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# Challenges in whole-genome annotation of pyrosequenced eukaryotic genomes

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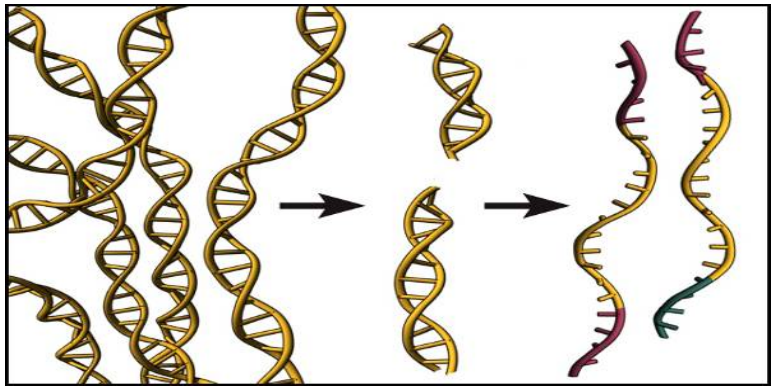
# Introduction

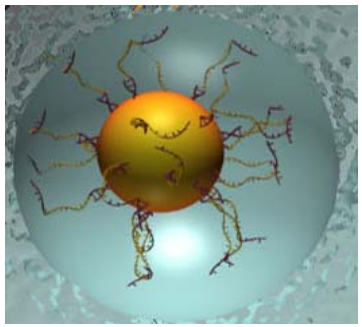
- Pyrosequencing technologies such as 454 and Solexa sequence DNA at much higher rate and lower cost than traditional Sanger technology.
- 454 is now mature enough to be used for **eukaryotic** genome sequencing and assembly.
- What will be the effect on **annotation**??

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

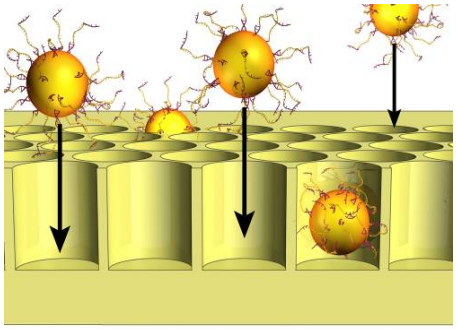
# 454 technology



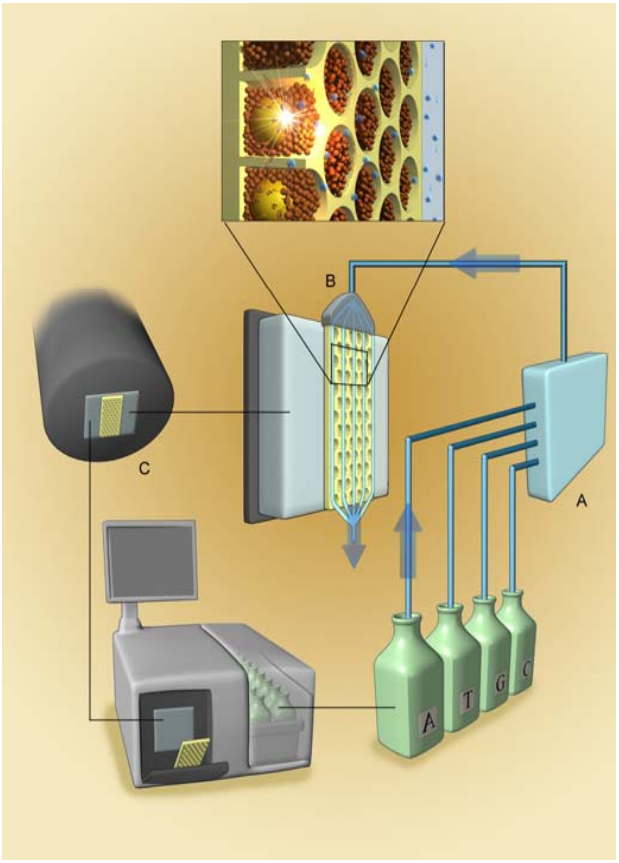
1. prepare adapter-ligated ssDNA library



2. clonally amplify on 28 µm beads



3. Load beads and enzymes in PicoTiterPlate™




4. Sequence by synthesis on the 454 Instrument

Images from Stephen Kingsmore, NCGR

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# 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

CGCACCCCCTCATATAAG

R T P S Y K

454 error

CGCACCCCCCCTCATATAAG

R T P L I *

What is the effect of the stutter on automatic annotation?

