

# Synthetic Yeast 2.0.

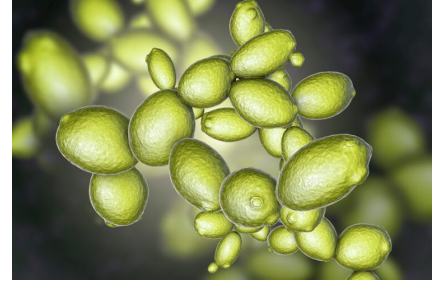
**Anniina Savolainen, Ida Uotila, Inka Mattila, Karoliina Laine, Senni Lehtonen**  
**29.3.2021**



Aalto-yliopisto  
Aalto-universitetet  
Aalto University

# Introduction to synthetic Yeast 2.0

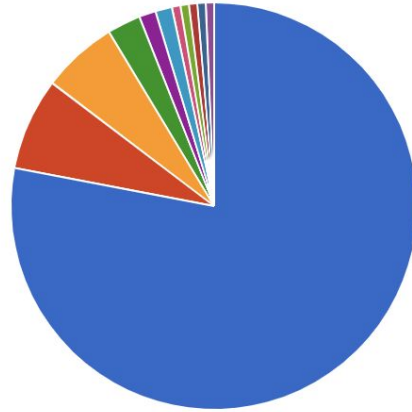
- **International project aiming to redesign the yeast genome**
- **First eukaryotic synthetic organism**
- **Saccharomyces cerevisiae as the basis for synthetic genome**
  - Well understood genome
  - 7 of 16 Chromosomes synthesized (II, III, V, VI, IX, X, XII)
- **Significance and impact of the yeast 2.0**
  - Can be used to answer in many questions related to fundamental properties of chromosomes, genome structure, evolution, gene content etc.
  - Yeast 2.0 can be optimized for desired functions e.g. to produce drugs, fuels, biomolecules
  - To reduce genomic contents and stabilize the genome



# Chromosome I and the region

## Chromosome I

- Not synthesized yet
- Length 230218 bases
- Strain S288C

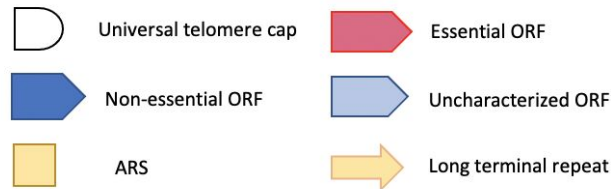
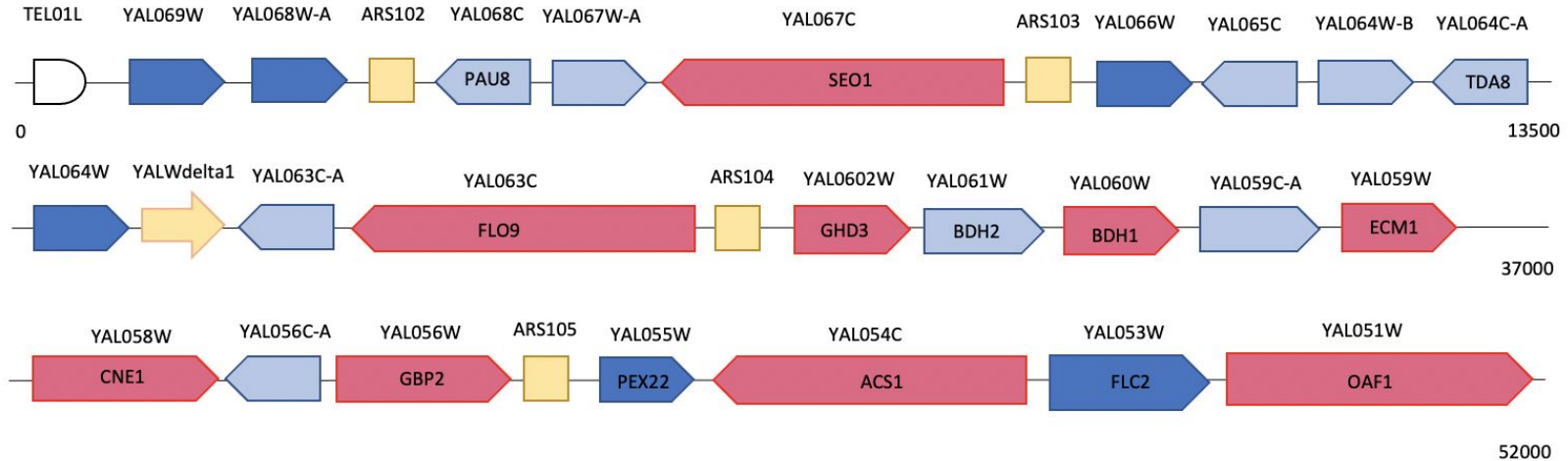


