Genetic modification on *Saccharomyces cerevisiae*: Project Sc. 2.0

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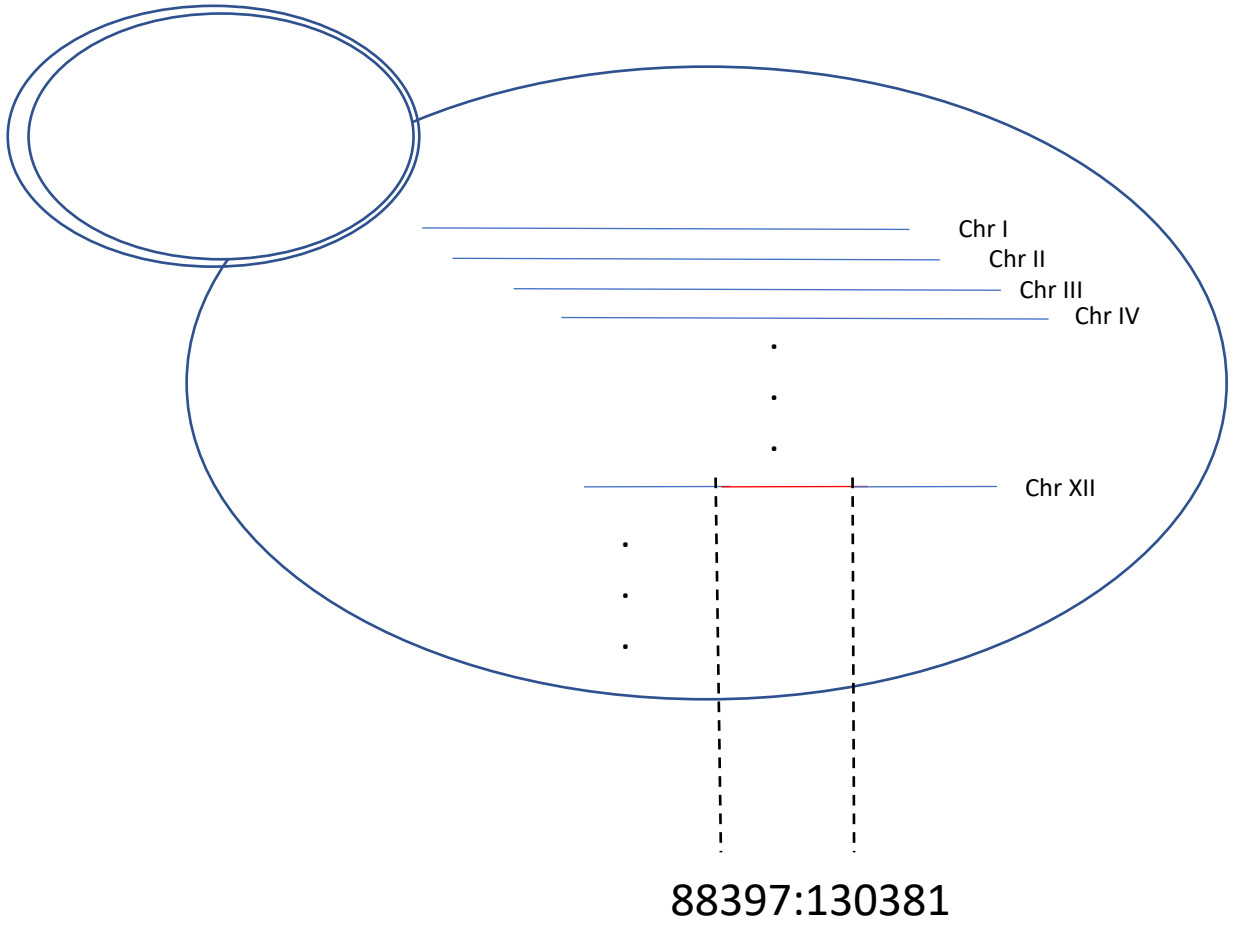
Yeast 2.0

