Synthetic genome

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Yeast 2.0

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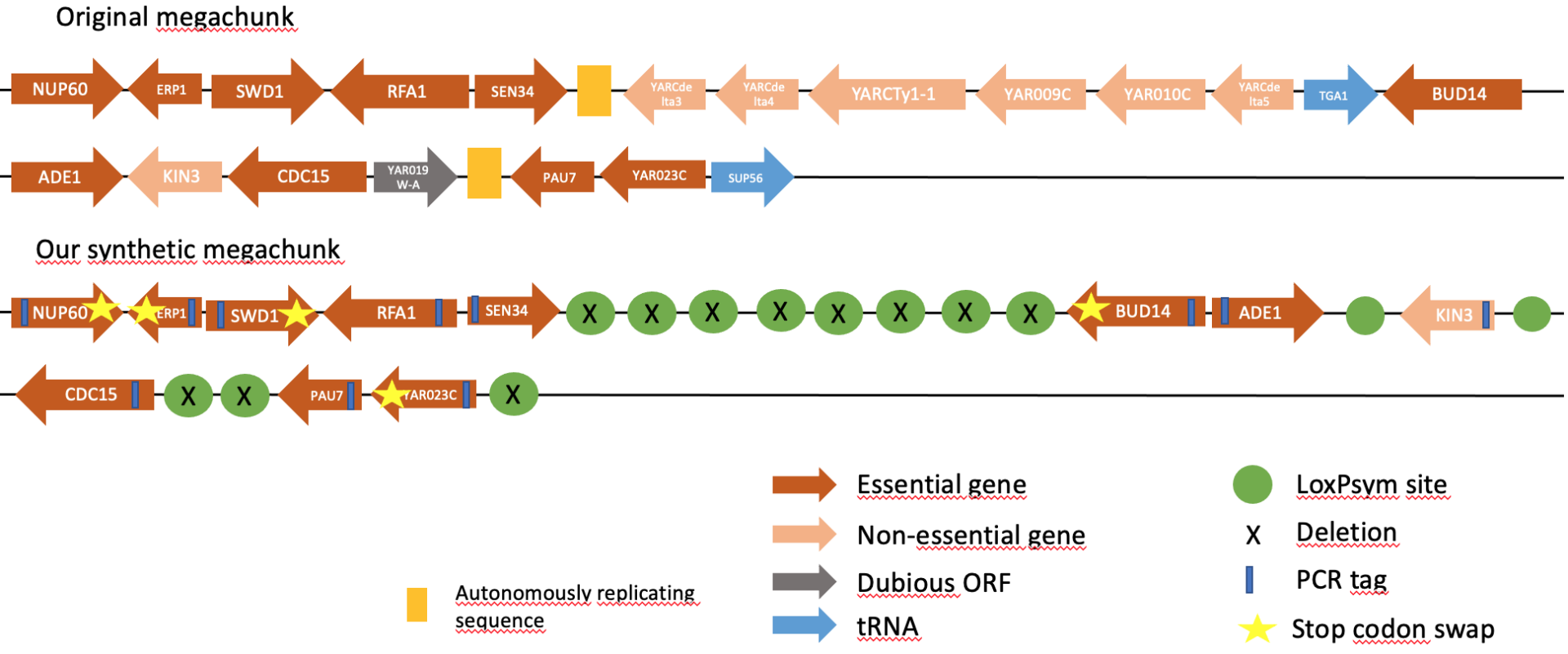
What is yeast 2.0?

