Title
Challenges in Whole-Genome Annotation of Pyrosequenced Eukaryotic Genomes

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Challenges in whole-genome annotation of pyrosequenced eukaryotic genomes

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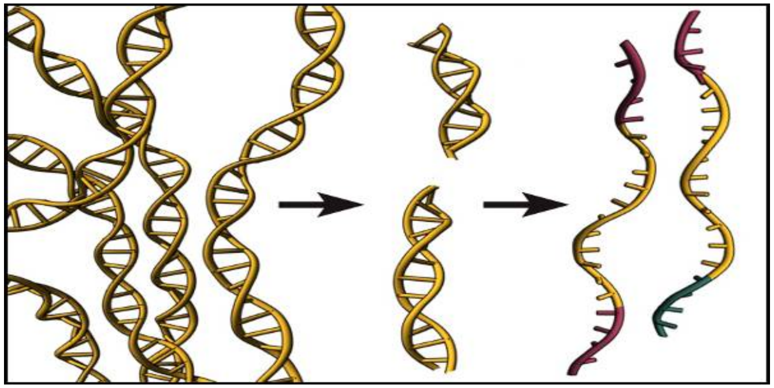
Introduction

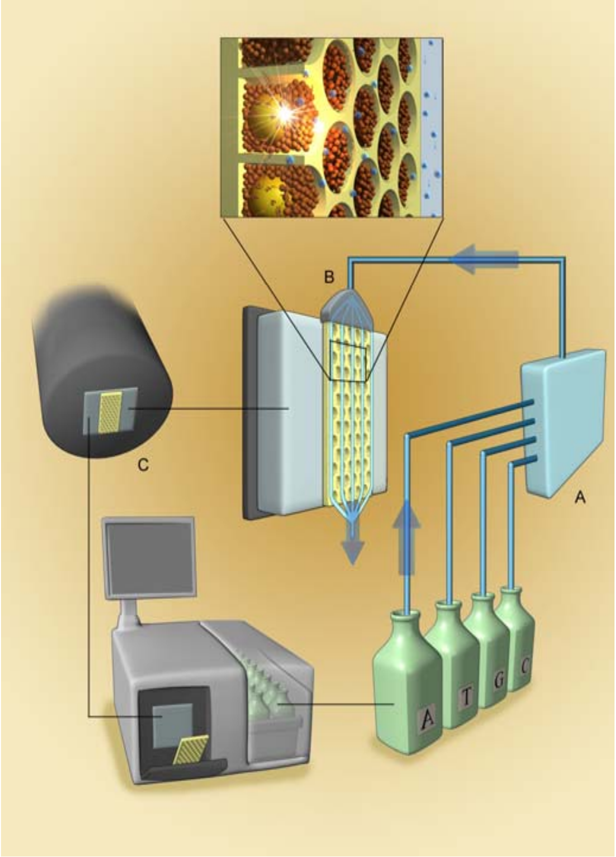
- Pyrosequencing technologies such as 454 and Solexa sequence DNA at much higher rate and lower cost than traditional Sanger technology.
- 454 is now mature enough to be used for **eukaryotic** genome sequencing and assembly.
- What will be the effect on **annotation**??

1. A simple experiment to assess assemblies that use 454 reads.
2. Successful production annotation of 2 assemblies that use 454.

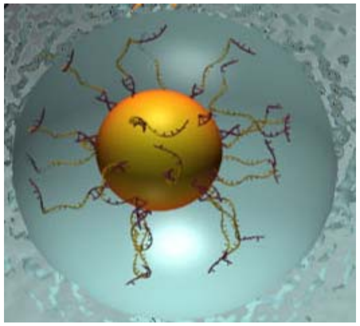
454 technology



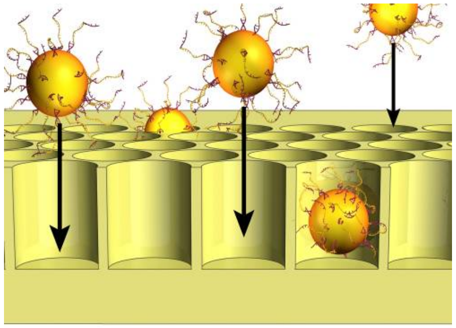
1. prepare adapter-ligated ssDNA library



