Synthetic Yeast 2.0

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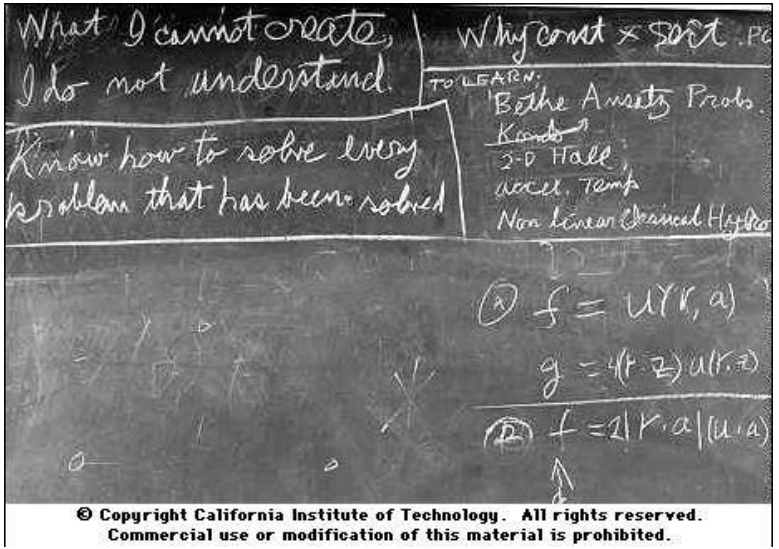
Introduction

- **Beginning of chemical DNA synthesis in 1970s, tech. progress allows for whole genome synthesis today**

- **Synthetic genomes with novel properties can inform about how genome sequences enable all aspects of cellular function and life** (Zhang et al., 2020)

- **Design principles for synthetic genomes:**
  1. (Near) wild-type phenotype and fitness
  2. Lack of destabilizing elements
  3. Genetic flexibility (Dymond et al., 2011)

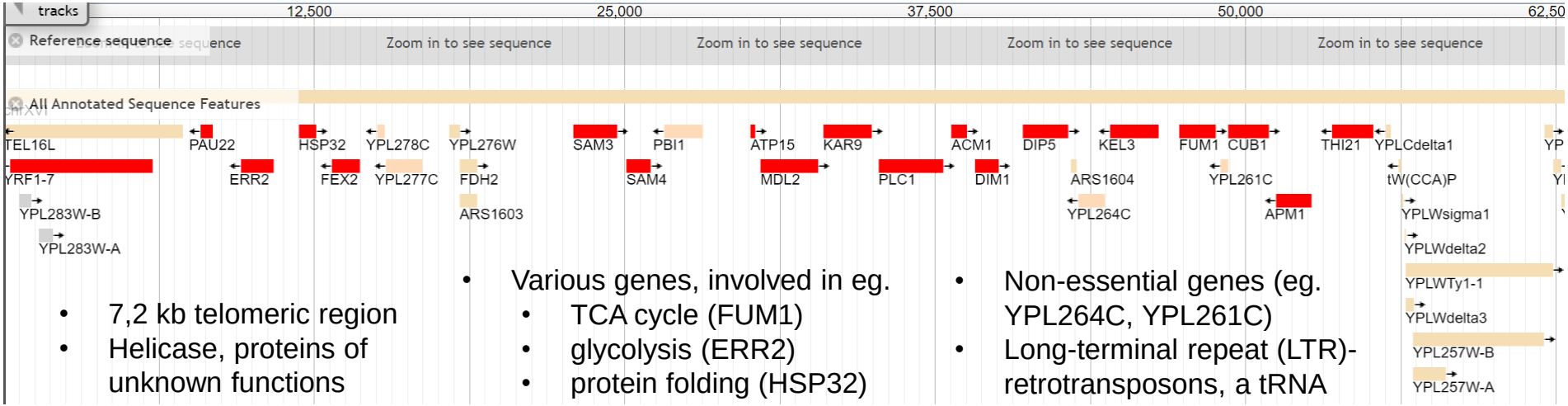
- **Genome design and synthesis from scratch**



"What I cannot create, I do not understand"
- R. Feynman

Selected region

- **Chromosome 16, 0-62 kb**
  - Not completely synthesized yet (Synthetic yeast 2.0 / Collaborators, 2021)
  - Contains various types of DNA, including telomeric region, essential and non-essential genes, transposons and a tRNA gene (The *Saccharomyces* Genome Database, 2021)



