Artificial Yeast Sc2.0

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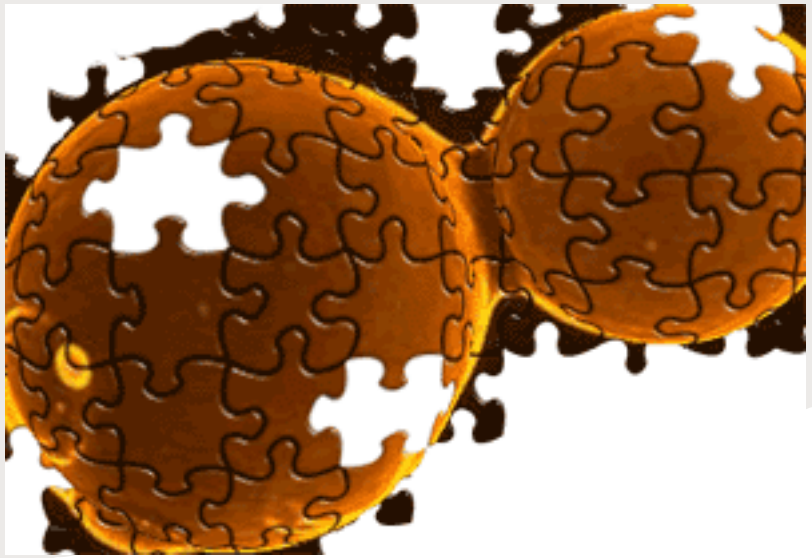
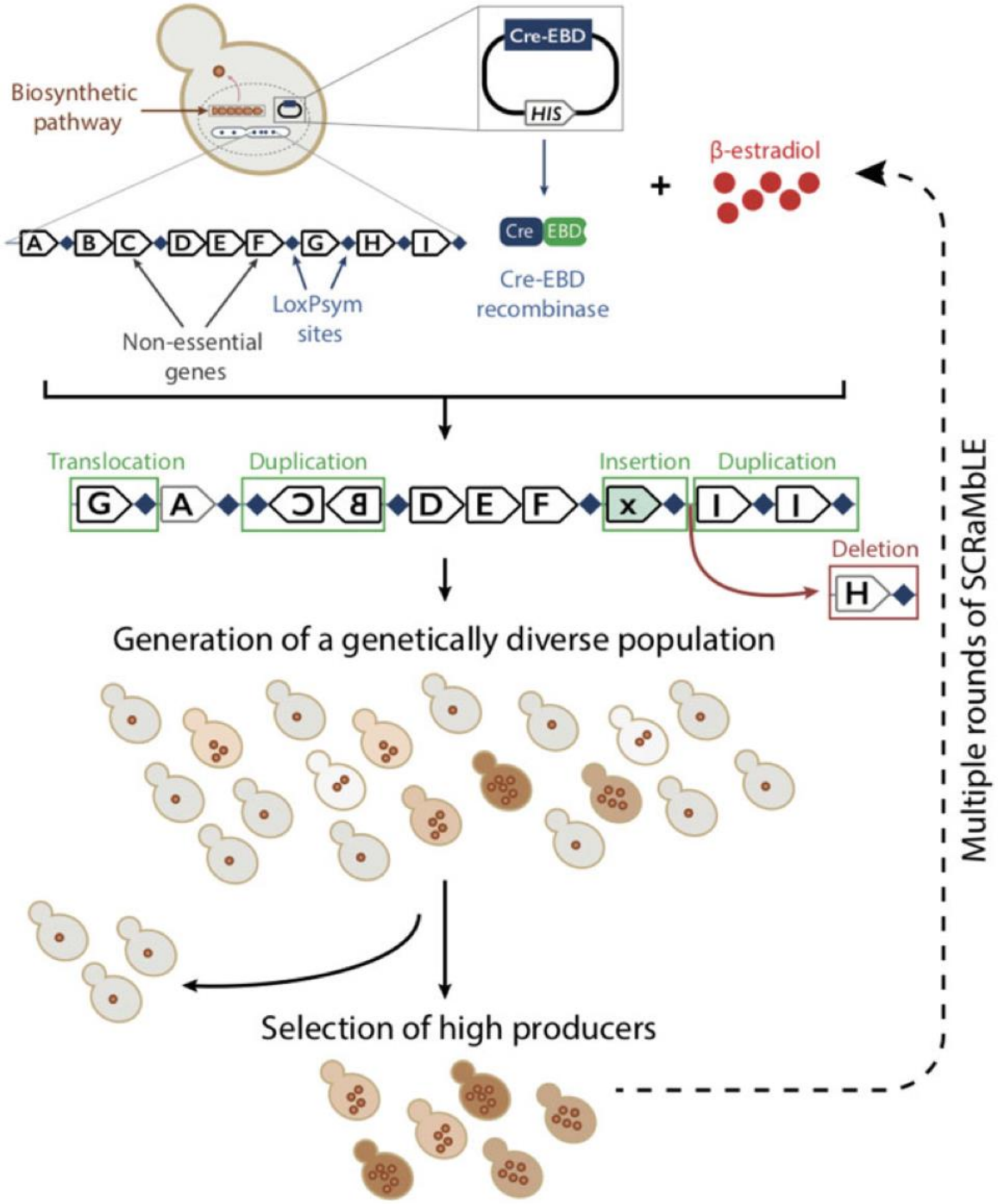
Yeast 2.0