4. Sequence by synthesis on the 454 Instrument



2. clonally amplify on 28 µm beads



3. Load beads and enzymes in PicoTiterPlate$^{\text{TM}}$

Images from Stephen Kingsmore, NCGR

454 vs. Sanger

	Sanger	**454**
Mbp per run	0.3	100
US$ per kbp	$1.0	$0.1
Read length (nt)	800	240
Paired ends distance (kb)	40	3
Error rate (%)	**0.1**	**0.5**

High coverage and depth of coverage

Poor assembly of repetitive regions

Many small gaps

Frameshifts genes

Data from Stephen Kingsmore, NCGR

Homopolymer stutter

Real sequence

CGCA**CCCCC**TCATATAAG

R T P S Y K

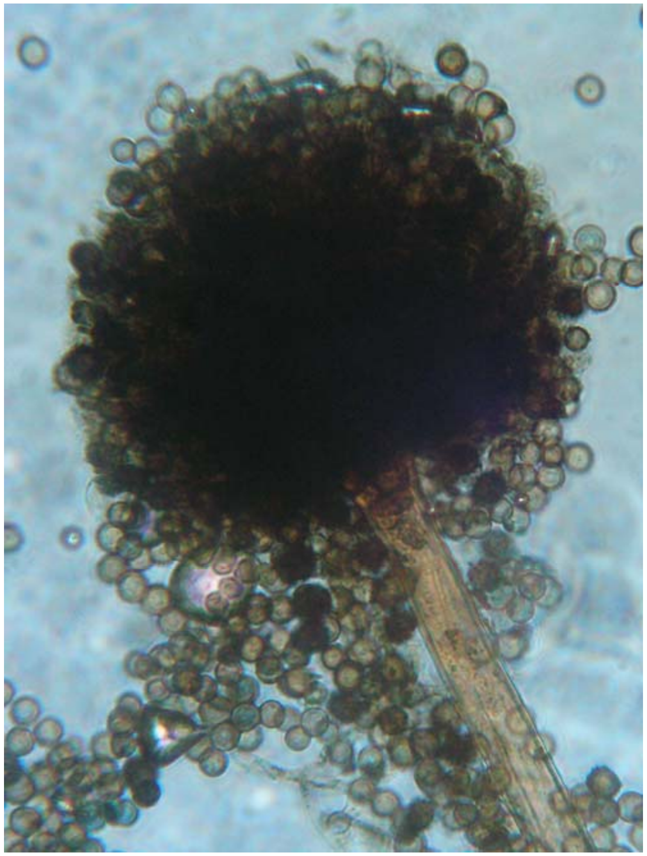
454 error

CGCA**CCCCCC**TCATATAAG

R T P **L I ***

What is the effect of the stutter on automatic annotation?

