YEAST 2.0

Group 10: Martin Ahlberg, Eeva Kaipia, Jimi Oikarinen, Jussi Pietiläinen

https://www.oculyze.net/are-yeast-cells-eukaryotic-or-prokaryotic-your-fyi-guide/

Contents

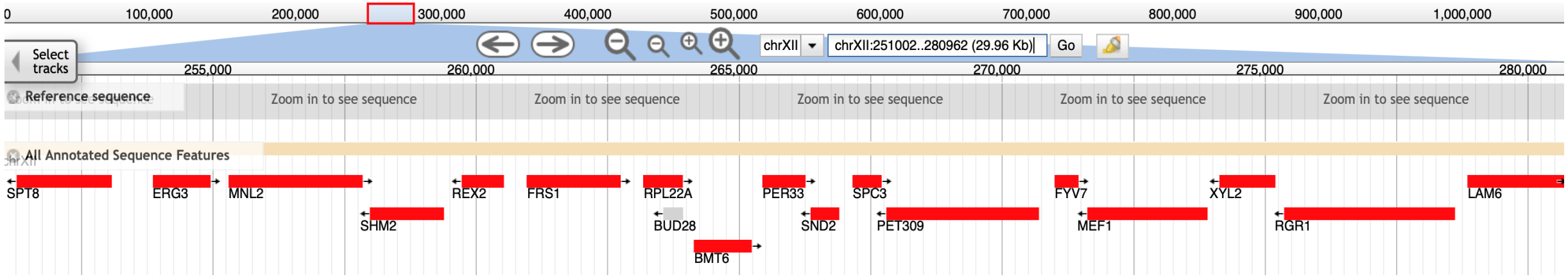
Background

- The aim of Yeast 2.0 is to design a plan to synthesize yeast genome from scratch for *Saccharomyces cerevisiae*
  - Goal is to reduce genomic instability
- Native chromosomal DNA is modified with the addition of 30–60 kb megachunks
  - Minimum conservative changes with considerable impacts
- The changes are made by for example:
  - The addition of PCR tags
  - Replacing of STOP codons
  - Removal of non-essential genes or introns

Computer programs used

- Database for yeast (*Saccharomyces*) genome: https://www.yeastgenome.org/
- Essential genes database: http://origin.tubic.org/deg/public/index.php/search/eukaryotes
- Process diagram: https://app.diagrams.net/

Megachunk construction



Essential genes: REX2, FRS1, SPC3, RGR1

Non-essential genes: SPT8, ERG3, MNL2, SHM2, RPL22A, BMT6, PER33, SND2, PET309, FYV7, MEF1, XYL2, LAM6

