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**Aalto University
School of Chemical
Engineering**

Yeast 2.0

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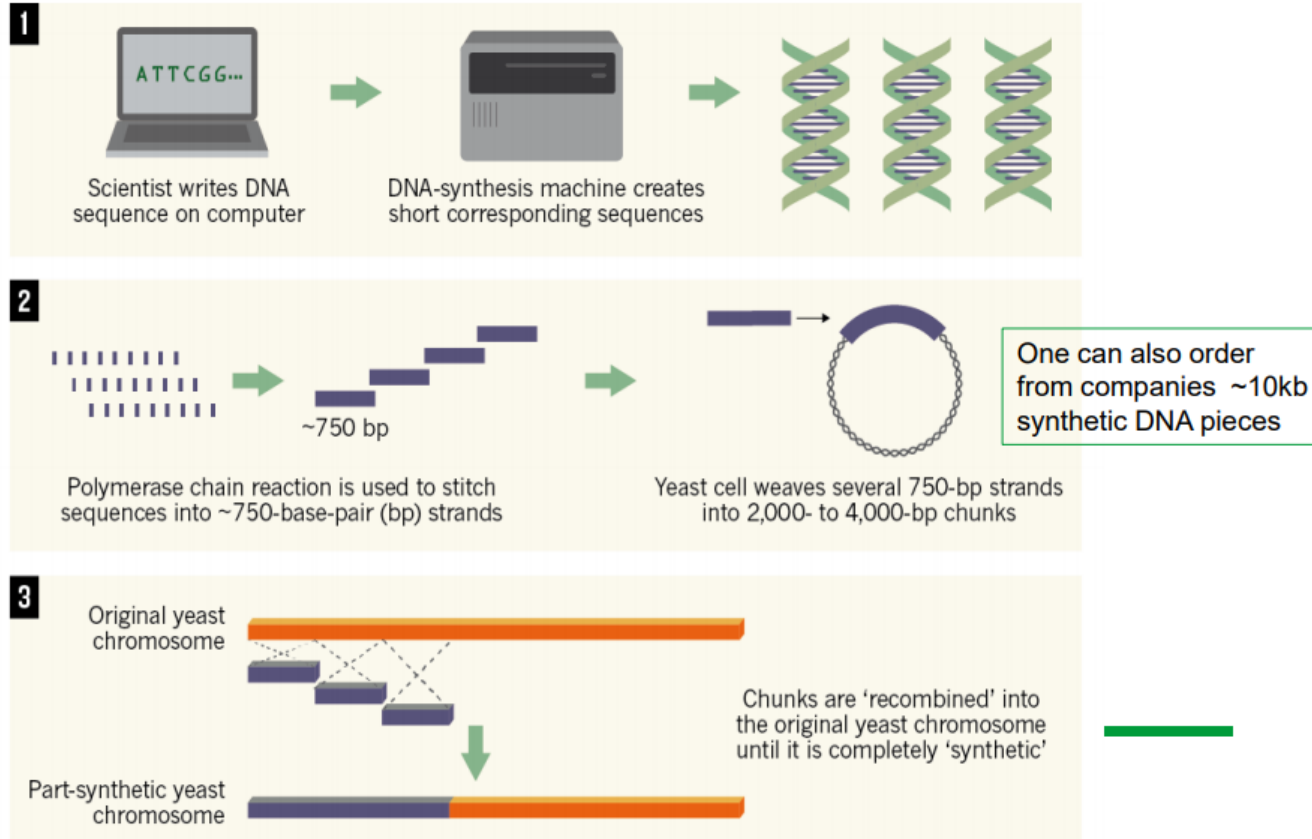
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Introduction

- Synthetic yeast 2.0 is the first attempt to build an artificial yeast (*S. cerevisiae*) genome from a scratch. The synthetic DNA is introduced to the wild-type genome in 30-60 kb pieces by replacing sequences.

- It is called synthetic because all the original pieces are replaced with synthetic ones.

