Yeast 2.0

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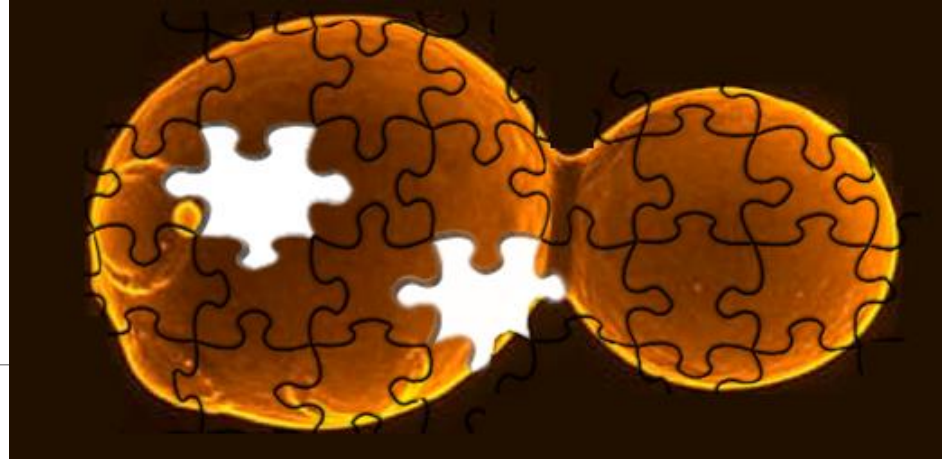


Synthetic yeast: DNA is designed and created by humans

Significance:

- What is the minimum viable genome?
- How do synthetic chromosomes perform in meiosis?
- Which parts in the genome are irreplaceable?
- Studying evolution using SCRaMbLE
- Industrial application

Improvements

- At present several changes are always made at once due to time and funding limits. In an ideal situation we could hundreds of different versions of the synthetic yeast so that impacts of individual changes could be studied.

Synthetic Yeast construction principles

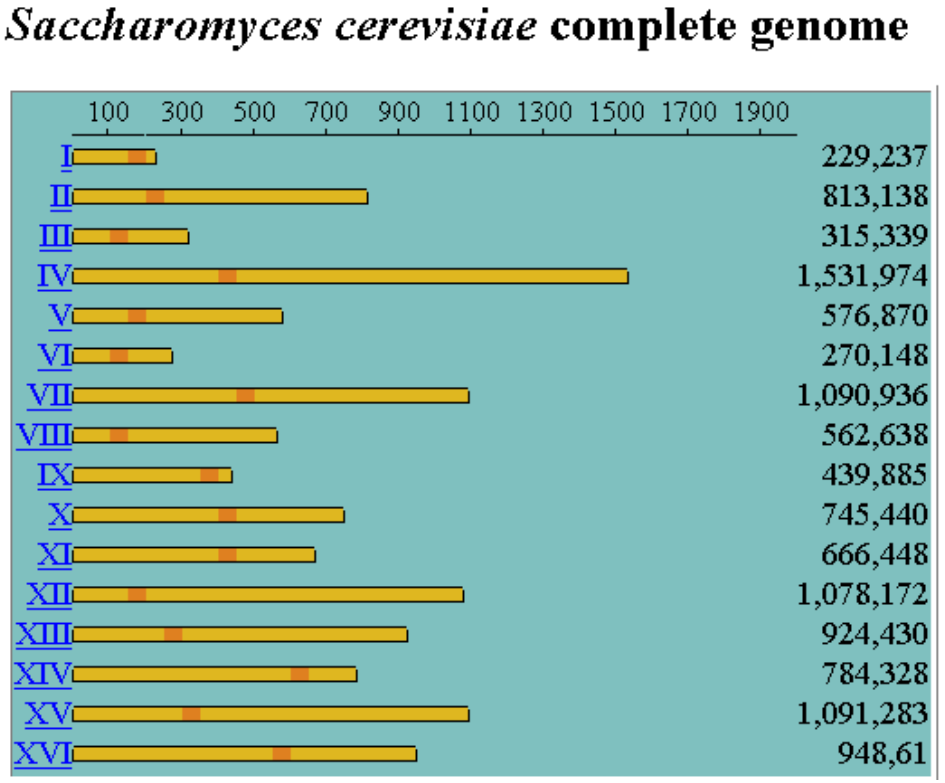
1. Keep yeastiness
2. Maintain fitness
3. Increase genomic stability -> delete transposons and relocate tRNA genes
4. Increase genetic flexibility -> enable SCRaMbLE
5. Try to minimize genome size -> remove nonessential genes and decrease telomere size
6. Step by step construction

