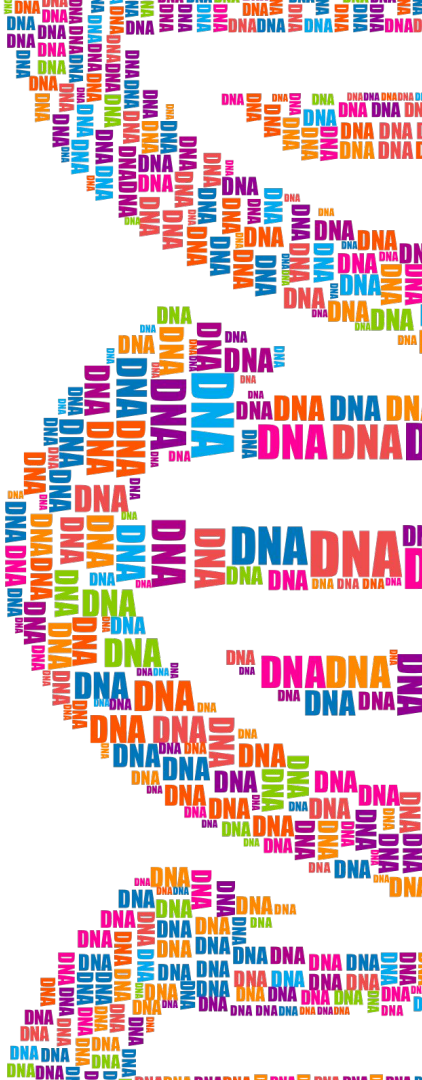
28.3.2022

Yeast 2.0

Hiltunen, K., Mattila, S.,
Oksa, K. & Raiskio, E.



Introduction

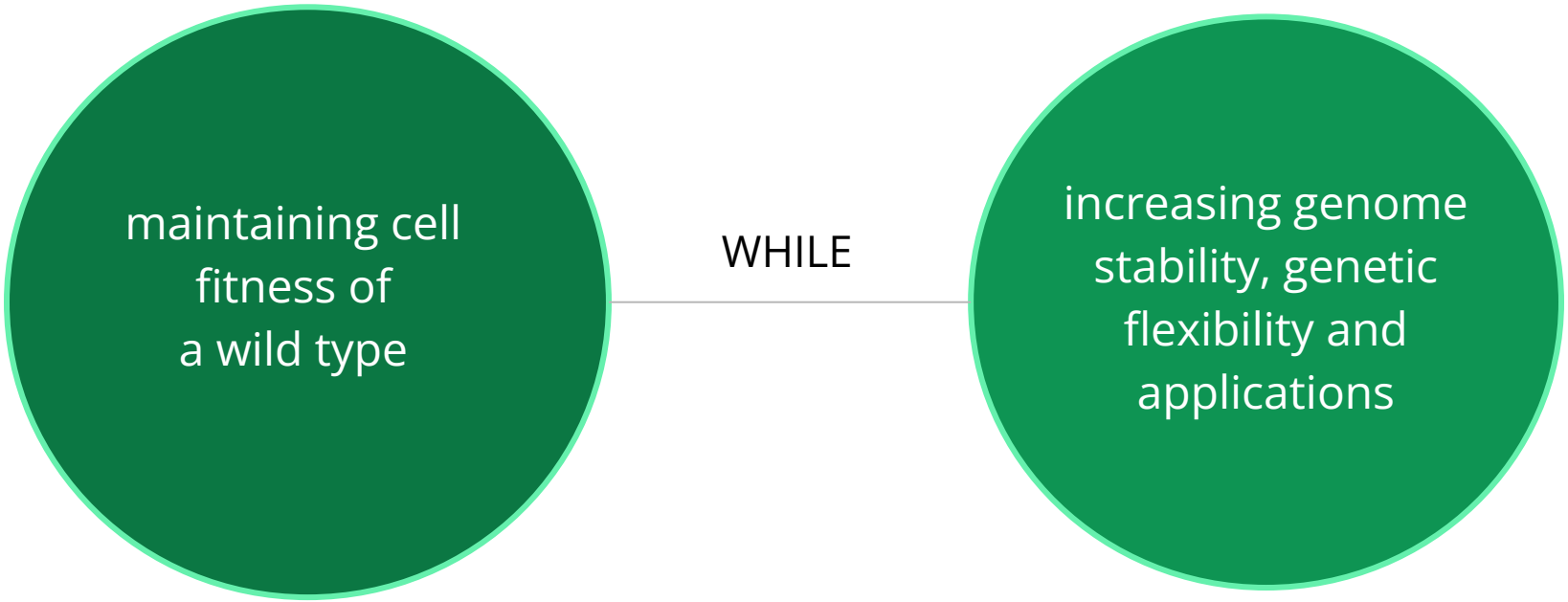
Yeast 2.0.