Dubious ORFs: BUD28

Functions of essential genes

REX2: 3′-5′ RNA exonuclease

FRS1: Translational quality control

SPC3: Subunit of signal peptidase complex

RGR1: Subunit of RNA polymerase II mediator complex

Functions of non-essential genes

SPT8: Transcriptional regulation

ERG3: Double bond formation

MNL2: Putative mannosidase

SHM2: Cytosolic serine hydroxymethyltransferase

RPL22A: Ribosomal 60S subunit protein

BMT6: Methyltransferase

PER33: ER protein

SND2: ER targeting protein

PET309: Translational activator

FYV7: rRNA maturation

MEF1: Translational elongation

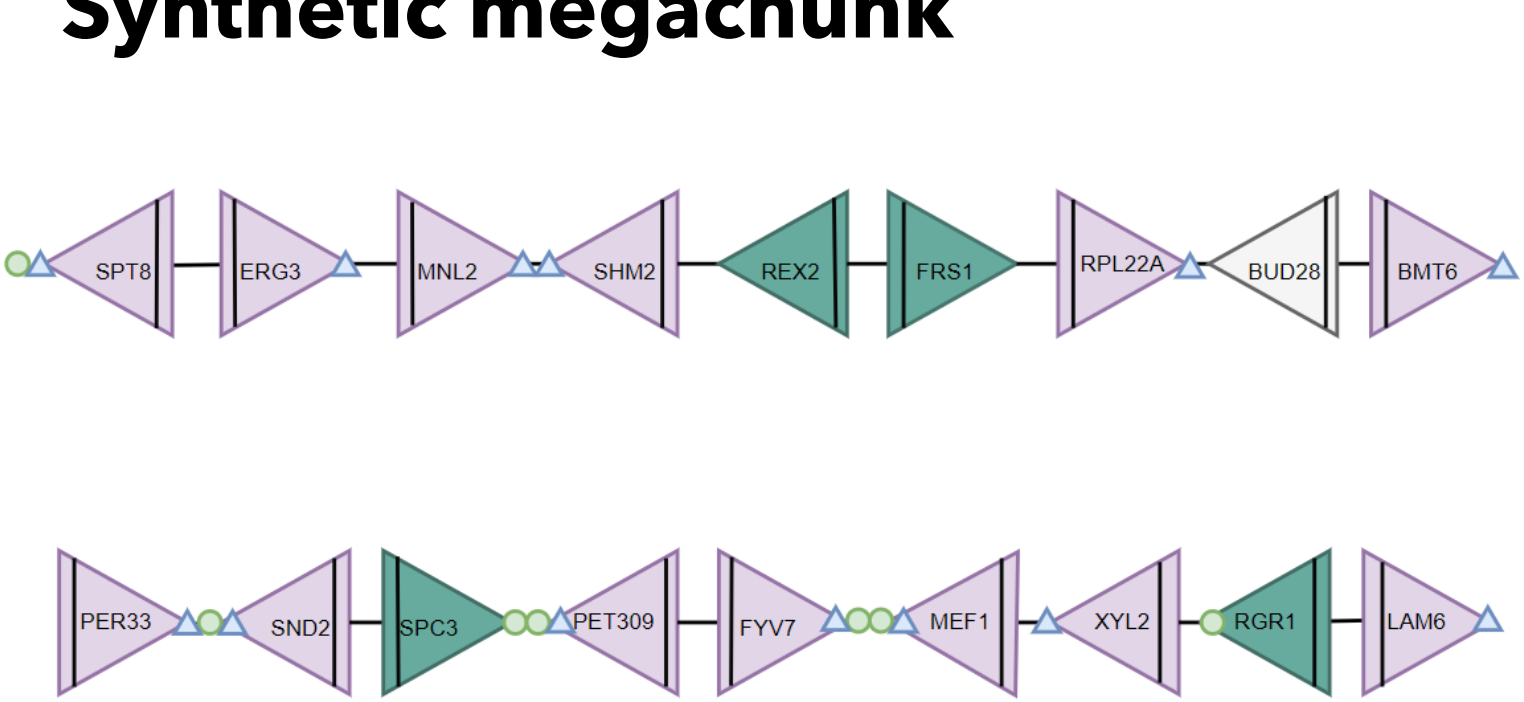
XYL2: Xylitol dehydrogenase

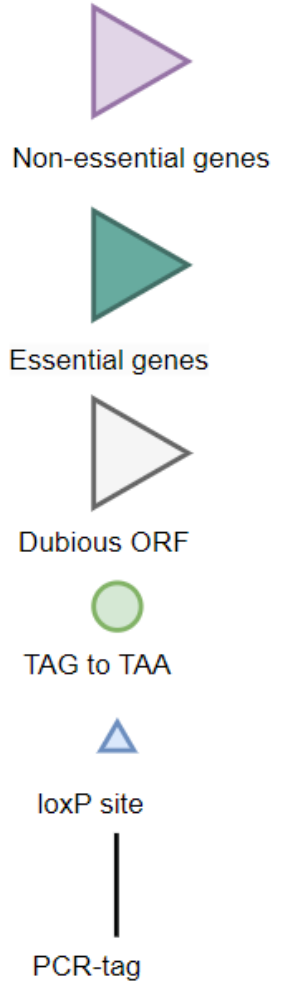
LAM6: Sterol transporter

Why we chose the genes

- Essential genes: Viability
- Non-essential genes: Needed for SCRaMbLE
- Dubious ORF: Deletion can be tricky
- RPL22A intron removed

