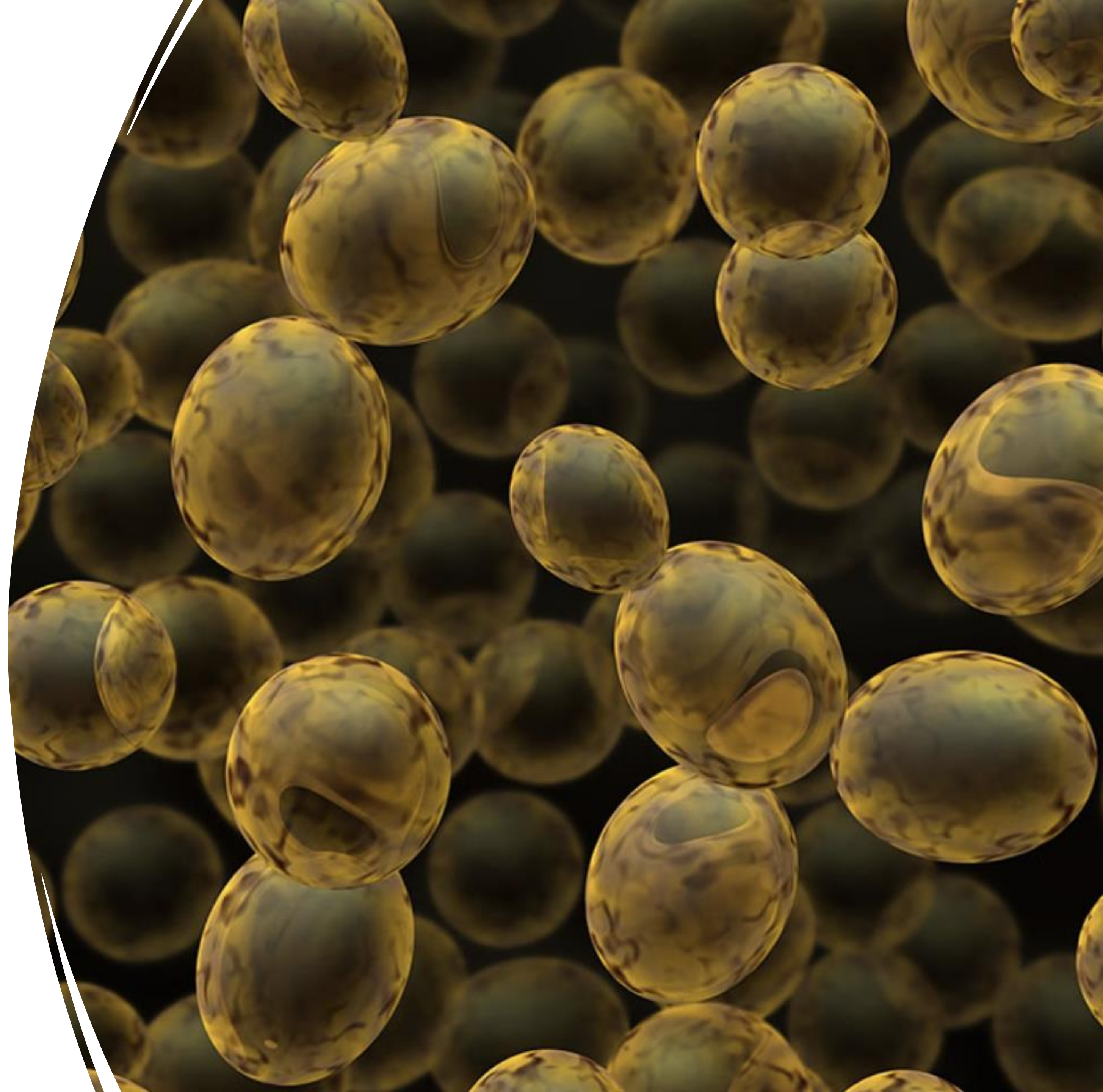
Yeast 2.0

CHEM-E8125 Synthetic Biology

Jenni Klemola, Lotta Rosenlöf,
Susa Salminen, Emma Vaara



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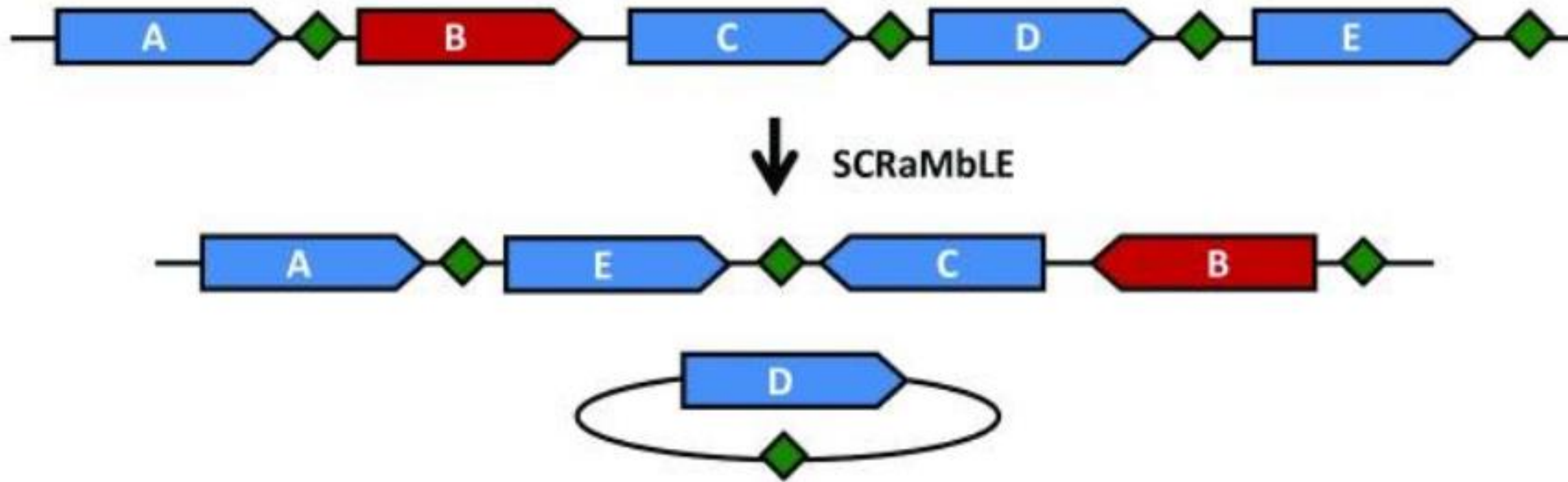
About Yeast 2.0 project

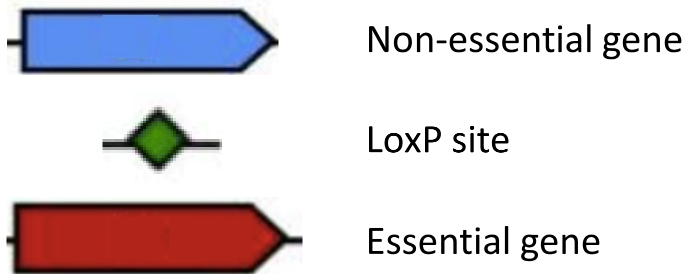
- Synthesizing and redesigning the genome of *Saccharomyces cerevisiae* by engineering the architecture of the genome but making minimal alterations to the gene sequences
- Goal is to make the genome
  - More condense by e.g. removing non-essential genes and repetitive elements
  - More stable by e.g. removing transposons
  - More genetically flexible by e.g. making SCRaMbLE possible
- Cells with designed genomes have industrial applications, e.g. they can be modified easily
- Provides understanding about essential genetic features and genetic combinations

SCRaMbLE mechanism

- **S**ynthetic **C**hromosome **R**earrangement **a**nd **M**odification **b**y **L**oxPsym-mediated **E**volution
- Inducible system that rearranges the genome at LoxPsym recombination sites
  - In Yeast 2.0 these sites are added to the genome
  - Activation of site-specific Cre recombinase gene is needed for the induction of SCRaMbLE
- SCRaMbLE can cause inversions, deletions, translocations and duplications
- A high genetic diversity can be achieved rather easily, fast and cheap by using SCRaMbLE

