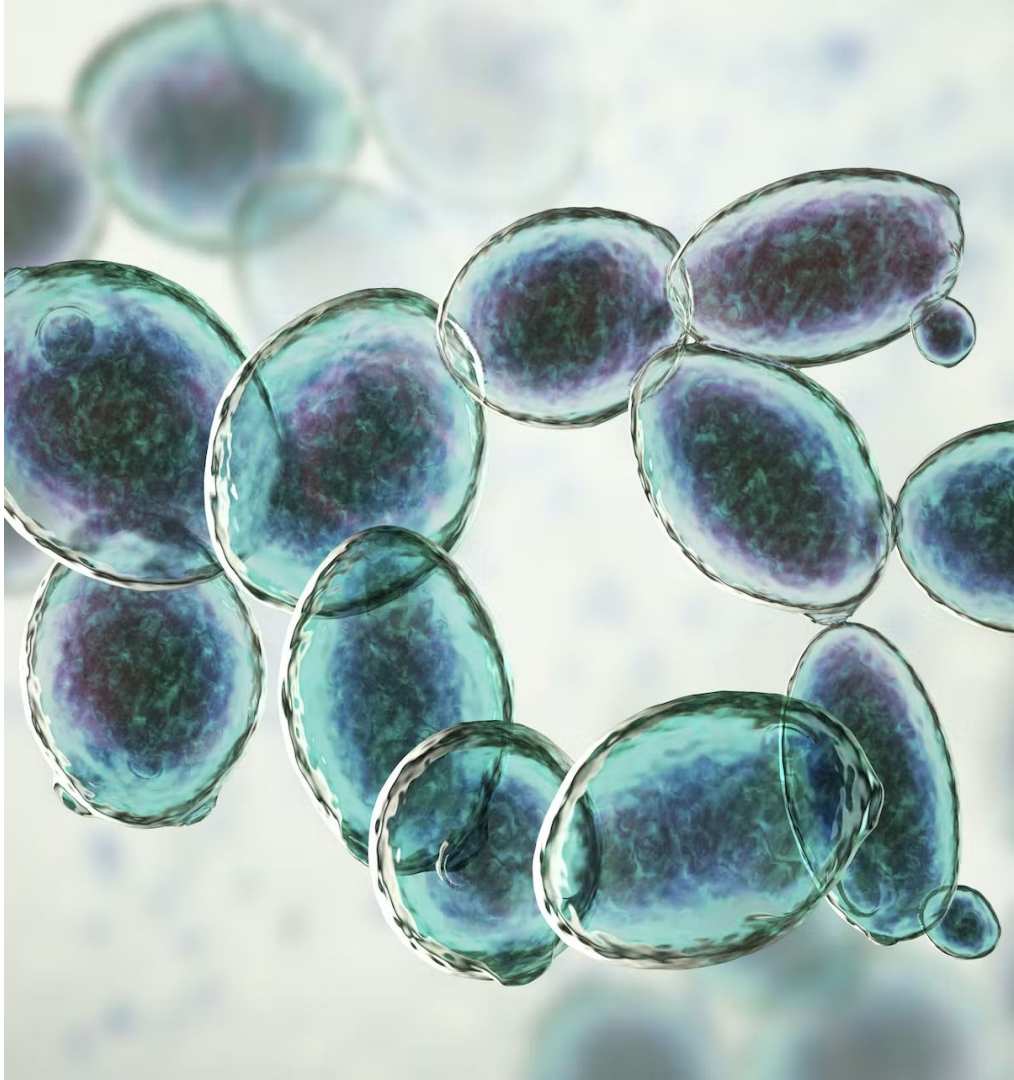
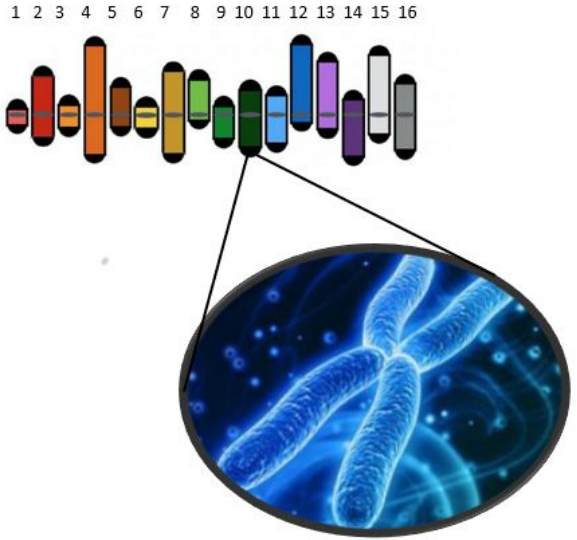
Yeast 2.0