Synthetic megachunk

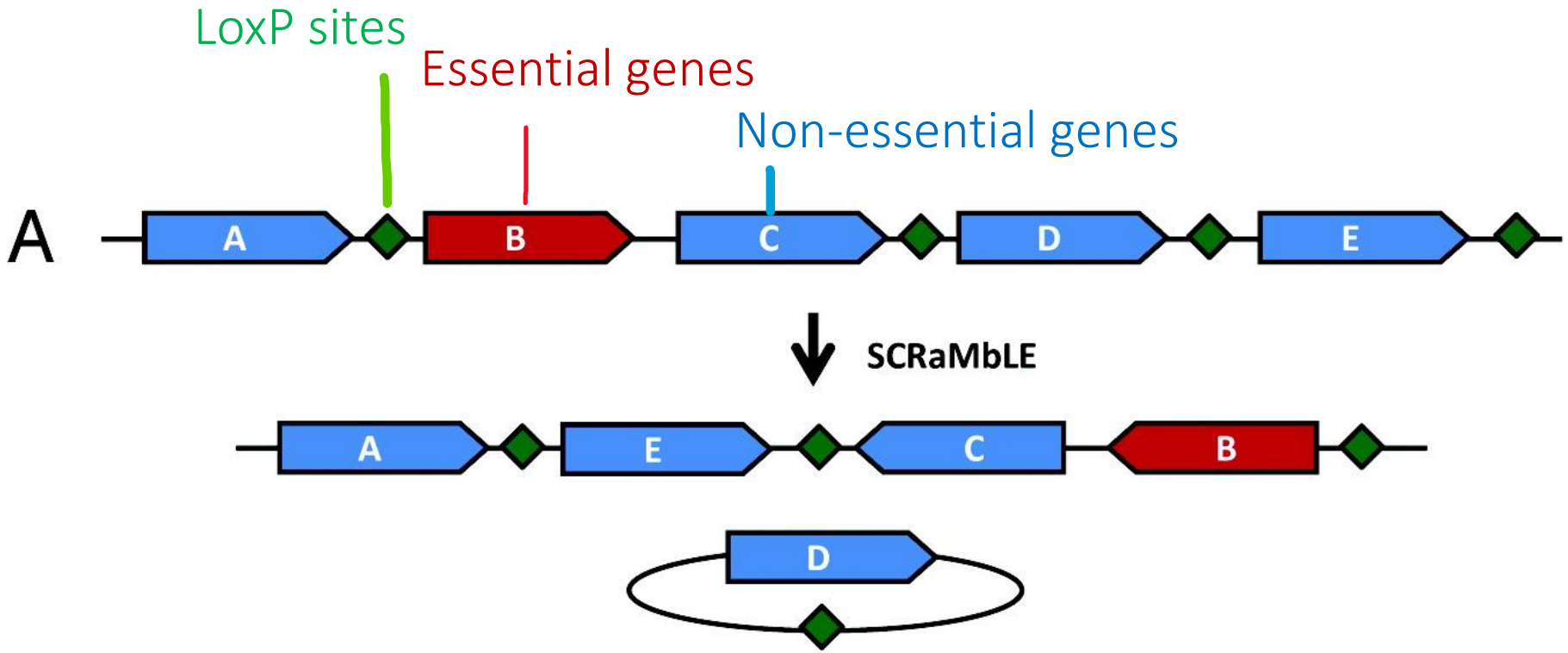




SCRaMbLE

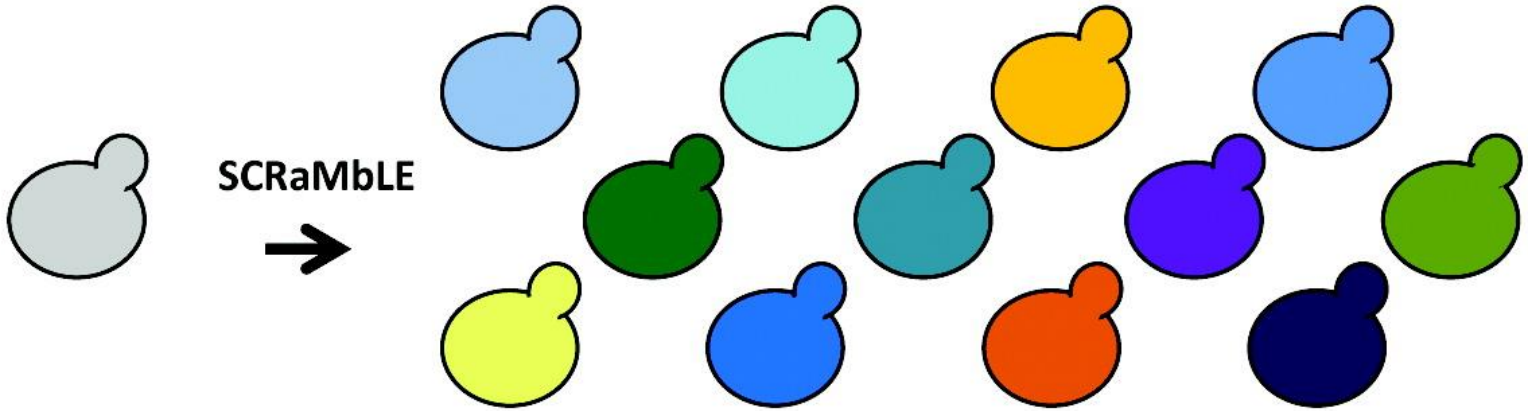
Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution

- Used to generate genetic diversity by recombination of genetic elements at specific sites, called loxP sites, by the activity of Cre recombinase
- LoxP sites are introduced in the 3'-UTRs of all non-essential genes
- Rearrangements can introduce deletions, duplications, inversions and translocations
