selectable markers

a. URA3

orotidine 5'-phosphate decarboxylase

$\text{O-SP} \rightarrow \text{UMP (uridylic acid)}$

URA3

b. mutants in URA3 (ura3-)

1. require uracil for growth

2. resistant to 5'-fluoroorotic acid

$\text{5FOA} \rightarrow \text{5'-F uracil}$

URA3

3. 5'-fluoro-uracil is toxic to cells

4. 5-FOA is a suicide substrate

5. Positive and negative selection

$\text{ura3} + \text{URA3} \rightarrow \text{positive selection by}$

growth on medium without uracil

$\text{ura3} \rightarrow \text{negative selection by}$

growth on medium with 5-FOA

c. Kanamycin resistance

NPT II - neomycin phosphotransferase II
**YFG**

40 bp Marker 40 bp

**Genome**

**YFG**

"Fancy Method"

**Plasmid**

![Diagram]

primer ATG U1 TAG U2

↓ PCR

ATG U1 TAG U2 KAN MX4 D2 TAG D1 TAA

upstream extend homology to 40

downstream extend homology to 40

R CR

Replacement
Functional tests

1. Deletion collection
2. Dominance
3. Complementation test
4. Epistasis
5. Suppressors
6. Enhancers
Viable strains

- 1570 are slow growing (12-9070 of wild-type growth rates)
  - enriched in ribosomal proteins
  - mitochondrial function

- assay under different conditions
  - ionic stress
  - high stress
    - salt hypersensitive 62 loci
    - alkali hypersensitive 128 loci
    - Nystatin resistance 11 loci

- visual screen of 4401 Δ strains
  - 673 with morphological differences

\[\text{wt} \quad \text{pointed} \quad \text{large} \quad \text{elongated} \quad \text{small} \quad \text{other} \quad \text{clumped}\]
Yeast deletion collection

1. Bar-coded (unique 20bp barcode)
2. Replace ORF w/ KAN

Experiments
1. 18.7% of genes essential in lab
2. With five environmental conditions, ~15% are slow growing
3. Chemical screens
   a. Pool all strains
   b. Grow in "chemical"
   c. Purify DNA.
   d. PCR or sequence tag

Starting: 

\[\text{After treatment:} \]
\[\text{[---]} \]
\[\text{[---]} \]
<table>
<thead>
<tr>
<th>Name</th>
<th>Website/company/reference</th>
<th>Resource</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharomyces Genome Database</td>
<td><a href="http://www.yeastgenome.org/">http://www.yeastgenome.org/</a> (Cherry et al. 2012)</td>
<td>Gene annotations, data from high-throughput screens, and publications with links to other databases and sequence information</td>
</tr>
<tr>
<td>Yeast deletion project</td>
<td><a href="http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html">http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html</a> (Brachmann et al. 1998; Winzeler et al. 1999)</td>
<td>Yeast community cooperative project to systematically delete each nonessential ORF</td>
</tr>
<tr>
<td>Yeast deletion collection</td>
<td>Thermo Scientific</td>
<td>Yeast strains with a deletion of a specified ORF can be purchased as individual strains or as a collection</td>
</tr>
<tr>
<td>Designer deletion strains</td>
<td>Thermo Scientific (Brachmann et al. 1998)</td>
<td>Set of strains developed for efficient one-step gene replacement using common yeast markers</td>
</tr>
<tr>
<td>pRS plasmid collection</td>
<td>American Type Culture Collection (Sikorski and Hieter 1989) (Christianson et al. 1992)</td>
<td>Low-copy (CEN), high-copy (2-μm), and integrating plasmids containing common yeast markers</td>
</tr>
<tr>
<td>Yeast GFP-tagged ORFs</td>
<td>Life Technologies (Huh et al. 2003)</td>
<td>Yeast strains containing individual ORFs fused to GFP at the C terminus</td>
</tr>
<tr>
<td>Yeast TAP-tagged ORFs</td>
<td>Thermo scientific (Ghaemmaghami et al. 2003)</td>
<td>Yeast strains containing individual ORFs fused to a TAP tag at the C terminus</td>
</tr>
<tr>
<td>Yeast GFP Fusion Localization Database</td>
<td><a href="http://yeastgfp.yeastgenome.org/">http://yeastgfp.yeastgenome.org/</a></td>
<td>Database of ORF-GFP fusions with images searchable by ORF name or cellular location</td>
</tr>
<tr>
<td>S. cerevisiae strain sequence alignment</td>
<td><a href="http://www.yeastgenome.org/cgi-bin/FUNGI/alignment.pl">http://www.yeastgenome.org/cgi-bin/FUNGI/alignment.pl</a></td>
<td>Bioinformatics tool for aligning sequences within S. cerevisiae strains</td>
</tr>
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<td>Yeast sequence strain alignment</td>
<td><a href="http://www.yeastgenome.org/cgi-bin/FUNGI/showAlign">http://www.yeastgenome.org/cgi-bin/FUNGI/showAlign</a></td>
<td>Bioinformatics tool for aligning sequences among yeast species</td>
</tr>
<tr>
<td>DNA Replication Origin Database</td>
<td><a href="http://cerevisiae.oridb.org/">http://cerevisiae.oridb.org/</a> (Nieduszynski et al. 2007; Siow et al. 2012)</td>
<td>Bioinformatics tool for searching location of ARS across the yeast genome</td>
</tr>
</tbody>
</table>
Complementation Test

