A. Plasmids

1. High copy number
   a. endogenous plasmid: \(2\mu\)
   b. 50-100 copies/cell

2. ARS plasmids
   a. AT rich sequence
   b. autonomous replication
   c. 50-100 copies/cell
   d. selectable marker
      1. \(\text{KAN}^R\)
      2. \(\text{URAS}\)

\(\text{orotidine 5'-phosphate deaminase}\)

\(\text{URAS} \downarrow\)

\(\text{uracil} \rightarrow \text{uridine monophosphate} \downarrow \text{to grow} \downarrow \text{uracil}\)
selectable markers

a. URA3

orotidine 5'-phosphate decarboxylase

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

\[ \text{URA3} \]

b. mutants in URA3 (ura3\(^{-}\))

1. require uracil for growth

2. resistant to 5-fluoroorotic acid

\[ 5\text{FOA} \rightarrow 5\text{-F uracil} \]

\[ \text{URAC3} \]

3. 5-fluoro-uracil is toxic to cells

4. 5-FOA is a suicide substrate

5. Positive and negative selection

\[ \text{Ara3} + \text{URA3} \rightarrow \text{positive selection by growth on medium without uracil} \]

\[ \text{Ara3} \rightarrow \text{negative selection by growth on medium with 5\text{FOA}} \]

c. Kanamycin resistance

\[ \text{NPT II} - \text{neomycin phosphotransferase II} \]
Artificial chromosomes

Loss rates  
Loss rate  

**Normal chromosome III (530 kb)**  
$4 \times 10^{-6}$  

**Deletion III (150 kb)**  
$1.4 \times 10^{-5}$  

Artificial chromosomes  

<table>
<thead>
<tr>
<th>Size</th>
<th>Event/division</th>
</tr>
</thead>
<tbody>
<tr>
<td>104 kb</td>
<td>$2 \times 10^{-9}$</td>
</tr>
<tr>
<td>55</td>
<td>$1.6 \times 10^{-2}$</td>
</tr>
<tr>
<td>11</td>
<td>$1.1 \times 10^{-1}$</td>
</tr>
<tr>
<td>Circular 55</td>
<td>$2.8 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

Questions

1. **Loss vs. nondisjunction?**
   - Loss increases while NJ remains the same

2. Why is length important?
Yeast deletion collection

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

Experiments
1. 18.77% 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

After treatment
Why

1. Loss of function
2. Gene is identified

ATG

... KAN Hz ...

20bp

bar code

5916 genes deleted (96.5% of genes)

1,105 essential on rich (aglucon) medium

1,105 4811

1. 62% have homolog in another organism
   1. 67% have homolog
2. 17% have paralog
2. 8.5% have paralog

1. haploid
2. heterozygous and homozygous diploid
First synthetic yeast chromosome revealed

US-based project recruited dozens of undergraduates to stitch DNA fragments together.

Ewen Callaway

27 March 2014

It took geneticist Craig Venter 15 years and US$40 million to synthesize the genome of a bacterial parasite. Today, an academic team made up mostly of undergraduate students reports the next leap in synthetic life: the redesign and production of a fully functional chromosome from the baker’s yeast Saccharomyces cerevisiae.

As a eukaryote, a category that includes humans and other animals, S. cerevisiae has a more complex genome than Venter’s parasite. The synthetic yeast chromosome — which has been stripped of some DNA sequences and other elements — is 272,971 base pairs long, representing about 2.5% of the 12-million-base-pair S. cerevisiae genome. The researchers, who report their accomplishment in Science¹, have formed an international consortium to create a synthetic version of the full S. cerevisiae genome within 5 years.

“This is a pretty impressive demonstration of not just DNA synthesis, but redesign of an entire eukaryotic chromosome,” says Farren Isaacs, a bioengineer at Yale University in New Haven, Connecticut, who was not involved in the work. “You can see that they are systematically paving the way for a new era of biology based on the redesign of genomes.”