- Synthesizing and optimizing the genome of baker's yeast (*Saccharomyces cerevisiae*)
- Aim: to create an "artificial" yeast species that is better suited for biotechnological applications than the natural form
- Removing parts of DNA and adding useful parts to make the genome more efficient
- Potential to facilitate the manufacture of biotechnological products and contribute to the development of new therapies and medicines.
- Can serve as a model to study the complex relationships between genotype and phenotype

Building better yeast, *Nature Communications*, *9*, **2018**, S. 1939.
I. S. Pretorius, J. D. Boeke, Yeast 2.0-connecting the dots in the construction of the world's first functional synthetic eukaryotic genome, FEMS yeast research, 18, 2018.

Yeast 2.0 modified region



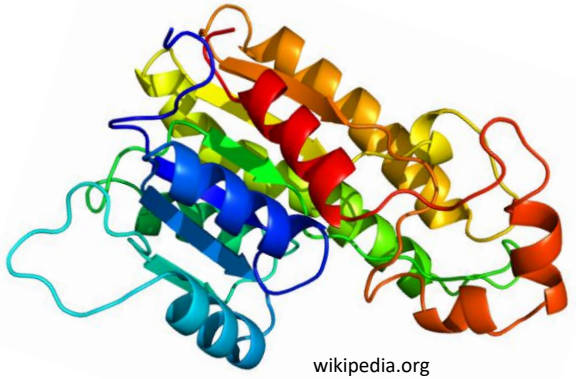
<u>42 kb length</u> region to be synthetically replaced

Contain essential genes for:

- Proteins assembly
- Structural protection

Region content

Chromosome XII - 88397:130381



wikipedia.org

Heat shock protein (HSP104)

After Heat or Environmental stresses

- Resolubilization
- Refolding
- Dissociation

Of aggregates of damaged proteins

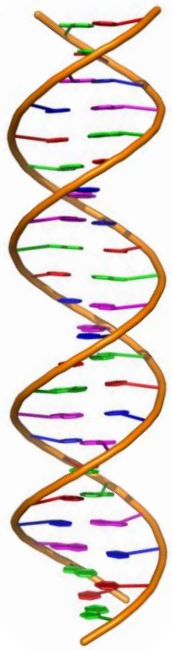
HSP family chaperone (SSA2)

Involved in:

- Protein folding
- Vacuolar import of protein
- Degradation of short-lived proteins

Other genes in the region

Code for proteins



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Unknown function

Cytoskeletal actin organization

Kinases

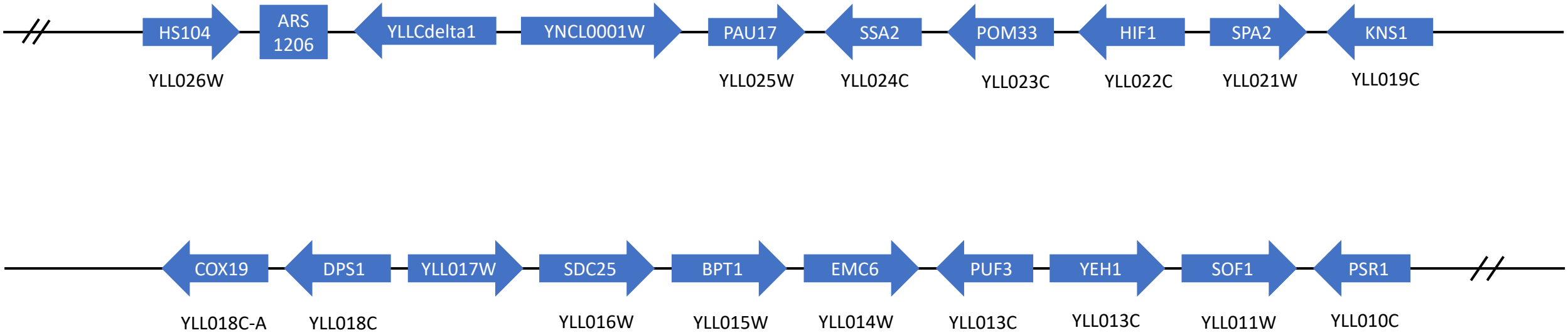
Mitochondrial

Transmembrane transport

.
.
.

