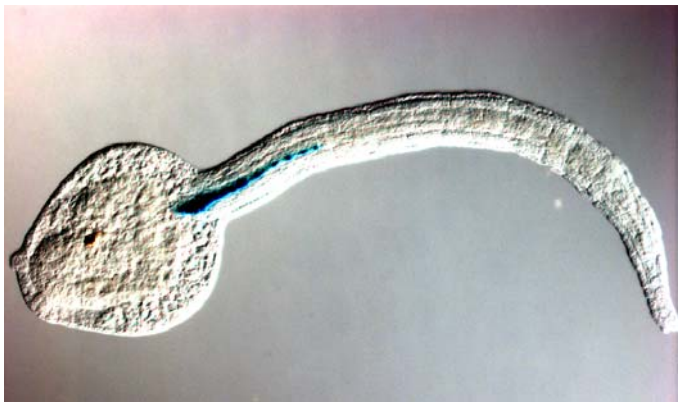


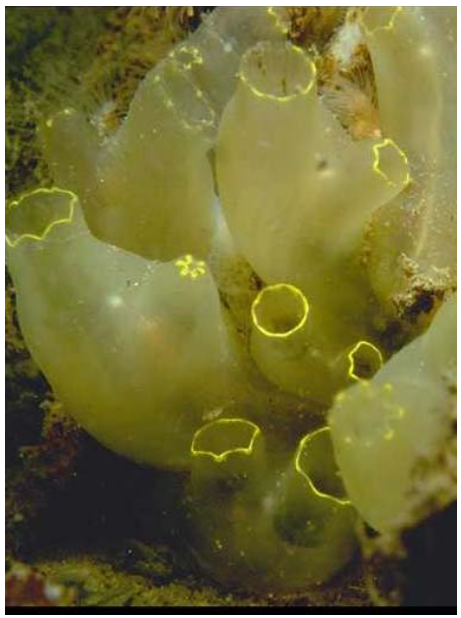


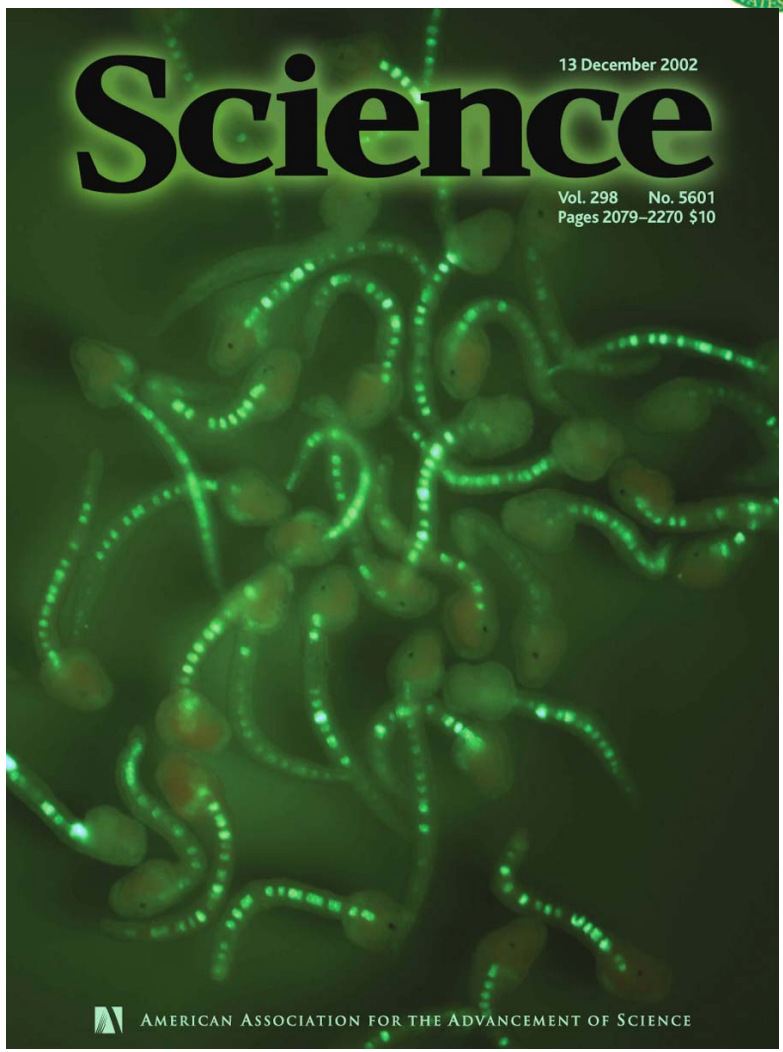
Ciona intestinalis
A Primitive Chordate

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE



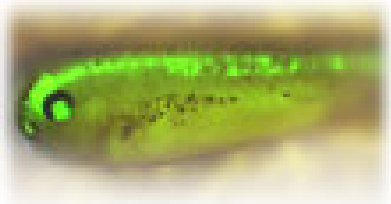




Xenopus tropicalis

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE



- Close relative of the well-studied *X. laevis*, a major model organism for developmental biology
- Favorite system for toxicology (EPA)
- Coordinated with
  - WashU BAC map project
  - cDNA projects at NIH, Sanger
  - other projects from international frog research community
- 7x coverage by early ‘05

Fungi (rots and plant pathogens)

Trichoderma reesei **possesses a host of carbohydrate degrading enzymes and is used extensively in industrial processes.**

Phakopsora pachyrhizi & P. meibomiae

Soybean rust was recently found in US.

White rot fungi like ***P. chrysosporium*** **are uniquely able to degrade lignin, the second most abundant natural polymer and a major component of biomass**

Highly repetitive sequence

Sudden Oak Death

- In partnership with the USDA, NSF, VBI, California Oak Mortality Taskforce, County Ag Commissioners, City of Walnut Creek, WC Chamber of Commerce.
- 4 TV Stations; various print media

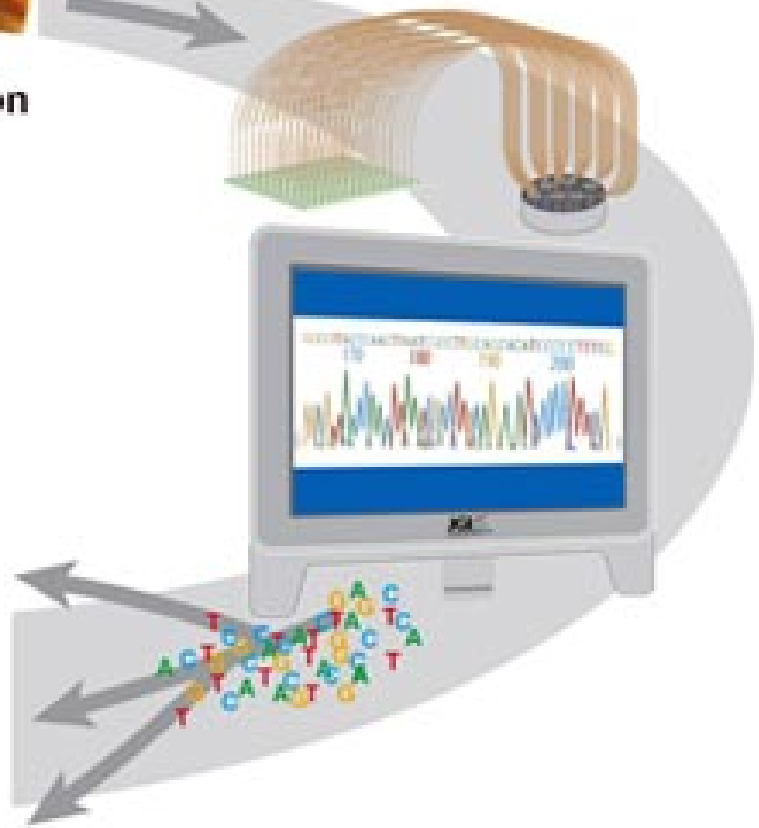
California Agriculture

Cave Bear

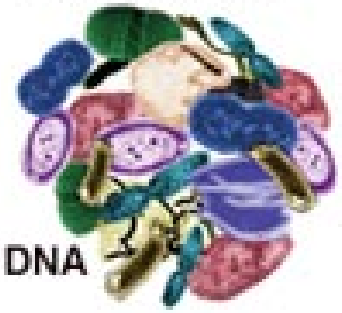
40,000-year-old DNA

DNA Extraction
(tooth)

High Throughput
DNA Sequencing



DNA
Comparisons



94% Microbe/Other DNA

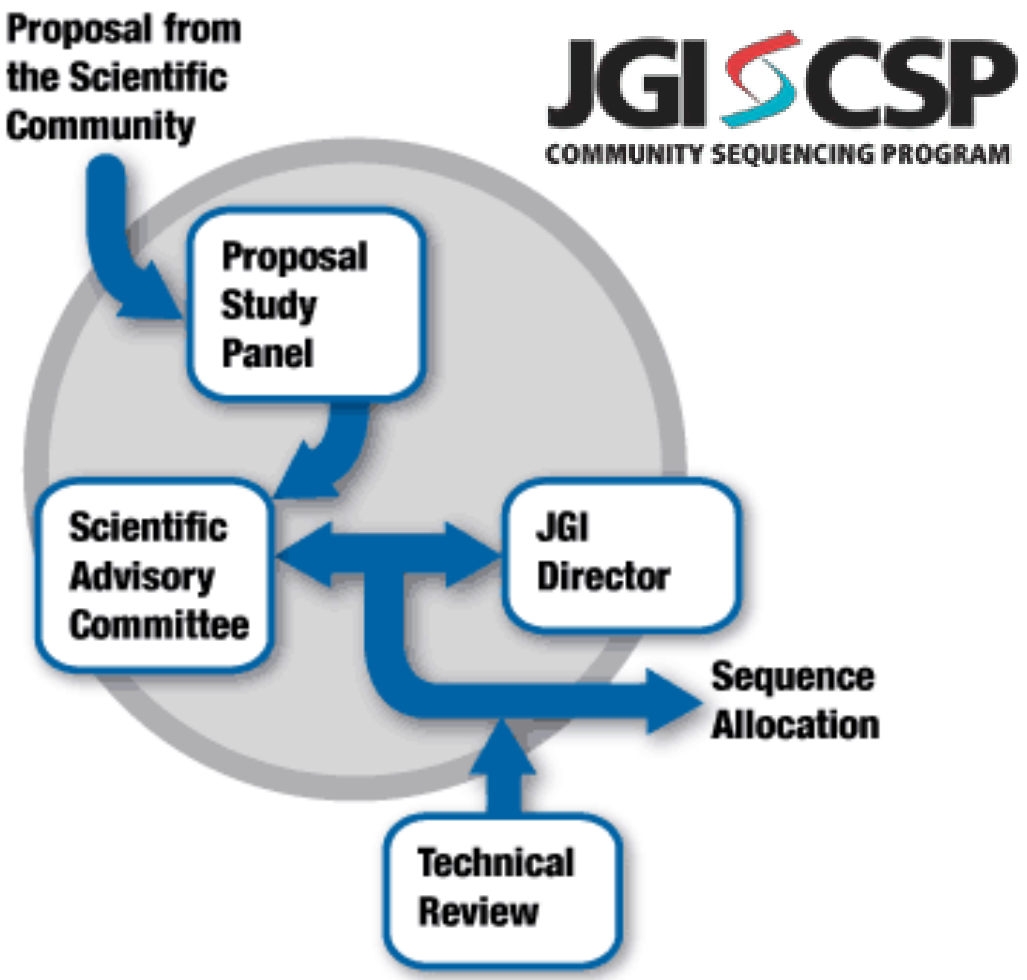
6% Cave Bear DNA

JGI Community Sequencing Program
"Non-traditional User Facility"

