Yeast 2.0

Group 6

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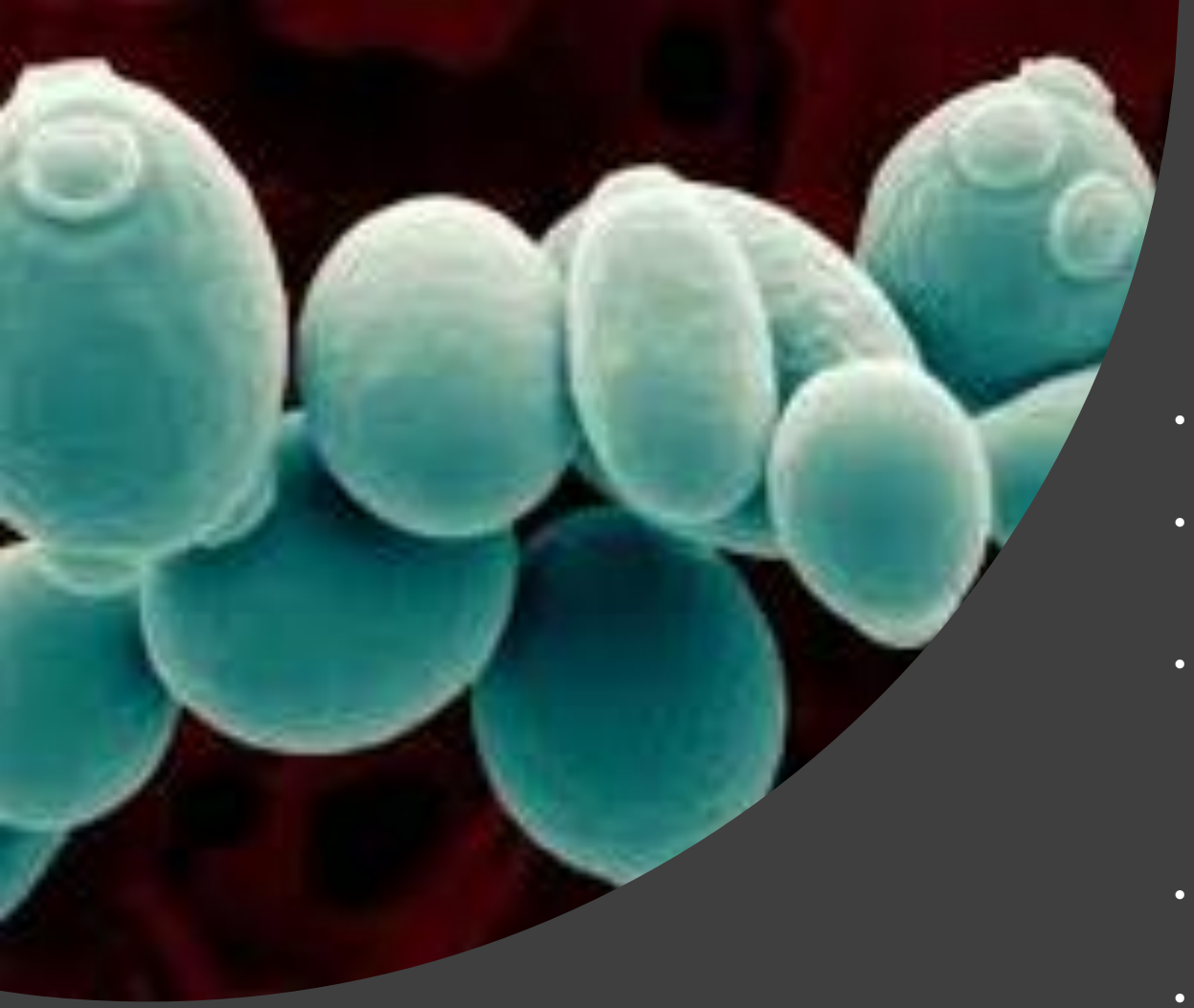
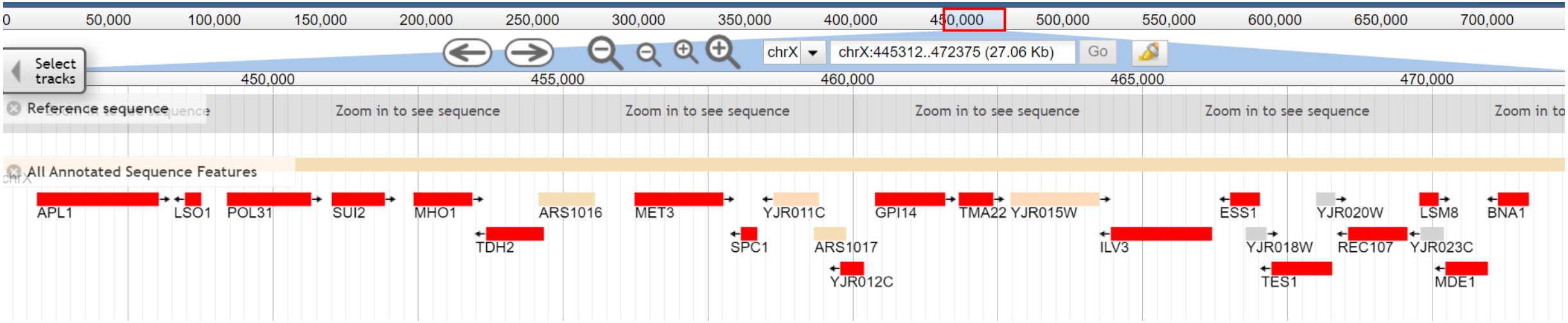