- 7,2 kb telomeric region
- Helicase, proteins of unknown functions

- Various genes, involved in eg.
  - TCA cycle (FUM1)
  - glycolysis (ERR2)
  - protein folding (HSP32)
  - RNA processing (DIM1)
  - Cell signaling (PLC1)

- Non-essential genes (eg. YPL264C, YPL261C)
- Long-terminal repeat (LTR)-retrotransposons, a tRNA gene

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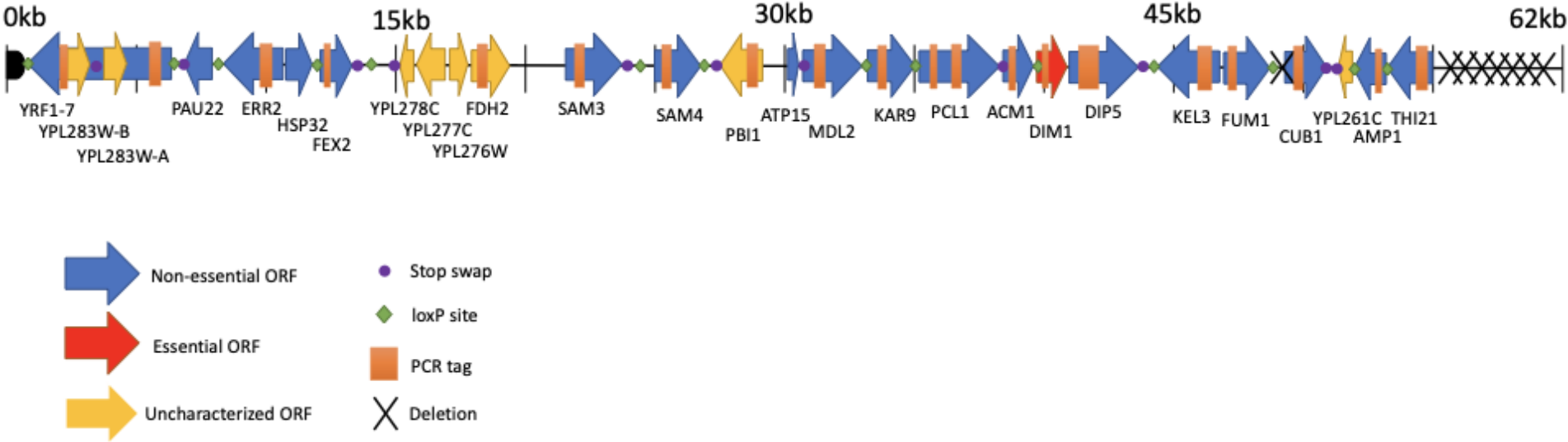
Picture from https://browse.yeastgenome.org/

Modifications

- Removal of transposons and 1 non-essential gene
- Addition of loxP sites
- Relocation of tRNA to "neochromosome"
- Replacement of all TAG stop codons with TAA
- Addition of PCRtags for SCRaMbLE
- No introns on this region → no need for deletion