The project began a few years ago, when Jeff Boeke, a yeast genetecist at New York University, set out to synthesize the baker’s yeast genome with much more drastic alterations than those demonstrated by Venter and his team in 2010.

The group at the J. Craig Venter Institute in Rockville, Maryland, had chemically synthesized short strands of DNA and stitched them together to create a version of the 1.1-million-base-pair DNA genome of the bacteria Mycoplasma mycoplotes, which was then inserted into a recipient cell². Venter’s team wrote a few coded ‘watermarks’ into the genome sequence, which spelled out the names of the team members, as well as several famous quotes. But besides these tweaks and a few other changes, the synthetic M. mycoplotes genome was identical to its blueprint.

By contrast, Boeke and his team thought that by stripping the genome of certain features to test their importance, they could justify the enormous cost and effort of synthesizing whole yeast chromosomes.

“I wasn’t skeptical about whether it could be done,” Boeke says. The question, he explains, was: “How can we make this different from a normal chromosome and put something into it that’s really going to make it worthwhile?”

![CONSTRUCTING LIFE](image)

**CONSTRUCTING LIFE**

Researchers have synthesized a fully functional chromosome from the baker’s yeast Saccharomyces cerevisiae. At 272,971 base pairs long, it represents about 2.5% of the organism’s 12-million-base-pair genome.

1. Scientist writes DNA sequence on computer → DNA-synthesis machine creates short corresponding sequences

2. Polymerase chain reaction is used to stitch sequences into ~750-bp-long strands → Yeast cells weave several 750-bp-long strands into 2500- to 4,000-bp chunks

3. *Build-a-genome*

   The scientists decided to omit certain DNA sequences from their synthetic chromosome, such as elements with the ability to move around the genome, known as transposons, and sections within genes that do not encode proteins, called introns. They also inserted a ‘scrambling’ system — which shuffles and removes genes — to provide a way of testing whether a given gene is essential to survival.

The initial plan was to contract commercial DNA-synthesis companies to create large chunks of the yeast’s first order, a 90,000-base-pair chunk of DNA corresponding to a portion of the S. cerevisiae chromosome to arrive.

“I’ll be dead long before this project will ever be finished,” Boeke remembers thinking. So he turned his microscope on chromosome-length stretches of DNA.
Complementation Test

mut1, mut2, mut3, …, mutx all have same phenotype

1. \[
\begin{align*}
\text{mut1} & \quad \text{MUT} \\
\text{mut} & \quad \text{MUT} \\
\text{mut} x & \quad \text{MUT}
\end{align*}
\]

wild-type recessive

2. \[
\begin{align*}
\frac{\text{mut1}}{+} & \quad \frac{+}{\text{mut2}}
\end{align*}
\]

mutant \quad \text{wild-type}
fail to complement \quad \text{complement}

one gene alleles \quad two genes

3. caycrats
intra-allelic complementation

aspartate transcarbamylase (12 subunits)

\[
\begin{align*}
\text{CC} & \quad \text{CC} \\
\text{CC} & \quad \text{RR} \quad \text{RR}
\end{align*}
\]
Suppressors

Types
a. Informational correct the phenotypic defects of a mutation without restoring wild-type sequence
b. Interaction

c. Bypass

Informational suppression
- Alter the genetic apparatus
  - tRNA suppressors
    1. stop codons
    2. frameshift
  - upf/nmd genes (smg in C. elegans)

Properties
1. Allele-specific but not gene-specific
2. Dosage-dependent
3. Strong alleles are deleterious
Informational Suppressors

1. tRNAs tyrosine
   \[ \text{UAU} \rightarrow \text{UAG as tyrosine} \]
   \[ \text{UAC} \]

   \[ \text{UAG} \]

2. anticodon mutation
3. other parts of tRNA

Yeast efficiency is \( \approx 10-15\% \)

\[ \text{ade}^2 \rightarrow \text{auxotroph auxotroph} \]

\[ \text{Selection} \]