- **Synthetic biology aims to redesign and reconstruct biological systems for new, useful end goals.**
- **Yeast 2.0 is ongoing project that designs and synthesizes a complete eukaroyotic genome - *Saccharomyces cerevisiae.***
- **Yeast 2.0 makes it possible to study different properties of chromosomes, evolution and genetics in general.**

Our megachunk design

- **Megachunk = synthetic 30-50 kB region in a selected chromosome**
- **Chromosome I**
- **Randomly chosen 30 kB megachunk between 151-181 kB**
- **Alterations:**
  - Deletion of transposons
  - Relocation of tRNA genes
  - Stop codon swap
  - PCR tags
  - Removal of non-essential genes

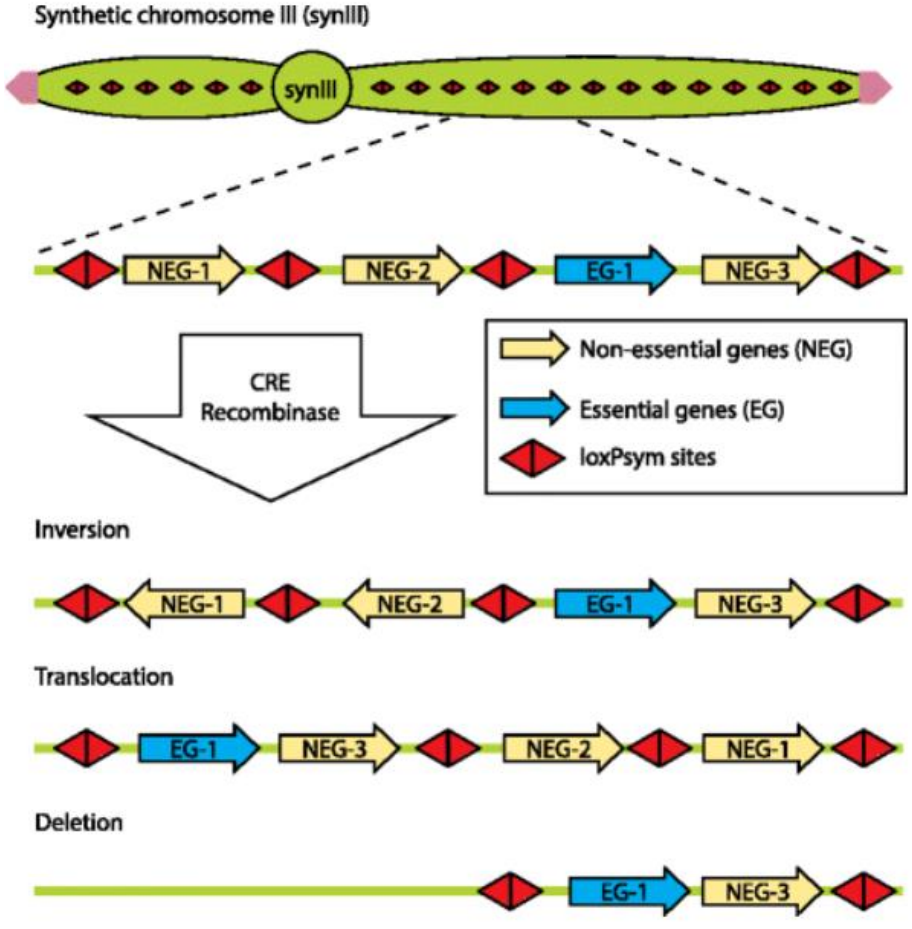
Our megachunk design



SCRaMbLE

= Synthetic Chromosome Recombination and Modification by LoxP-mediated Evolution

- **Used to rapidly reorganise the genome**
- **Initiated by the induction of Cre expression**
- **LoxP sites facilitate the recombination of genetic elements**
- **Can be used to target multiple synthetic chromosomes simultaneously**



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Neochromosome

- **Neochromosome is a separate centromeric plasmid where tRNA genes are removed from the synthetic chromosomes**