Modifications

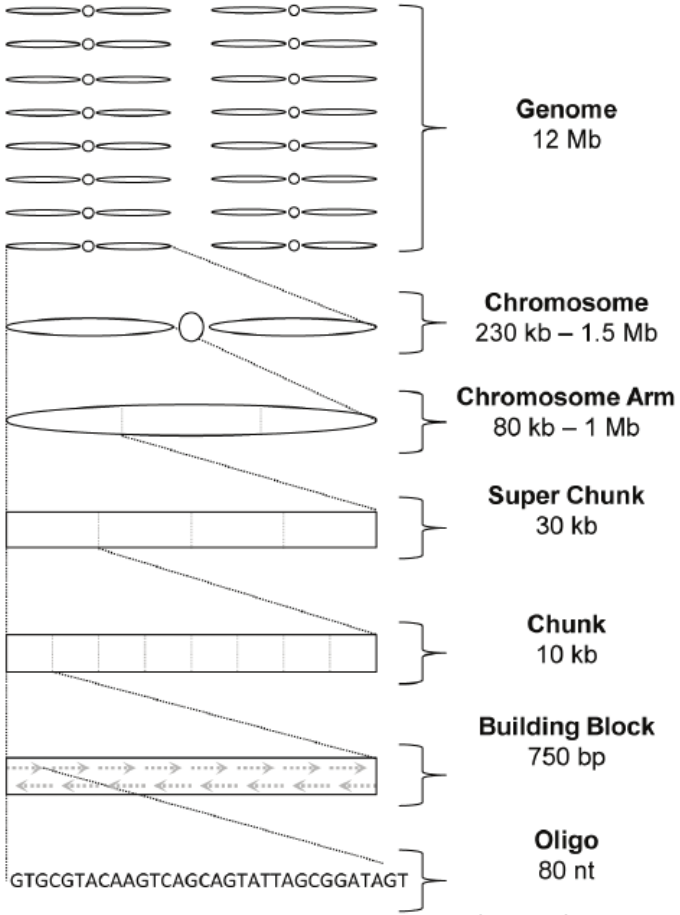


Software and Databases (links in the underlined names)

- **BioStudio for genome designing**
  - Editing in both nucleotide and chromosome level
  - Simultaneous access for all researchers in the project to examine and modify the genome
- **GeneDesign for designing of genes or building blocks**
  - Features e.g. manipulation of codons, designing the restriction sites and designing of the oligonucleotides
- ***Saccharomyces Genome Database***
  - Browsing the yeast genome
  - Analysis of genome through different datasets
- **Synthetic Yeast 2.0**
  - Design principles of the project
  - Data from already synthesized chromosomes
- **Database of Essential Genes for the survival of an organism**

Wet lab

- **Oligonucleotides are assembled by polymerase chain assembly into building blocks or minichunks**
- **Minichunks are combined into chunks by e.g. in vitro recombination or homologous recombination in yeast**
- **Chunks are bound into megachunks by restriction enzyme cutting and ligation**
- **Megachunks are introduced into the genome by homologous recombination and selectable markers such as URA3 and LEU2**



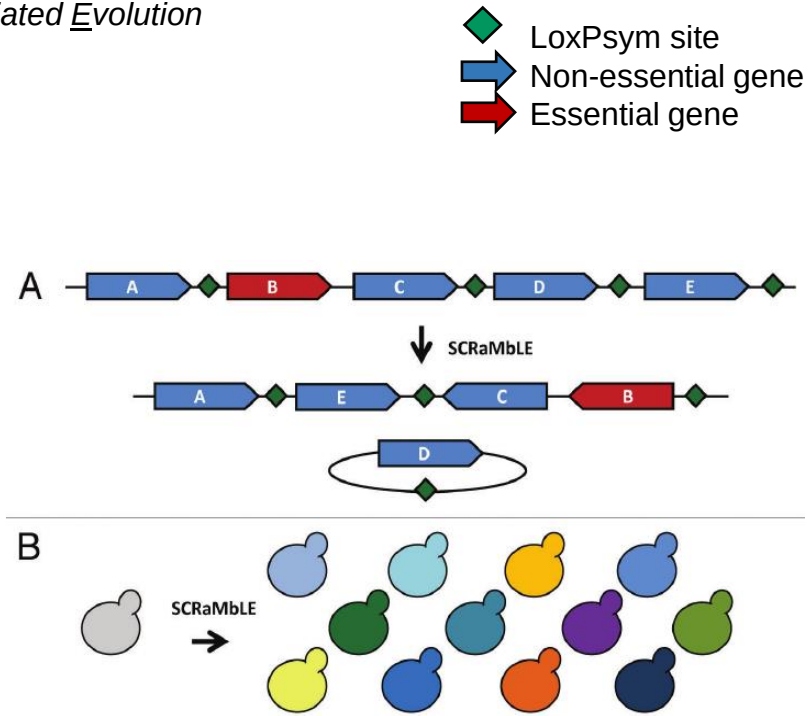
Dymond & Boeke, 2012

SCRaMbLE

Synthetic Chromosome Recombination and Modification by LoxP-mediated Evolution

- **Non-directional LoxPSym sites allow for recombination in either orientation**
  - 3`UTR of non-essential genes
  - LTRs
  - tRNAs
  - Flanking centromere
  - Adjacent to telomere

  > translocations, inversions, deletions
- **SCRaMbLE (Cre recombinase) induction via b-oestradiol leads to increased genetic diversity and allows for selection of desired phenotype**
- **Genome analysis via PCRtags**
  - and/or comparative genome hybridization, molecular karyotyping, whole-genome sequencing