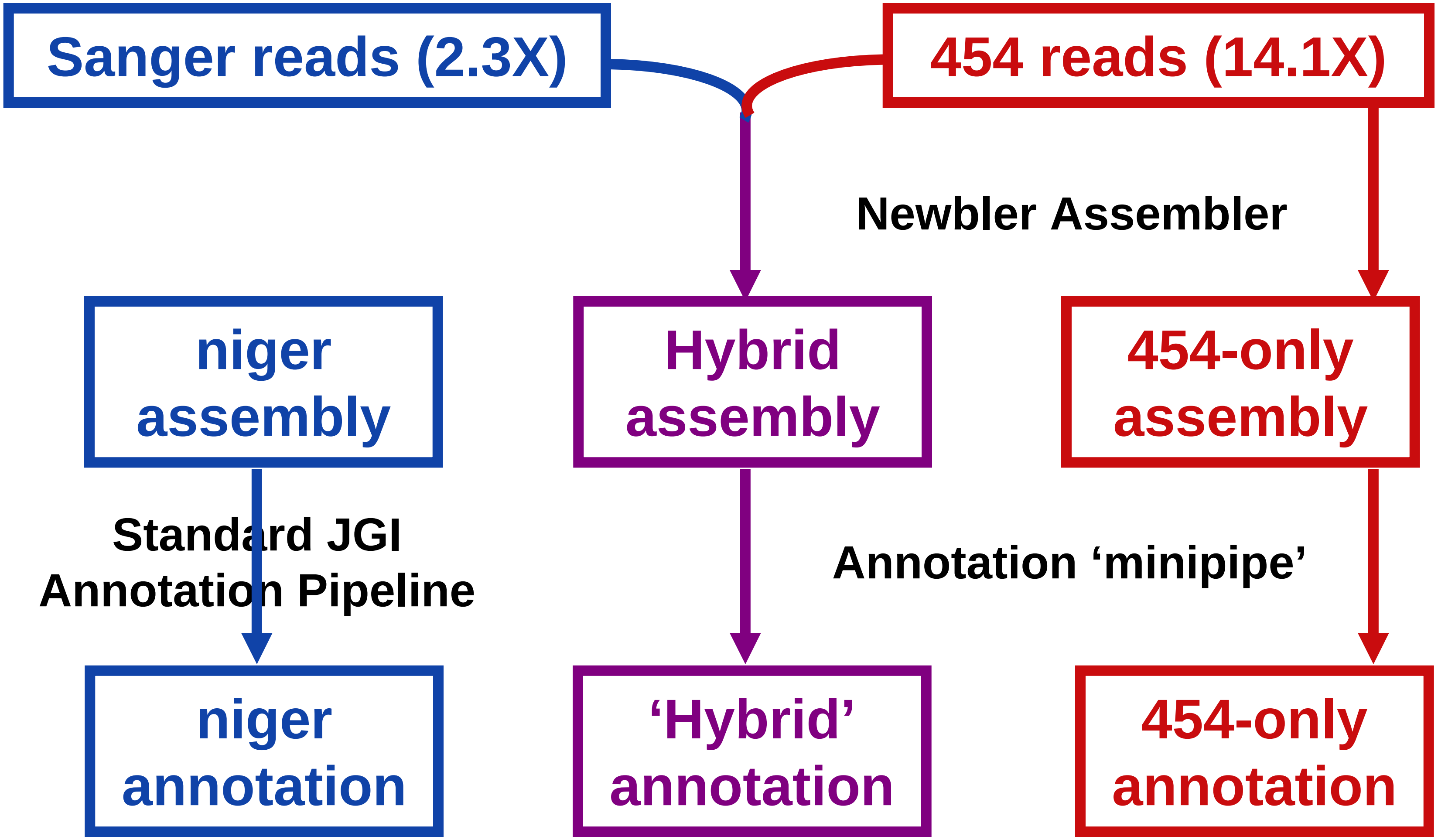
The test bed



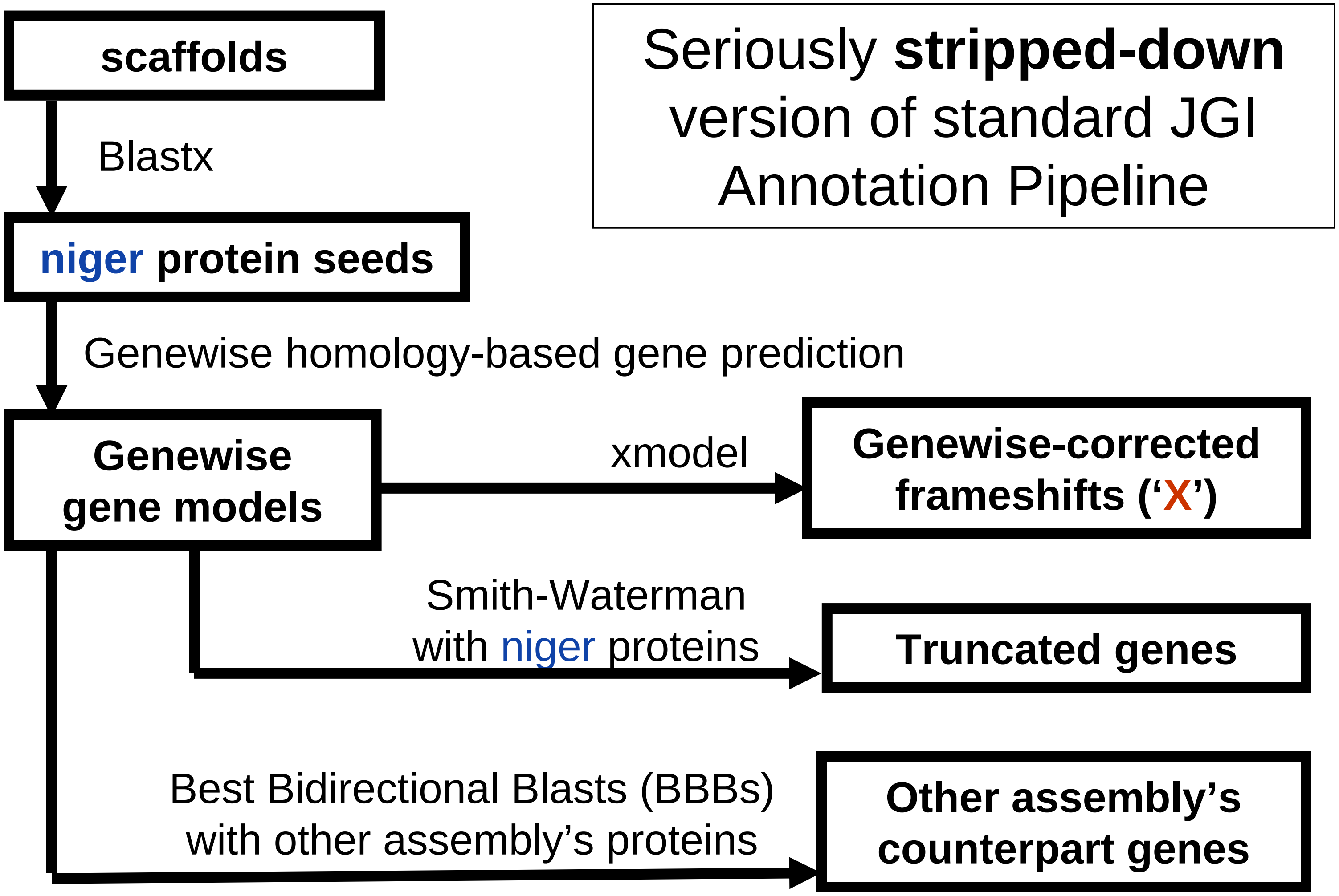
- *Aspergillus carbonarius*
- Ascomycote fungus
- Small (< 40Mbp)
- Haploid
- Well-known close relative: *Aspergillus niger* genome sequenced and annotated by JGI 2006

Photo from Scott Baker, PNNL

Experimental design



What is a 'minipipe' ?



Seriously **stripped-down** version of standard JGI Annotation Pipeline

scaffolds

Blastx

niger protein seeds

Genewise homology-based gene prediction

Genewise gene models

xmodel

Genewise-corrected frameshifts ('X')

Smith-Waterman with niger proteins

Truncated genes

Best Bidirectional Blasts (BBBs) with other assembly's proteins

Other assembly's counterpart genes

Aspergillus assemblies

	niger	**Hybrid**	**454-only**
Assembly size (Mbp)	37.2	34.9	32.2
# scaffolds	143	873	78
N50/L50 (# / Mbp)	6 / 2.0	8 / 1.8	10 / 0.9
Total gap space (Mbp)	2.4 (6%)	2.5 (7%)	0.5 (2%)
# gaps		556	1482
Ave. gap size (nt)		4420	367
Std. dev. gap size (nt)		6068	294

minipipe results

	Hybrid	**454-only**
# Genewise models	9730	9595
Model density (# / Mbp)	279	297
Models with ‘X’ (frameshift)	1048 (11%)	1161 (12%)
# aligned niger proteins	10494 (94%)	10406 (93%)
# niger proteins < 80% covered (truncated)	2710 (16%)	4115 (27%)

Sanger fixes 454 errors

BBBs between the 2 annotations		**454-only**	
		w/o 'X'	**w/ 'X'**
Hybrid	**w/o 'X'**	8359	238
	w/ 'X'	34	912