Select for growth on adenine medium

\[ \text{Screen} \]

Screen for white colonies
Informational suppression

smg - C. elegans

upf - yeasts

unc-54  im-29  emb-30  unc-76
myosin

nonsense codons \rightarrow 11 bp deletion

1. mRNA show increased instability

2. In presence of smg/upf mutations, mutants mRNAs show increased stability

3. NMD - nonsense mediated decay

4. Not all nonsense alleles are suppressed

Why?
Intrachute suppressor

Correct phenotypic defect by two mutant proteins restoring function

β-tubulin - α tubulin

Properties

1. Allele specific and gene specific

2. Suppressor mutation often has phenotypic

Bypass suppressor

Correct phenotypic defect by another pathway or later in the pathway by acting
3. bypass suppressors

\[ \downarrow \text{mut} \quad \text{act down stream or} \]
\[ \downarrow \quad \text{in a second pathway} \]

alleles non-specific

2. Enhancers, false non-complementation

a. Example w/ tubulin

\[ \alpha - \text{tub mut} \quad \text{wt} \quad \text{Resistance} \]
\[ + \]

\[ \beta - \text{tub mut} \quad \text{wt} \quad \text{Resistance} \]
\[ + \]

\[ \alpha - \text{tub mut} \quad + \quad \frac{+}{\beta - \text{tub mut}} \quad \text{mutant ?} \]

\[ \beta - \text{tub mut} + \alpha - \text{tub mut} \rightarrow \text{poison product interaction} \]

dual allele specificity
D. Multi-copy suppression

1. Use high copy number plasmid

or 2. Regulatable promoter (GAL1)

mut^{+s} + genomic library \rightarrow \text{rescue of phenotype}

1. Works best with loss of function alleles rather than null alleles

2. This suggests that \text{\uparrow dosage stabilizes protein}
Adenine biosynthesis

\[ \text{ADE3} \quad \text{ADE4} \quad \text{ADE5} \quad \text{ADE7} \quad \text{ADE6} \quad \text{ADE8} \]

\[ P_{-} \text{ribose} \rightarrow P_{-} \text{ribosylaminc} \rightarrow P_{-} \text{ribosylglycinamide} \]

\[ P_{-} \text{ribosyl}-PP \]

\[ \text{ADE2} \]

\[ P_{-} \text{ribozymalingo} \rightarrow P_{-} \text{ribosylsuccinc} \rightarrow \text{carboxamidine} \]

\[ \text{imidaazole} \quad \text{carboxylde} \quad \text{ADE1} \quad \text{amino imidaazole} \quad \text{( CAIR)} \]

\[ \text{( CAIR)} \]

ade 2, ade 1 accumulate pigments that are red

\[ \Delta \text{ade3} \quad \text{white} \]

\[ \Delta \text{ade6} \quad \text{white} \]

\[ \Delta \text{ade 2} \quad \text{red} \quad \text{white} \]

\[ \text{ade 3-101} \quad \text{white} \]

\[ \text{ade 2} + \text{ade 6} \quad \text{Epistasis} \]

\[ \text{ade 2} + \text{ade 3} \]

\[ \text{ade 3-101} + \text{ade 2} \]
synthetic lethality

adc2 ade3 mutx

High copy 2μ

MUTx

mutagenize to 100% viability

Plate 2000 colonies

→ look for all red colonies

→ most colony will sector

→ if all red then plasmid is required for viability

Expectations

mutX mutY = dead

mutX MUTY viable

MUTx mutY viable
Generalizations of synthetic lethality

1. The majority of synthetic lethal relationships occur among genes acting a single pathway (intrinsic)

2. Some synthetic relationships occur in biochemically distinct circuits - may be functionally redundant for example repair + recombination