mut 1, mut 2, mut 3 \cdots mut x
all have same phenotype

1. \frac{mut 1}{MUT} \quad \frac{mut x}{MUT}
   \text{wild-type recessive}

2. \frac{mut 1}{+} \quad \frac{+}{mut 2}
   \begin{align*}
   \text{mutant} \\
   \text{fail to complement}
   \end{align*}
   \begin{align*}
   \text{wild-type} \\
   \text{complement}
   \end{align*}
   \begin{align*}
   \text{one gene allele}
   \end{align*}
   \begin{align*}
   \text{two genes}
   \end{align*}

3. caversa
   \begin{align*}
   \text{intra-alletic complementation}
   \end{align*}
Adenine biosynthesis

\[ \text{ADE} 3 \]
\[ \rightarrow \text{PRPP} \rightarrow \text{P-ribosylaminic} \rightarrow \text{P-ribosylglycinamide} \]
\[ \text{ADE} 2 \]
\[ \rightarrow \text{P-ribosylaminoimidazole (CAIR)} \]

\[ \Delta \text{ade} 3 \]
\[ \Delta \text{ade} 6 \]
\[ \Delta \text{ade} 2 \]
\[ \text{ade} 3 \text{-101} \]

\[ \text{ade} 2 \text{ j ade} 6 \]
\[ \text{ade} 2 \text{ j ade} 3 \]
\[ \text{ade} 3 \text{-101 j ade} 2 \]

accumulate pigments that are red

\[ \Delta \text{ade} 3 \]
\[ \Delta \text{ade} 6 \]
\[ \text{ade} 2 \text{-101} \]

Epistasis
cdc4 → cdc7 → 7 4 → 7 → 4 → 4, 7

why do you get an arrest

→ x → y → z → divide substrates

→ negative control

cdc9 (ligase)

incomplete DNA synthesis

cdc10 (DNAase ORC)

α - irradiation

blocks

1. mutants
   - drug
   - phenotype
   - suppressor

2. complements
3. pathways
4. Phenotyping

seeks out damage and says wait
rad (radiation sensitive) relieved the sensing mechanism

→ continue through cell cycle
→ death

DNA damage → mitotic 9, 17, 24 Hrs 3
DNA replication → s phase Pol G Rfc5p Dpb11

spindle anaphase checkpoint ??

↓ Damage

substrate-product x

negative control

if one can find relief of dependence = control mech
Genetic Screens

1. interesting phenotypes
2. Forward
3. Reverse

a) silent cassettes
b) mothers switch not daughters
c) both cells switch
MATING TYPE IN *S. cerevisiae*

1. Each produce a diffusible peptide hormone
2. Each mating-type has a receptor for the peptide

Diploid

1. Cannot mate
2. Enter meiosis \(\rightarrow\) sporulate.

A. Mating is stable in lab strains (het-)
B. Wild-type strains can switch mating-types (HO)

\(\text{Four meiotic} \quad \circ \rightarrow \circ \rightarrow \text{products} \rightarrow \circ \quad \text{can mate with sibs} \)

c. Ask about the paxtin
**a-specific genes**  
1. **a-factor**  
2. **a-factor receptor**

- **α factor**

- Mating occurs in G1
- α factor can be secreted and diffuse
- Polarization
  - Contact
  - Fusion

A cell responding to high levels of α factor via receptor (STE2) activates G protein

Gα, Gβγ

STE4, STE18

GEE scaffold  
**CDC24**  
**CBB**  
**FAR1**  
Binds p21 activated kinase

STE20  
**STE5**
actin polymerization

1. Two class of sterile
   MT - specific
   MT - non-specific
MATα vs MATα

chr.3

1. Proteins
   MAT A1 - homodomain protein
   MAT A2 - unknown function
   MAT a1 - homodomain protein

2. Phenotypes
   a mat a1: sporulation defective
   a mat a2: no phenotype
   Δ mat a2: decreased mating efficiency

3. Mechanism
   a. a1 + a2: repress haploid specific genes
   b. a2 + aα: repress a-specific genes
   c. a1: activate a-specific genes

α/α: no α, a specific gene expression
test with α-factor as it will cause MATa to arrest in G1 but not MATα

1. Mothers switch
2. Pairs of cell switch
3. Most but not all mother switch

act between G1 + S

c. Is there exchange of information?