- Random recombination of genes in the synthetic DNA leads to strains with high genetic diversity
- Cre recombinase is expressed on a separate plasmid under an inducible promoter LoxP mediated evolution of the yeast can be started whenever the need arises
- Easy generation of genetic diversity assures fast evolution of strains for different applications



B

Induction of SCRaMbLE in synthetic strain



Wetlab

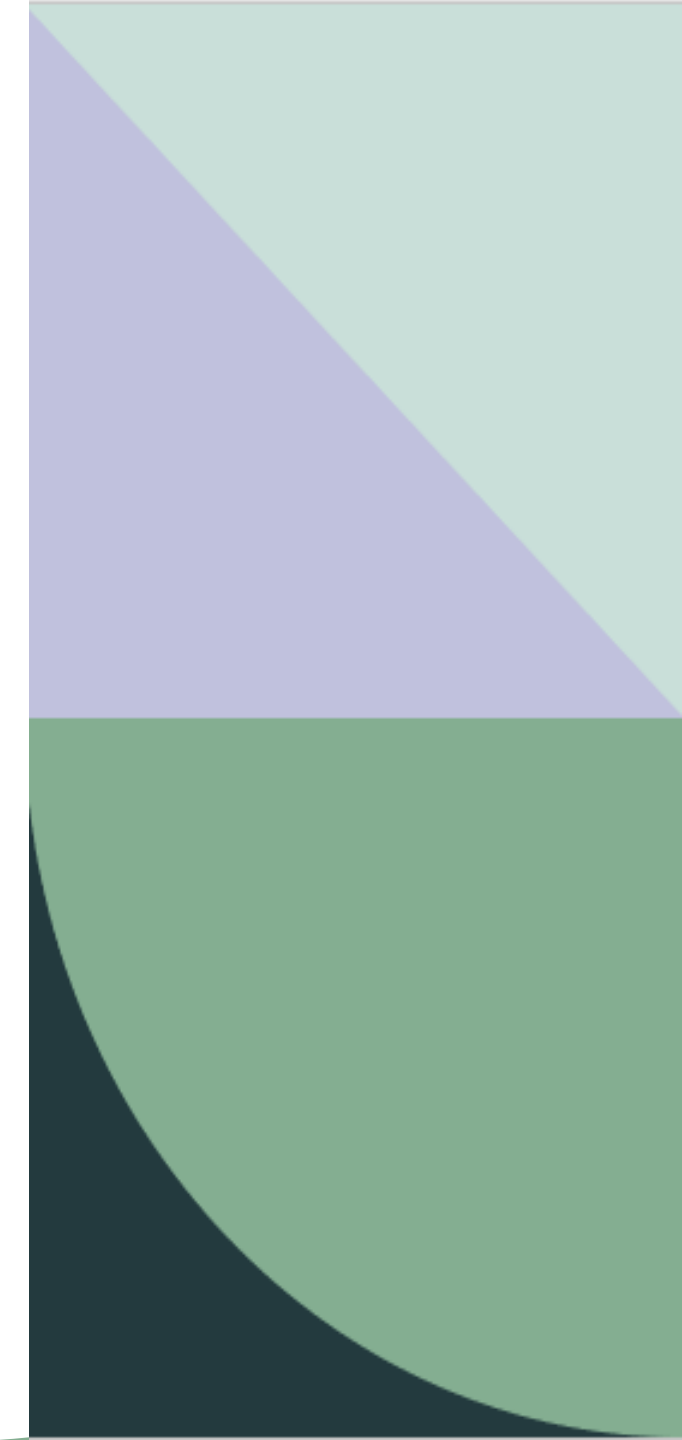
If DNA is ordered:

3X 10KB DNA SEQUENCES ARE ORDERED

1ST 10KB WITH LEU2 MARKER, 2ND 10KB WITH URA3 MARKER, 3RD 10KB WITH LEU2 MARKER

SAME PROCEDURE BUT FASTER

COSTS MORE?



Wetlab

If DNA is synthesized:

DNA OLIGOS WITH OVERLAPS ARE GENERATED BASED ON THE DESIGNED SEQUENCE

BUILDING BLOCKS (750BP) FROM THE OLIGOS ARE STITCHED TOGETHER WITH PCR

RESTRICTION ENZYMES AND LIGATION ARE USED TO COMBINE BLOCKS TO MINICHUNKS (3 KB) AND MINICHUNKS TO CHUNKS (10 KB)

THE CHUNKS ARE TRANSFORMED INTO THE YEAST CELL AND INTEGRATED INTO THE NATIVE CHROMOSOMAL LOCATION BY HOMOLOGOUS RECOMBINATION

EACH CHUNK HAS AN AUXOTROPHIC MARKER (*LEU2* & *URA3*) WHICH IS ALTERNATED AT THE END OF EVERY OTHER CHUNK

Further developments

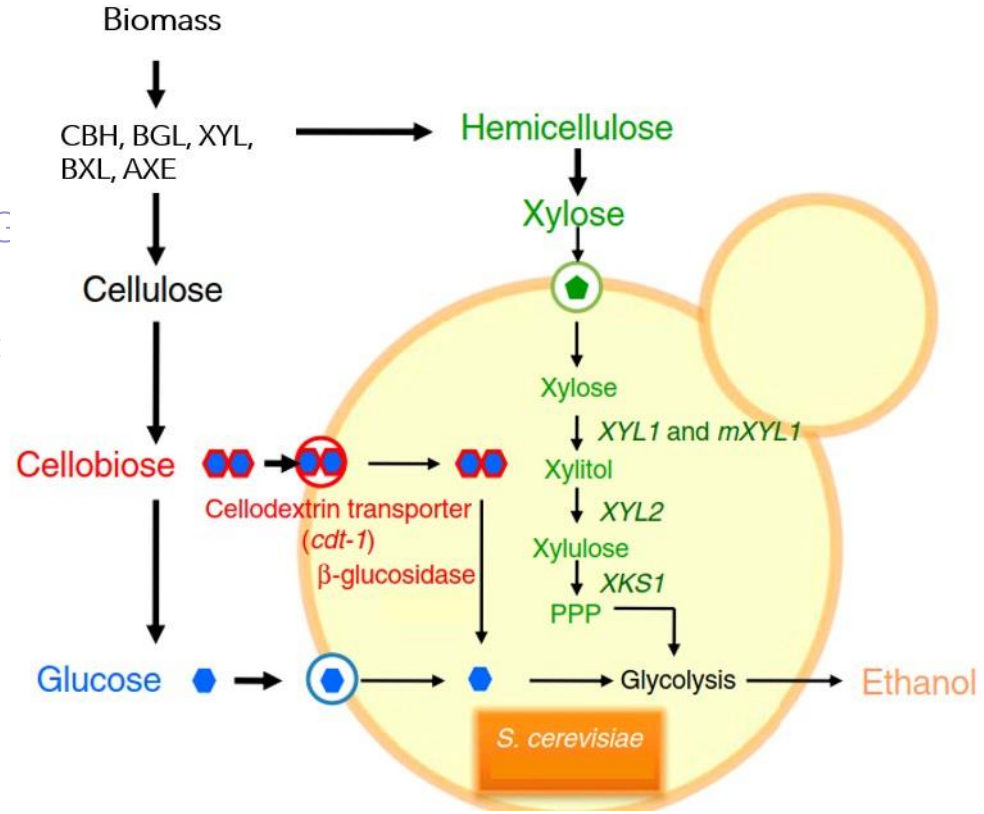
- Screening → Undesirable changes in gene expression?
  - Unlikely since non-essential genes not needed for basic function
- Gene deletions to optimize for an application