- Alterations that are done: PCR-Tags are incorporated, telomeres are synthesized, introns and non-essential genes are removed, stop codon TAG is replaced with TAA, transposons are deleted, tRNA genes are relocated
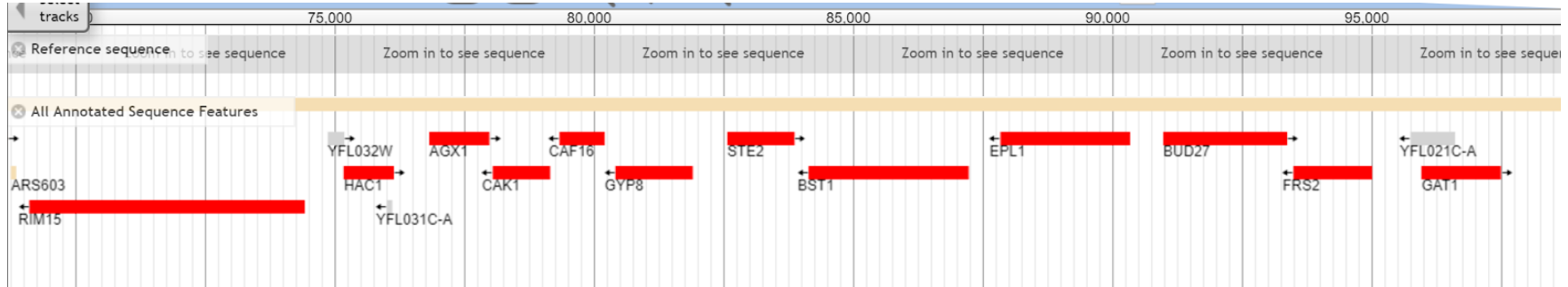
- Yeast 2.0 can be used to answer many questions about chromosomes, genomes structure and evolution, RNA splicing, etc...

- Yeast 2.0 can be industrially significant. It can be genetically engineered to produce useful compounds in greater quantities and to have a better tolerance for stress factors (e.g. pH, temperature, pressure…)

- The artificial yeast could be used to produce biofuels better. This could happen by adding genes to the genome that enhances the production.

Construction of Yeast 2.0

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-base-pair (bp) strands (~750 bp) → Yeast cell weaves several 750-bp strands into 2,000- to 4,000-bp chunks

One can also order from companies ~10kb synthetic DNA pieces

3 Original yeast chromosome → Part-synthetic yeast chromosome

Chunks are 'recombined' into the original yeast chromosome until it is completely 'synthetic'

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Synthetic design of a ~30kb region ('Megachunk')

chrVI:68657..98398 (29.74 Kb)

Essential genes : CAK1, EPL1, FRS2
Non - essential genes: RIM15, HAC1, AGX1, CAF16, GYP8, STE2, BST1, BUD27, GAT1
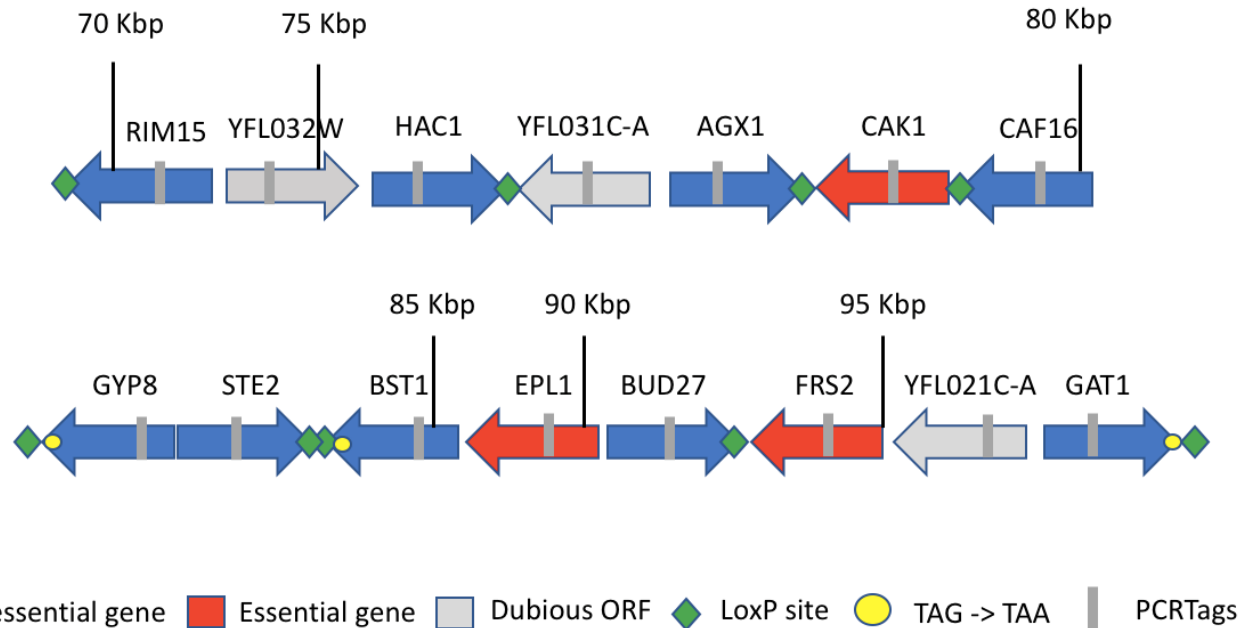**Dubious ORFs:** YFL031C-A, YFL021C-A
Autonomously replicating sequence ARS603
No introns in this region, no tRNA genes and no transposon