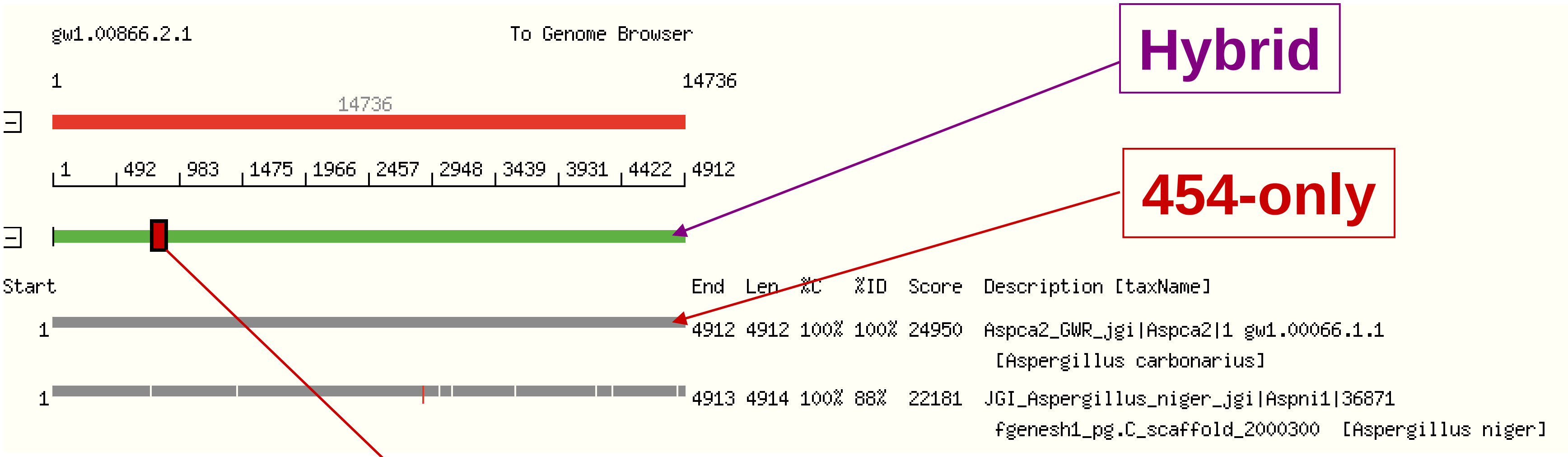
Genes w/ IS corrected by Sanger

Uncorrected genes + real pseudogenes

Advancing Science with DNA Sequence

An error, but not in the hybrid

JGI Portal view



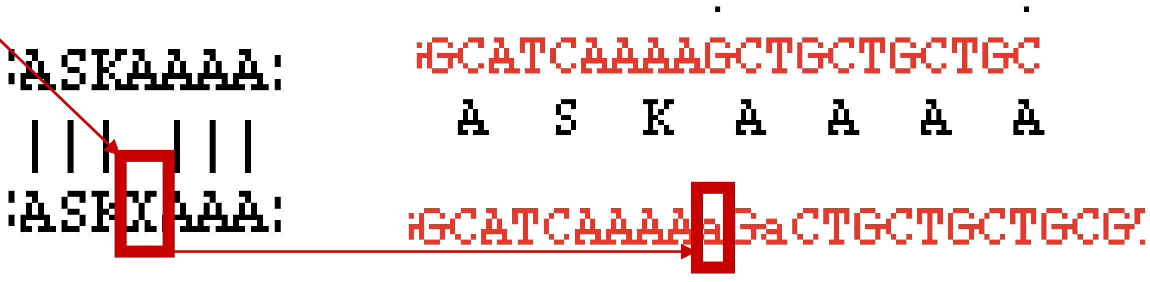
```
gw1.00866.2.1                              To Genome Browser

1                                                   14736
                   14736

 1    492  983  1475 1966 2457 2948 3439 3931 4422 4912

Start                                               End  Len  %C   %ID  Score  Description [taxName]
   1                                                4912 4912 100% 100% 24950  Aspca2_GWR_jgi|Aspca2|1 gw1.00066.1.1
                                                                                [Aspergillus carbonarius]
   1                                                4913 4914 100% 88%  22181  JGI_Aspergillus_niger_jgi|Aspni1|36871
                                                                                fgenesh1_pg.C_scaffold_2000300  [Aspergillus niger]
```