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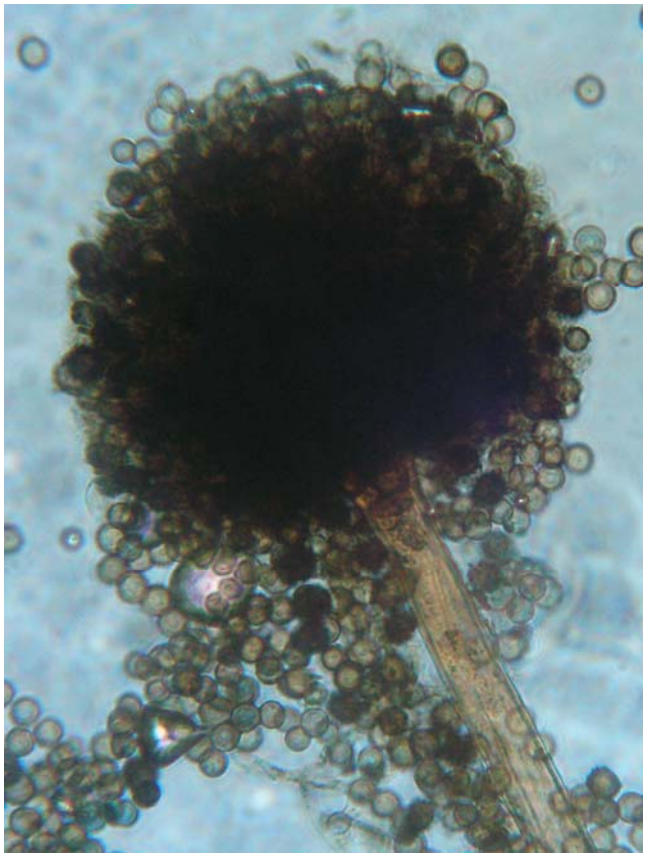
# The test bed



- *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

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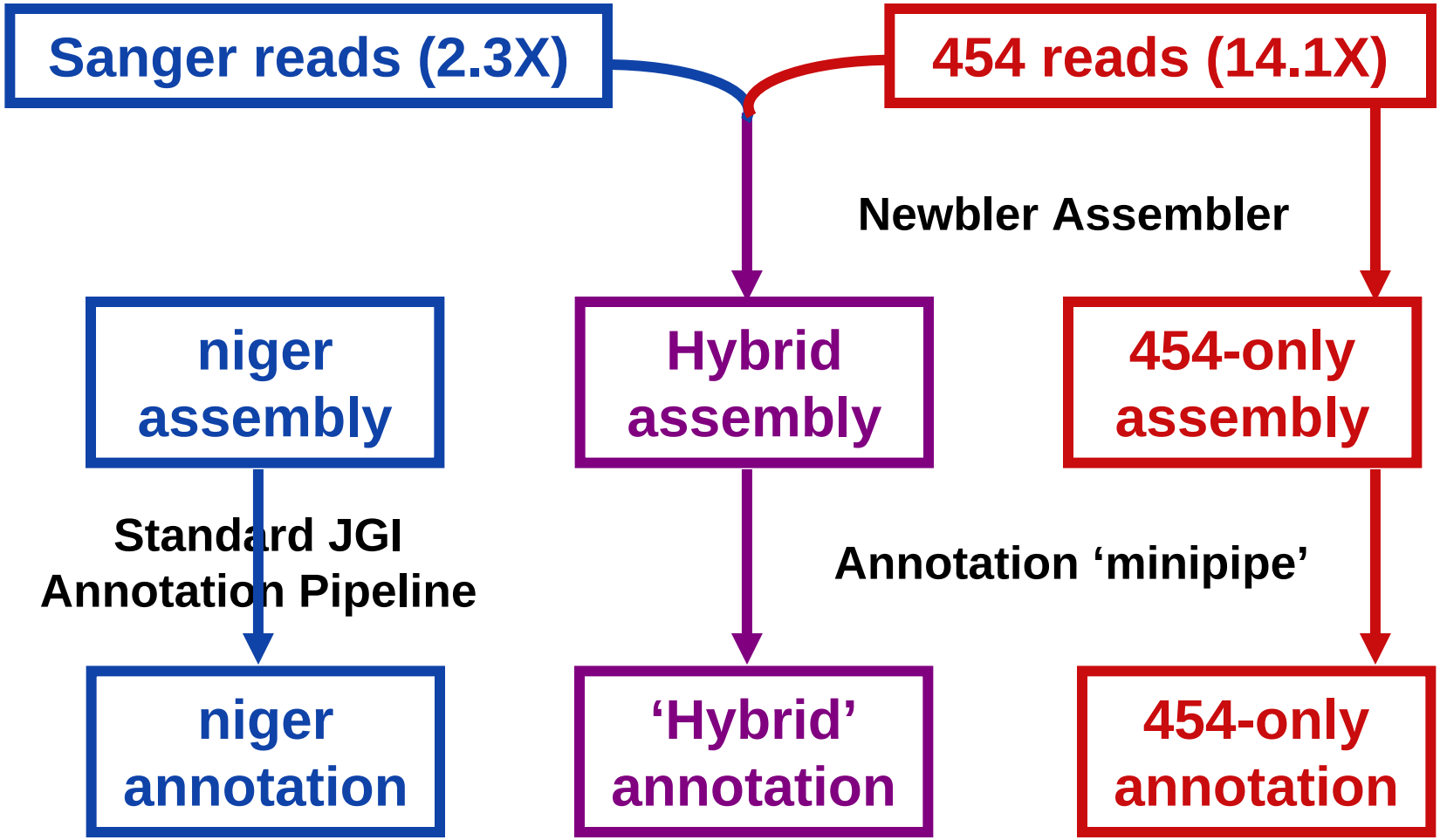
# Experimental design