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

- **Allocation of ~15gigabases/year for sequencing to advance the frontiers of science supported by DOE**
- **56 Proposals received in Feb. '04 totaling 100Gb in requested sequencing (equivalent to the current WW sequencing capacity)**
- **150 Proposals received in Feb. '05.**
- **SAC approved 23 projects beginning Fall '04**
- **New RFP Spring '06**



JGI

DOE-Joint Genome Institute
http://www.jgi.doe.gov

img

integrated microbial genomes
http://img.jgi.doe.gov/v1.1/main.cgi

PhIGs

Phylogenetically Inferred Groups
http://phigs.jgi-psf.org/

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Genomics, Gene Expression and other Studies in Soybean Rust

Martha Lucía Posada-Buitrago Ph.D

Genomics Division
Evolutionary Genomics

DOE- Joint Genome Institute
Lawrence Berkeley National Laboratory

Soybean Rust

Caused by two species of fungi:

Phakopsora pachyrhizi

aka “Old World” or “Asian” isolate

More aggressive pathogen.

Phakopsora meibomiae

aka “New World” or “American” isolate

Not as aggressive

Soybean rust hosts

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

LEGUMES (Papilionoideae)

Cultivated Crops:

- ***Glycine max* (soybeans)***
- ***Phaseolus lunatus* (lima and butter beans)***
- ***Phaseolus vulgaris* (green beans, kidney beans)**
- ***Vigna unguiculata* (cowpeas)***
- ***Cajanus cajan* (pigeon peas)**
- ***Pachyrhizus erosus* (yam bean, jicama)***

Ornamental plants:

Hyacinth bean, lupine, royal poinciana

Wild hosts:

Kudzu, sweet clover

Kudzu infected with soybean rust

Photos by Reid D. Frederick

Soybean Rust in the World

Phakopsora pachyrhizi

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

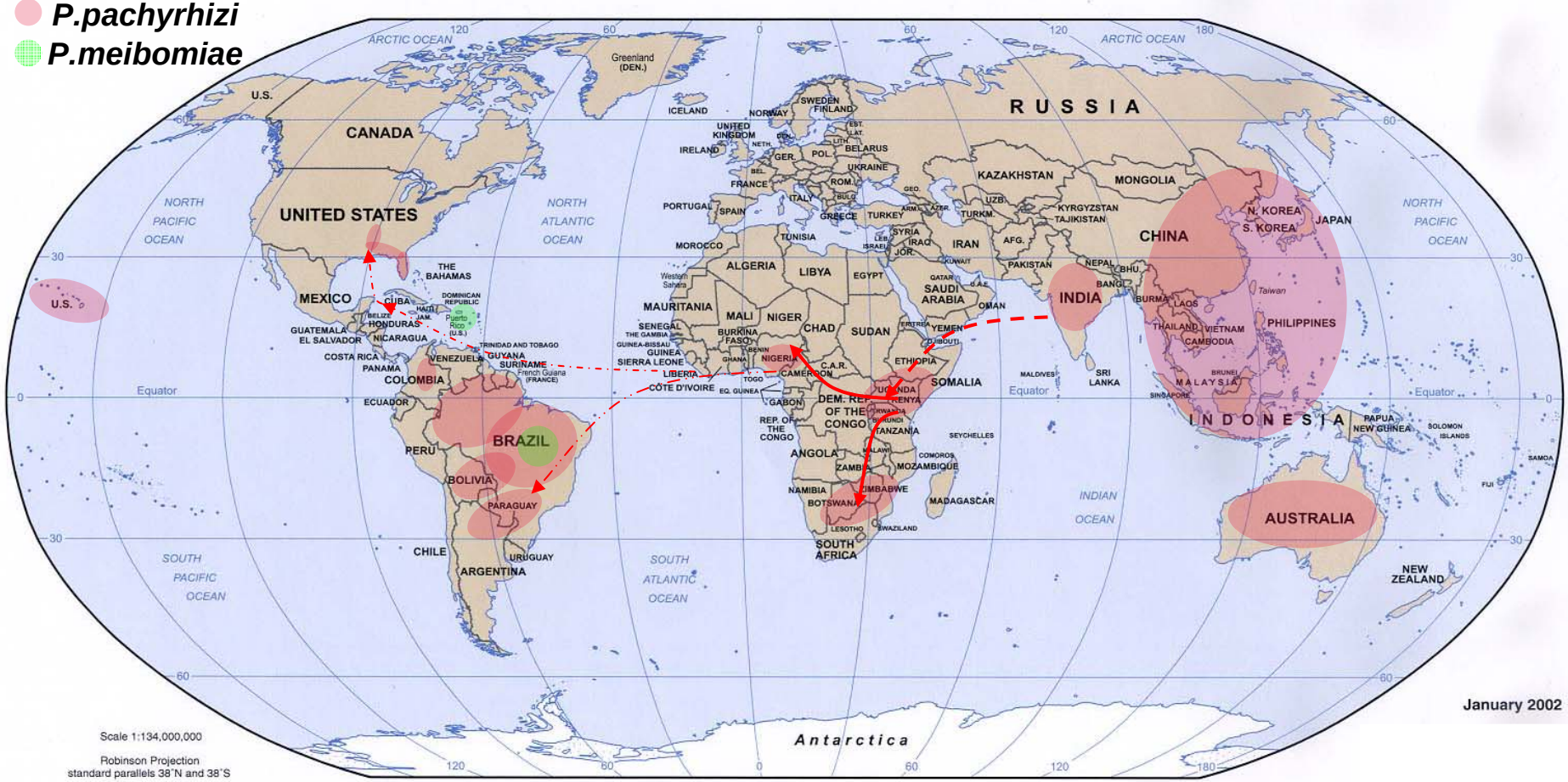
Japan	1904	
Kenya	1997/1998	Thought to be windborne from Asia
Nigeria	1997/1998	
Rwanda	1997/1998	
Zimbabwe	1997/1998	
South Africa	2001	Thought to be windborne from Africa
Paraguay	2001/2002	
Brazil	2002	
Argentine	2002	
Bolivia	2003	
Colombia	2004	Hurricane Ivan
USA	Oct 2004	

Soybean Rust in the World

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

- *P.pachyrhizi*
- *P.meibomiae*



Soybean Rust Effects

Premature defoliation

Yield decrease characterized by:

- **Increase in number of unfilled pods/plant**
- **Decrease in number of normal pods/plant**
- **Decrease in number of seeds/plant**
- **Decrease in weight of seed/plant**
- **Decrease in 1000-seed weight**
- **Decrease in germinability of seed**

Soybean fields (Zimbabwee)

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Photos by Reid D. Frederick

Symptoms

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Photos by Reid D. Frederick

Symptoms

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Infected cotyledons




Infected stem





Infected pods


Photos by Christine Stone

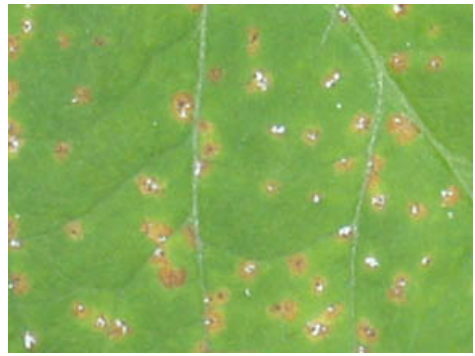
Symptoms

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Infected leaves


9 dpi

12 dpi

15 dpi

18 dpi

Photos by Christine Stone

GENOME SEQUENCING PROJECT

Phakopsora pachyrhizi
Phakopsora meibomiae

Initial Genome Project Strategy

Random shotgun libraries:

General 3kb insert size in vector pUC18,
Mid-size 8-10kb insert in vector p21
Fosmid (40kb insert size) in pCC1FOS

cDNA libraries from different stages of *P.pachyrhizi* (in pSPORT1)

Sequencers:

ABI3730
MegaBACE 4000

Informatics:

Reads processing by Phred
Reads assembly by Phrap
Verification
Genome annotation

	Library (Insert size)	Bases sequenced
P. pachyrhizi	3 Kb	146.60 Mb
	8 Kb	264.28 Mb
	40 Kb	5.75 Mb
Total		416.63 MB
P. Meibomiae	3 Kb	125.20 Mb
	8 Kb	5.97 Mb
Total		131.17

DOE-JGI Data by 27.05.05

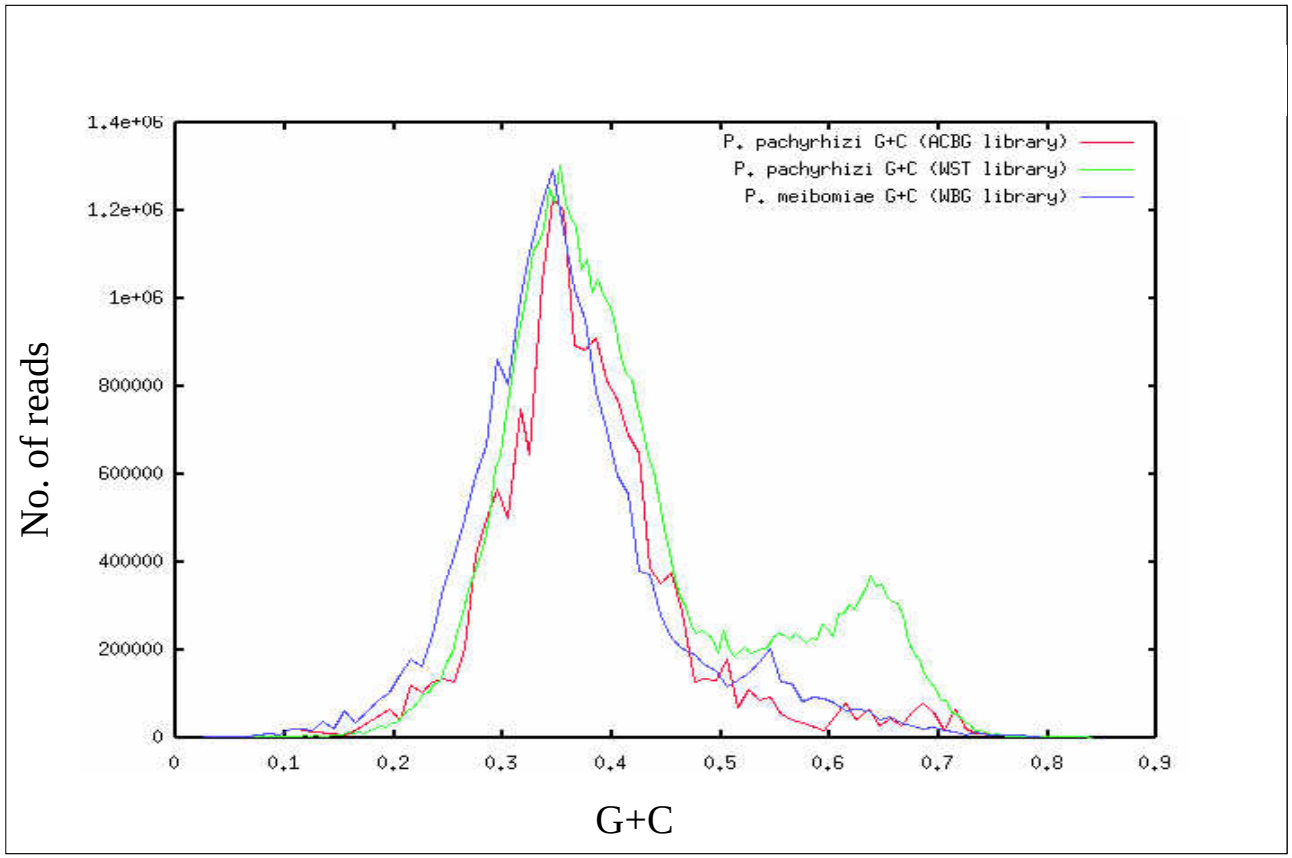
Several independent methods were used to estimate the genome size. Although there were considerable uncertainties associated with most of the methods, they consistently yielded a genome size above 500 MB.