Yeast 2.0 represents a major step forward in the understanding of genetics and the potential applications of synthetic biology.

- **Better understanding** of genetics and genes interactions
- **New applications:** it's possible to engineer yeast to perform even more complex tasks.
- **Improved biosecurity:** better tools for detecting and preventing the spread of harmful pathogens.
- **Ethical considerations:** a significant milestone in ethical considerations around the creation of new forms of life.





SCRaMbLE

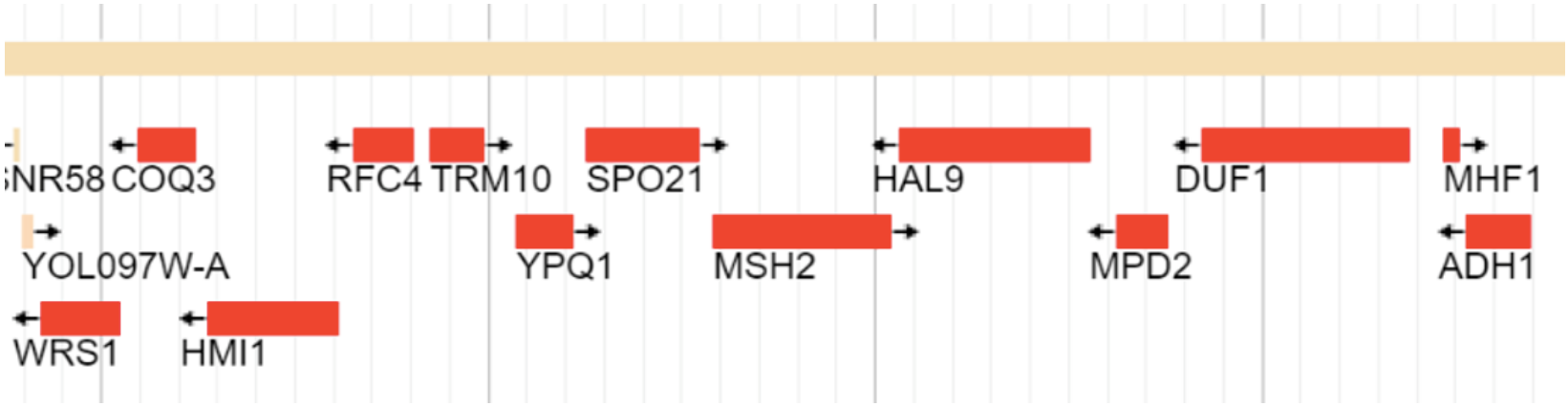
Synthetic Chromosome Rearrangement and Modification by loxP-mediated Evolution (SCRaMbLE) - a recently developed system for diversifying gene expression through genome shuffling.

Site-specific recombinases are a family of DNA modifying enzymes that can recognize and drive recombination between two specific DNA sites to generate deletion, inversion, or integration of DNA fragments between the target sites:

- loxP sites – targets;
- Cre recombinase.

Original megachunk of our choice in chrXV

~ 136 kb – 161 kb



Gene of interest: *MSH2*

Essential genes: *WRS1*, *HMI1, RFC4, TRM10, YPQ1, SPO21, HAL9, MPD2, MHF1*

Non-essential genes: *COQ3*, *DUF1, ADH1*

Essential genes in the megachunk

Essential Gene	Description
WRS1	Cytoplasmic tryptophanyl-tRNA synthetase
HMI1	Mitochondrial ATP-dependent DNA helicase
RFC4	Subunit of replication factor C complex
TRM10	tRNA methyltransferase
YPQ1	Vacuolar membrane transporter for cationic amino acids
SPO21	Component of the meiotic outer plaque of the spindle pole body
HAL9	Transcription factor containing a zinc finger
MPD2	Member of protein disulfide isomerase family
MHF1	**Component of the heterotetrameric MHF histone-fold complex**

Why *MSH2*?

