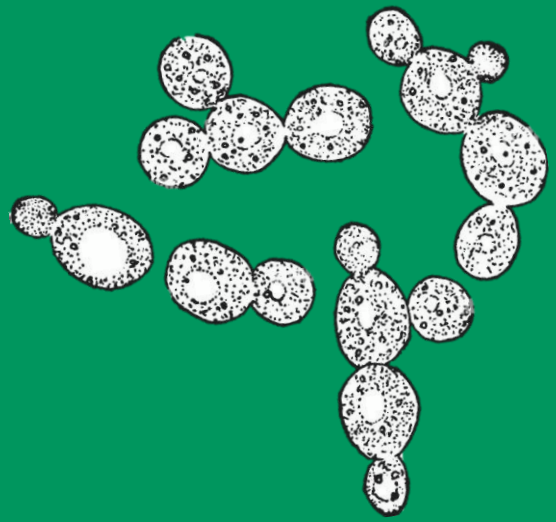
Synthetic Yeast 2.0

Group 13



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Submitted on 25.3.2023

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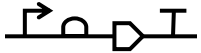
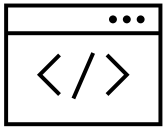
Outline

Yeast 2.0

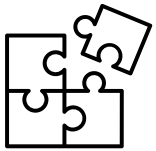


Design

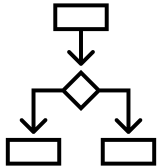


Programs

Wetlab



SCRaMbLE



Further development

What is Yeast 2.0?

Synthetic Yeast 2.0

- The aim of project is to reconstruct a fully synthetic *Saccharomyces cerevisiae* yeast chromosome
- *S. cerevisiae* was chosen for the project because it is the most researched and understood genome
- 7 out of 16 chromosomes were successfully synthesised

By learning how to synthesize the genome, we can:

- **Answer** the fundamental questions about chromosomes properties
- **Develop** more efficient biofuel, medicines and other fermentation-related industries

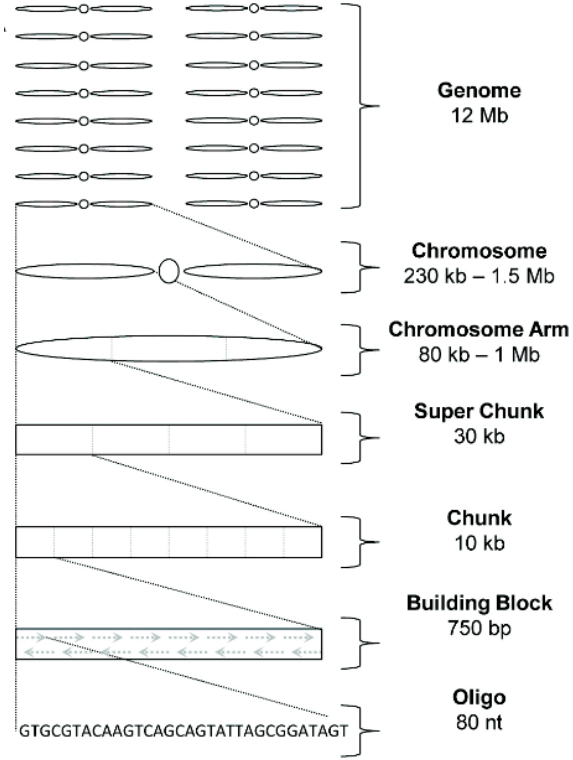
Synthetic Yeast 2.0 logo and slogan
https://syntheticyeast.github.io

Synthetic Yeast 2.0

- Changes to native genome are done using **“bottom-up” approach**. The alternations are done on a region approximately of 30 kb. This way, it is easier to monitor the viability and fitness of the cell.
- Assembly is done step-wise:

Building blocks *750 bp* → Mini-chunks *3 kb* → Chunks *10 kb* → Mega-chunks *30–50 kb* → Introduction to wild-type genome

- Usually, 3–6 x 10 kb chunks are introduced at a time



The yeast genome division into increasingly smaller segments
https://doi.org/10.4161/bbug.19543