3. Each gene has only a small number of synthetic interactions.

A. redundant
B. buffering
What copy number of a gene causes proliferation defects?

most genes reach ~ 75 copies/cell

115 genes < 10 copies → lethality
55 genes < 5 copies → at greater #s

dosage sensitive genes

DSG

Single gene changes are not sufficient
→ suggest "mass action"
Synthetic lethal screens with temperature-sensitive alleles

↓

ts allele in so grow at 21° but not 37°

↓

↓

screen for failure to grow at 21° often gives additional genes in the pathway

det both new mutations in y and elsewhere in pathway
Figure 2 Synthetic lethal relationships in the secretion pathway of S. cerevisiae.

<table>
<thead>
<tr>
<th>Step</th>
<th>Function</th>
<th>Number of genes</th>
<th>Same step</th>
<th>Different step</th>
<th>Not secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Translocation to Golgi</td>
<td>23</td>
<td>8</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Maturation in ER</td>
<td>16</td>
<td>10</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Vesicle budding</td>
<td>12</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Vesicle fusion</td>
<td>16</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Transport to Golgi</td>
<td>11</td>
<td>2</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Fusion w/ plasma membrane</td>
<td>30</td>
<td>43</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>Retrieval</td>
<td>20</td>
<td>15</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Vacuolar targeting</td>
<td>16</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Endocytosis</td>
<td>20</td>
<td>12</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>Endosome to vacuole</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>173</td>
<td>116</td>
<td>68</td>
<td>53</td>
</tr>
</tbody>
</table>

Miticotic arrest defective

\[
\begin{align*}
\text{Mad / Bub} & \quad \text{budding uninhibited by benomyl} \\
& \quad \text{Andrew Hoyt} \\
\text{Andrew Murray} & \\
\downarrow & \\
\text{mad1} & \quad \text{bub1} \\
& \quad \text{2} \\
& \quad \text{3} \\
& \quad \text{Mps1} \\
\text{cdc20} & \quad \text{kinetochore} \\
& \quad \text{mad2} \quad \text{mad1} \\
& \quad \text{APC} \\
\text{nud1} & \quad \text{bub3} \quad \text{mad3} \\
& \quad \text{bub1} \\
& \quad \text{skp1} \\
\text{bub2} & \quad \text{bfa1} \quad \rightarrow \text{Tem1 / SPB} \\
& \quad \text{benomyl} \\
& \quad \text{Hoyt} \quad 3 \times \text{benomyl (70 \text{ug})} \\
& \quad \text{Li, Murray} \quad \text{disturb} \quad \text{but not disynchr} \\
& \quad \text{15 \text{ug}} \\
& \quad \text{death} \quad \text{\rightarrow WT delay} \\
\quad \text{\rightarrow mutants progress} \\
\quad \text{\rightarrow arrest in prophase} \\
\quad \text{\rightarrow MPF, dwarf buds} \\
\quad \text{different genes} \quad \rightarrow \text{all nonessential}
\end{align*}
\]
Figure 4  Kinase-specific inhibition can be achieved by coupling chemistry and genetics. (A) Wild-type kinases are inhibited by a non-specific inhibitor. (B) Wild-type kinases are not inhibited by non-specific inhibitor analogs containing a sterically bulky functional group. (C) Engineered kinases show normal kinase activity. (D) Engineered kinases are inhibited by inhibitor analogs that contain a sterically bulky functional group. (E) Chemical structures of ATP and the general kinase inhibitor PP1. (F) Chemical structures of specific kinase inhibitors, IC<sub>50</sub> wt/mutant (nM).
Shokat alleles.

\[
\begin{align*}
\text{large hydrophobic residue} & \rightarrow \text{glycine alanine} \\
= \text{new pocket}
\end{align*}
\]

most kinases are unaffected because pocket is too small.