Estimation Method	Genome Size
cDNA Coverage	720 Mb
All-Pairs Read Alignment	500-800 Mb
Gene Density	300-700 Mb
Shotgun Fosmid Coverage	600-950 Mb

G+C content *in P. pachyrhizi* and *P. meibomiae*

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE



Phakopsora pachyrhizi and Phakopsora meibomiae G + C content estimation

The mean G+C content *in P. pachyrhizi* and *P. meibomiae* is 34-35%, estimated with the "G+C content program" (Chapman) on sequences from three different genomic libraries.

Fosmid sequencing

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Random fosmids

Stanford University:

Finished	87
Incomplete	28

Selected fosmids

Lawrence Livermore National Laboratory:

Probes designed for	120
Selected	50
To go	70
Sequencing	24
Finished	0

Probes designed based on ESTs selected by high similarity to “interesting” genes from other fungi and unknown genes highly expressed in germinating spores from *P. pachyrhizi.*

MITOCHONDRIAL GENOMES

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Known mitochondrial genome sequences were blasted against the entire set of reads. Potential mitochondrial sequences were assembled with the Phred Phrap Package. This resulted in single contig assemblies for both fungal mitochondrial genomes.

Genome analysis and annotation:

DOGMA Dual Organellar GenoMe Annotator (http:// bugmaster.jgi-psf.org/dogma).

tRNAscan-SE 1.21 (http:// www.genetics.wustl.edu/eddy/tRNAscan-SE/)

MacVector 7.1 (Accelrys)

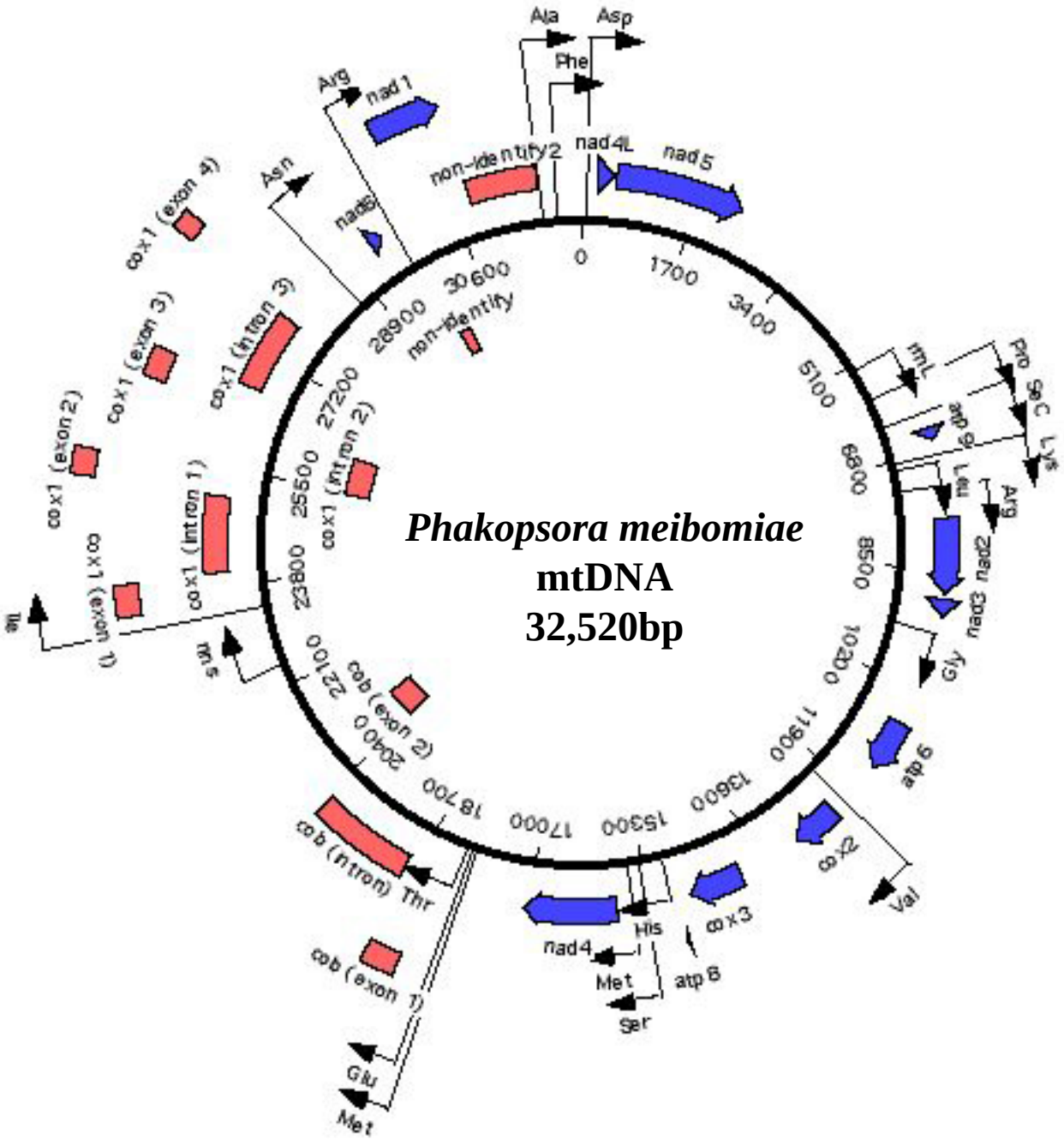
Blast algorithm

Mitochondrial Genomes

The complete nucleotide sequence of the mitochondrial (mt) genome was determined for *Phakopsora pachyrhizi* and *P. meibomiae.*

These 32 kb genomes contain the genes encoding ATP synthase subunits 6, 8, and 9 (atp6, atp8, and atp9), cytochrome oxidase subunits I, II, and III (cox1, cox2, and cox3), apocytochrome b (cob), reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunits (nad1, nad2, nad3, nad4, nad4L, nad5, and nad6), the large and small mitochondrial ribosomal RNAs (rrns and rrnl) and tRNAs for all amino acids.

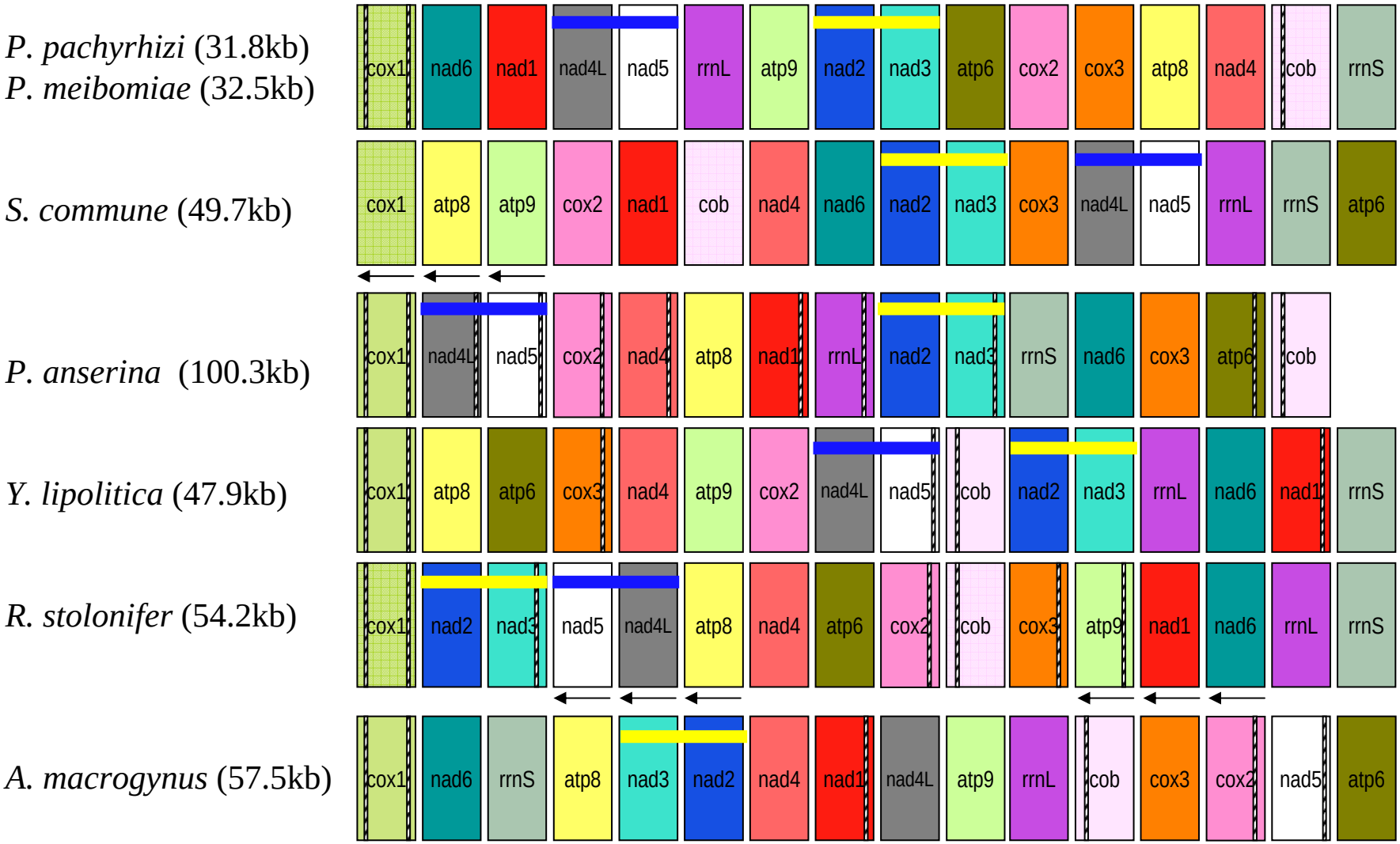
	P. Pachyrhizi	*P. meibomiae*
Size	**31.82 Kb**	**32.52 Kb**
G+C	**34.6 %**	**34.9 %**