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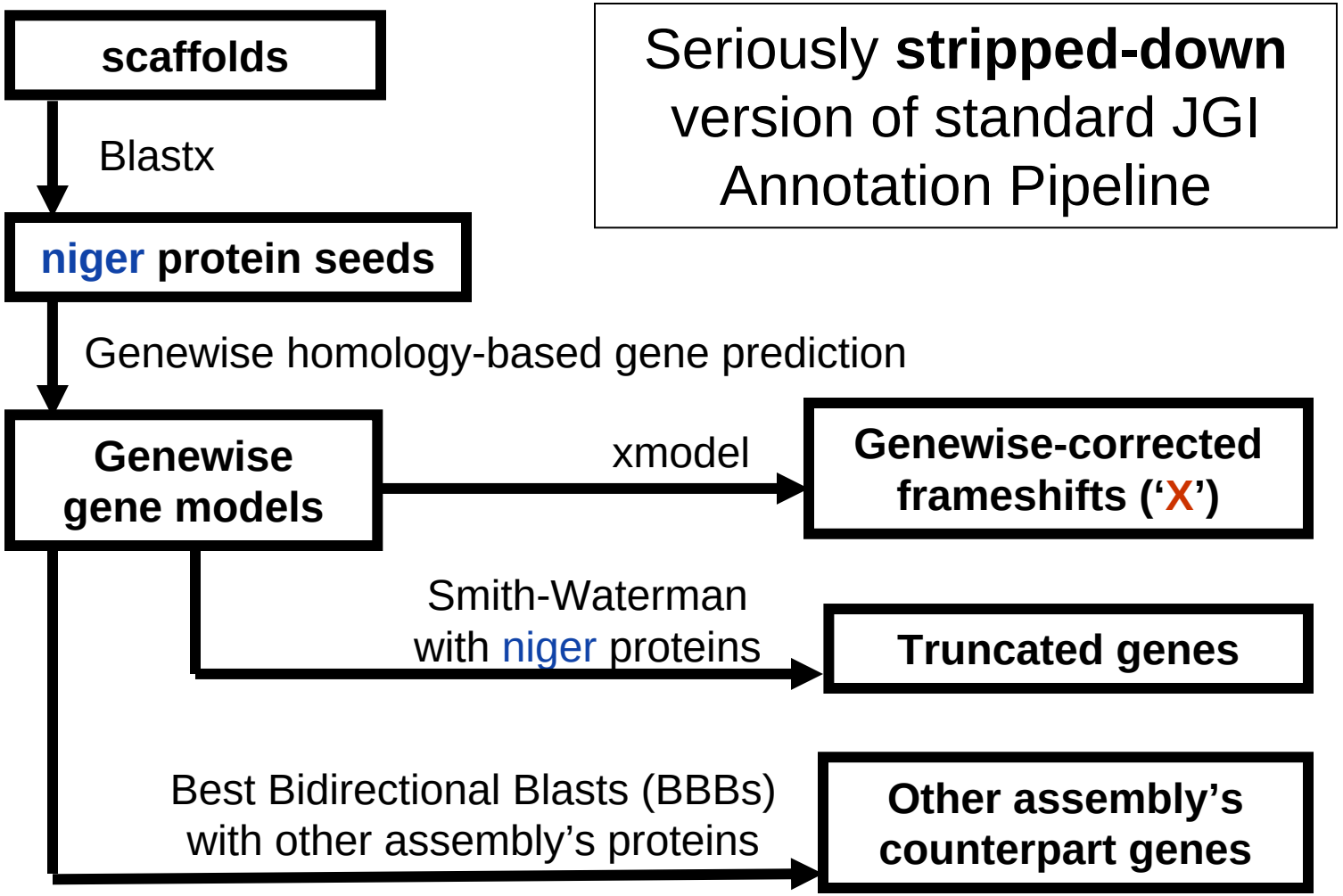
# What is a 'minipipe' ?