Non-essential

Targeted gene sequence

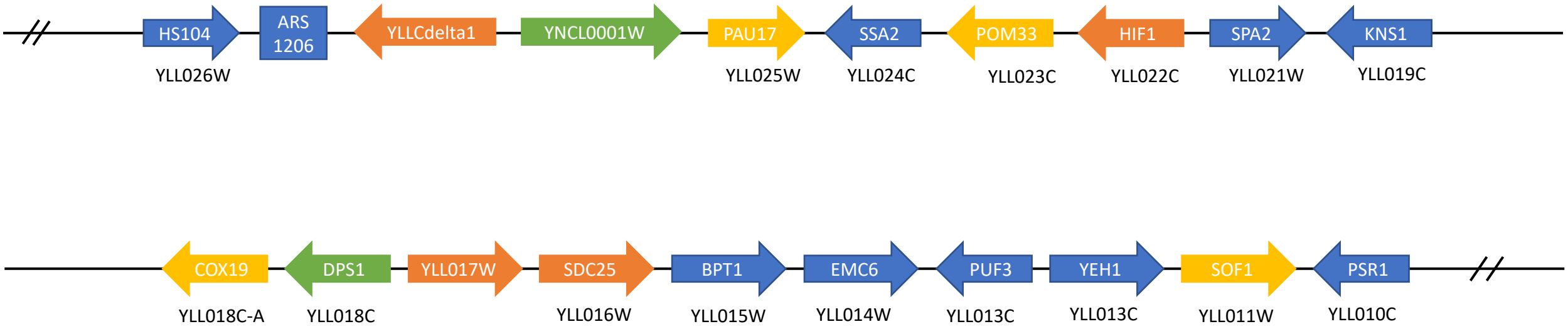


Needed modifications:

- Synthetic telomers
- Removal of introns
- Removal of non essential genes
- Replacement of TAG stop codons
- Deletion of transposons and SPR3 gene
- Relocation of tRNA genes

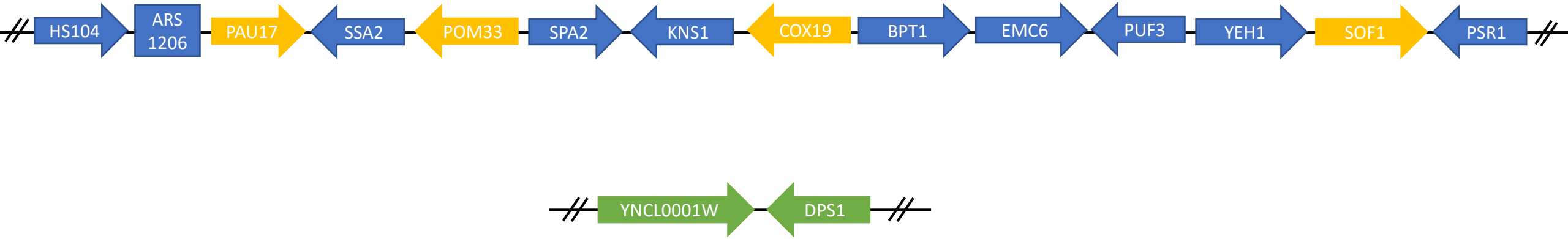
Targeted gene sequence

No telomers
No introns



- Essential genes
- Non-essential genes
- tRNA genes
- Genes with AUG stop codon

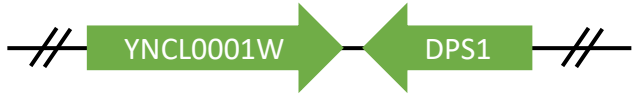
Synthetic megachunk



- Deletion of 3 non-essential genes
- Deletion of 1 transposon
- Removal of 4 TAG stop codons
- Relocation of 2 tRNA genes