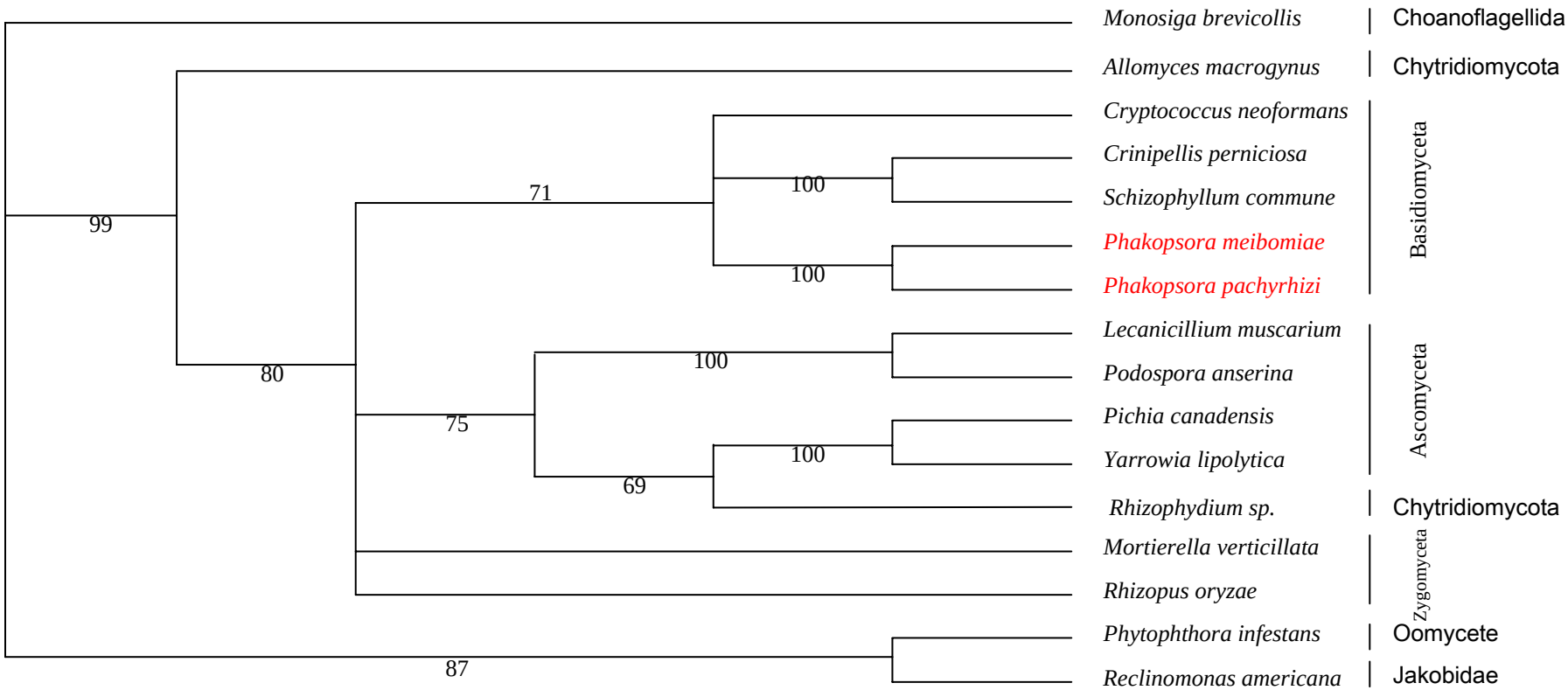
Phakopsora meibomiae

mtDNA

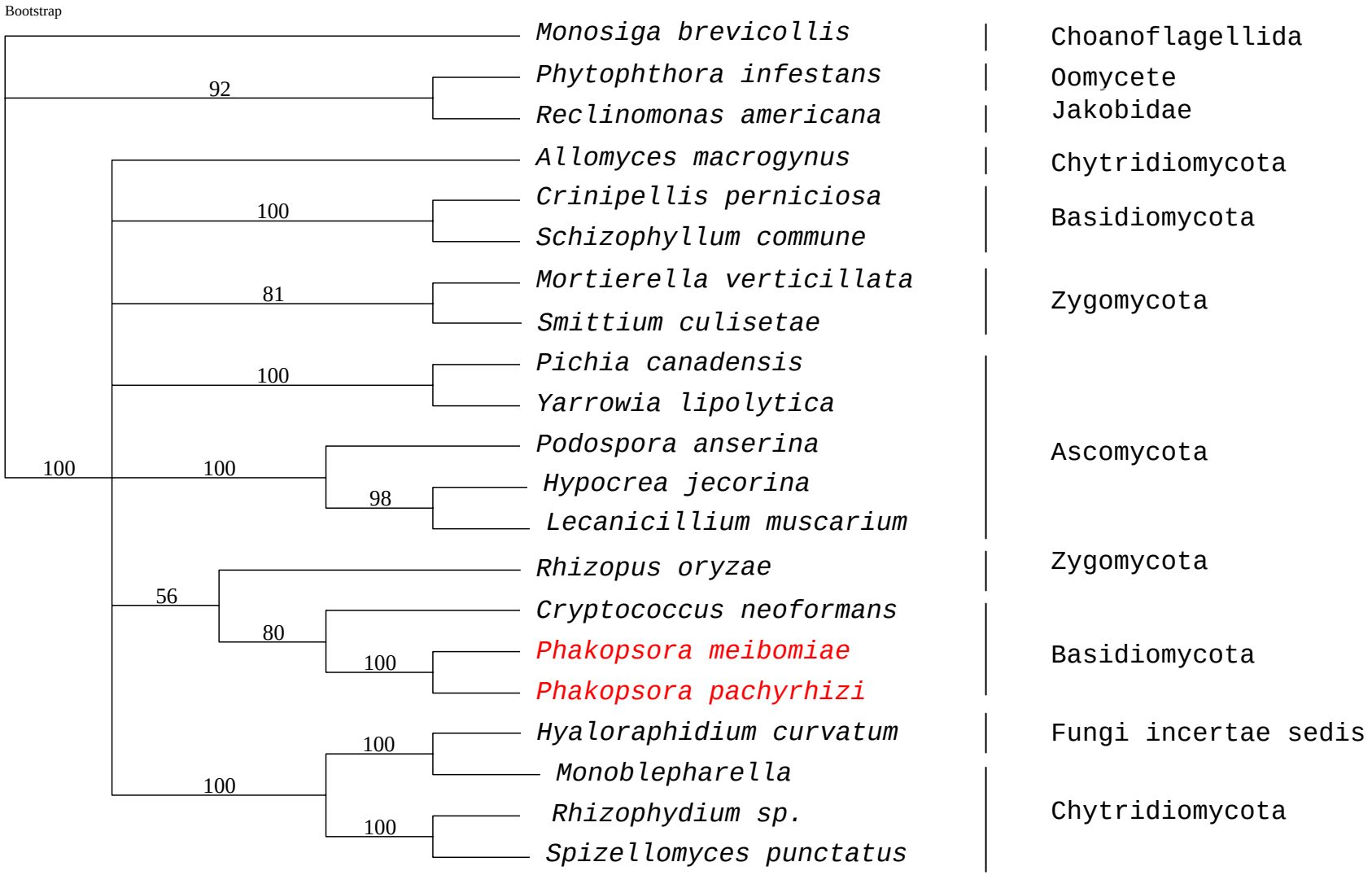
32,520bp



Comparison of mitochondrial genomes from the four phyla of fungi. Protein-coding and rRNA genes are represented by boxes; arrows indicate the direction of transcription. Lines within genes represent presence of intron(s).



Phylogenetic tree of 1582 amino acid position from four mitochondrial-encoded proteins from 16 taxa. The genes encoding cob, cox1, nad1 and nad5 are present in all organisms compared. Parsimony-bootstrap support was calculated from 100 replicates using Paup 4.0b10. *Monosiga brevicollis*, *Phytophthora infestans* and *Reclinomonas americana* were included as outgroups.



Phylogenetic tree of 1296 amino acid position from seven mitochondrial-encoded proteins from 21 taxa, including 18 species from all fungal phyla and *Monosiga brevicollis*, *Phytophthora infestans* and *Reclinomonas americana* as outgroups. The genes encoding cob, cox1, cox2, cox3, nad1, nad4 and nad5 are present in all organisms compared. Parsimony-bootstrap support was calculated from 100 replicates using Paup 4.0b10.

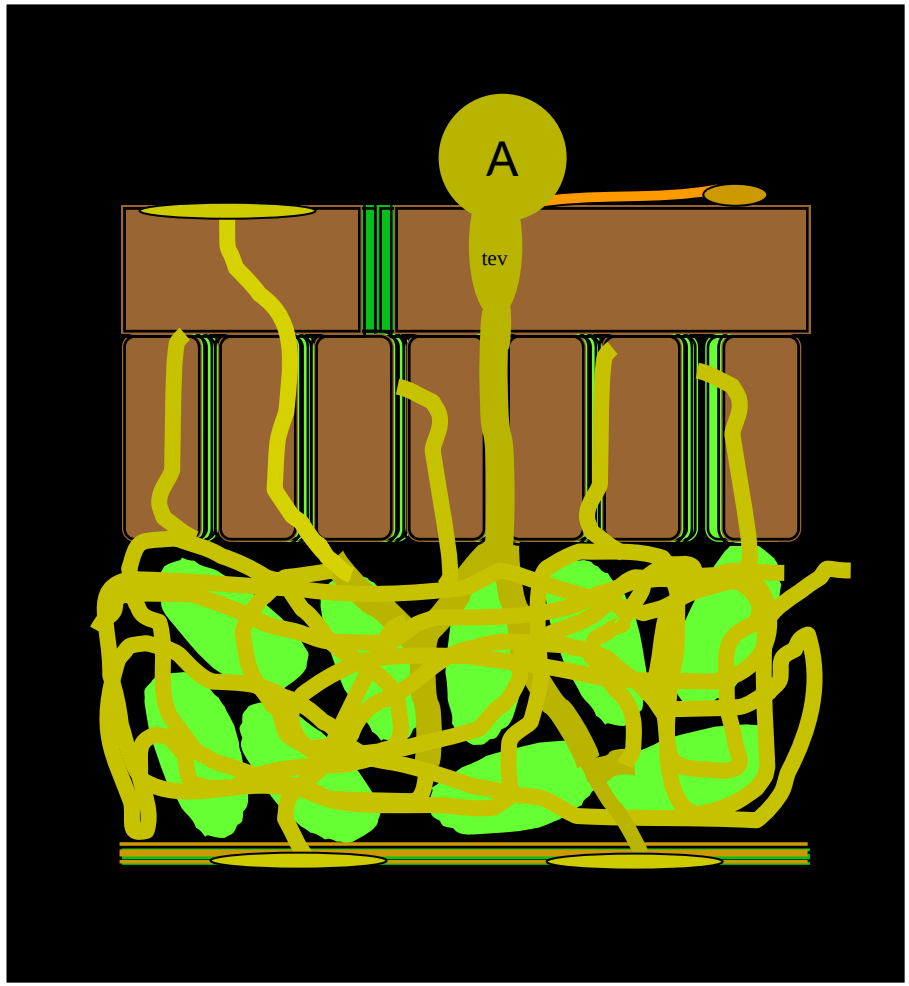
Gene Expression Studies

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

Interacción Susceptible

2 h	Appresoria begin developing
5 h	Appresoria expansion
7-12 h	Penetration through cuticle
12-16h	Increase in diameter
24 h	Primary hyphae emerging from tev
48 h	Intercellular hyphal growth (60µm from penetration site)
3-8 days	Intercellular hyphal growth (75-450 µm from penetration site)
9 days	Sporulation
14 days	Sporulation peak