S. cerevisiae chromosome I

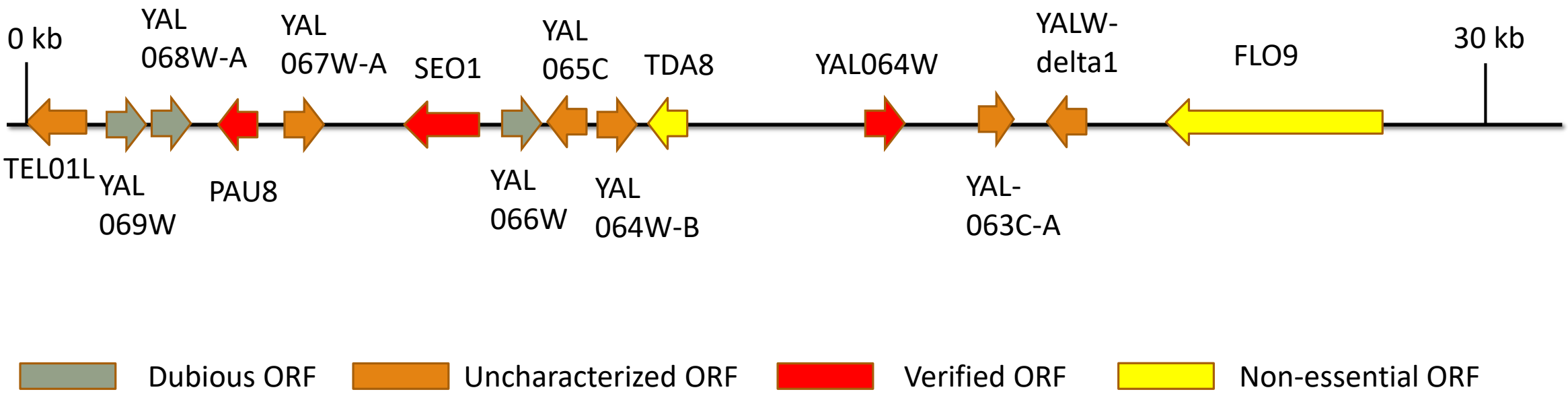
- Chr I has not been earlier synthesized
- ~230 kb -> modification from 0 to 30 kb
- No introns, transposons or tRNA genes

Modifications:

- PCRTag (new coding sequence, same amino acid sequence)
- Telomere cap (simple sequence repeat)
- Removal of non-essential genes
- Stop codons: TAG->TAA (unnatural amino acids)
- Addition of loxPsym sites (all of the ORFs)

