Synthetic Biology

Yeast 2.0 - chromosome II

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The selected region

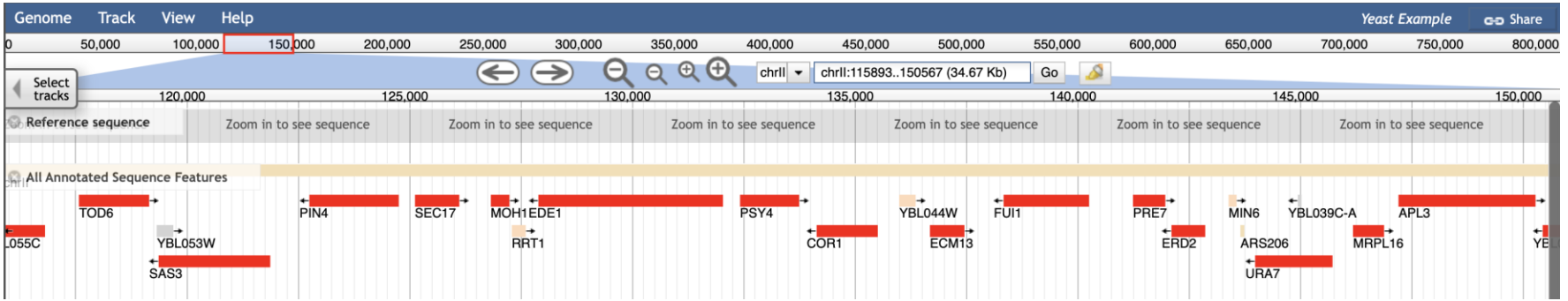
Chromosome II bases 115894 – 150835, size (34.94 Kb)

Contains:

- **17 known genes**
- **2 dubious open reading frames**
- **3 proteins with unknown function**
- **1 autonomously replicating sequence**
- **2 introns**
- **914 TAG stop codons in total in both strands**

The selected region

Chromosome II bases 115894 – 150835, size (34.94 Kb)



Essential genes: SEC17, COR1, PRE7, ERD2, MRPL16
Non-essential genes: YBL055C, TOD6, SAS3, PIN4, MOH1, EDE1, PSY4, ECM13, FUI1, URA7, APL3, YBL036C
Dubious ORFs: YBL053W, YBL039C-A
Autonomously Replicating Sequence: ARS206
Protein of unknown function: RRT1, YBL004W, MIN6

The essential genes

SEC17 – needed for vesicular transport between ER and Golgi

COR1 – subunit of QH2 cytochrome c reductase, needed in electron transport chain

PRE7 – subunit of proteasome 20S, which degrades proteins and therefore maintains cellular homeostasis

ERD2 – HDEL receptor, plays a role in keeping proteins in ER

MRPL16 – large subunit of mitochondrial ribosome

Software

Yeast genome browser was used to inspect the region
https://browse.yeastgenome.org/

The following tracks were used:

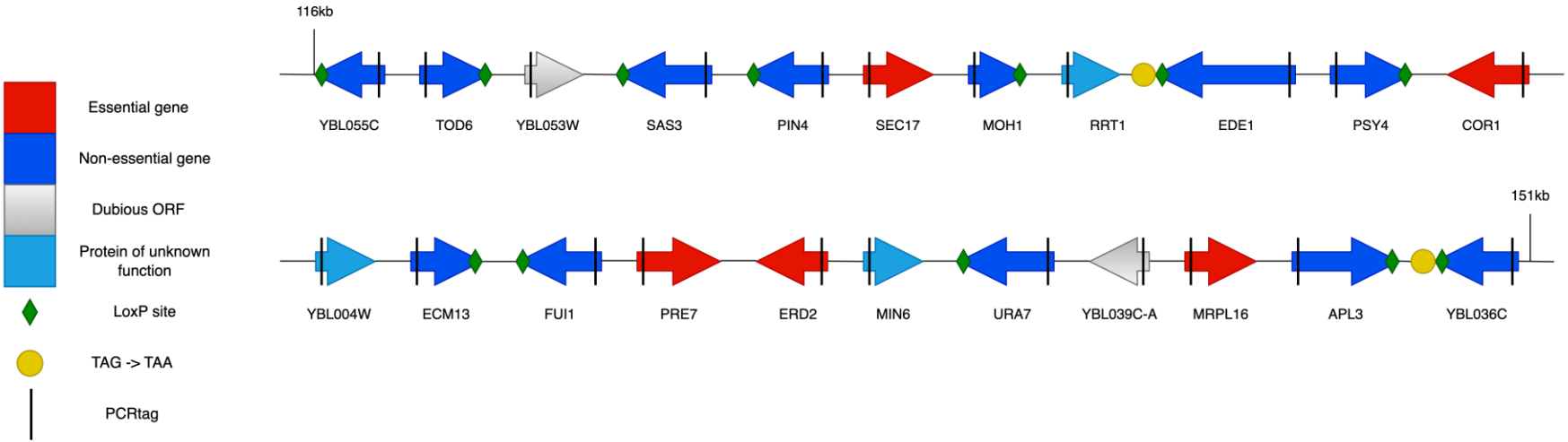
- **Reference sequence DNA**
- **All annotated sequence features**
- **Verified_introns**