Our design of a ~30kb region ('Megachunk')

**chrVI:68657..98398 (29.74 Kb)**

Softwares

- *Saccharomyces cerevisiae* genome database (SGD)
    - https://www.yeastgenome.org/

- SnapGene (planning, visualizing, documenting of molecular biology procedures)
    - https://www.snapgene.com/
- ApE - a plasmid editor (free download from multiple websites)
- BioStudio (eukaryotic genome design)
- Database of essential genes
    - http://www.essentialgene.org/

Wet Lab

- Hierarchical assembly plan:

- Construction of building blocks from oligos or buying ready-made chunks

- Smaller chunks are assembled into 'megachunks' (30-60 kb) through restriction enzymes and subsequent ligation

- Integration of 'megachunks' into yeast genome via homologous recombination

- Alternation between two selection markers (LEU2 and URA3)

Wet Lab
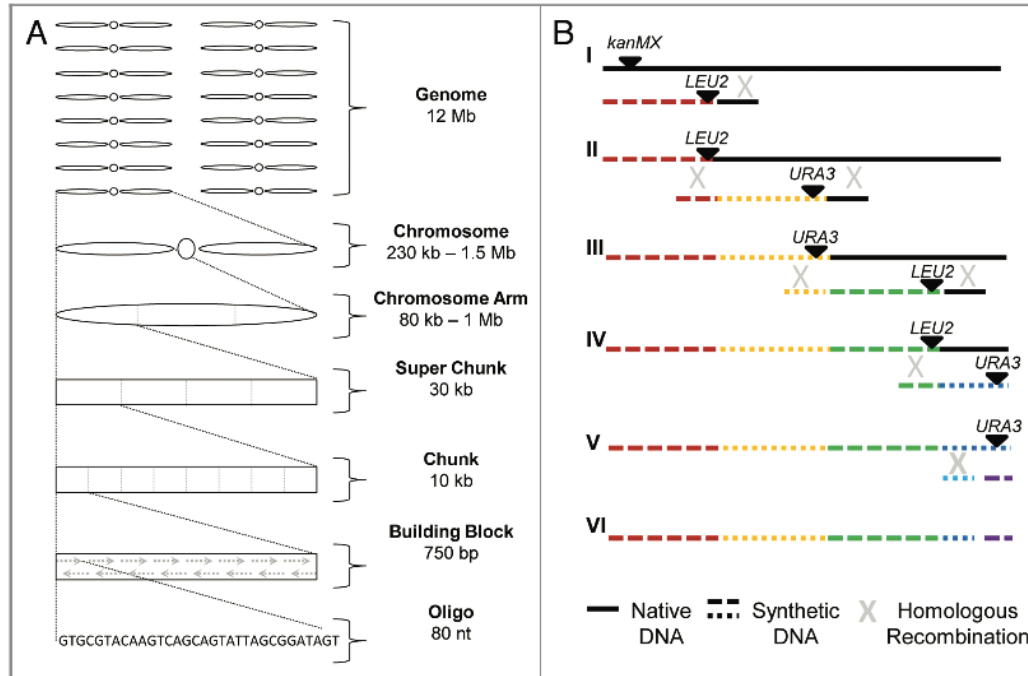
Genome modularity and integration of synthetic DNA

SCRaMbLE mechanism

- **S**ynthetic **C**hromosome **R**ecombination **a**nd **M**odification **b**y **L**oxP-mediated **E**volution

- Cre recombinase can recombine a pair of short target sequences called the *LoxP* sequences (both derived from bacteriophage P1)

- Recombination between loxPsym sites could lead to genome rearrangements

- Introduction of around 5000 symmetrical loxP sites 3 bp after the stop codon of nonessential genes and at major landmarks