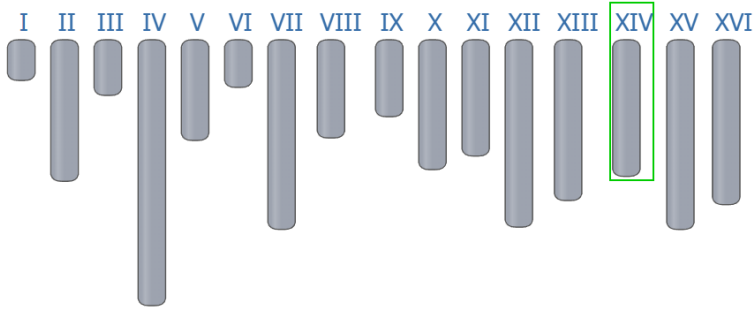
Our design & Modifications

What was chosen and why?

Chromosome XIV, strain S228c

- Contains the **KRE1**
  - Cell wall glycoprotein involved in beta-glucan assembly
  - Serves as a K1 killer toxin membrane receptor

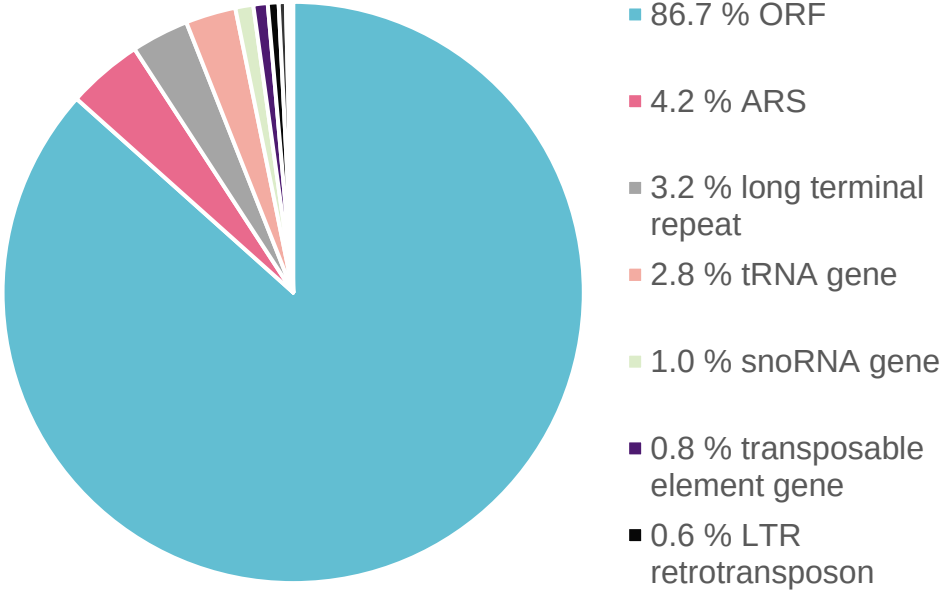
- **Studying the assembly of this gene might help scientists to identify K1 resistant cells, thereby:**
  - Developing more fit and robust cells
  - Use KRE1 for selective pressure for cultivation of transformed cells