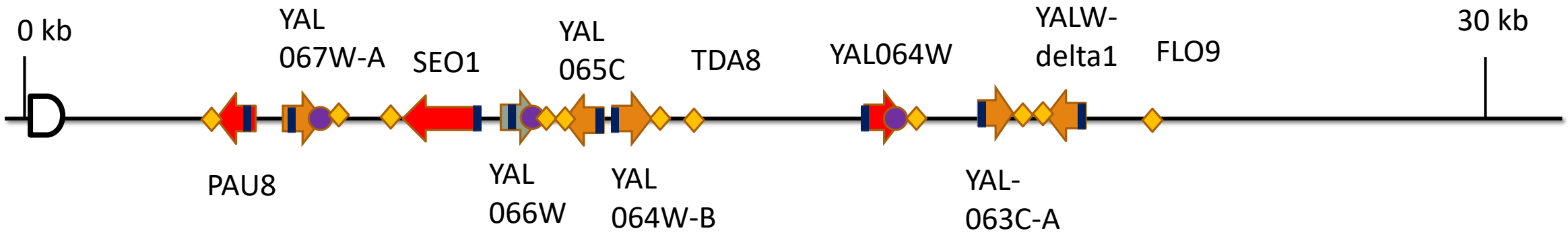


Chromosome I



FLO9: flocculation and adhesion
TDA8: biological role unknown

Synthetic chromosome I



Dubious ORF Uncharacterized ORF Verified ORF

Deletion of non-essential genes: FLO9 and TDA8

loxPsym site addittion

Stop codon TAG-> TAA: YAL067W-A, YAL066W, YAL064W

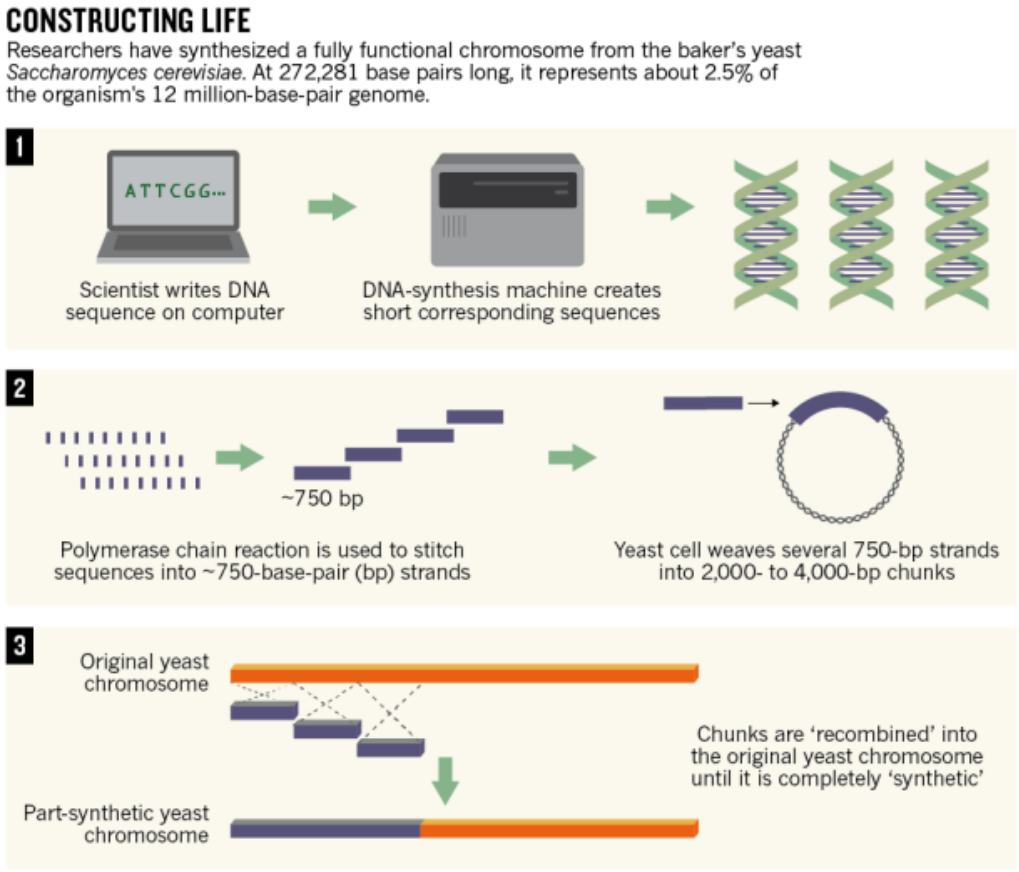
PCRTag

Telomere cap

Construction – bottom up approach

- 30-60 kb DNA pieces
- 750 bp building blocks
- 3 kb minichunks
- 10 kb chunks
- Recombination into yeast

After the cycle yeast fitness must be evaluated.



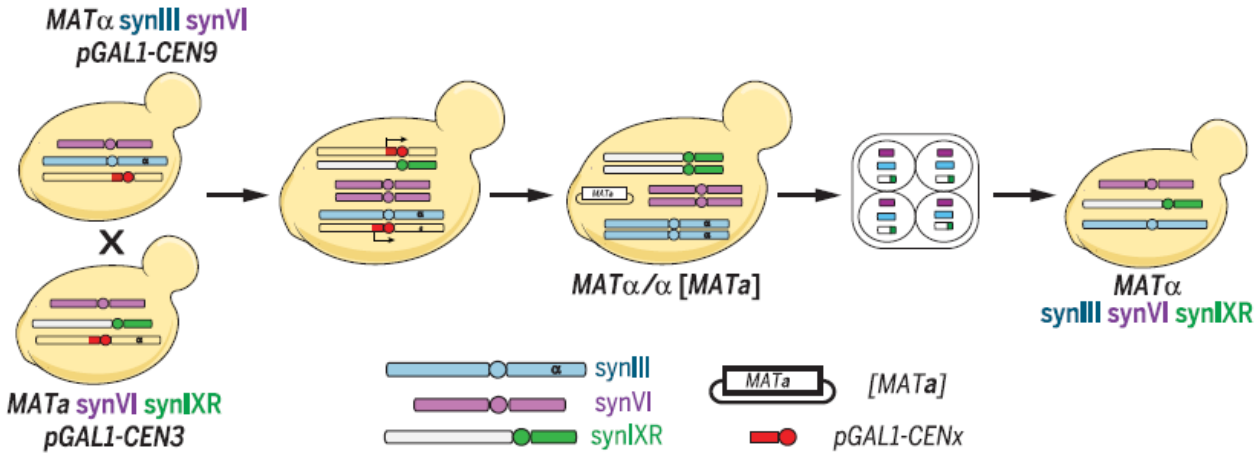
Combining synthetic chromosomes into one strain

- Mating and sporulation of different strains
  - Multiple crossovers challenge the recovery of non-recombinant progeny chromosomes
  - Increase of synthetic chromosomes makes it difficult to find spores that contain entirely full-length synthetic chromosomes
- Endoreduplication intercross can be used to overcome these problems

Endoreduplication intercross

- GAL1 promoter is used to destabilize the chromosome and to make it duplicate
- MATa was introduced to the cell to permit the sporulation

1. Strains are grown in galactose to induce GAL1 promoter
2. Chromosomes are destabilized and duplicated
3. Mating of two different yeast strains → intercross of chromosomes
4. Sporulation and dissection → New strains with multiple synthetic chromosomes



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