SCRaMbLE mechanism





Non-essential gene

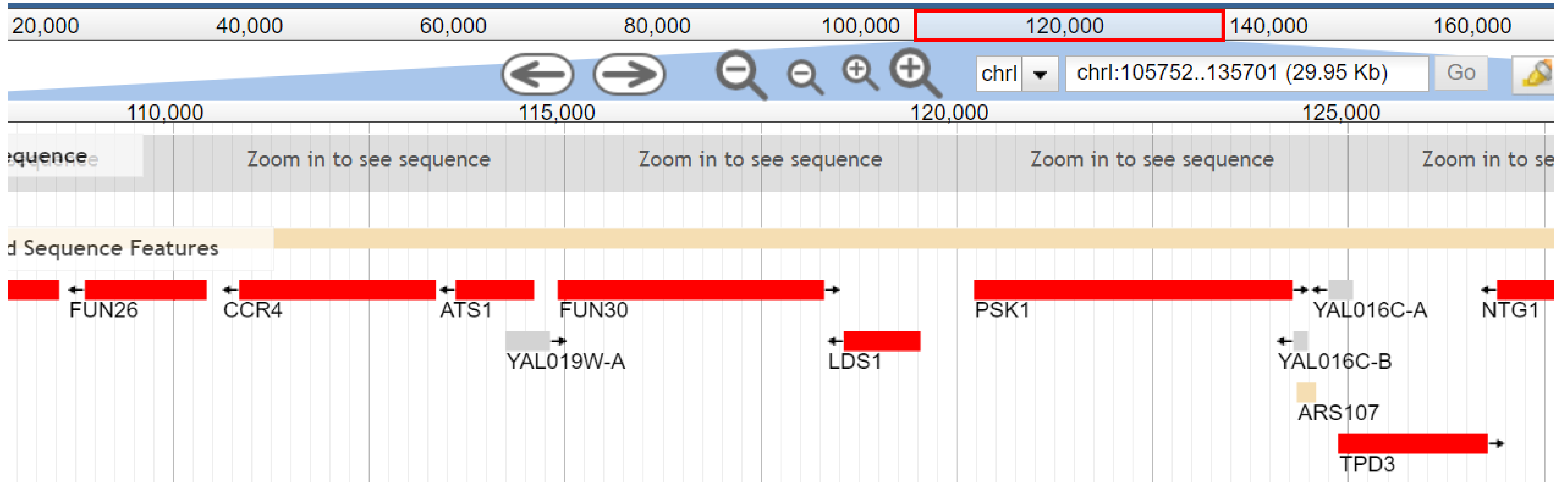
LoxP site

Essential gene

Dymond and Boeke, *Bioeng. Bugs.* **3** (2012) 168-171.

The chosen region

- 30kb region from chromosome I (~105,000-135,000)
  - None of the genes with known function (red in picture above) are essential according to the Database of essential genes
  - Dubious ORFs: YAL019W-A, YAL016C-A, YAL016C-B
  - Autonomously replicating sequence: ARS107
  - Unknown function: SWC3