S. cerevisiae S288C chromosomes
https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?chr=XIV&id=GCF_000146045.2

Chromosome XIV

- Not synthesized yet
- Composition



Strain: S288C
Length: 784333 bases
NCBI RefSeq: BK006947.3

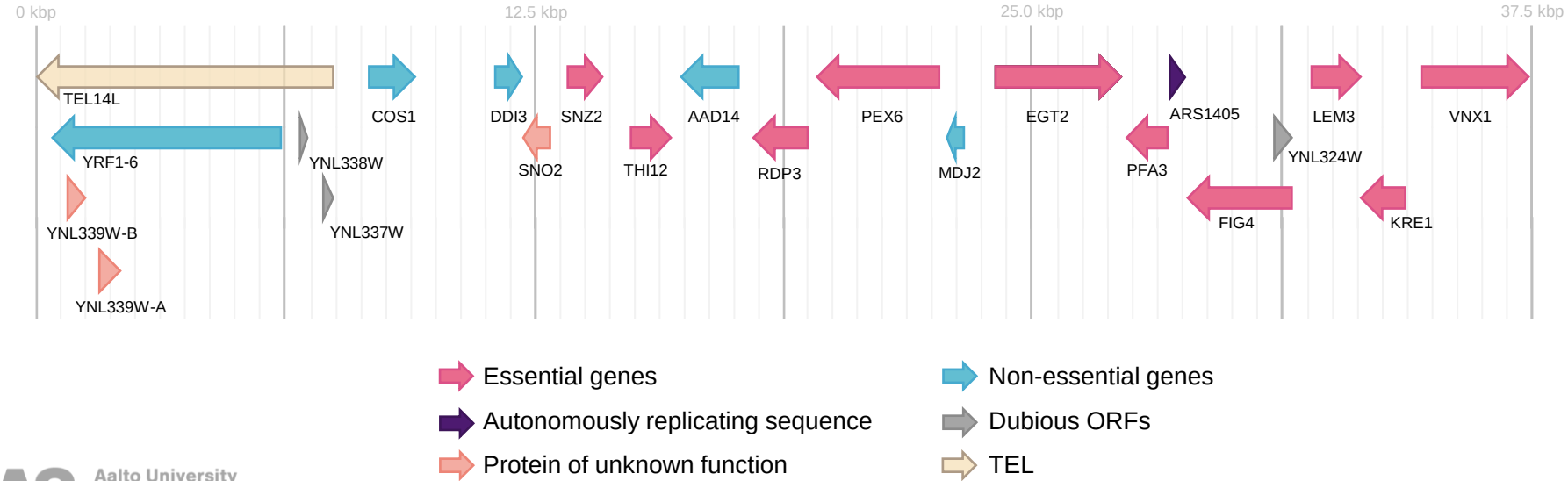
https://www.yeastgenome.org/contig/Chromosome_XIV

List of genes in the megachunk

TEL14L-	Telomeric region on the left arm of Chromosome XIV
YRF1-6	Helicase encoded by the Y' element of subtelomeric regions
YNL339W-B , YNL339W-A	Proteins of unknown function
YNL338W, YNL337W	Dubious open reading frame; unlikely to encode a functional protein,
COS1	Endosomal protein involved in turnover of plasma membrane proteins
DDI3	Cyanamide hydratase that detoxifies cyanamide
SNO2	Protein of unknown function
SNZ2	Protein involved in thiamine and pyridoxine biosynthesis;
THI12	Protein involved in synthesis of the thiamine precursor HMP
AAD14	Putative aryl-alcohol dehydrogenase;
RPD3	Histone deacetylase,
PEX6	AAA-peroxin;
MDJ2	Constituent of the mitochondrial import motor; associated with the presequence translocase
EGT2	Glycosylphosphatidylinositol (GPI)-anchored cell wall endoglucanase
PFA3	Palmitoyltransferase for Vac8p; required for vacuolar membrane fusion
ARS1405	Autonomously Replicating Sequence
FIG4	Phosphatidylinositol 3,5-bisphosphate (PtdIns[3,5]P) phosphatase; required for efficient mating and response to osmotic shock
YNL324W	Dubious open reading frame
LEM3	Membrane protein of the plasma membrane and ER
KRE1	Cell wall glycoprotein involved in beta-glucan assembly; serves as a K1 killer toxin membrane receptor
VNX1	Calcium/H+ antiporter localized to the endoplasmic reticulum membrane

Original megachunk

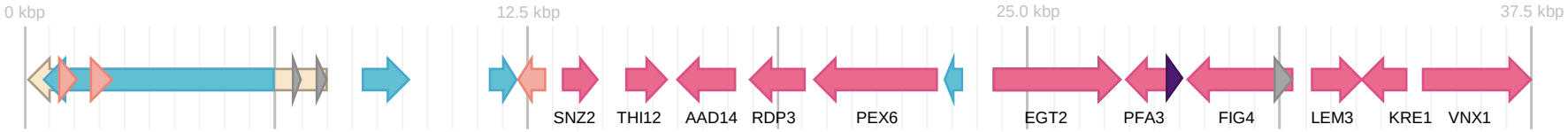
Chromosome **XIV, chunk between 0–37.5 kbp**