Katri Nieminen, Pinja Salminen, Viivi Mäkelä, Pihla Manninen



Our chosen region ~30 kb megachunk



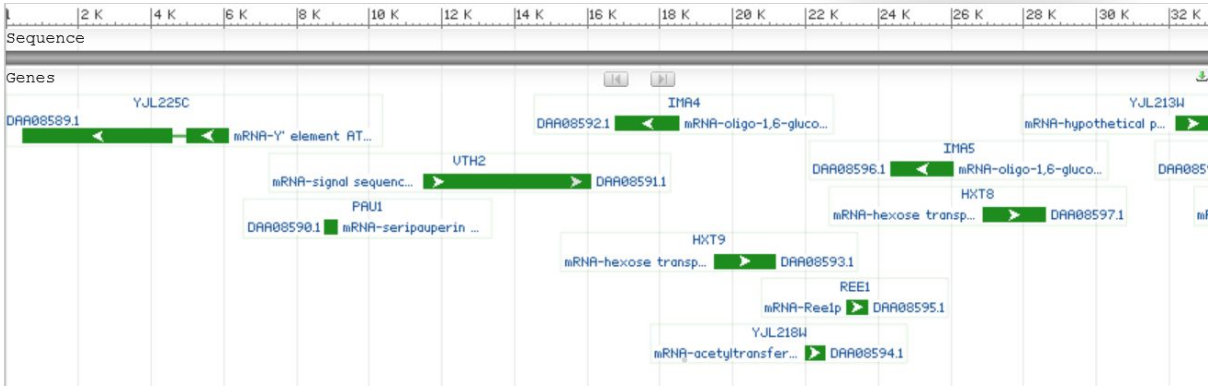
Saccharomyces cerevisiae S288C

Chromosome 10
1 bp → 33158 bp

TEL10L-ARS1001-**YJL225C**-ARS1002-**YJL223C**-**YJL222W**-ARS1003-
YJL221C-**YJL219W**-**YJL218W**-**YJL217W**-ARS1004-**YJL216C**-**YJL214W**-**YJL213W**

telomere: TEL10L
gene 1: **YJL225C,** (5664 bp) ATP-dependent helic.
gene 2: **YJL223C,** (362 bp) PAU1
gene 3: **YJL222W,** (4649 bp) VTH2
gene 4: **YJL221C,** (1769 bp) IMA4
gene 5: **YJL219W,** (1703 bp) HXT9
gene 6: **YJL218W,** (590 bp) acetyltransferase
gene 7: **YJL217W,** (596 bp) REE1
gene 8: **YJL216C,** (1745 bp) IMA5
gene 9: **YJL214W,** (1709 bp) HXT8
gene 10: **YJL213W,** (995 bp) hypothetical protein

blue = non-essential ORF
light blue = uncharacterized ORF
red = essential ORF



https://www.ncbi.nlm.nih.gov/nuccore/BK006943.2?report=graph

Closer look at the genes:

1. **ATP-dependent helicase**, predicted to enable helicase activity, predicted to be involved in DNA duplex unwinding.
   Uncharacterized: Biological function and role not yet well understood in cellular processes

2. **Seripauperin PAU1**, predicted to be a structural constituent of cell wall.
   Non-essential: encodes an enzyme which appears in metabolism when there is no oxygen available. Needed when alcohol is produced

3. **VTH2**, signal sequence-binding protein, enables signal sequence binding activity.
   Non-essential: Vth2 protein is involved in thiamine biosynthesis, but yeast can obtain thiamine from their environment so no significant change has been shown in growth or function when the gene is removed

4. **Oligo-1,6-glucosidase IMA4**, enables oligo-1,6-glucosidase activity.
   Essential: the gene encodes a protein involved in adenine synthesis. Adenine is obligatory in cell survival and growth and without the gene IMA4 the cell can not survive without exogenous adenine

5. **Hexose transporter HXT9**, enables hexose transmembrane transporter activity.
   Essential: Without the HXT9 gene, yeast cells would have a reduced ability to take up glucose, resulting in reduced growth and survival

Closer look at the genes:

6. **Acetyltransferase**, predicted to enable galactoside O-acetyltransferase activity.
   Non-essential: Enzyme that is not part of the key processes of the cell, so not essential for the survival of the cell, can be removed without harming the growth of the cell in normal growth conditions.

7. **REE1 (Ree1p)**, regulation of Enolase I.
   Essential: Involved in the regulation of enolase, which plays a part in glycolysis.