(Based on Koch et al. 1983; Keogh et al. 1980)

cDNA libraries

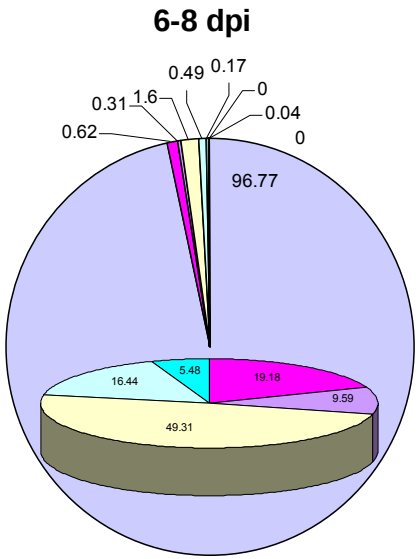
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

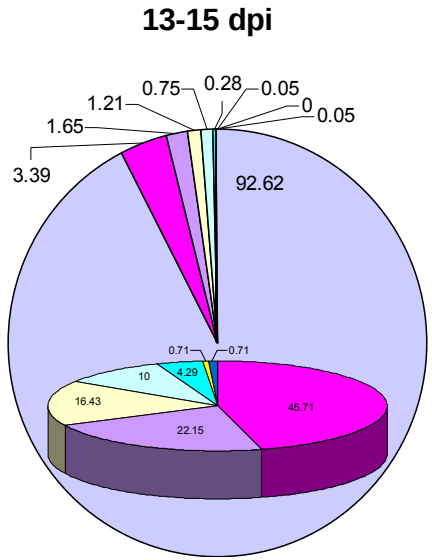
U.S. D.O.E. JOINT GENOME INSTITUTE

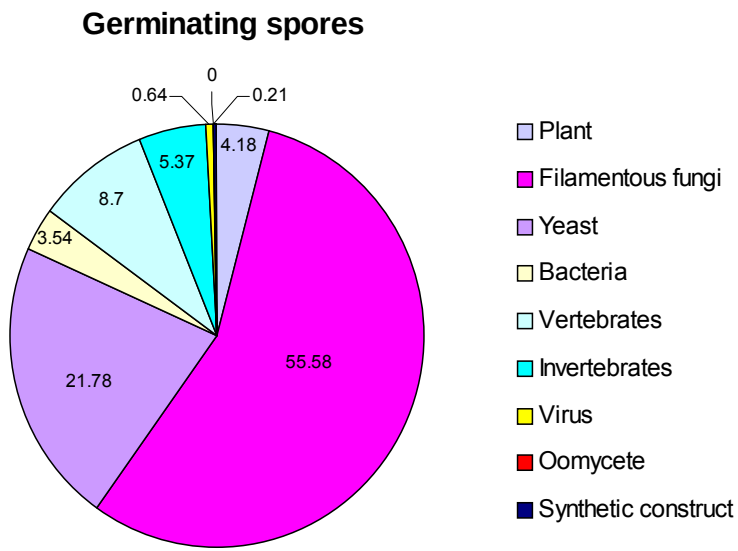
Germinating Spores	Resting spores	Hyphal growth	High sporulation
16 Hours on water surface	Kept at – 80°C	**6** **7** **8** Days after inoculation	**13** **14** **15** Days after inoculation

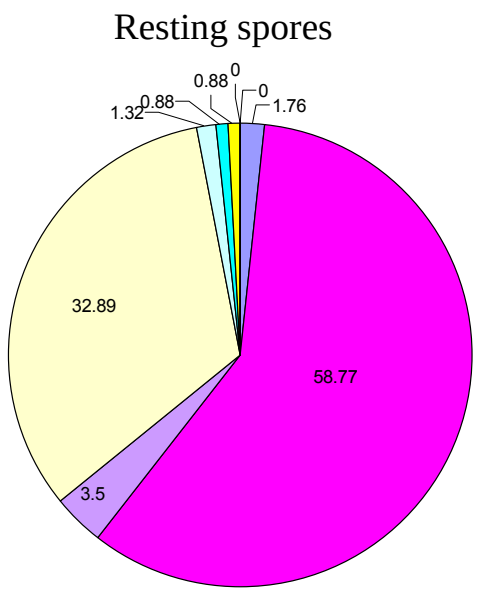
mRNA was extracted from infected leaf at each time point and pooled together for the construction of the cDNA libraries. Unidirectional cDNA libraries constructed in plasmid pSPORT1 (Invitrogen).

Description	ESTs	cDNAs	Libraries	Clusters	Consensus	Singlets
6-8 dpi	**6100**	**5374**	**1**	**1154**	**1278**	**1827**
13-15 dpi	**6023**	**4610**	**1**	**1291**	**1387**	**1356**
Resting urediniospores	**2295**	**1762**	**1**	**393**	**455**	**335**
Germinating urediniospores	**29601**	**18638**	**1**	**2686**	**3394**	**2142**
***Phakopsora pachyrhizi* v2.1**	**44019**	**30244**	**4**	**5105**	**6165**	**4961**









Percentage of similarity of cDNA clusters from the *Phakopsora pachyrhizi* germinating and resting spores libraries and the infected soybean leaf libraries (6-8 dpi and 13-15 dpi) to proteins from other organisms. Inner pies show the percentage of similarity of cDNA clusters to proteins from other organisms, excluding plant homologs.

cDNA functional categories

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

The cDNA clusters were classified into functional categories based on the BlastX hits and the Pfam hits, according to the Expressed Gene Anatomy database (EGAD, TIGR, Rockville, MD).

Approximately 23 % of the cDNA clusters from the 6-8 dpi and 13-15 dpi libraries and 40% from the germinating and resting spores libraries show similarity to hypothetical proteins or proteins of unknown function. Several homologs to pathogenesis related proteins (PR proteins) and defense proteins were identified in the infected leaf tissue libraries (Apidaecin, Beta defensin, Thaumatin, etc). In the GS library several homologs to pathogenicity proteins were identified. All the libraries show a high percentage of metabolism related proteins.

Real Time RT-PCR

Gene Selection

P. pachyrhizi **putative Heat-induced catalase, ATP-binding cassette (ABC) transporter, Plasma membrane (H+) ATPase and two constitutive genes, putative Alpha and Beta-tubulin, were selected from the ESTs from the germinated spores to study their gene expression during the infection cycle of** ***P. pachyrhizi*** **on soybean.**

RNA

Total RNA (40ng) from non-infected plant, germinating spores, infected leaf tissue from 1, 2, 4, 6, 8, 10, 12 and 14 dpi were used as template. Positive controls were performed using fungal DNA (25ng), while RNase treated RNA samples and no template were used as negative controls.

Real Time RT-PCR

Real Time RT-PCR was performed in the ABI Prism 7700 (Perkin Elmer) with 40ng of total RNA using the SuperScript One-Step RT-PCR with Platinum Taq Kit (Invitrogen), following the manufacturer's protocol for a 25µl reaction.

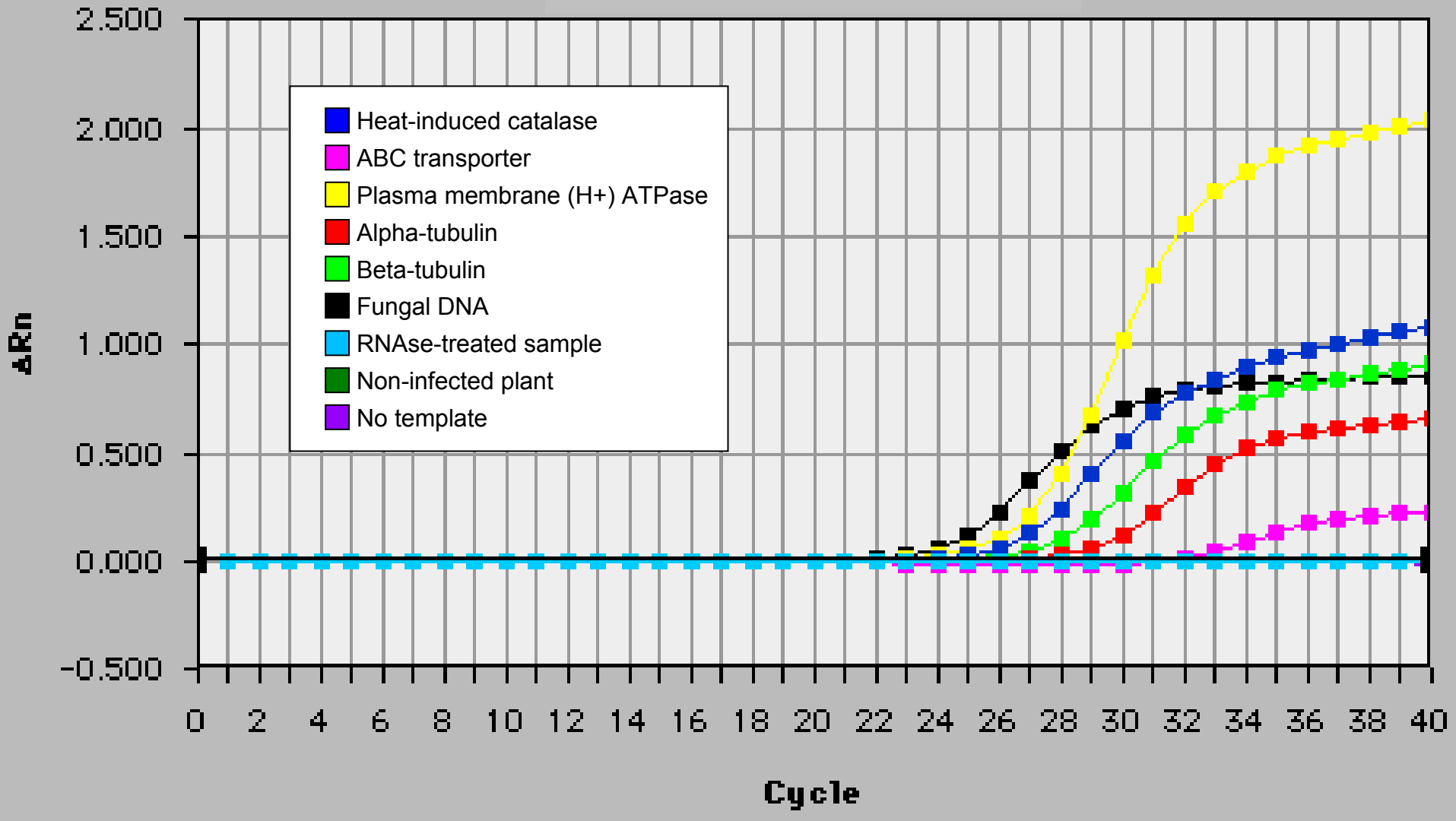
Primers and probes designed for Real Time RT-PCR assays