- ORF
- ARS
- long terminal repeat
- tRNA gene
- transposable element gene
- telomere
- LTR retrotransposon
- pseudogene
- centromere
- ncRNA gene
- snoRNA gene

Region of interest: 0-50000 bp of Chromosome I

# What is in the selected region?

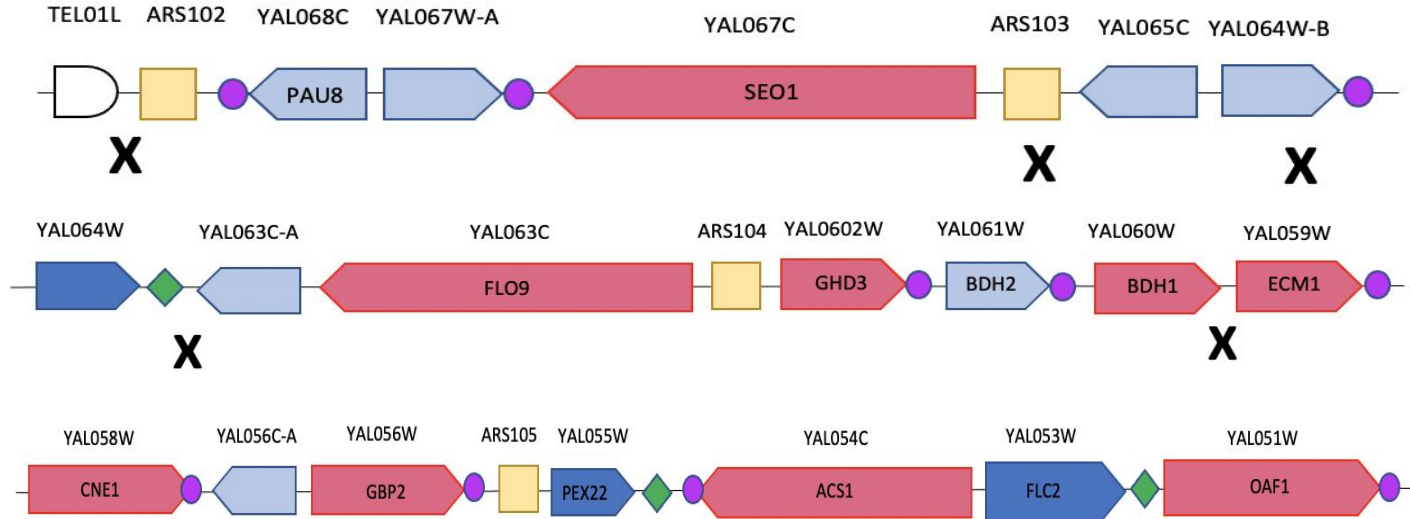
- Illustration of the region of interest



# Alterations to the selected region

- **Replacing stop codons e.g. TAG → TAA**
- **Synthetic telomere**
- **Removal of non-essential genes**
  - Non-essential genes: YAL064C-A
  - Dubious ORF: YAL066W, YAL068W, YAL069W, YAL059C-A
- **Removal of transposons**
  - YALWdelta1
- **Adding loxP sites for SCRaMbLE**
- **Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution**
  - To study different phenotypes by combinatorial rearrangements

# Illustration of design



Universal telomere cap



Codon swap



LoxPsym Site



Deletion



Aalto-yliopisto  
Aalto-universitetet  
Aalto University

# Methods for constructing the Synthetic Yeast 2.0.

## Hierarchical assembly

- Building Blocks (BBs) produced from oligonucleotides with PCR
- BBs are assembled into 2-4 kb minichunks or further to megachunks
- Native sequence is replaced with chunks by homologous recombination and alternating selection markers
- DNA integration is confirmed with PCRtags