- Inducible evolution and genome minimization

- Production of strains with a large genotypic diversity

- Cre expression control through galactose or estradiol induction

SCRaMbLE mechanism
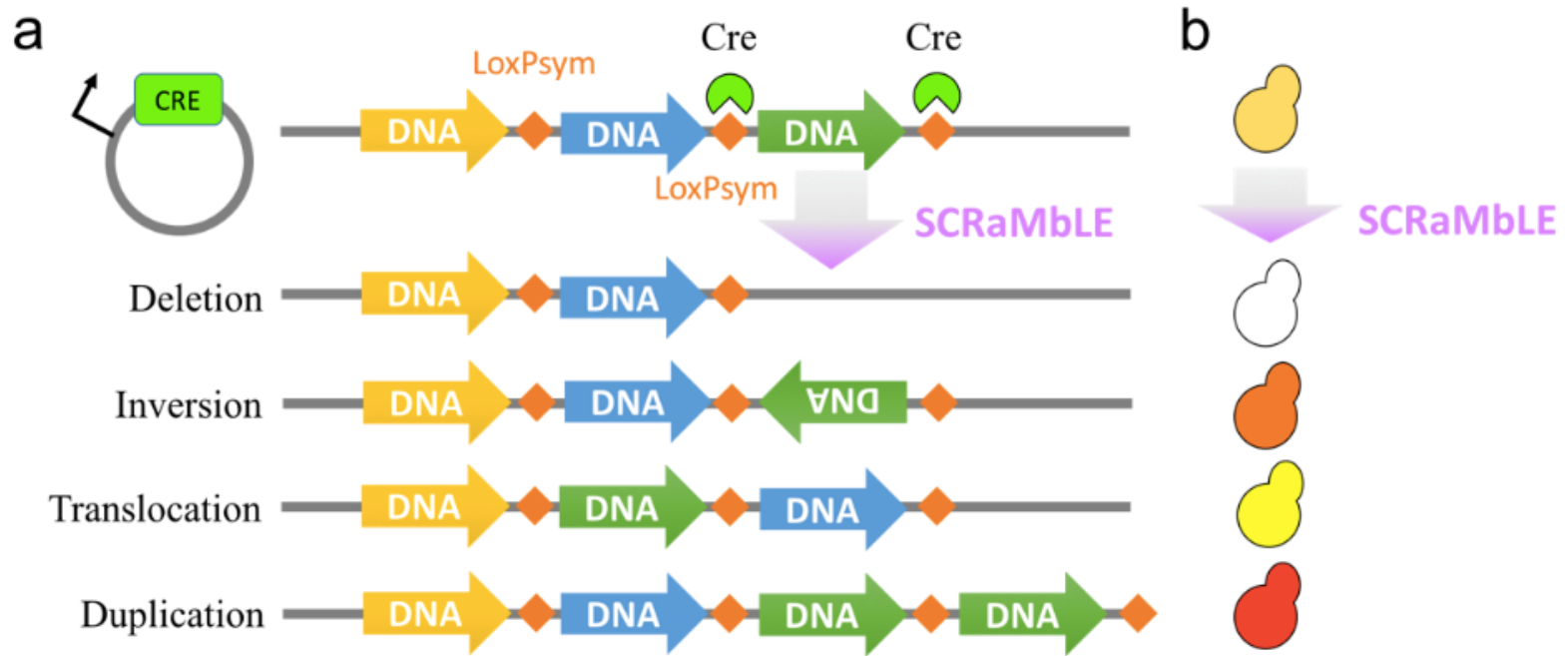
SCRaMbLE leads to diversity generating desired phenotypes

Possible problems

- Harmful to humans

- Harmful to environment

- Harmful dual-use e.g. bioterrorism

- Reduced fitness of the yeast

- Instability of the genome

References

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- Jia, B. *et al.* (2018) 'Precise control of SCRaMbLE in synthetic haploid and diploid yeast', *Nature Communications*, 9(1), p. 1933. doi: 10.1038/s41467-018-03084-4.

- Merja Penttilä, Synthetic yeast 2.0, Synthetic Biology (CHEM-E8125), Aalto University, School of Chemical Engineering, 2019, (course material)

- Synthetic Yeast 2.0, http://syntheticyeast.org/ 25.03.2019

- *Saccharomyces cerevisiae* Genome Database, https://www.yeastgenome.org/ 25.03.2019

- https://www.genscript.com/synthetic_yeast_genome.html 25.03.2019

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Any questions?