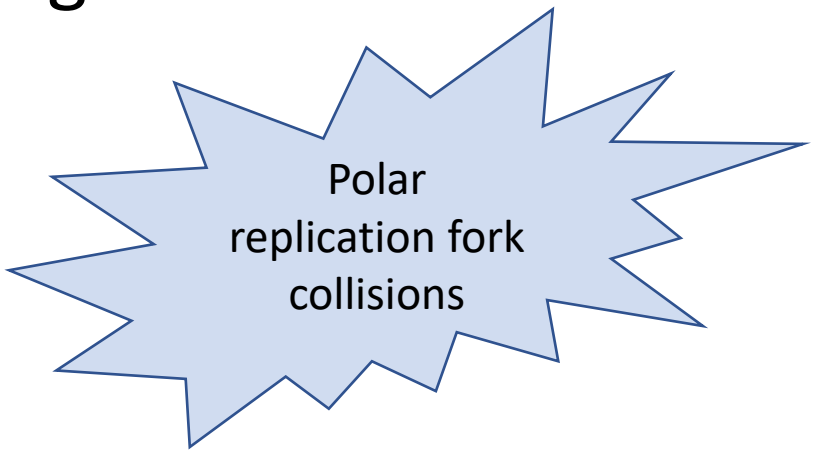
- Essential genes
- Non-essential genes
- tRNA genes
- Genes with AUG stop codon

Relocation of tRNA genes



- Rationale:

  Constant transcription of tRNA genes leads to more DNA breakage and instability



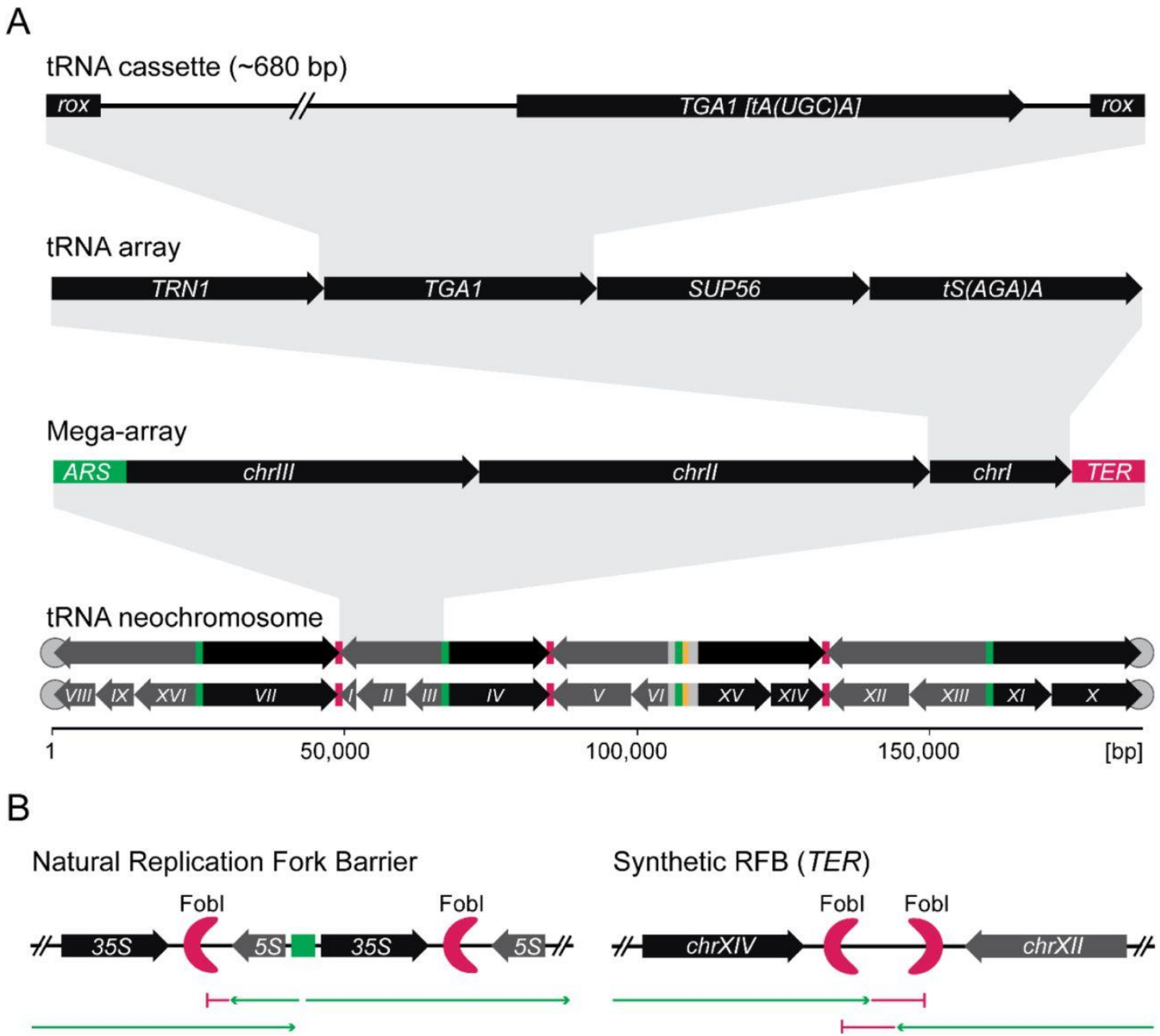
- Solution

tRNA neochromosome

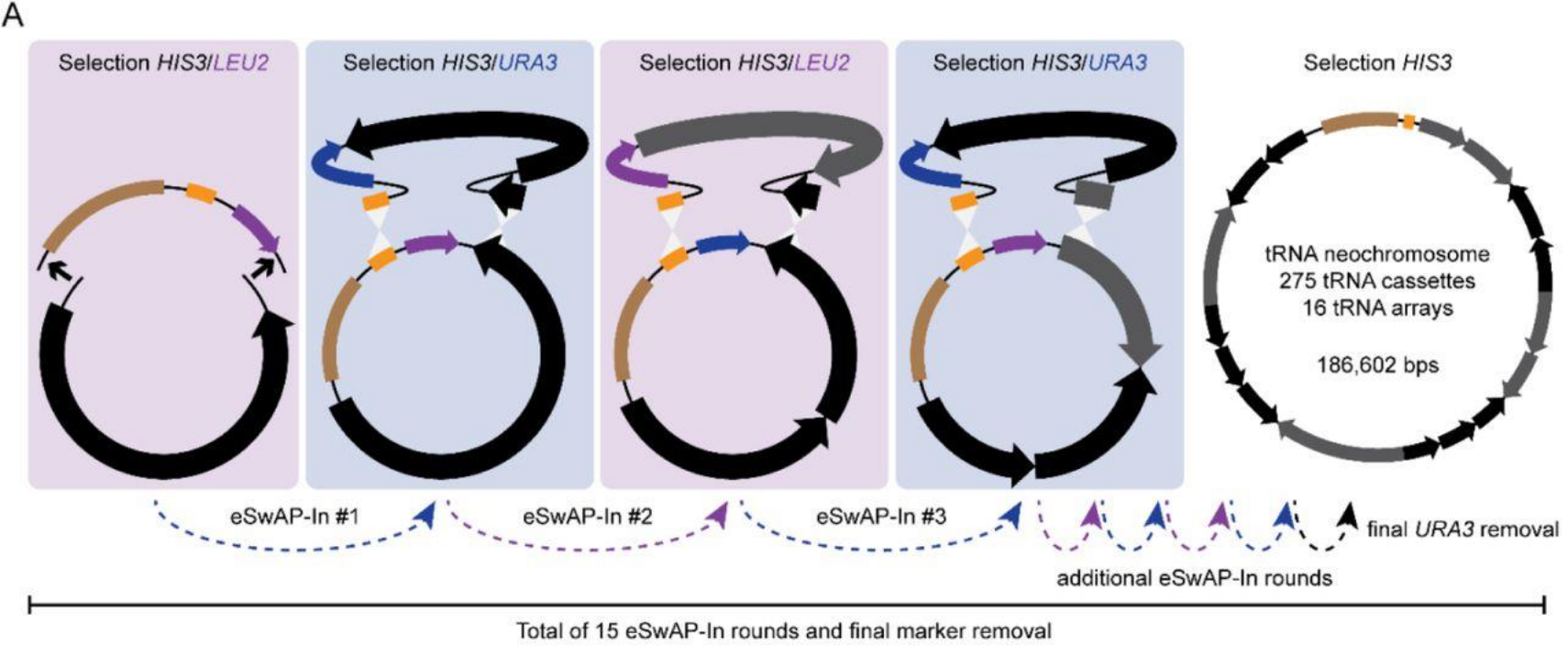
Schindler *et al., biorxriv,* **2022**. [https://doi.org/10.1101/2022.10.03.510608]