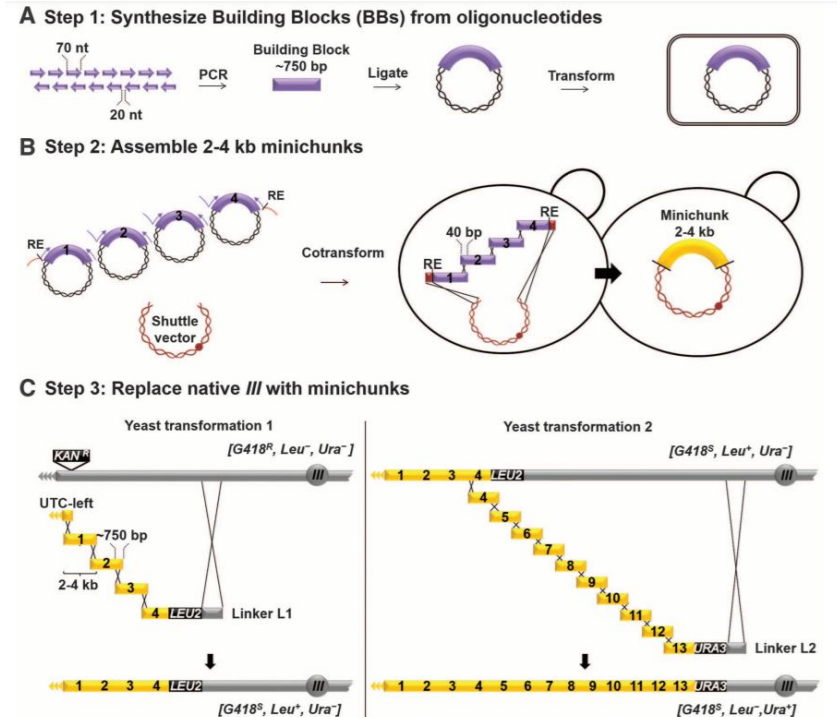


Fig. 1 Synthetic genome construction (Annaluru et al. 2014)

# Methods for constructing the Synthetic Yeast 2.0.

## Softwares for design

- **BioStudio Design**
- **Snap gene**
- **ApE - plasmid editor**
- **Database of essential genes**
  - [essentialgenes.org](http://essentialgenes.org)
- **SGD Saccharomyces genome database**
  - [yeastgenome.org](http://yeastgenome.org)
- **Gene Designer**
- **Gene Design**



# Utilization of the yeast 2.0

- Can be harnessed to produce valuable products such as drugs, biofuels and biomolecules by replacing nonessential genes with genes of interest.
- Facilitates the production optimization.
- Helps to understand properties and functions of chromosomes and individual genes.
- Fully synthesized genome allows direct testing and thus offers answers to evolutive questions.
- A platform for systematic studies of eukaryotic chromosomes.
- Getting into the unknown possibilities the detection of new functions such as expression of unnatural amino acids.

# Further development

## Synthetic yeast 3.0. project

- **Minimizing the yeast genome**
- **More radical changes to more compact genome**
- **All essential genes from each chromosome to centromeric plasmid (eArray) to avoid problem with SCRaMbLE**

## Challenges

- **Instability of genome**
- **Lack of knowledge**

# References

[https://www.yeastgenome.org/contig/Chromosome\\_I](https://www.yeastgenome.org/contig/Chromosome_I)

<http://syntheticyeast.org/>

Richardson, S. M., Mitchell, L. A., Stracquadanio, G., Yang, K., Dymond, J. S., DiCarlo, J. E., ... & Bader, J. S. 2017. Design of a synthetic yeast genome. *Science*, 355(6329), 1040-1044.

Annaluru, N., Muller, H., Mitchell, L. A., Ramalingam, S., Stracquadanio, G., Richardson, S. M., ... & Chandrasegaran, S. 2014. Total synthesis of a functional designer eukaryotic chromosome. *science*, 344(6179), 55-58.

Dai, J., Boeke, J.D., Luo, Z. *et al.* Sc3.0: revamping and minimizing the yeast genome. *Genome Biol* 21, 205 (2020). <https://doi.org/10.1186/s13059-020-02130-z>

**Thank you! :)**  
**Questions?**



Aalto-yliopisto  
Aalto-universitetet  
Aalto University