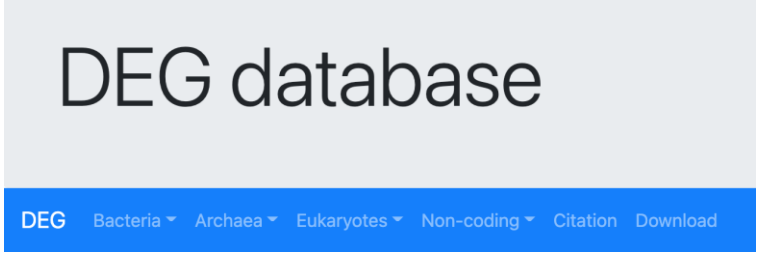
- **Two tRNA genes from our megachunk are removed**
  - TGA1
  - SUP56

- **tRNA genes tend to be sites of genomic damage and rearrangement**

Computer programs

- **Saccharomyces genome database, SGD**

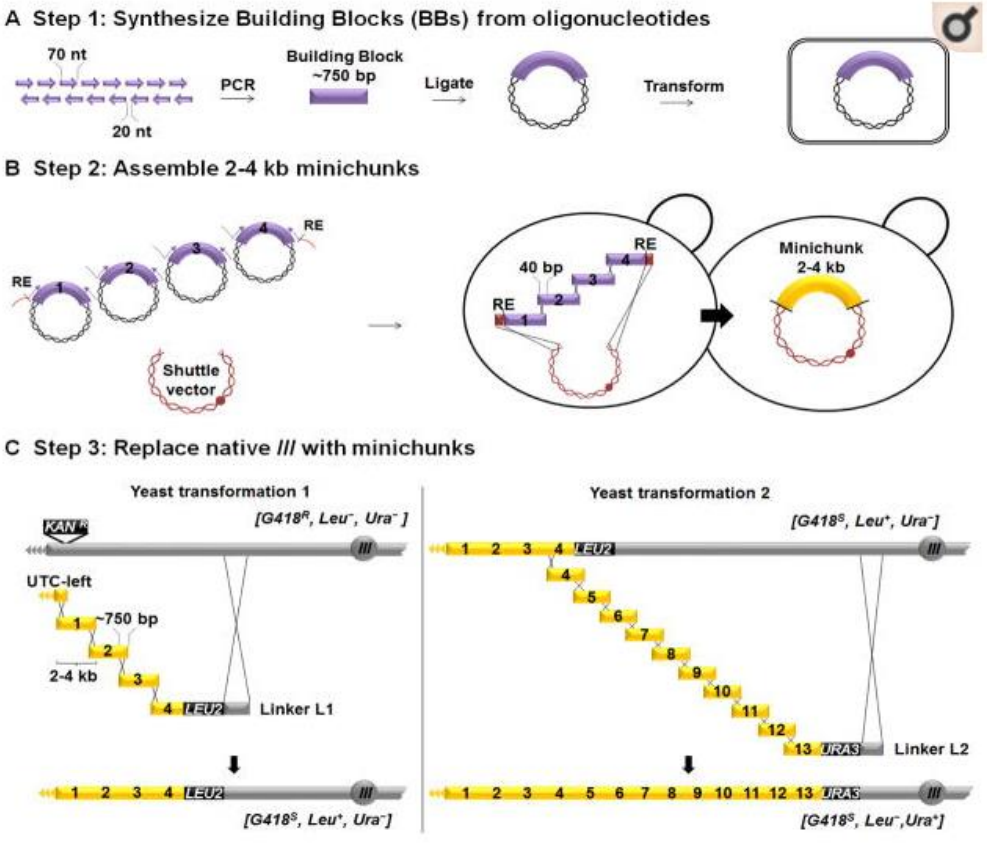
- **Database of essential genes, DEG**



Wet Lab Procedure

Goal: construction of megachunks (30-50kb) from building blocks and their introduction into wild type yeast genome.

1. **Design and synthesis of building block from oligonucleotides.**
2. **Assembly of minichunks through cutting with restriction enzymes and homologous recombination.**
3. **Replacement of native sequences through transformation with alternating selection for URA3 and LEU2 markers.**
4. **Confirmation of successful replacement with PCRtags.**



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