- can be used to answer questions about cell properties, such as
  - chromosome properties, genome organization, gene content, function of RNA splicing and many more

- allows direct testing of evolutionary questions

- could be used as a platform for new applications:
  - protein and biomolecule production in food, pharmaceuticals, materials and energy industry
  - study of foreign genetic elements
  - detoxification of waste compounds from chemical and agricultural sectors
  - model organism
  - etc.

GOAL


maintaining cell fitness of a wild type
WHILE
increasing genome stability, genetic flexibility and applications

Where to start?

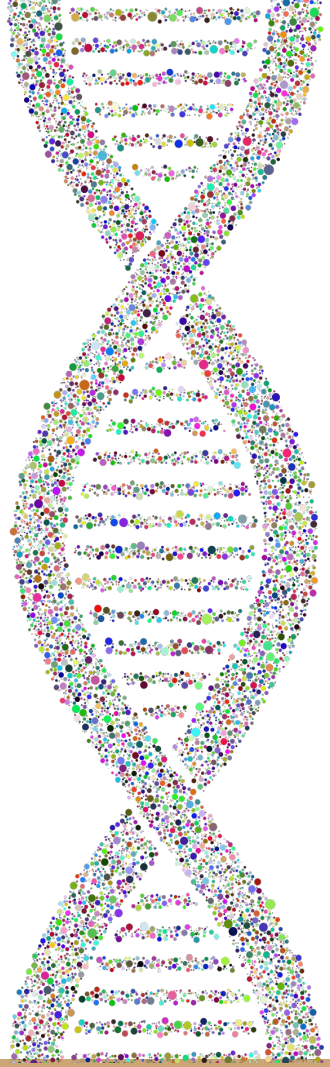
- Projects are most easier to start when you have a template
- For this, the S. cerevisiae (wt) chromosome I (160k...230k) was the simplest choice:

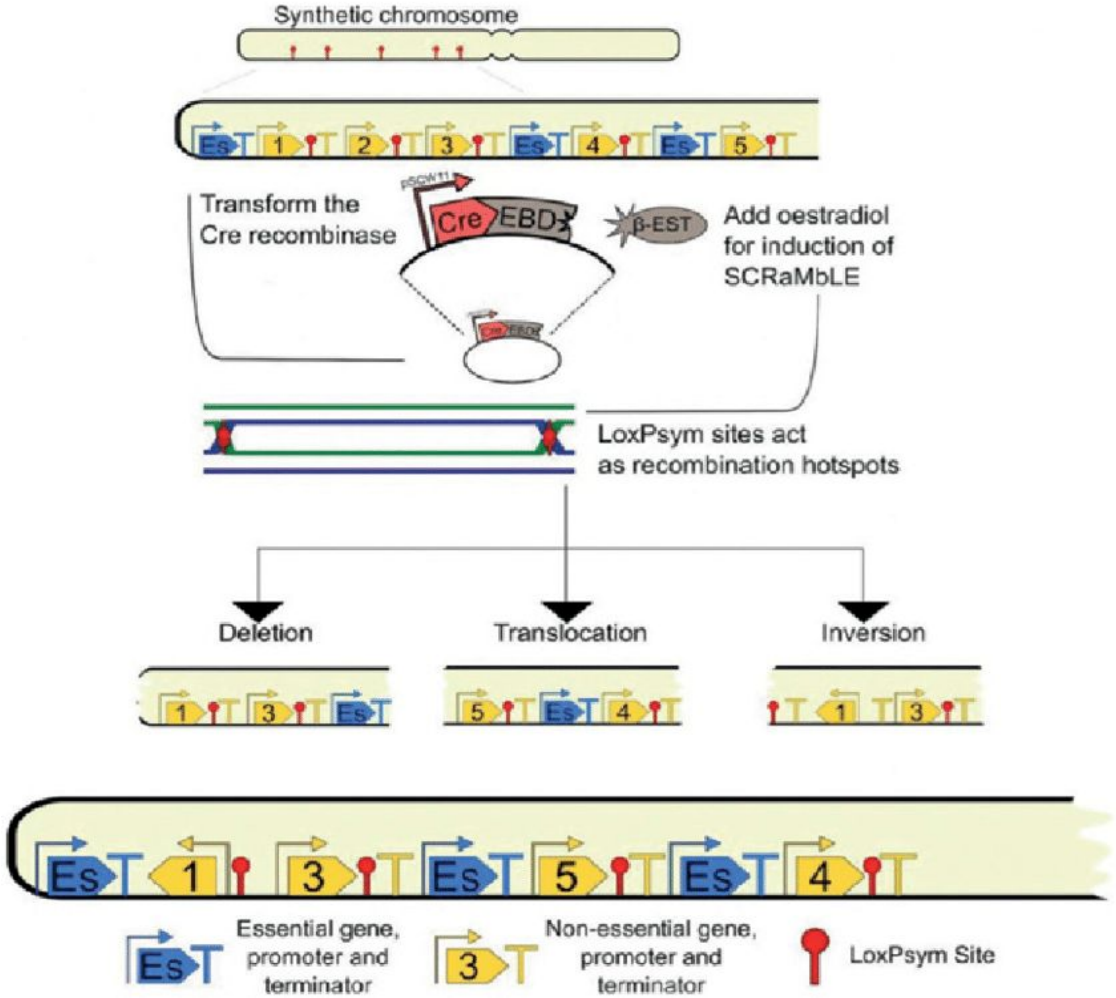
This region of chromosome I contains features that are great examples for edits