Gene functions

PMT2: transferres mannose residues from lipid carrier to proteins in ER

FUN26: nucleoside/nucleobase transmembrane transporter

CCR4: involved in mRNA poly(A) tail shortening

ATS1: needed for modification of wobble nucleosides

FUN30: involved in ATP-dependent chromatin remodeling

LDS1: involved in spore wall assembly

PSK1: serine/threonine protein kinase

Gene functions

TPD3: needed for cell morphogenesis and transcription by RNA polymerase III

NTG1: involved in DNA repair

SYN8: endosomal S NARE protein

DEP1: involved in histone deacetylation

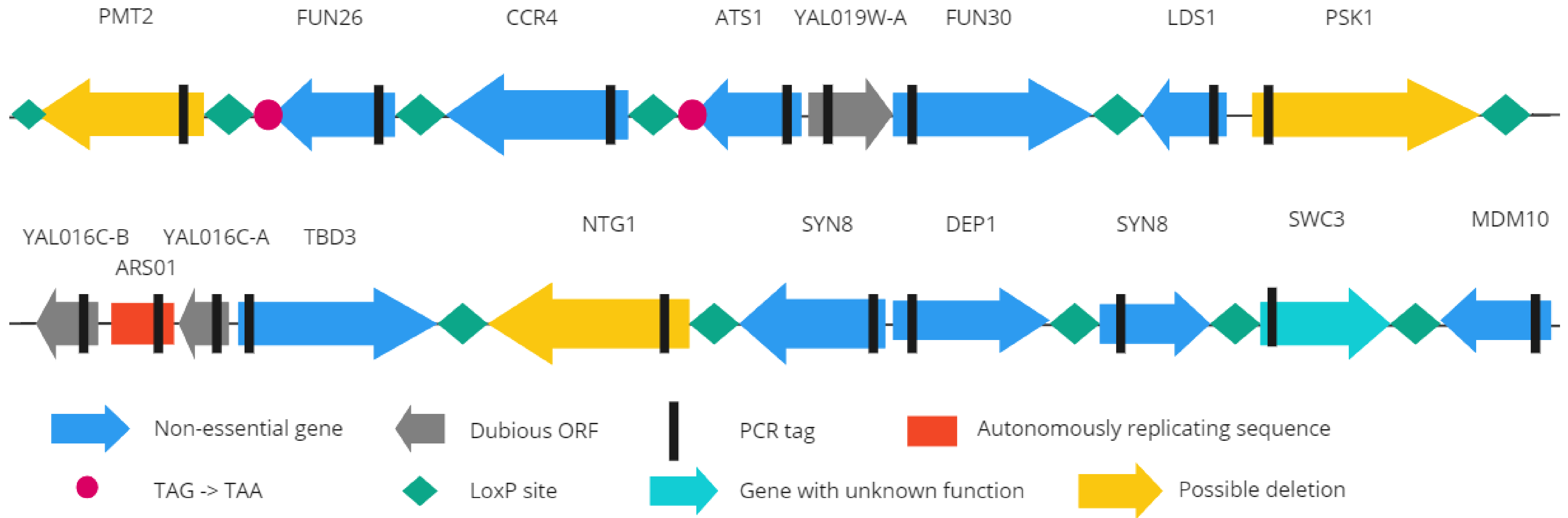
CYS3: involved in converting homocysteine to cysteine

MDM10: subunit of molecular tether between mitochondria and ER
and involved in the assembly of outer membrane beta-barrel proteins

A closer look to the essentiality of few genes

- *PMT2*
  - There are multiple other protein O-mannosyltransferases in yeast and *PMT2* has also a paralog, *PMT3*
  - To some extend the O-mannosyltransferases can replace the functions of each other but it is essential to have some functioning O-mannosyltransferase protein
- *PSK1*
  - Has a paralog *PSK2* in chromosome XV, which is results from duplication
- *NTG1*
  - Has a paralog, *NTG2*, that is capable of the same function, so if *NTG1* is deleted, *NTG1* can replace it
  - However, *NTG1* and *NTG2* are the only genes that have the DNA N-glycosylase activity, so it is potentially essential to have one of them present
- Possible deletions
  - *PMT2*, *PSK1* and *NTG1* have paralogs that are capable of the same functions, so these genes can be deleted from the synthetic megachunk if the paralogs are retained in the genome