TAG stop codons were found using "Add sequence search track"

What is done

- Introns (in SEC17 and ERD2) and non-essential genes are removed
- TAG stop codons are replaced with TAA
- PCRtags are incorporated into most open reading frames
- loxP sites are added everywhere where deletions were made

Synthetic megachunk



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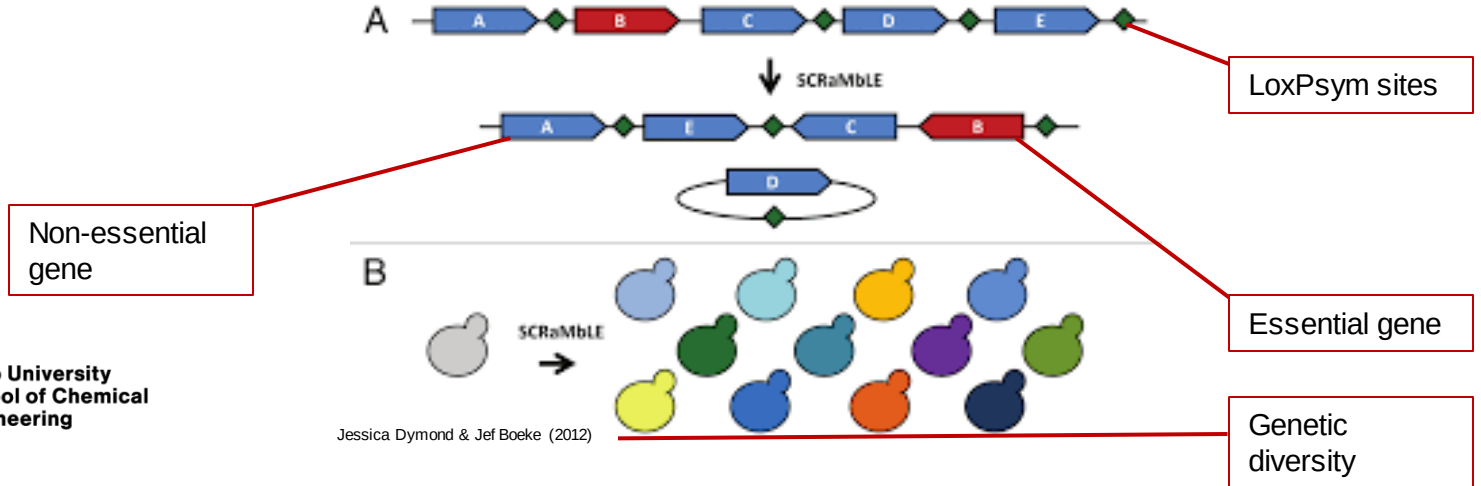
Wet lab procedure

- DNA is purchased in 2-4 kb minichunks
- Minichunks have overlapping regions for homologous recombination
- Two transformation steps are used
- Temporal selection markers (LEU2 and URA3) are used to verify that native DNA sequence has been replaced

SCRaMbLE mechanism

- ***S**ynthetic **C**hromosome **R**earrangement and **M**odification by **L**oxP-mediated **E**volution*
- Implemented in the yeast genome
- Scramble rearranges or removes regions of the synthetic yeast chromosomes when the cell is given a chemical stimulus
- Continued Scramble leads to rearranged genome that only contains the genes required for lab-based growth
- Enables to explore what genes and genome arrangements are essential and which ones are not