S. cerevisiae chromosome XIV sequence

Original megachunk

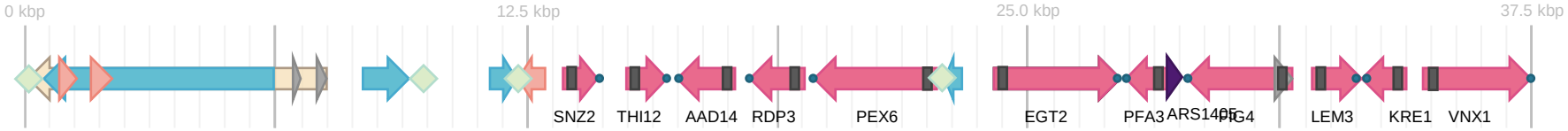
Chromosome **XIV, chunk between 0-37.5 kbp**



- Essential genes
- Non-essential genes
- Autonomously replicating sequence
- Dubious ORFs
- Protein of unknown function
- TEL

Editing

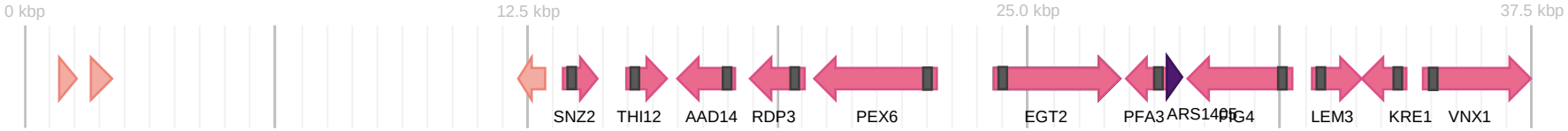
- **Deletion** of non-essential genes, introns and repeats (LTRs, Sub-TELs and transposons)
- **Re-location** of tRNA genes
- **Addition** of loxPsym sites
- **Re-coding** stop codons (TAG -> TAA) and including PCRTags



- Essential genes
- Autonomously replicating sequence
- Protein of unknown function
- LoxPsym site
- PCRtags
- Non-essential genes
- Dubious ORFs
- TEL
- Codon swap

Constructed megachunk

- **Deletion** of non-essential genes, introns and repeats (LTRs, Sub-TELs and transposons)
- **Re-location** of tRNA genes
- **Addition** of loxPsym sites
- **Re-coding** stop codons (TAG -> TAA) and including PCRTags



Essential genes
Autonomously replicating sequence
Protein of unknown function
LoxPsym site
PCRtags
Non-essential genes
Dubious ORFs
TEL
Codon swap

Databases & Softwares

Databases & Softwares

Databases

- ***S. cerevisiae* genome database:** https://yeastgenome.org/
  - Includes biological information about *Saccharomyces cerevisiae*

- **Yeast 2.0:** https://syntheticyeast.github.io/
  - To learn about the designs of Sc 2.0

Softwares & Other tools

- **BioStudio:** https://metacpan.org/pod/Bio::BioStudio::Git
  - The archive of genetic codes

- **DNA Atlas:** https://www.dnaatlas.com/
  - To construct sequences and plasmid maps

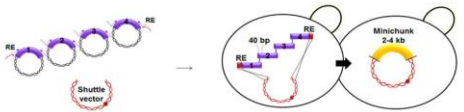
- **ORF finder:** https://web.expasy.org/translate/
  - Translation of nucleotide to a protein sequence