Yeast 2.0 - background

- Yeast is attractive for synthetic biology since it is flexible, robust, and fast growing
- Yeast 2.0 is an attempt to design a synthetic yeast genome from scratch into *Saccharomyces cerevisiae* to minimize genomic instability that is a result from the repetitive DNA sequences in yeast and induce genetic flexibility
- The goal is to make conservative and minimal changes to the wild-type genome by replacing native chromosomal DNA through the insertion of ~ 30-60 kb sized megachunks
    - Megachunks are assembled from 3-6 ~ 10 kb sized chunks that are assembled by restriction enzyme cutting and ligation
- The synthetic DNA sequences are introduced into yeast cells, where cellular machinery finishes building the chromosomes
- The alterations done in the yeast 2.0 genome are: the incorporation of PCRTags, the synthesis of synthetic telomere sequences, the removal of introns and non-essential genes, replacing stop codons (TAG) with TAA, deletion of transposons and relocation of tRNA sequences
- Yeast 2.0 could bring various benefits industrially and environmentally, such as for the production of bioethanol, bioplastics, and other high-value chemicals

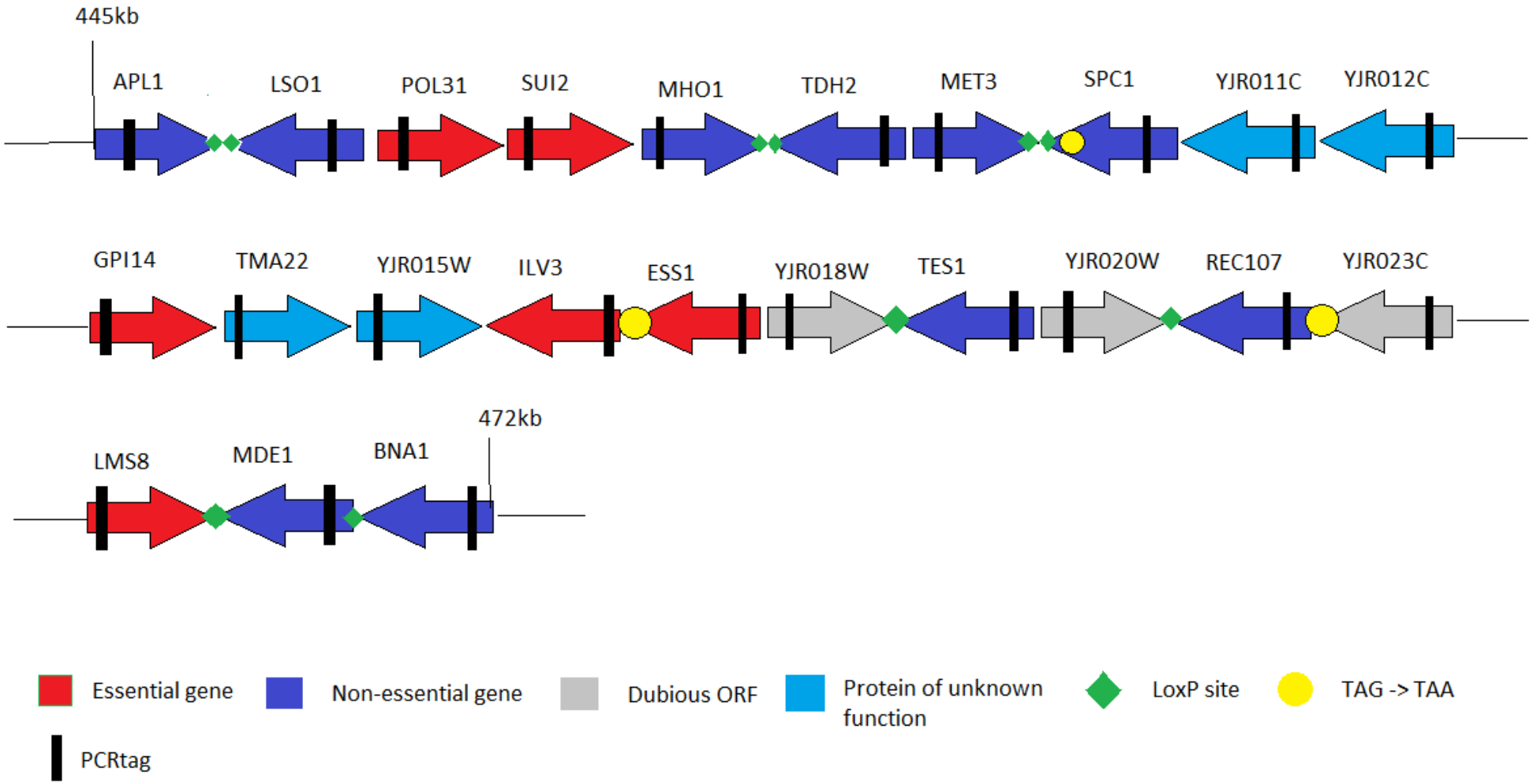
~30kb region of our choice

We selected ~445kb-475kb region in chromosome X



Essential genes: POL31, SUI2, GPI14, ILV3, ESS1, LSM8
Non-essential genes: APL1, LSO1, MHO1, TDH2, SCP1, MET3, TES1, REC107, MDE1, BNA1
Dubious ORFs: YJR018W, YJR020W, YJR023C
Autonomously Replicating Sequence: ARS1016, ARS1017
Putative protein of unknown function: YJR011C, YJR015W
Protein of unknown function: YJR012C, TMA22

Synthetic megachunk



Computer programs/databases

- *Saccharomyces* Genome Database (https://www.yeastgenome.org/)
- Database of Essential Genes (http://origin.tubic.org/deg/public/index.php)
- Sequence visualization and analysis --> DNA atlas

Wet lab procedure

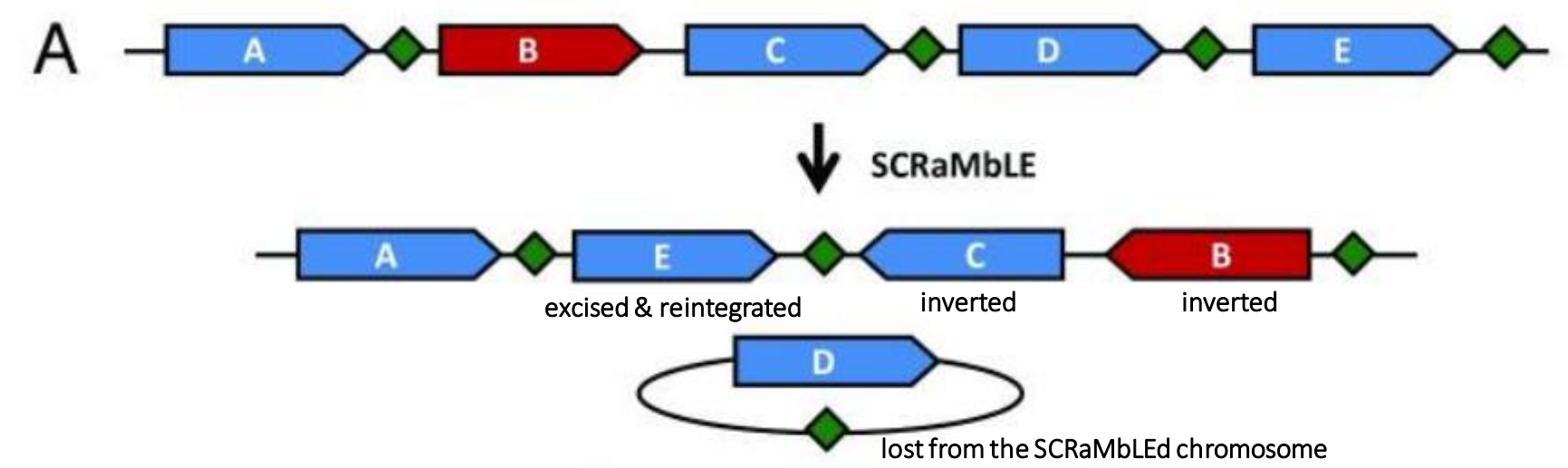
- The wanted short DNA sequences are either ordered or synthesized
- PCR is used to compile these sequences into building blocks (750bp)
- Restriction enzymes and ligation are used to combine blocks to chunks etc.
- Blocks (750bp) --> minichunks (3kb) --> chunks (10kb) --> megachunks (30kb)
- The megachunks are transformed into the yeast cell with homologous recombination
- Markers LEU2 and URA3 are alternated at the end of each chunk and megachunk