8. **Oligo-1,6-glucosidase IMA5**, enables oligo-1,6-glucosidase activity.
   Non-essential: Codes enzyme isopropylmalate isomerase, which is involved in biosynthesis of amino acid leucine, but it can be obtained from nutrition as well.

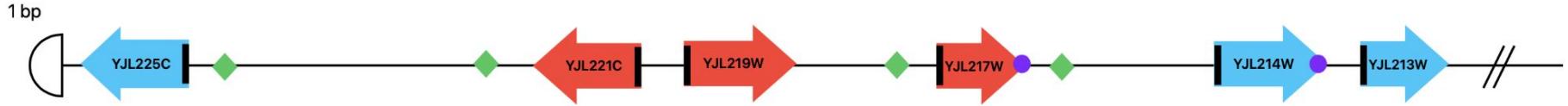
9. **Hexose transporter HXT8**, enables fructose (and glucose, mannose) transmembrane transporter activity. Involved in hexose transport.
   Uncharacterized: Has been identified and partially characterized, but more research is still needed to understand its function.

10. **(Uncharacterized protein) hypothetical protein**, predicted to enable hydrolase activity, acting on carbon-nitrogen bonds.
    Uncharacterized: Has been identified and partially characterized, but more research is still needed to understand its function.

Alterations to the yeast chromosome

Original yeast chromosome

Modified yeast chromosome



= universal telomere cap
= uncharacterized ORF
= non-essential ORF
= essential ORF
= codon swap from TAG to TAA
= loxPsym site
= PCRTag

Alterations at nucleotide level:
→ Removal of introns (2 in total from genes 4 and 7)
→ Adding PCRTags
→ Codon swap from TAG to TAA

Lab construction

1. Sequences are synthetized with DNA-synthesis machine

2. PCR is used to build 750 bp strands

3. yeast cell weaves 750 bp strands to 2-4 kbp strands

4. 2-4 kbp strands (minichunks) are combined to to the original yeast chromosome by homologous recombination

5. Markers LEU2 and URA3 are alternated in the ends of a minichunk and one marker is left to the final megachunk

Scramble-mechanism and significance of yeast 2.0

Scramble-mechanism

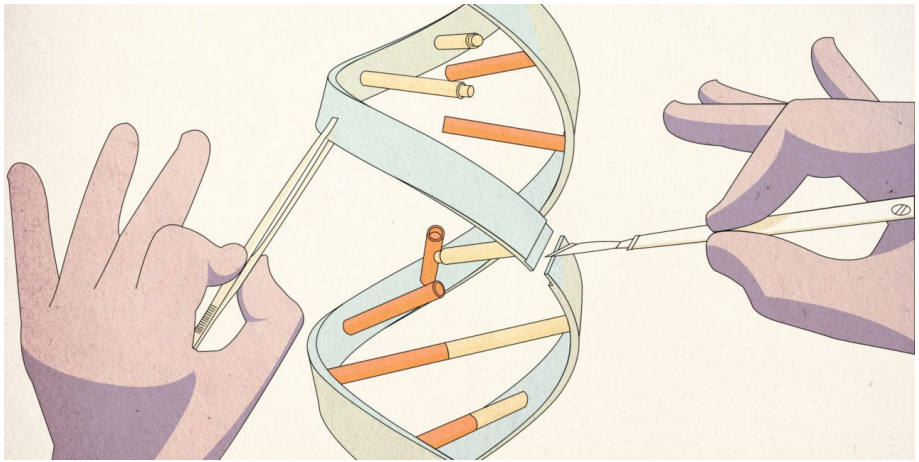
- To improve the function of a target
- Randomly recombining genetic elements to create novel combinations of genetic information

Significance and impact

- Advanced our understanding of genetics and opened new possibilities for biotechnology application
- Provided a platform for studying the basic principles of genetics and genomics

Future and further development

- **Improve the efficiency, robustness and reliability**: achieved by incorporating new genetic elements or by removing unnecessary ones
- **Genomic stability**: must remain stable over multiple generations for it to be useful in biotechnology applications -> remains stable over long periods of time and under various environmental conditions.
- **Broaden the scope**: synthesis and engineering of other yeast species to expand the range of applications and possibilities for biotechnology



Knowing genome information, engineering the yeast for different applications is possible

- **Biofuel production**, optimizing the yeast strains for maximum biofuel production
- **Pharmaceutical production (insulin, vaccines)**, optimized to produce specific pharmaceuticals and improve the yield and efficiency of the production process.

Thank you!

References

- https://www.yeastgenome.org/
- http://origin.tubic.org/deg/public/index.php/query/eukaryotes/degac/DEG2001.html
- https://www.ncbi.nlm.nih.gov/nuccore/BK006943.2?report=graph