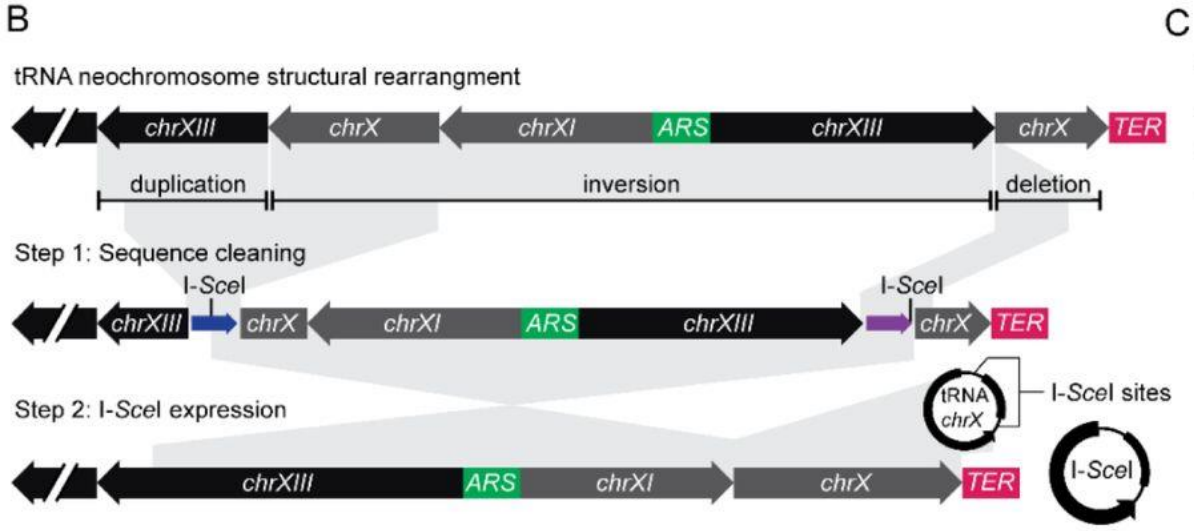
Design of a tRNA neochromosome

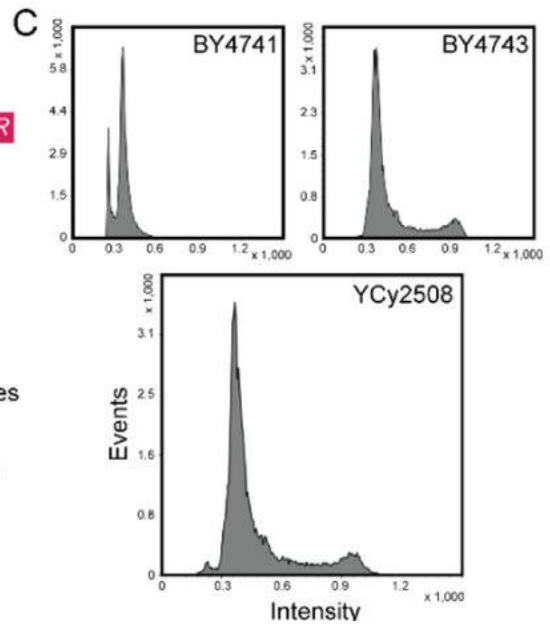
- Orthogonality
- tRNA genes do not face the replication fork