- Homologous and highly similar to the human *MSH2*
- Codes for DNA mismatch repair protein
- Null mutant –> defects in DNA repair
- Mutations in the human *MSH2* linked to Lynch syndrome, breast cancer, and ovarian cancer

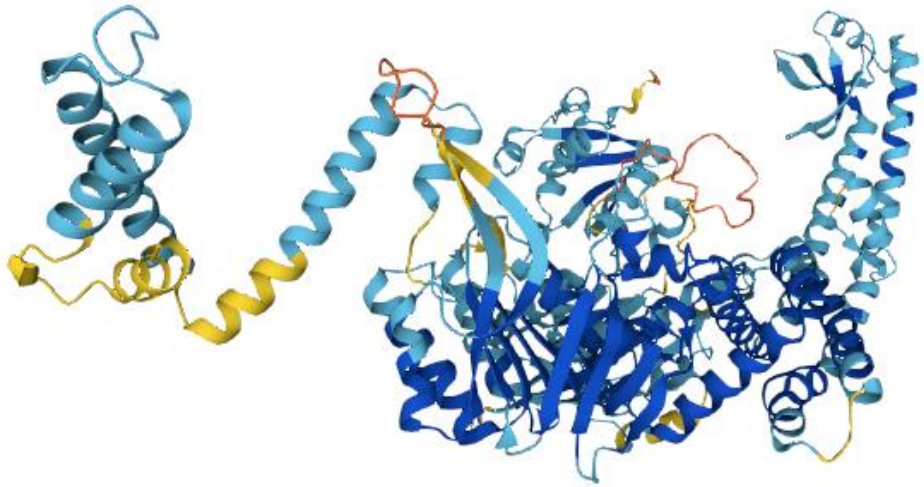
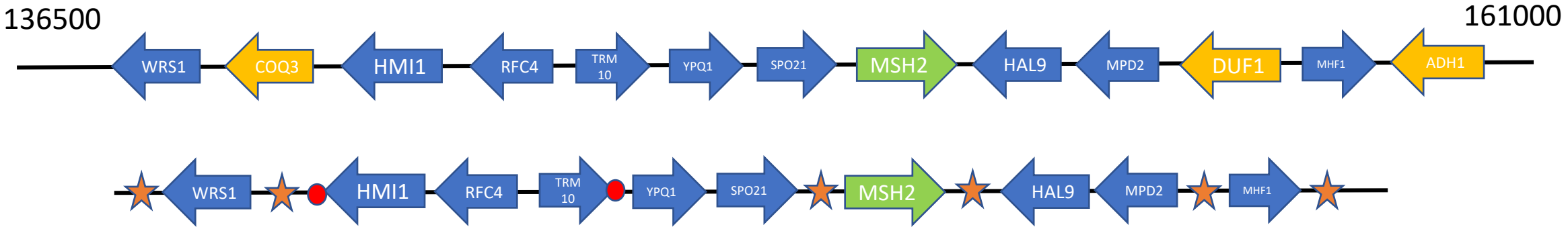


Illustration of the Megachunk design



TAG -> TAA

LoxPsym site

ORF — Essential ORF

ORF — Non- Essential ORF

MSH2 — Gene of interest

What was done?