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

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# *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 |

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# 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%) |

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# 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**

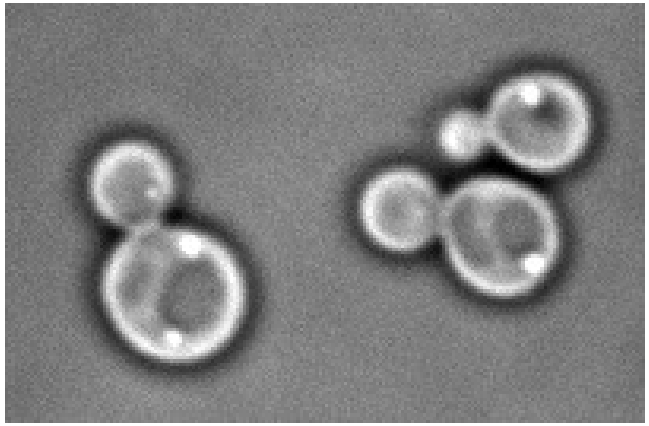
**Uncorrected genes + real pseudogenes**

# An error, but not in the hybrid

JGI Portal 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



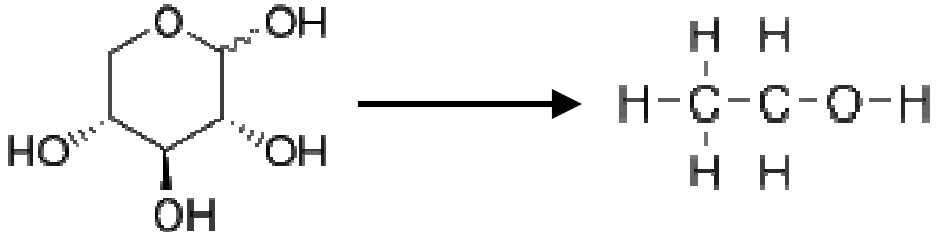



Photo from Dana Wohlbach, U. WI

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# 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

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# 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% |

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# 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!**

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# 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.

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# Acknowledgments


## JGI

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  - Xueling Zhao
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## Our collaborators

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

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# JGI Annotation Pipeline

*Genome assembly*

Repeat Library → **Repeat Masking**

EST/FLcDNA → **Data Mapping**

Training predictors → **Gene Prediction**

External gene sets → **Annotation**

Experimental data → **Validation**

Manual curation → **Release**

→ ***Mini-portal*: Blast/Download**

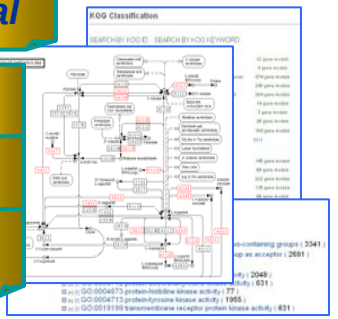
→ **Genome Browser**, **Protein Pages**

→ ***Full Portal***

**GO**

**KOG**

**KEGG**



**Automated Pipeline**

**Staged Portal Release**