- use mata- mutant
  mata- mutant

"Mutants are healed by switching"

d. Cloning the genes

1) complementation

HMR, HML are "silent" copies of mating-type information
MATα ≠ MATα
they encode different proteins

MATα encodes Matα1

MATα encodes Matα2

MATα encodes Matα1

MATα encodes Matα2

Three sets of genes are regulated:

α-specific asg

α-specific xsg

haploid-specific hsg

\( a = \Delta \)

\( a_1, a_2 \rightarrow \text{UNA1} \)

\( a_1, a_2 \)

α-specific α-factor α-specific

α2 → aspecific

α1+α1 → MAT α/α specific
How are HMRα and HMLα kept silent?

- HML
- HMR

- Remove a cassette
- Insert α-cassette
- Remove old cassette
- Switch from α → α

- silent
- HAT
- Rine + Herskowitz

- cis-acting sites not present at HAT
  - E = essential
  - I = important

1. Turn on HMR, HML → sterile
   - α-factor resistant
Screen

\[ \alpha \rightarrow \text{mat} \rightarrow \text{a} \]

\[
\text{mat} \rightarrow \text{a} \text{ cells as diploids} \\
\text{M} \rightarrow \text{A}
\]

\[
\text{mat} \rightarrow \text{a} \text{ cells as a cells}
\]

HMR mat a HMR sir mate as a cells

screened 675,000 colonies for mating as a cell \( \rightarrow 296 \)

SIR 1 73

SIR 2 13

\[ \text{NAD-dependent histone deacetylase} \]

SIR 3 31

interacts w/ sir 2, sir 4, histone tails

SIR 4 24

recruited by Rap 1

Unknown 17
sir2, sir3, sir4 → non-mating
decrease of HMR, HML

sir1 → "leaky" still able to mate
null alleles are "leaky"
genetically equivalent cells have different
phenotypes.

look at phenotype of single cells

α-factor medium

a
no division - sensitive

a
division - resistant

α/a
resistant

a sir2
resistant

a sir3
resistant

a sir4
resistant

a sir1-1
20S: 80R

α sir1Δ
21S: 79R

α sir1Δ + sir1
resistant

Questions:
1) threshold response of sir1 mutants
2) ask if there only two types of cells

↓ return to medium
↓ without α-factor
↓ return to
α-factor
MOTHERS SWITCH AND DAUGHTERS DO NOT

1. Mothers transcribe HO gene

   AGTTECAAGTTT  →  HO endonuclease that cuts
   CCCGAACAGAT    →  gene conversion using HMR, HML as templates
   AATT

2. What allows mothers (or prevents daughters) to transcribe HO?
   a. regulator of HO transcription
      Swi1- Swi6 complex
      Swi5
   b. Swi5 is transcriptionally active in S, G2, M and constitutive expression allows daughters

   5 genes required for HO expression → SNF
c. SWI5 is found in both M and D's.

d. What activates SWI5 in mothers?

**Genetic Screen for daughters that switch**

1. \( Ho^+ \) selects (receptor gene) for \( \alpha \)-factor

mutagenize

\[ \text{\( \alpha \)-factor agar} \]

\[ \text{wild-type} \quad \text{mut} \quad \text{a} \]

\[ \quad \alpha \quad \alpha \quad \alpha \quad \alpha \quad \alpha \quad \alpha \]

\[ \text{arrest} \quad \text{aa} \quad \text{aa} \quad \text{arrest with} \quad \text{4 shmoos} \]

\[ \text{shmoos} + \quad \text{budding cells} \quad \text{ashl} \quad \text{HO} \quad \text{I-HO} \]

1. Show ash1 is recessive

2. Clone by complementation - GATA TRY Family

3. Daughters transcribe HO

4. Ash1 protein is only in daughters nucleus

5. O/E \( \rightarrow \) inhibit switching in mothers.
Localization of Ash1 m RNA

a. looked for defective URS1 function

\[ \text{HO} \rightarrow \text{ADE2} \rightarrow \text{white} \]

\[ \text{HO} \rightarrow \text{CUN1} \rightarrow \text{canavanine} \]

↓ look for inactive HO promoter

\[ \text{urs}1 \text{ urs}2 \rightarrow \text{red} \rightarrow \text{can} \text{R} \]

\[ \text{urs}1 \text{ urs}2 \rightarrow \text{can} \text{R} \]

\[ \text{Swi-Snf complex mutants} \]

chromatin remodeling complex.

add 3rd gene

\[ \text{URS1} - \text{binds} \text{Swi} \]

\[ \text{urs}2 \rightarrow \text{GAL-HO} \rightarrow \text{lacZ/β-gal} \]

direct mother specific HO

1) dependent on SWI/SNF genes except SWI5

3000 canR red colonies

315 β-gal+ → SHE gene

222 SWI5 mutants characterized 46 → 5 comp. groups
SHE1  Myosin 4 (class V myosin)

SHE5  Bni1 (alin)

SHE2  RNA binding protein, interacts with She3

SHE3  Adapter between She2 and Myo4

SHE4  Myosin binding domain

mRNAs move along actin cables to the daughter bud.
sas (something about silencing)

20% male

80% nonmating

all mate

non mating $\rightarrow$ 2 genes

SAS 2

3

- 3 old genes

rescue w/ library $\rightarrow$

new gene

new gene