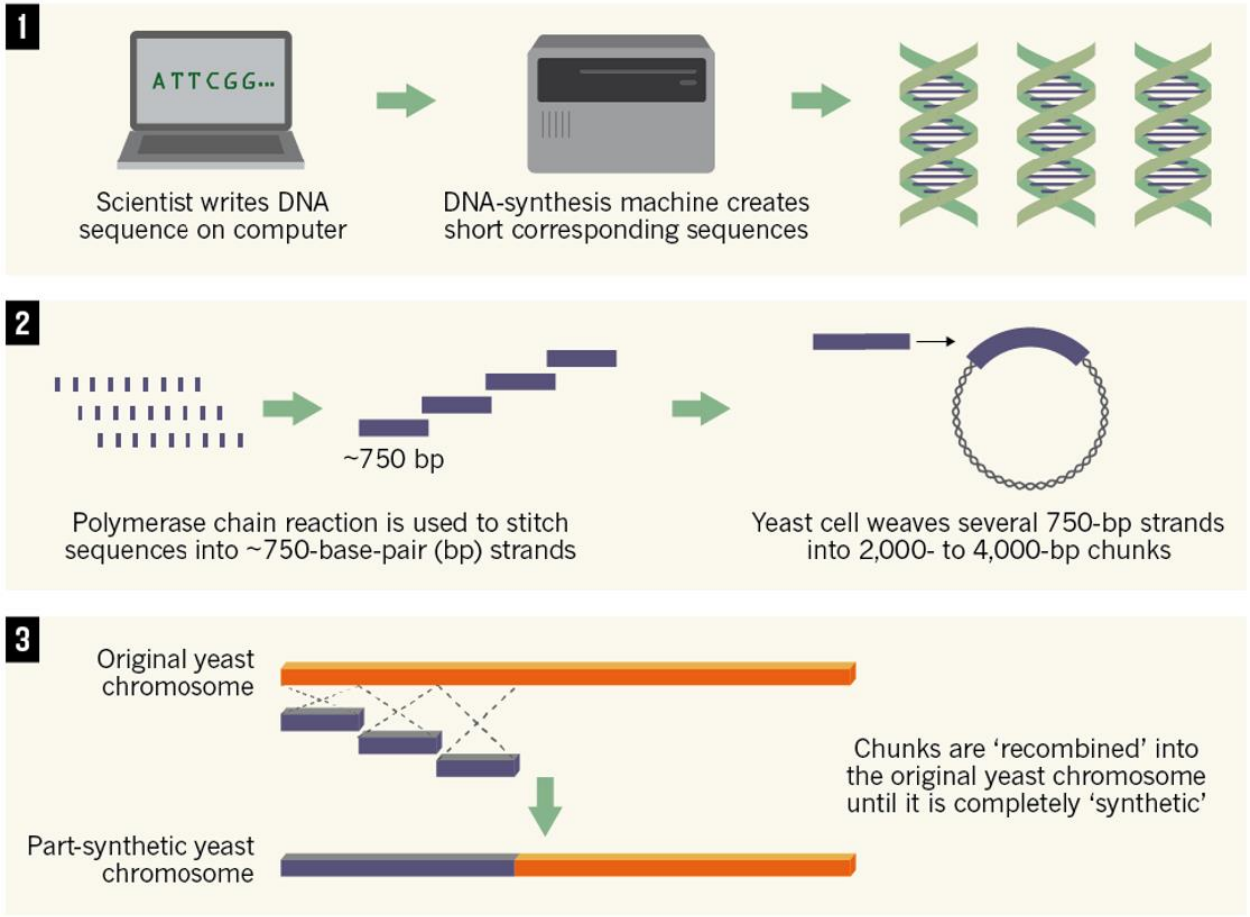
- Non-essential genes were deleted
- TAG-stop codons were changed to TAA
- LoxP sites
  - Deletions with LoxP, sites remain after deletion
  - Added around the gene of interest
  - Added around the megachunk

Computer programs

The *Saccharomyces* Genome Database (SGD)	Benchling	Sequence Polishing Library
• Biological information and search and analysis tools to explore it	• Planning, analysis and construction of plasmids, genes and DNA	• Optimization of codons

Wet lab construction method

- Short sequences of DNA are synthesized
- DNA sequences are combined into ~750bp building blocks using PCR
- With ligation and restriction enzymes, building blocks are first assembled into ~3kb minichunks, subsequently into ~10kb chunks and finally into 30-50kb megachunks
- Integration of the megachunk into yeast genome occurs through homologous recombination
- Megachunks are added by alternating auxotrophic genetic markers e.g., URA3 and LEU2 to ensure the integration
- Synthetic sequences are recombined into the genome until the chromosome is completely synthesized



Callaway, E. 2014. First synthetic yeast chromosome revealed. *Nature*.

What would you use the yeast for or develop further? How?

Yeast 3.0 :

- Relocation of repetitive genes in 2.0 had only minuscule effect on cell growth → bigger changes might be viable
- Shortening the genome
- Relocation of essential genes to a centromeric plasmid (eArray)
- More insight into how much of the yeast genome is still redundant, and in general what is the
- minimal genome to still yield viable cells in specific circumstances

new opportunities for expanding the use of yeast and improving its performance in these industries

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