Putative Gene	Forward Primer	Fluorogenic Probe	Reverse Primer	Amplicon
Heat-induced Catalase	CCTGGTGTAGAGCCTTCTGCA	FAM- ACCCAGTCCTTCAATCGAGGCTATTTTCC-TAMRA	TGACGATGGGTGTCAGGGT	70
ABC Transporter	GAAACATTGGATGTACAACCTGGA	FAM- CCCTATACTCGATTGATTGGTGGACTGCTTG-TAMRA	TCGAGTCGTGCAGCTCATTT	76
Plasma membrane (H+) ATPase	TCGTTCACACGGCTGGTTT	FAM- TTTATGGAGAGACCATCGGCGGCTT-TAMRA	AGCAATCAGAAAAGCGCCC	68
Alpha-tubulin	CCAAGGCTTCTTCGTGTTTCA	FAM- TCGTTTGGAGGCGGACTGGTTCA-TAMRA	CAAGAGAAGAGCGCCAAACC	65
Beta-tubulin	CCCCGTGCAGTTTTGATTG	FAM- TTGGAACCAGGAACCATGGATTCGG-TAMRA	CCAAAAGTCCCGGATCGA	64

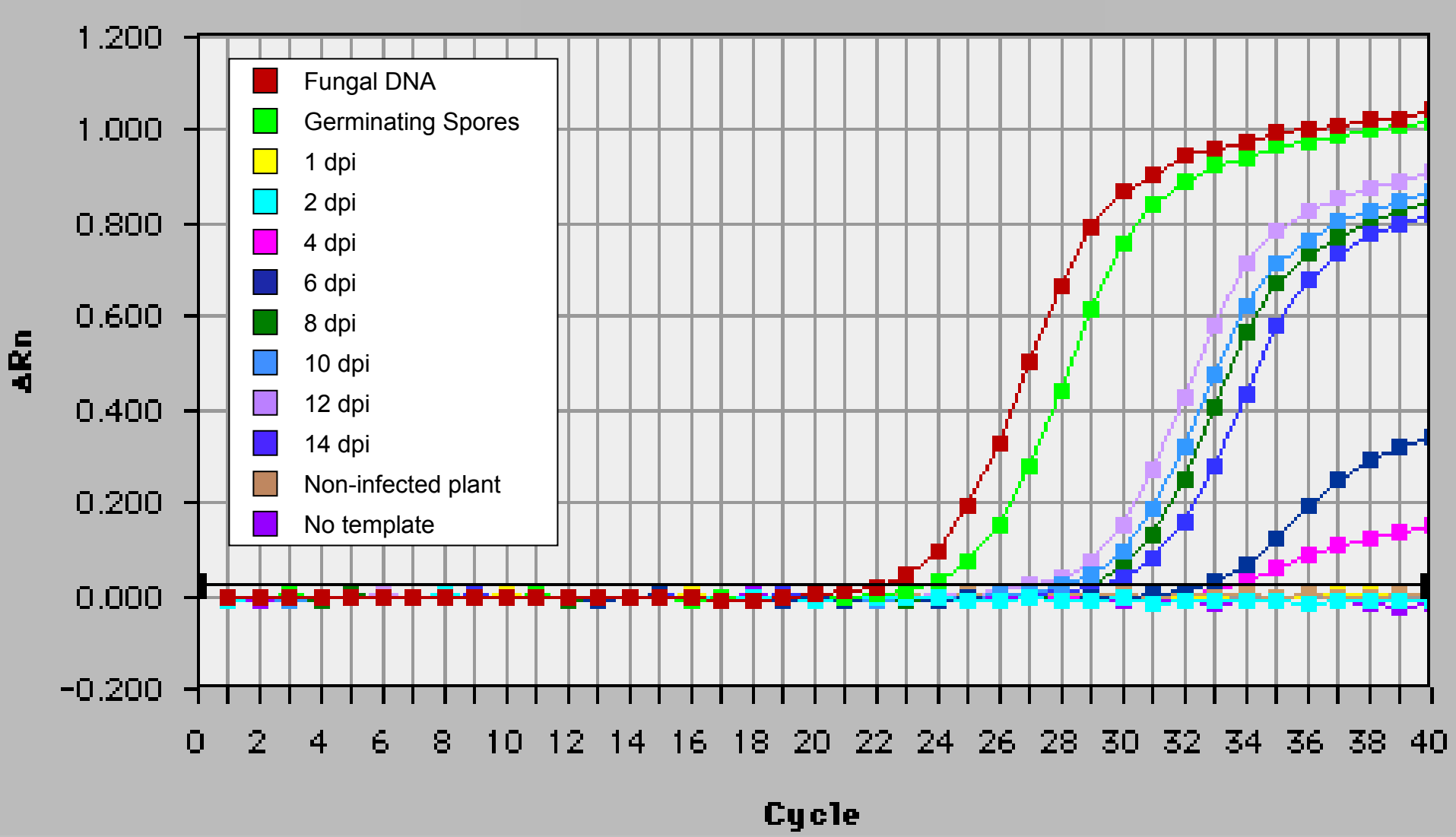
Putative genes of *P. pachyrhizi* selected from the germinating spores cDNA library. Primers and probes were designed using Primer Express 1.0 (Perkin Elmer). Primers (Operon); Probes (Synthegen).

Thermal cycling conditions (ABI 7700):

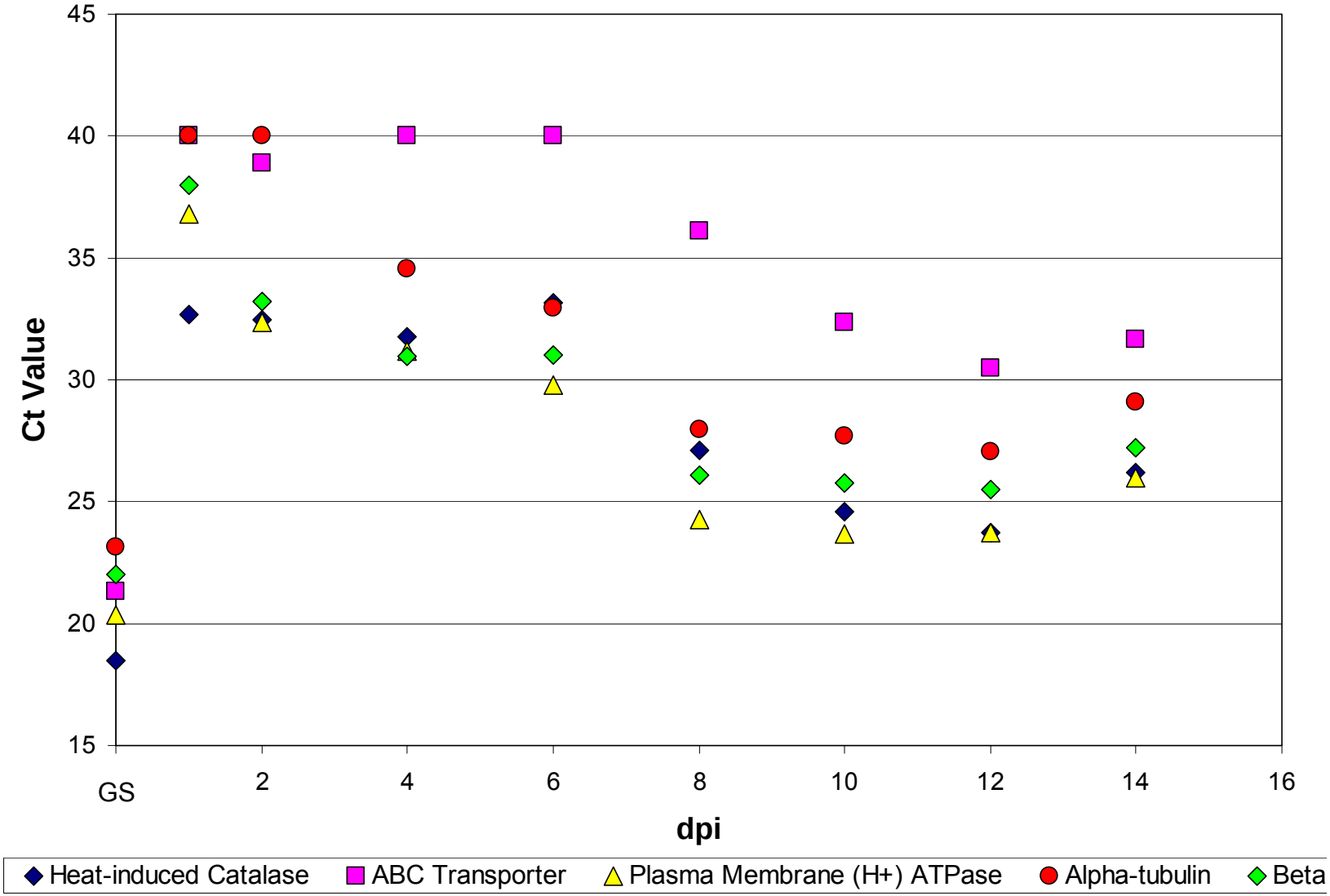
50°C for 15 min (hold)
95°C for 10 min (hold)
40 cycles: 95°C for 15 s
60°C for 1 min



Real Time RT-PCR spectra for 10dpi. Fungal DNA (positive control), RNase treated sample and no template (negative controls).