Hybrid

454-only

Hybrid

454-only

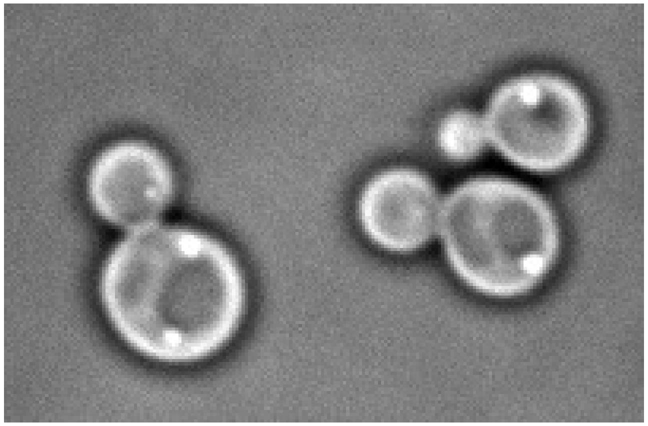


protein view

gene view

Production annotation of hybrid assemblies

- Yeasts *Candida tenuis* and *Spathaspora passalidarum*
- do xylose -> ethanol
- Tiny haploid genomes, few introns
- Well-known close relative: *Pichia stipitis* genome released by JGI 2006



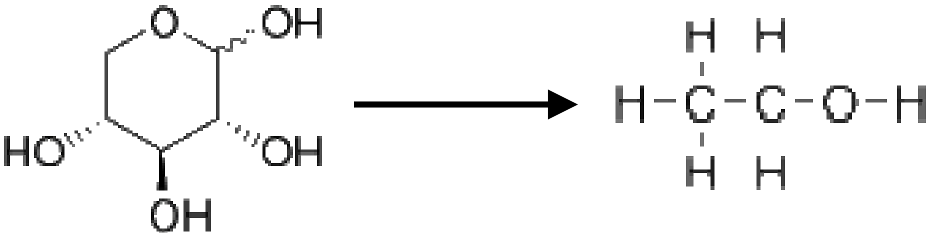


Photo from Dana Wohlbach, U. WI

454 vs. Sanger, round 2

	Sanger	**Old 454**	**New 454**
Mbp per run	0.3	100	450
US$ per kbp	$1.00	$0.10	$0.02
Read length (nt)	800	240	**450**
Paired ends distance (kb)	40	3	**20**
Error rate (%)	0.1	0.5	**??**

Data from Joann Mudge, NCGR

Quality of yeast assemblies

	Pichia	Spatha	tenuis
Assembly size (Mbp)	15.4	13.3	10.7
# scaffolds	9	47	25
N50/L50 (# / Mbp)	4 / 1.8	4 / 1.7	3 / 1.2
Total gap space (Mbp)	0.0 (0%)	0.3 (2%)	0.2 (2%)
‘X’ rate (frameshifted)	2.0%	3.4%	2.4%
% Pichia proteins aligned		95.7%	94.6%
% Pichia aligned proteins <80% covered (truncated)		3.2%	5.8%

Production yeast annotations

	Pichia	**Spatha**	**tenuis**
# genes	5841	5726	5452
Gene density (# / Mbp)	378	431	507
Avg. gene length (nt)	1627	1472	1459
Avg. protein length (aa)	493	478	477
# exons / gene	1.4	1.3	1.2
% genes w/ Pfam	62.4	71.3	73.4
% genes w/ SwissProt	88.3	91.9	92.3

Unexceptional -- A GOOD THING!

Conclusion

- 454 techonology poses challenges to both assembly and annotation.
- Hybrid assembly helps resolve many of these challenges, including correction of many 454 sequence errors.
- The JGI Annotation Pipeline successfully annotated 2 yeasts of bioenergy significance.
- Hybrid assemblies of small eukaryotic genomes can be suitable substrates for production annotation.