Synthetic megachunk



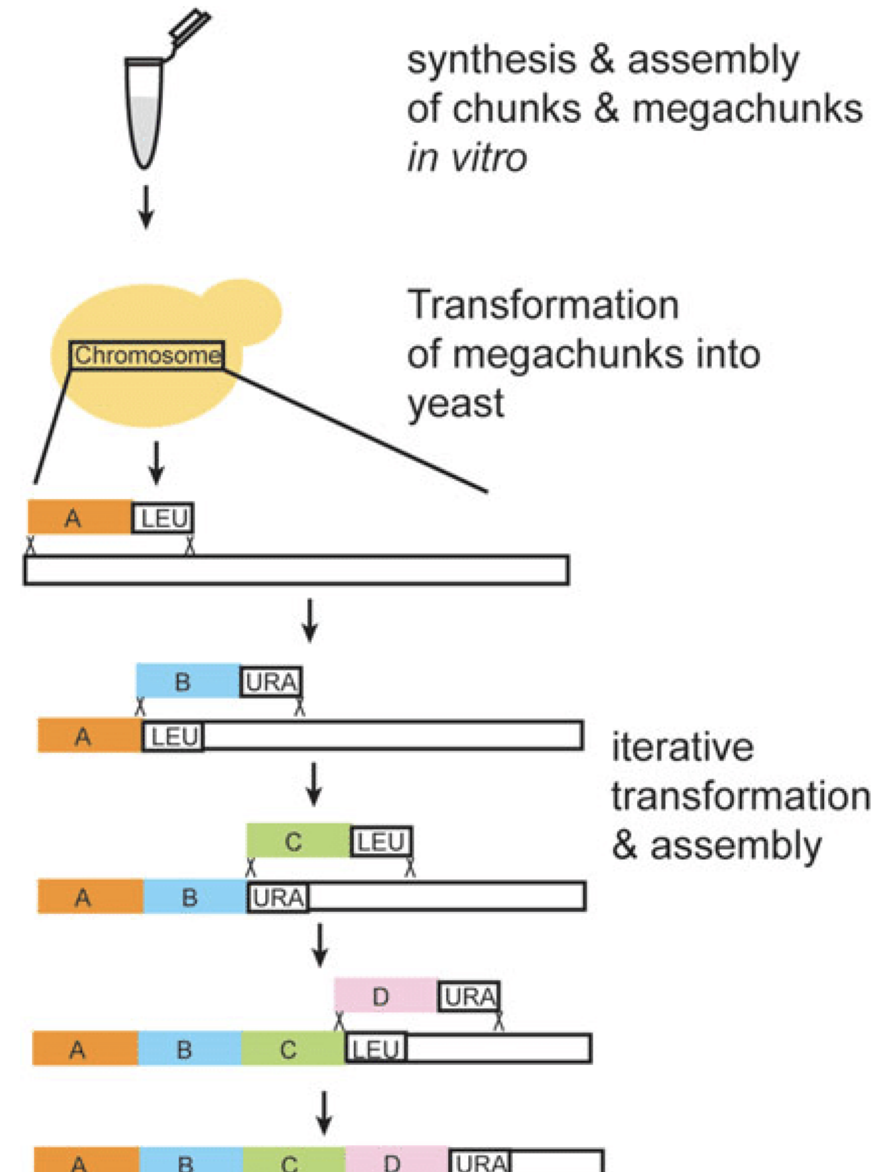
Used computer programs and databases

Yeast genome database,
https://yeastgenome.org/

Database of essential genes,
https://tubic.org/deg/public/index.php

Wet lab procedures

- Hierarchical assembly plan;
- **PCR** to assemble wanted DNA sequences into building blocks
- **Restriction enzymes & Ligation** to combine building blocks into chunks
- **(750 bp) Blocks -> (3 kb) minichunks -> (10 kb) chunks -> (30-50kb) megachunks**
- **Megachunks (30 kb)** are finally transformed into the yeast cell with homologous recombination.
- Markers LEU2 and URA3 can be used as auxotrophic marker for yeast.



https://www.researchgate.net/figure/Synthetic-yeast-chromosome-assembly-The-Figure-shows-the-key-steps-of-S-cerevisiae_fig1_293827090

Future prospects & problems

- Biopharmaceutical production by heterologous biosynthesis
  - Construction of a fully-synthetic eukaryotic genome designed for specific purposes
- Industrial fermentation of bioethanol and biobutanol
  - From agricultural products and by-products
- Engineering yeast to be used in biotechnology for a more sustainable future
  - Using yeast to produce e.g. jet fuel, spider silk, and animal free milk
- Production levels are limited because of e.g. metabolic burden to yeast, toxicity of pathway intermediates and products, precursor availability, and cofactor imbalance
- Predicting the phenotype from genotype, due to yeast species having significant genetic variability
- Possibly harmful to humans or the environment

References

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