Real Time RT-PCR spectra for Alpha-tubulin



Expression patterns of five putative genes over the infection cycle of *P. pachyrhizi* on *G. max* generated using Real Time RT-PCR. C_T (threshold cycle) is the cycle in which a significant increase in ΔR_n is detected. Germinating spores (GS) were used as a positive control. dpi: days post inoculation

Specific objectives

- Develop a suppression subtractive hybridization (SSH) library of the resistant interaction and identify transcripts/ESTs (Expressed Sequence Tags)

Suppression subtractive hybridization (SSH) cDNA library

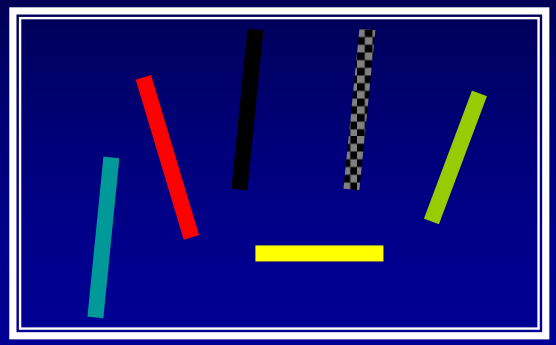
Two libraries:

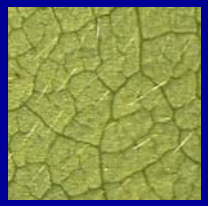
pooled RNA from t = 1, 6, 12, 24, 48hpi

(each from first trifoliate, from 4 plants)

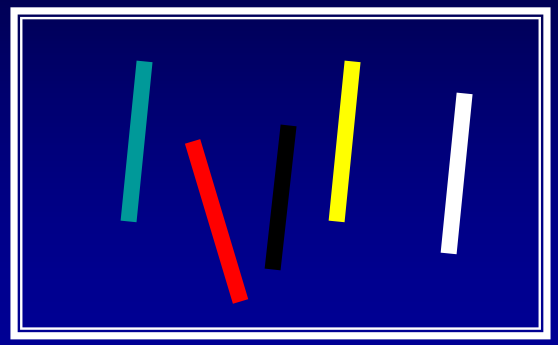
- Forward Subtraction:
  - Tester = Komata/HW94 [Resistant/immune]
  - Driver = Komata/TW72 [Susceptible]
- Reverse Subtraction:
  - Tester = Komata/TW72 [Susceptible]
  - Driver = Komata/HW94 [Resistant/imune]

Suppression subtractive hybridization (SSH) cDNA library

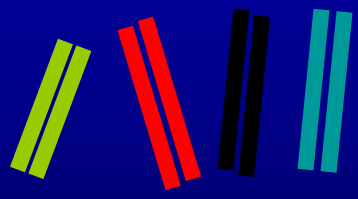




Resistant rxn
"Tester"



Susceptible rxn
"Driver"
(overabundance)



Suppress "common" transcripts & pcr-select "uncommon" from tester

Activator or up-regulated gene in a resistant reaction

Suppression subtractive hybridization (SSH) cDNA library

- Our unique approach:

  "driver"= susceptible interaction

- This should identify not just the general "defense-related" genes of typical pathogen invasion, also genes that are differentially turned on that prevents the disease from progressing

- Suppression should allow for the identification of unique, rare gene expression

Suppression subtractive hybridization (SSH) cDNA library

Results:

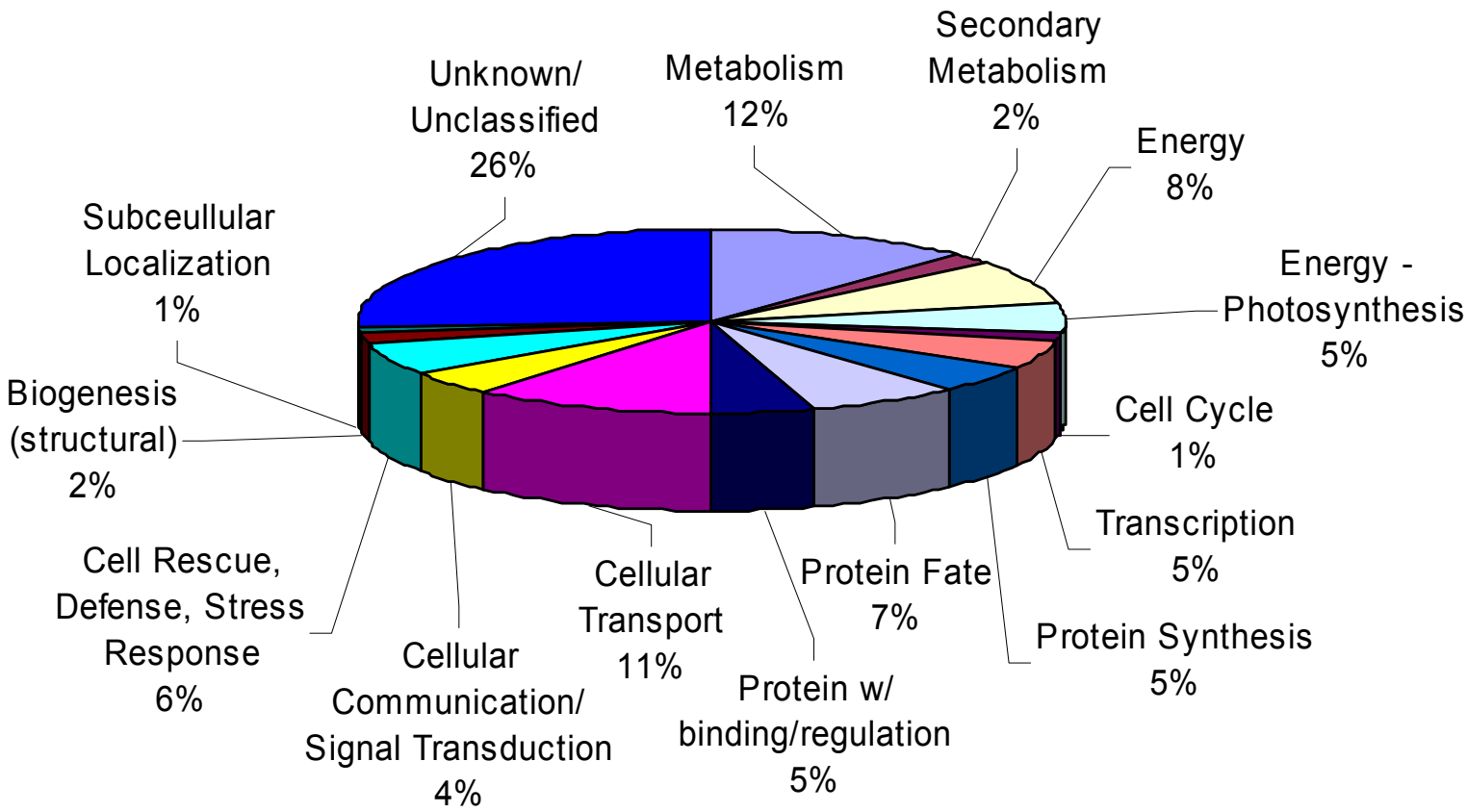
Forward Subtraction Library:

- 1056 clones sent for single-pass sequencing
  [Nucleic Acid Facility (NAF) at USDA-ARS-ERRC in Wyndmoor, PA]
- 45 clones did not sequence
- Due to method (blunt-digest) clones with multi-inserts (~15%), 1182 ESTs
- Insert sizes of EST ranged from 52nt to >600nt, no full-length transcripts were identified
- A low-redundant subset of 979 EST

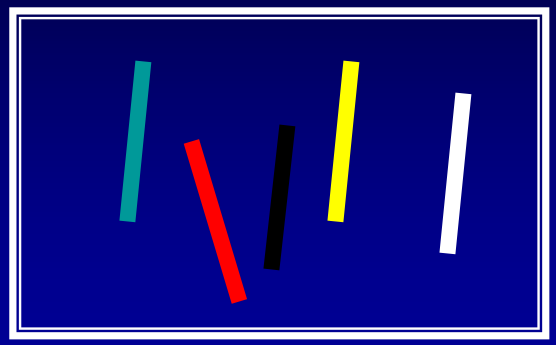
Subtractive suppressive cDNA library Data Analysis

- Comparative genomics using sequence-similarity tool BLAST (Basic local alignment search tool)
  - BLASTx = protein database
  - EST = dbEST
  - Unigene = compiled cluster of sequences from ESTs/mRNA/genomics projects
- Further analysis into Functional Categories (MIPS- Munich Information Center for Protein Sequences)

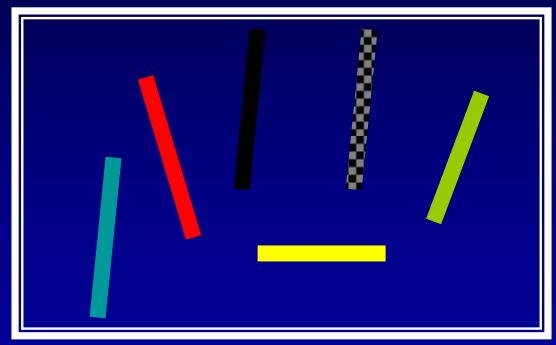
Results: Forward Subtraction
Functional Categories



Suppression subtractive hybridization (SSH) cDNA library

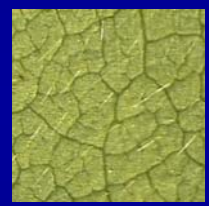


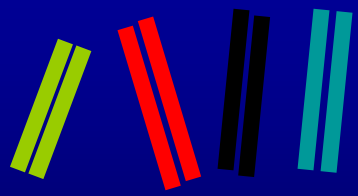
Reverse subtraction



Susceptible rxn
"Tester"

Resistant rxn
"Driver"
(overabundance)