Schindler *et al., biorxriv,* **2022**. [https://doi.org/10.1101/2022.10.03.510608]







Method

- E-SwAPin Method
- Linearization

Schindler *et al., biorxriv,* **2022**. [https://doi.org/10.1101/2022.10.03.510608]

PCR Tags

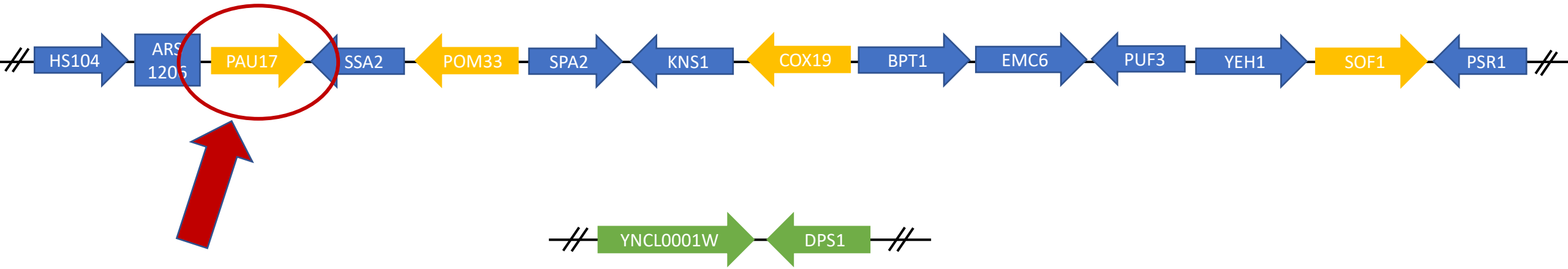
Unique sequence that is added to the ends of the PCR primers

- **Rationale:**
- Allows us to check that the synthetic substitution of the genome has occurred
- Allows us to monitor changes to the genome after SCRaMbLE etc.

- **Considerations:**
- Usually ~60% different from native sequences (minimum 33%) (Ensures they do not interfere with PCR synthesis)
- Melting temperatures between 58 °C and 60 °C

Mitchell, L. A., Phillips, N. A., Lafont, A., Martin, J. A., Cutting, R., & Boeke, J. D. (2015). qPCRTag Analysis - A High Throughput, Real Time PCR Assay for Sc2.0 Genotyping. Journal of Visualized Experiments, 99. https://doi.org/10.3791/52941

Synthetic megachunk



Giving examples of primers for this region

PCR Primer Sequences for PAU17 / YLL025W

primer	start	len	product size	tm	seq
primer-left-0	356	22	596	59.03	ACACTGCTGTACCAAACTAGGA
primer-right-0	951	21	596	59.10	GTTGCCAACCCAATCATGTCT
primer-left-1	436	22	569	57.01	TGGTTTCGACGGTATAGATGAA
primer-right-1	1004	20	569	58.86	ACACCACTGCTACCAAGGAA
primer-left-2	369	21	581	57.57	AAACTAGGATTTGACGCCCTT
primer-right-2	949	22	581	58.23	TGCCAACCCAATCATGTCTAAA
primer-left-3	333	20	670	59.05	CGCTCTATCTGCAGACGGTA
primer-right-3	1002	21	670	58.95	ACCACTGCTACCAAGGAAGAA
primer-left-4	323	20	694	58.74	CCATCTCCAGCGCTCTATCT
primer-right-4	1016	20	694	57.74	GGTTAGACAGCAACACCACT

Computer tools

SGD	*Saccharomyces cerevisiae* whole genome
Benchling	Molecular biology experiments design (primers, plasmids, synthetic sequence...)
NCBI gene bank	Sequence, information about a gene of interest
UNIprot	Sequence, information about a protein of interest
GenScript	DNA primer design

Thank you!

Are there any questions?