\[M \rightarrow G \text{ analog sensitive allele}\]
A Euploid cells
Chromosomes

A B C

Complex subunits

Protein complex

Chaperone

QC

B Aneuploid cells
Chromosomes

A B C

Complex subunits

Protein complex

QC

Fig. 1. Aneuploidy causes proteotoxic stress. (A) Cells use protein quality-control (QC) and feedback mechanisms to maintain subunit stoichiometries of complexes whose subunits are encoded by different chromosomes. The protein quality-control machinery ensures accurate folding and maintains complex subunits that lack a binding partner in a soluble state. Eventually, excess and misfolded subunits must be degraded, as illustrated here by the yellow subunit that has been produced in relative excess. (B) Changes in chromosome number in aneuploid cells (shown here as disomy of the green chromosome) lead to a genomic imbalance that results in stoichiometric protein imbalances. Every subunit encoded by an unbalanced chromosome that functions in a protein complex lacks its binding partner(s) and must rely on cellular chaperones to maintain solubility and, if no binding partner is found, on the cellular proteases for its eventual degradation. This can lead to an increased burden on the protein quality-control systems and the exhaustion of the cellular protein quality-control machinery.
1. Aneuploidy

70-90% of human tumors have aneuploidy.

What are the consequences?

Amon Lab

1. Make disomic strains (1n+1)
2. Monitor chromosome loss of a YAC
3. Monitor proliferation on medium with low levels of microtubule poison (benomyl)

4. Findings - Most aneuploids show phenotypic
   a) 10/13 disomes show increased loss
   b) 8/13 disomes are supersensitive to benomyl

5. What other cellular processes are affected?
a) forward mutation rates

3/13 show increased mutations at several loci.

b) measure DNA repair

Rad52-GFP foci form after treatment with DNA damaging agent (pleomycin)

In wild-type → foci disappear upon removal

7/13 disomies, the foci remain

Mechanisms

1. Extra DNA or imbalance (dosage-sensitive) in proteins

2. Add YAC with human DNA
   no phenotypes

3. Trisomy 2n+1 (less imbalance)
   4/5 are not sensitive to DNA damage
   3/3 do not lose the YAC
Are the effects due to many genes or a single gene on the disomic chromosome?

Mate

\[
\begin{array}{c}
00000000 \\
0000000 \\
0000000 \\
0000000
\end{array}
\begin{array}{c}
\bigcirc \\
\bigcirc \\
\bigcirc \\
\bigcirc
\end{array}
\rightarrow
\begin{array}{c}
00000000 \\
0000000 \\
0000000 \\
0000000 \\
0000000 \\
0000000 \\
0000000 \\
0000000
\end{array}
\]

Yeast deletion \times \text{disome collection}

1. \begin{enumerate}
   \item \text{fitness/growth was associated with loss of particular genes and particular disomes}
   \begin{enumerate}
     \item \text{\Delta SSD1, disome VIII}
     \item \text{\Delta PCA32, disome XI}
     \item \text{endocytosis}
     \item \text{\Delta EDE1, disome IX}
     \item \text{protein transport}
     \item \text{\Delta 3 different COG genes, disome XVI (retrograde Golgi)}
   \end{enumerate}
\end{enumerate}
EDE1 - coxl protein recruited to endocytic patches as an early step

PRK1 - protein kinase that regulates endocytic proteins
- maps to chromosome IX

\[ \text{Aprk} \quad \Delta \text{edel} = \text{no longer shows synthetic fitness defect} \]

In this case, ↑ dosage of one gene causes ↓ fitness of one disome.

Are there genes that when deleted affect the fitness of multiple disomes?

5/11 possible A's cause ↓ fitness of multiple disomes.

TPS1 - trehalose 6-phosphate synthase
The role of trehalose is protection from stress.
Δ MNN10  Golgi mannose1 transglucerase caused ↓ fitness in 8/16 disomes
Δ RVS167  actin associated endocytosis and exocytosis protein
   3 disomes ↓ fitness
   2 disomes ↑ fitness
Δ UBP3  deubiquitinase
   10/13  ↓ fitness
   2/13  synthetic lethality
Δ of Genes affecting protein trafficking are enriched in this assay
test with \( \alpha \)-factor as it will cause \( \text{MATa} \) to arrest in \( G1 \) but not \( \text{MATa} \).