Wet lab procedure

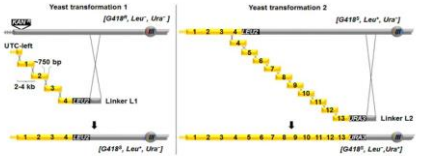
Wet lab procedure

1. Synthesis of building blocks

2. Assembly of minichunks



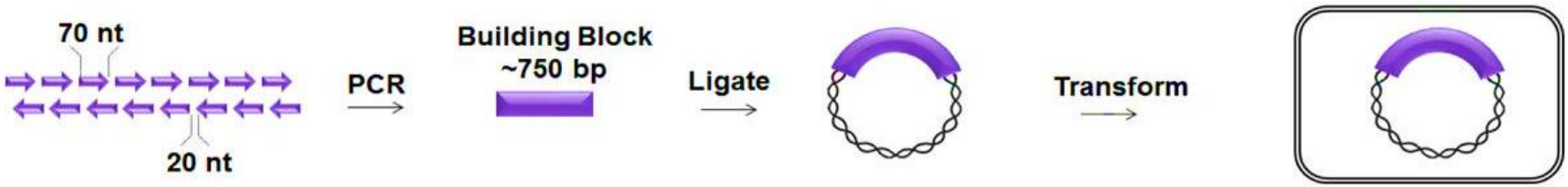
3. Replacement with minichunks



4. Confirmation using PCRtags

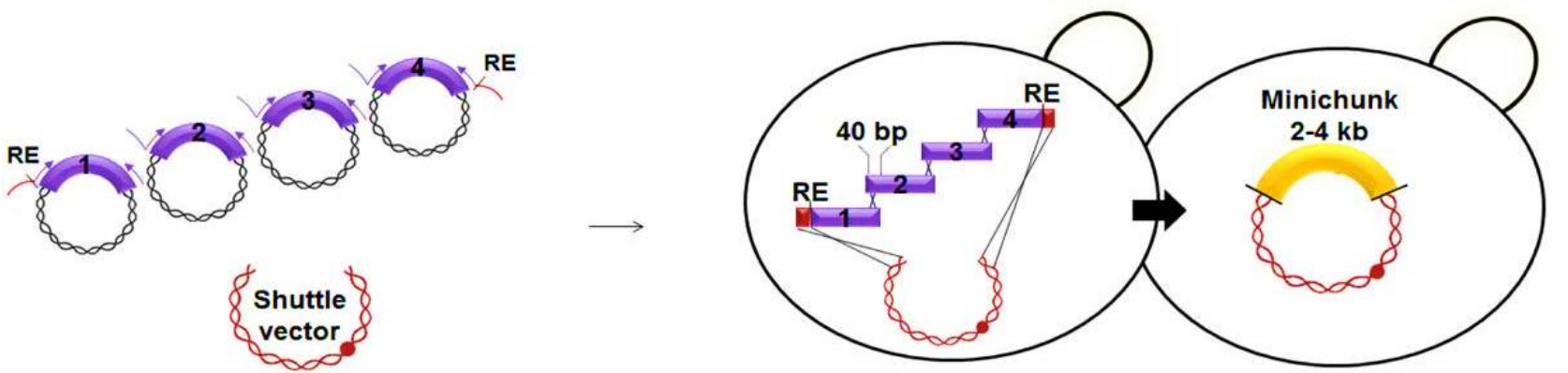
https://doi.org/10.1126%2Fscience.1249252