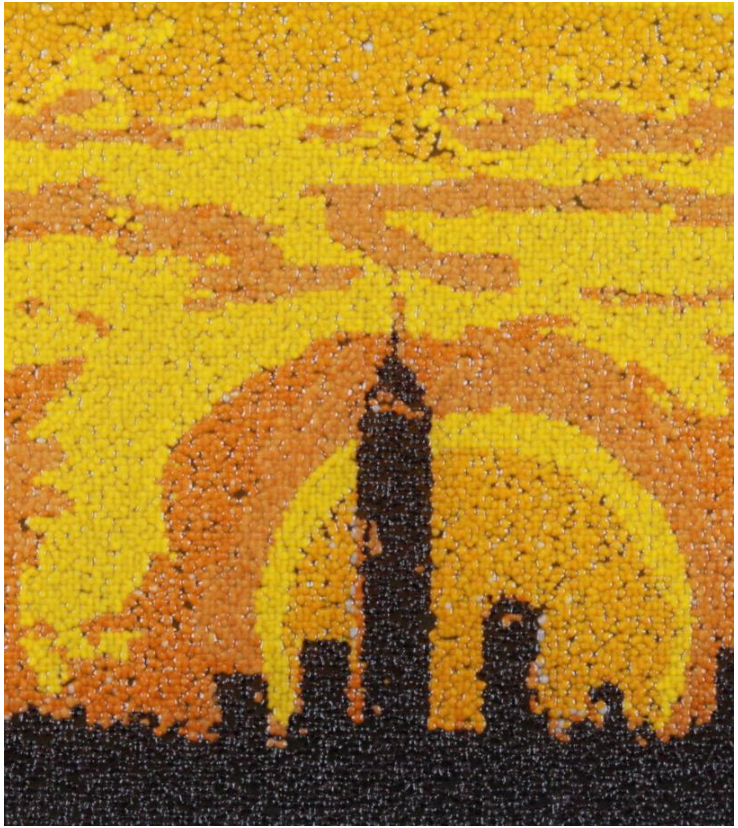
Dymond & Boeke, 2012

Importance of Yeast 2.0

"The synthetic genome is designed to increase genome stability and genetic flexibility while maintaining cell fitness near that of the wild type." (Wu et al,. 2017)

Yeast 2.0

- **A model for future synthetic cell lines/organism**
- **A platform for industrial strains with highly specialized functions**
- resveratrol, insulin precursors, vaccines HPV
- Insertion of gene variants improving ethanol tolerance:
    - *Growth*: MOG1 (nuclear import protein)
    - *Survival*: MGS1 (genome maintenance) (Haas et al., 2019)

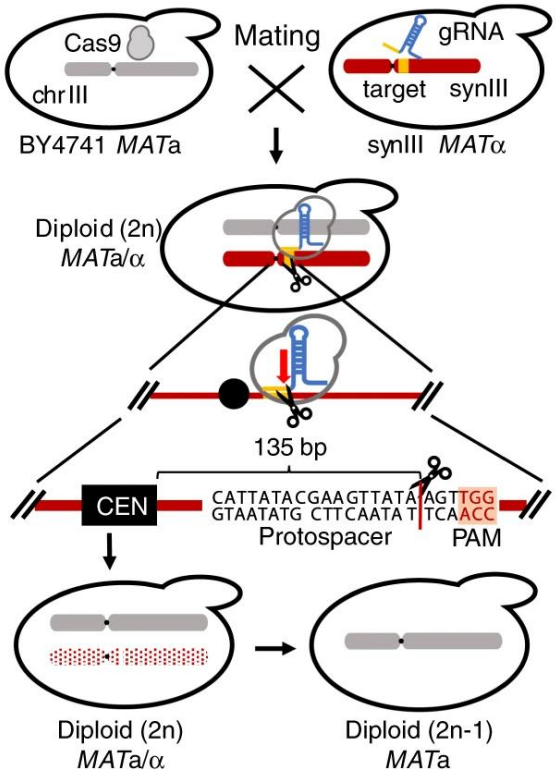


Jasmine Temple, NYU Langone Health (Yeastart.org)

Further development

- **Synthetic fuel, biodiesel from biomass, food ingredients, protein drugs** (Nielsen, 2016)

- **Yeast 3.0** (Dai et al., 2020)
  - **Engineer regulatory sequences**
  - **Utilize genetics tools and directed evolution**
    - CRISPR-Cas9
  - **Change more DNA sequences**



Xu et al., 2020

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Thank you! Q&A

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