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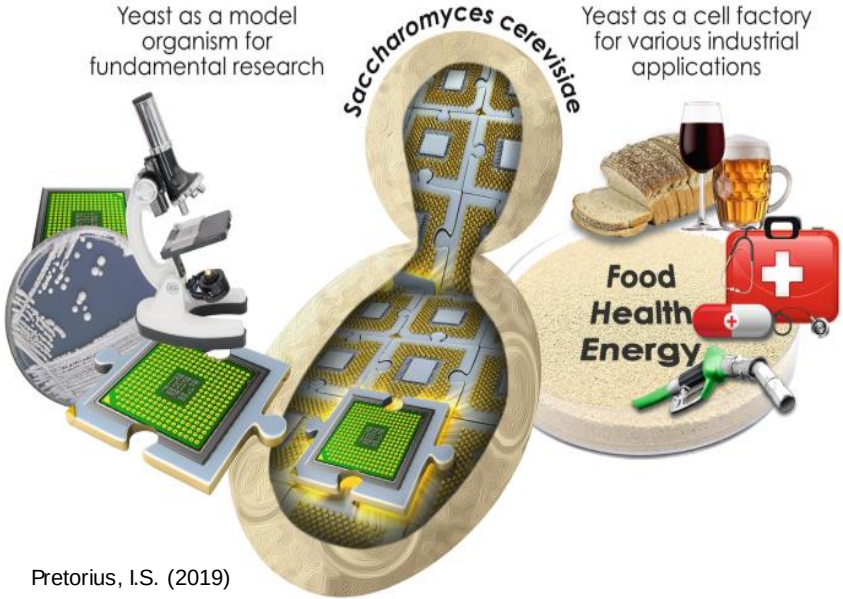
Significance and impact of yeast 2.0

- Deeper understanding of how complex eukaryotic cells work
- Conserving only the desired genes in the genome
- Yeast 2.0 has the possibilities to help overcome some of the world's biggest challenges that relate to e.g. health, food, water, energy, and the economy.
- Enables a better future for the whole planet

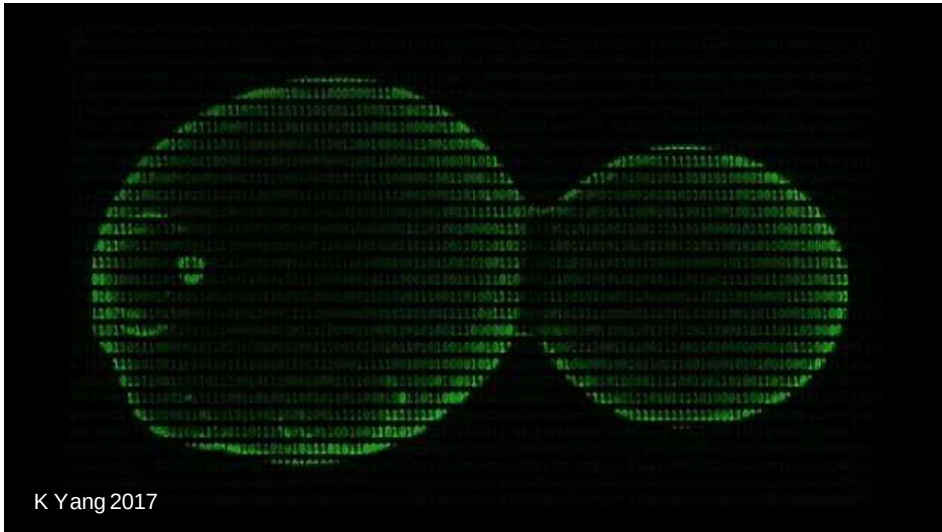


Pretorius, I.S. (2019)

Developing further

- Different versions of yeast 2.0 could be made for different purposes. For example by adding genes for maximizing secretion of heterologous protein production (SEC61 in yeast is needed in heterologous protein production)

- Yeast 3.0 has been proposed and has a more compact genome
- It will be explored how much the yeast genome can be compacted for future easier examinations



Sources

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