1. Synthesis of building blocks



https://doi.org/10.1126%2Fscience.1249252

2. Assembly of minichunks



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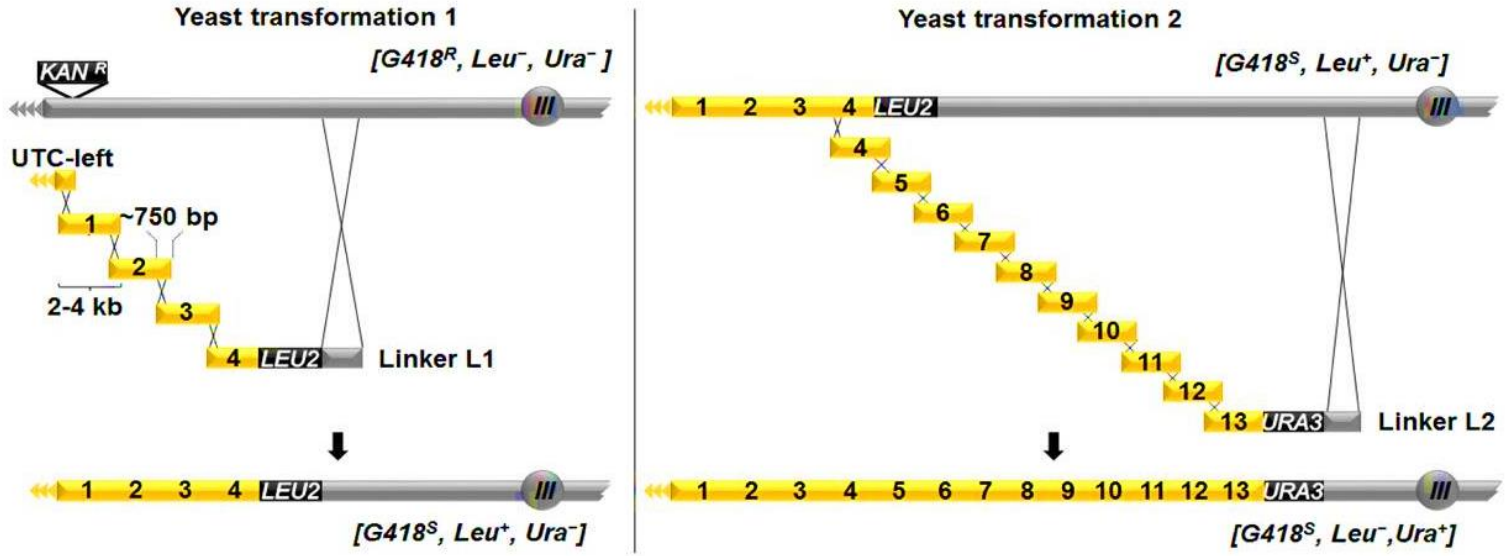

https://doi.org/10.1126%2Fscience.1249252

SCRaMbLE

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

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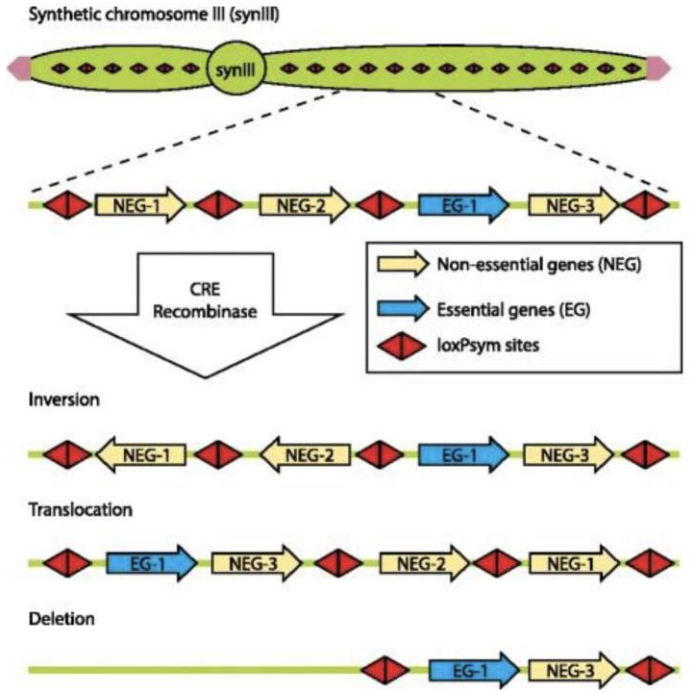
SCRaMbLE

- **Genetic engineering technique** that allows for the creation of novel genetic variations
- Generates combinatorial genomic diversity through rearrangements at designed recombinase sites
- Synthetic chromosome is designed with multiple **LoxP sites**, specific DNA sequences recognized by Cre recombinase enzyme
- Cre recombinase recognizes and recombines the LoxP sites, results in different combinations of DNA sequences
- Enables a **variety of random mutants in a short time**
- Used in yeast to generate a library of genetically diverse strains with novel phenotypes
- Has potential applications in biotechnology and synthetic biology



The pathway of Scramble (Annalaru et al, 2015).

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https://doi.org/10.4161/bbug.19543

Further Development

Further developments

Yeasts 3.0

- Research initiative constructing a comprehensive and high-quality genome scale model of yeast *S. cerevisiae*.
- Builds upon the work of the Yeast 2.0 project.

Biochemical and food industry

- The production of synthetic fuels, utilization of biomass for biodiesels
- Food ingredients
- Protein drugs

Thank you!
Questions?

References

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