Acknowledgments

JGI

- **Assembly**
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- **Annotation**
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  - Asaf Salamov
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  - Xueling Zhao
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Our collaborators

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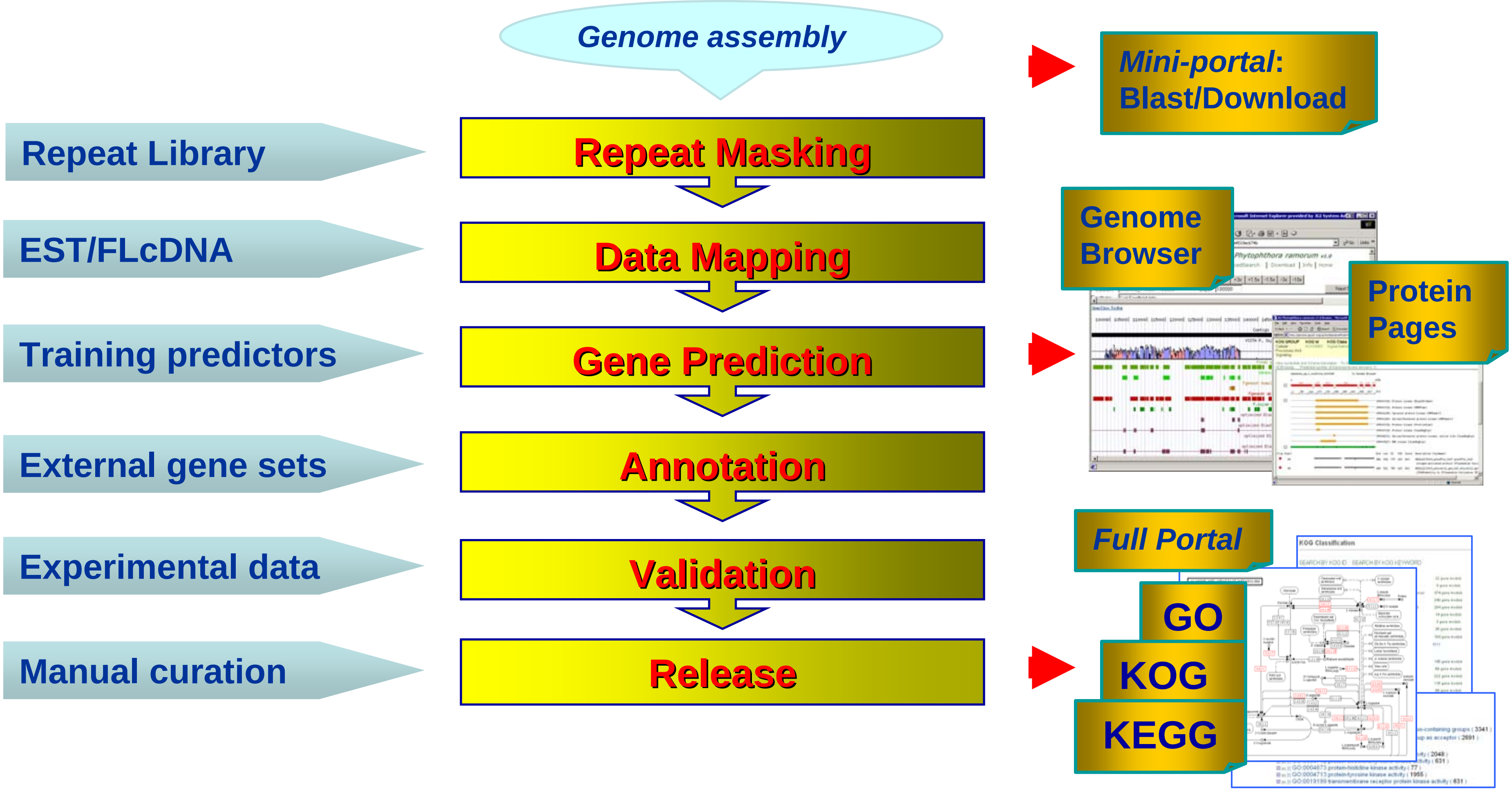
  - Scott Baker
  - Giancarlo Perrone
- **Yeasts**
  - Audrey Gasch
  - Dana Wohlbach
  - Tom Jeffries
- **NCGR**
  - Stephen Kingsmore
  - Joann Mudge

JGI Annotation Pipeline



Genome assembly

Repeat Library
EST/FLcDNA
Training predictors
External gene sets
Experimental data
Manual curation

Repeat Masking
Data Mapping
Gene Prediction
Annotation
Validation
Release

Mini-portal: Blast/Download

Genome Browser
Protein Pages

Full Portal
GO
KOG
KEGG

Automated Pipeline

Staged Portal Release