→Ethanol production from biomass

- Synthetic pathway needs to be introduced
- Enzymes for biomass breakdown
- The chunk could be made longer to accommodate the genes or genes could replace transposons

Ethanol production from biomass - What needs to be done?

- We want the yeast to break down biomass (cellulose, hemicellulose) to glucose and xylose by itself
  - Genes encoding following cellulolytic enzymes: Cellobiohydrolase 1 & 2 (CBH 1 & 2), endoglucanase (EG β-glucosidase (BGL)
  - Genes encoding the following hemicellulolytic enzymes: Xylanase (XYL), β-xylosidase (BXL), acetylxylan esterase (AXE)
- Glucose metabolized without problem
- Synthetic xylose metabolic pathway
  - Gene encoding xylose reductase (*XYL1*) introduced
  - *XYL2* (xylitol dehydrogenase) and *XKS1* (xylulokinase) already present in the yeast -> could be replaced with genes from another organism for efficiency
- Instead of importing glucose the yeast could be made to import cellobiose (glucose dimer) with *cdt1* transporter
  - To overcome glucose repression



Modified from Ha et al. (2011)

Encountered problems

- Determining if gene deletions are beneficial
  - Contradicting information regarding functioning e.g. chemical resistance

References

- https://www.yeastgenome.org/
- http://origin.tubic.org/deg/public/index.php/search/eukaryotes
- https://app.diagrams.net/
- Jessica Dymond & Jef Boeke (2012) The Saccharomyces cerevisiae SCRaMbLE system and genome minimization, Bioengineered, 3:3, 170-173, DOI: 10.4161/bbug.19543
- Building better yeast. *Nat Commun* 9, 1939 (2018). https://doi.org/10.1038/s41467-018-04159-y
- Synthetic biology (Course CHEM-E8125), spring 2023 Lecture slides Prof. Merja Penttilä
- Chu, B. C., & Lee, H. (2007). Genetic improvement of *Saccharomyces cerevisiae* for xylose fermentation. *Biotechnology advances*, *25*(5), (2007), 425-441. https://doi.org/10.1016/j.biotechadv.2007.04.001
- Wei, N., Oh, E. J., Million, G., Cate, J. H. D & Jin, Y.-S. (2015). Simultaneous utilization of cellobiose, xylose, and acetic acid from lignocellulosic biomass for biofuel production by an engineered yeast platform. *ACS synthetic biology*, Vol.4 (6), p.707-713. https://doi.org/10.1021/sb500364q
- Claes, A., Deparis, Q, Foulquié-Moreno, M. R. & Thevelein, J. M. (2020). Simultaneous secretion of seven lignocellulolytic enzymes by an industrial second-generation yeast strain enables efficient ethanol production from multiple polymeric substrates. *Metabolic engineering*, Vol.59, p.131-141. https://doi.org/10.1016/j.ymben.2020.02.004
- Ha, S.-J., Galazka, J. M., Kim, S. R., Choi, J.-H., Yang, X., Seo, J.-H., Glass, N. L., Cate, J. H. D., Jin, Y.-S. & Ingram, L. (2011). Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation. *Proceedings of the National Academy of Sciences*, Vol.108 (2), p.504-509. https://doi.org/10.1073/pnas.1010456108