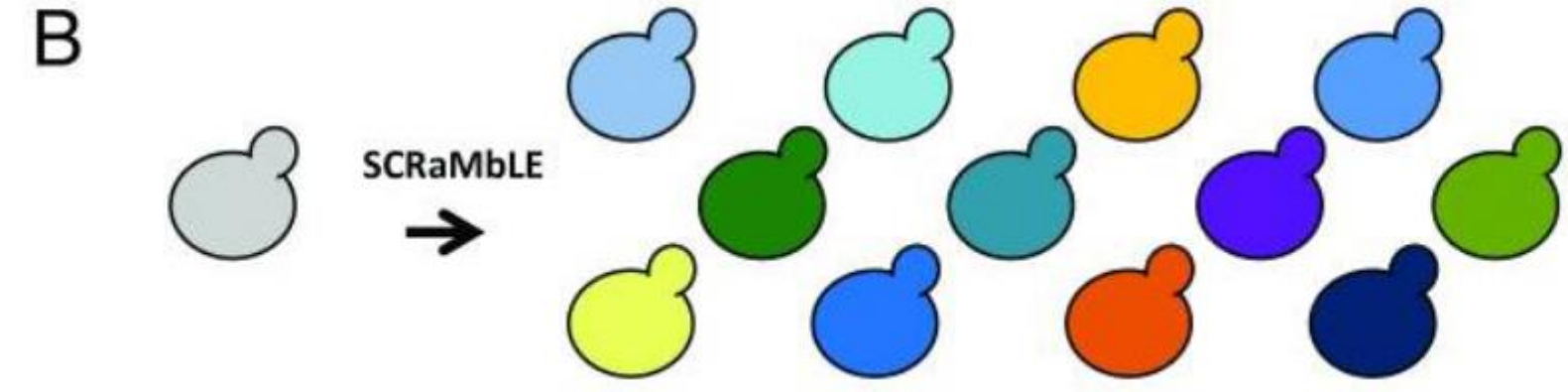
SCRaMbLE mechanism

Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution

- Generates combinatorial genomic diversity through rearrangements at designed recombinase sites
- Strains with high genetic diversity

- genes within synthetic chromosomes can be randomly removed and rearranged inside yeast cells when they are given a specific chemical stimulus:
  - SCRaMbLE requires yeast cells to contain a plasmid expressing Cre recombinase
  - Cre binds and recombines pairs of loxPsym DNA-sites -> recombination can lead to genome rearrangements





Green diamonds: LoxPsym sites
Blue arrows: non-essential genes
Red arrows: essential genes

Gray: Induction of SCRaMbLE in a synthetic strain
Colors: represent genetic diversity

Dymond & Boeke, 2012

Future prospects

- Butanol production
- 2 more ILV genes need to be altered
- In addition numerous other changes needed

--> might not be doable in the frame of yeast 2.0

Problems faced

- It was difficult to determine which genes can be omitted from the chunk as many of their functions are not known
- In order to actually make the changes we wanted several different chromosomes and regions would need to be targeted
- Difficult to know what kind of effects different changes have

References

- Yeastgenome.org
- http://origin.tubic.org/deg/public/index.php (for finding essential genes)
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