Suppress "common" transcripts & pcr-select "uncommon" from tester

Repressor/other regulated gene in a susceptible reaction

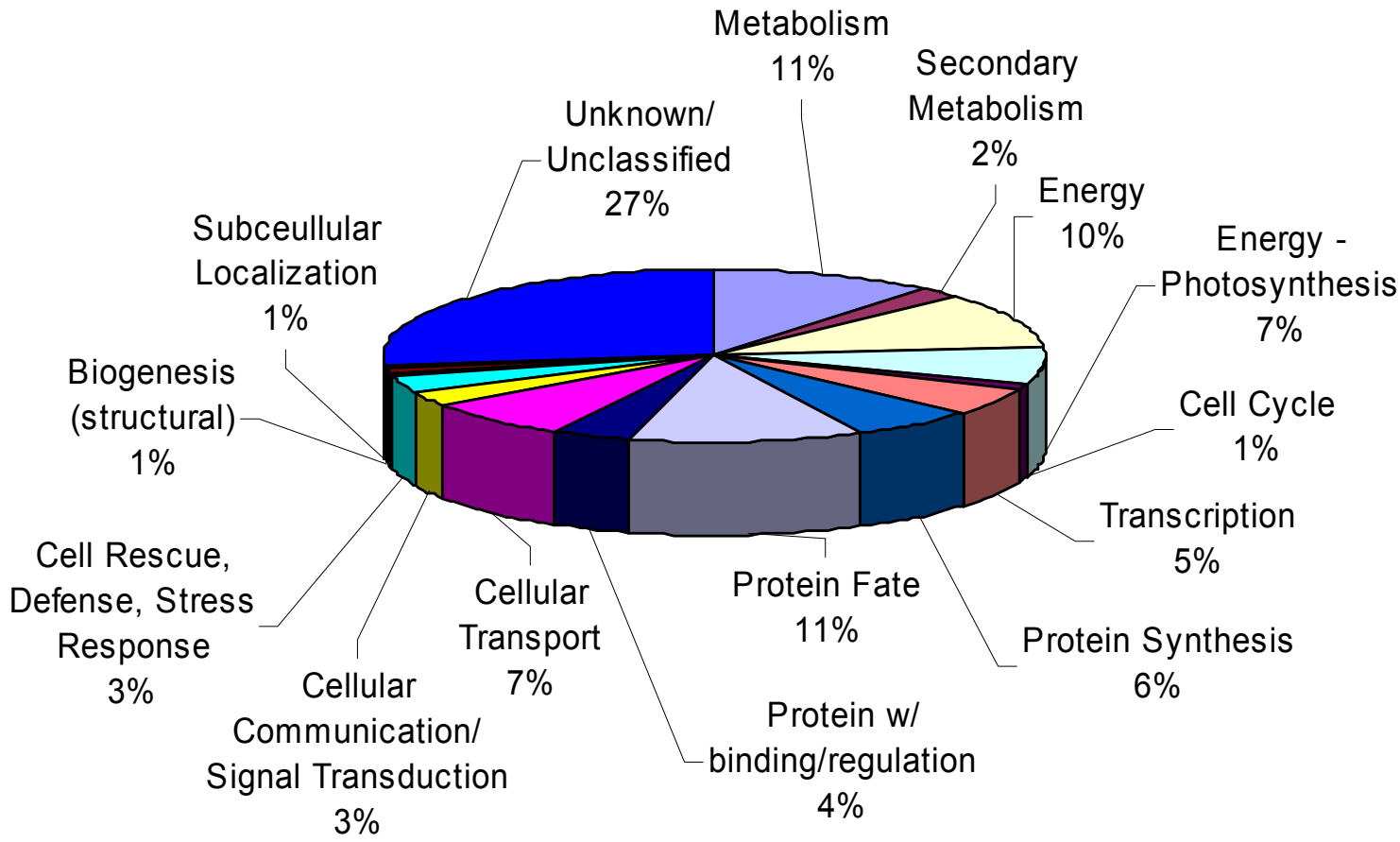
Suppression subtractive hybridization (SSH) cDNA library

Results:

Reverse Subtraction Library:

- 672 clones sequenced (NAF)
- 12% with multi-inserts, 590 ESTs sequence edited & computer analyzed
- 555 low redundant ESTs further analyzed and grouped into functional categories

Results: Reverse Subtraction
Functional Categories



Specific objectives

- Develop a suppression subtractive hybridization library of the resistant interaction and identify transcripts/ESTs (Expressed Sequence Tags)
- Protein profiling of germinating and resting urediniospores from *P. pachyrhizi*

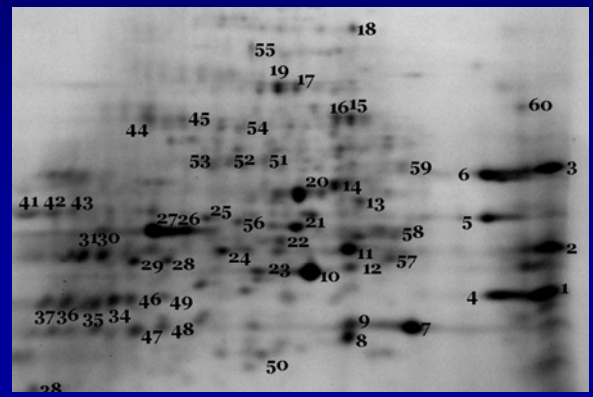
Enriched extracellular proteins from germinating and resting urediniospores

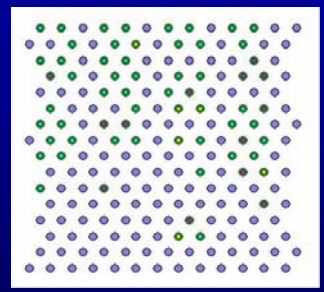
- Vacuum infiltrate leaflets
- Low spin, collect infiltrate - 45µm filter
- Concentrate, dialysis, acetone-precipitation

USDA-ARS-FDWSRU & USDA-ARS-ERRC)

Enriched extracellular proteins from germinating and resting urediniospores

- 2-D protein gel
- Pick spots for MALDI
- In-gel trypsin-digestion
- MALDI/TOF-TOF mass spectrometry ABI4700

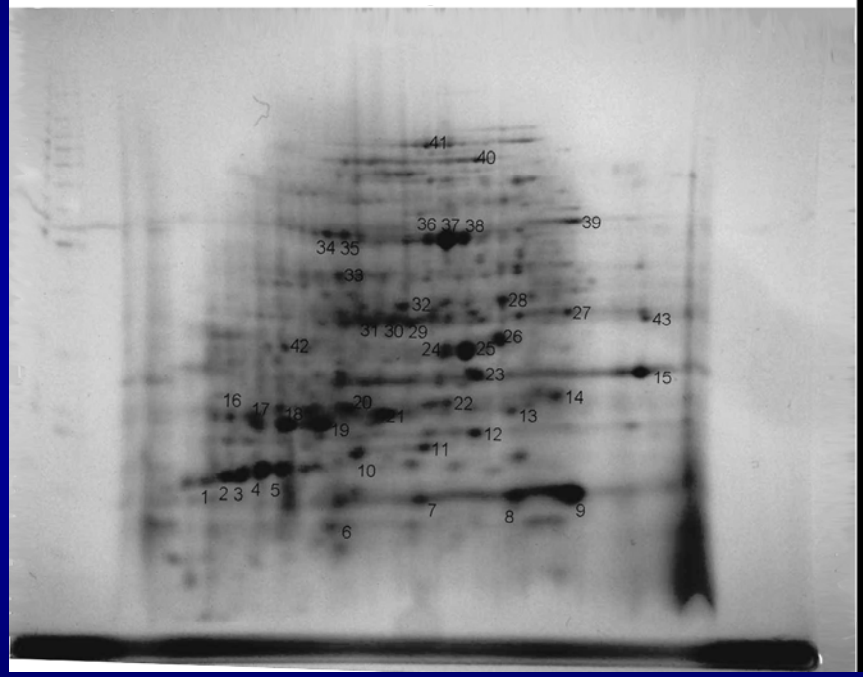




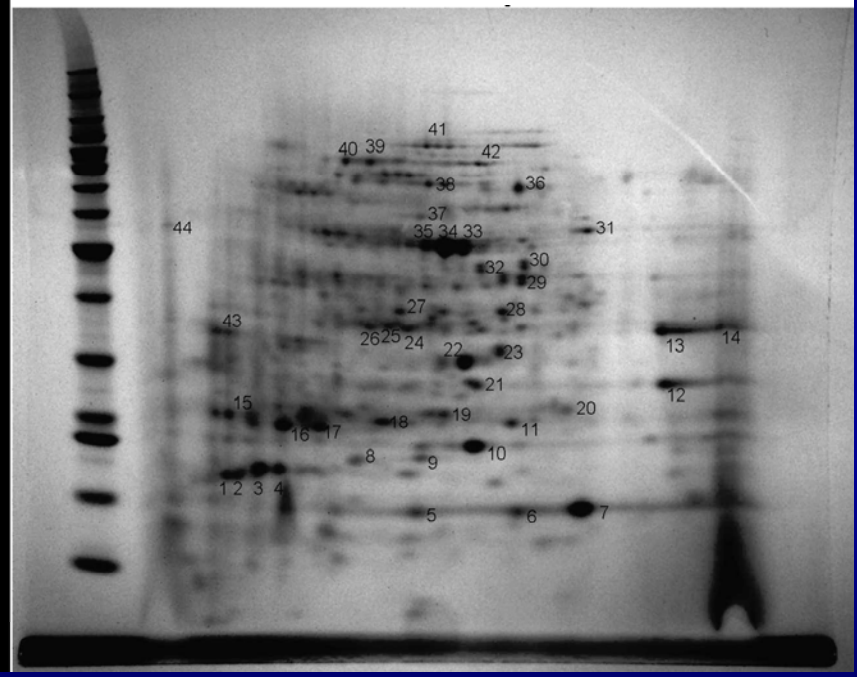
USDA-ARS-FDWSRU & USDA-ARS-ERRC)

Protein profiling with 2D-gel and MALDI/TOF-TOF mass spec 12hpi

Germinating spores



Resting spores



Selected spots were blasted against the “nr” database and the EST database (six reading frames)

USDA-ARS-FDWSRU & USDA-ARS-ERRC)

Enriched extracellular proteins from soybean leaves from resistant and susceptible interaction

Time points: 0h, 12h, 24h, 48h, 72h, 6dpi

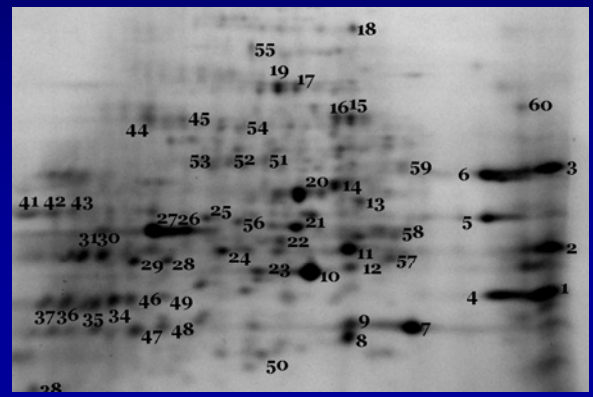
Treatments: Mock, HW94-1, TW72-1

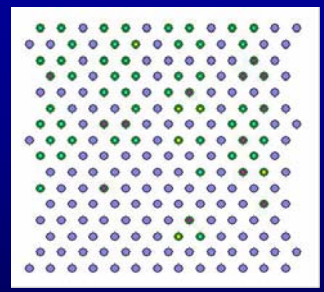
Soybean cv.: *G. max* cv. Komata

- Vacuum infiltrate leaflets
- Low spin, collect infiltrate - 45µm filter
- Concentrate, dialysis, acetone-precipitation

Enriched extracellular proteins from soybean leaves from resistant and susceptible interaction

- 2-D protein gel
- Pick spots for MALDI
- In-gel trypsin-digestion
- MALDI/TOF-TOF mass spectrometry ABI4700





USDA-ARS-FDWSRU & USDA-ARS-ERRC)

Acknowledgements

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

U.S. D.O.E. JOINT GENOME INSTITUTE

USDA/ARS/FDWSRU

Reid D. Frederick
Jane J. Choi
Christine L. Stone
Craig Austin
Laura Ewing

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

Jeffrey L. Boore
Peter Brokstein
Nick Putman
Harris Shapiro
Jarrod Chapman

Lawrence Livermore National Laboratory

Laurie Gordon

- This work was performed under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48, Lawrence Berkeley National Laboratory under Contract No. DE-AC02-05CH11231 and Los Alamos National Laboratory under Contract No. W-7405-ENG-36.