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 \( \text{mata}^- \) mutant
   - use \( \text{mata}^- \) mutant

"Mutants are healed by switching"

D. Cloning the genes
   1) complementation

HMR, HML are "silent" copies of mating-type information
How are HMRα and HMLα kept silent?

Rine + Herskowitz

cis-acting sites not present at MAT

E = essential    I = important

Trans-acting
1. Turn on HMR, HML \rightarrow sterile

\alpha-factor resistant
in ho− background

Screen

\[ \alpha \rightarrow \text{mata} \rightarrow \alpha \]

\[ \frac{\text{mata} \rightarrow \text{mate as } \alpha \text{ cells as diploids}}{\text{MATα}} \]

\[ \text{mata} \rightarrow \text{mate as a cells} \]

HMR mata1 HMR sir mate as a cells

screened 675,000 colonies for mating as \[ \alpha \] cell \[ \rightarrow \] 296

SIR1 73

SIR2 13

SIR3 31

SIR4 24

Unknown 17

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

interacts w/ sir2, sir4, histone tails

recruited by Rap1

""
sir2, sir3, sir4 → non-mating
depression 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 0 shmo

α
division - resistant 088

aA
resistant

a sir2
resistant

a sir3
resistant

a sir4
resistant

a sir1-1 205 : 80R

a sir1A 215 : 79R

a sir1A + sir1 resistant

Questions
1) Threshold response of sir1 mutants
2) Ask if there only two types of cells

↓ return to medium 8

↓ return to medium without α-factor

↓ return to α factor
Establish repression
sir 2, 3, 4 maintain double mutant

sensitive parent
resistant parent

14 days

4 x 10^-3 change in stable/cell division

MOTHERS SWITCH AND DAUGHTERS DO NOT

1. Mothers transcribe HO gene

- HO endonuclease that cuts
- Double stranded break
- Gene conversion using HMR, HML as templates

2. What allows mothers (or parents daughters) to transcribe HO?

   a. Regulator of HO transcription

      Swi4 - Swi6 complex

      Swi5

   b. Swi5 is transcribed only in S, G2, M and constitutive expression allows daughters

   5 genes required for HO expression -> SNF
c. Swi5p is found in both M and D's.

d. What activates Swi5 in mother's?

Genetic Screen for daughters that switch

1. HO⁺ ΔstecΔ (receptor gene) for α-factor

mutagenise \[ α - factor \text{ agar}\]

wild-type \[ μ\text{+} \] mut \[ a \]

\[ α \] \[ α \] \[ a \] \[ a \] \[ a \] \[ a \] \[ a \]

\[ α \text{ arrest} \] \[ α \text{ arrest} \] \[ a \text{ arrest with} \] 4 shmoos

shmoos + budding cells \[ \text{ashl} \]

1. Show ash1 is recessive

2. Clone by complementation - GATA THRE Family

3. Daughters transcribe HO

4. Ash1 protein is only in daughters nucleus

5. OE → inhibit switching in mothers.

All mRNA is localized to daughters.
Localization of Ash1 mRNA

a. Looked for defective URS1 function

- HO active
  - ADE2 → white
  - CAN1

- Look for inactive HO promoter
  - sw16
  - sw15
  - sw14
  - ur51 ur62

- URS1-binds Swi

- add 3rd gene

- URS2 → GAL-HO → lacZ/β-gal

1) dependent on SWI/SNF genes except SWI15

- 3000 canR red colonies

- 222 sw15 mutants characterized 46 → 5 comp. groups
SHE1  Myosin 4 (class V myosin)
SHE5  BN11 (formin)
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.