Including: Transposable elements, fitness-decreasing and -increasing genes, indicator elements, non-coding regions...

SCRaMbLE

- Used to rearrange synthetic yeast chromosomes
- Can also be used to delete unnecessary genes
  - Goal to reach minimal synthetic genome
- For mechanism to work, it requires
  - Adding bidirectional loxPsym sites
  - Cre recombinase (usually in plasmid)
- Can remove repetitive regions, destabilizing regions, and introns, which can be replaced with designed sequences
- Can be induced post-translationally or transcriptionally





Source: Ellis, T. (2019) DOI:10.1042/BIO04103006

Alterations

Synthetic telomere replaces the original longer telomere

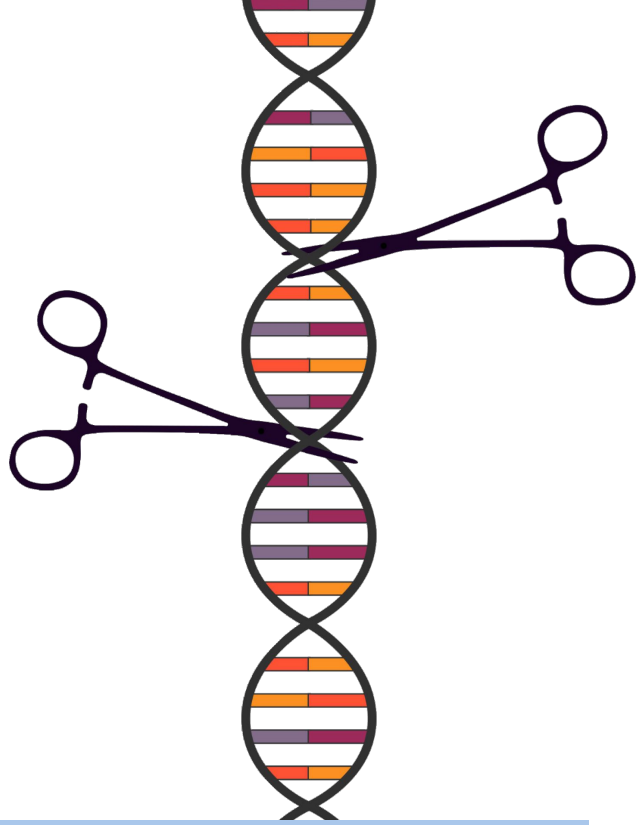
- synthetic repetitive sequence

Replacing all TAG stop codons with TAA

- subsequent introduction of new unnatural amino acids

Removal of non-essential genes and introns

- dubious ORFs
- non-essential ORFs



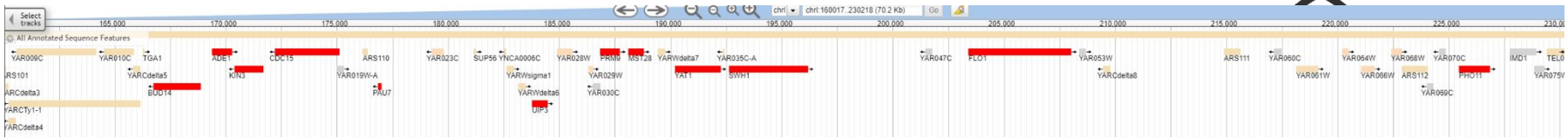
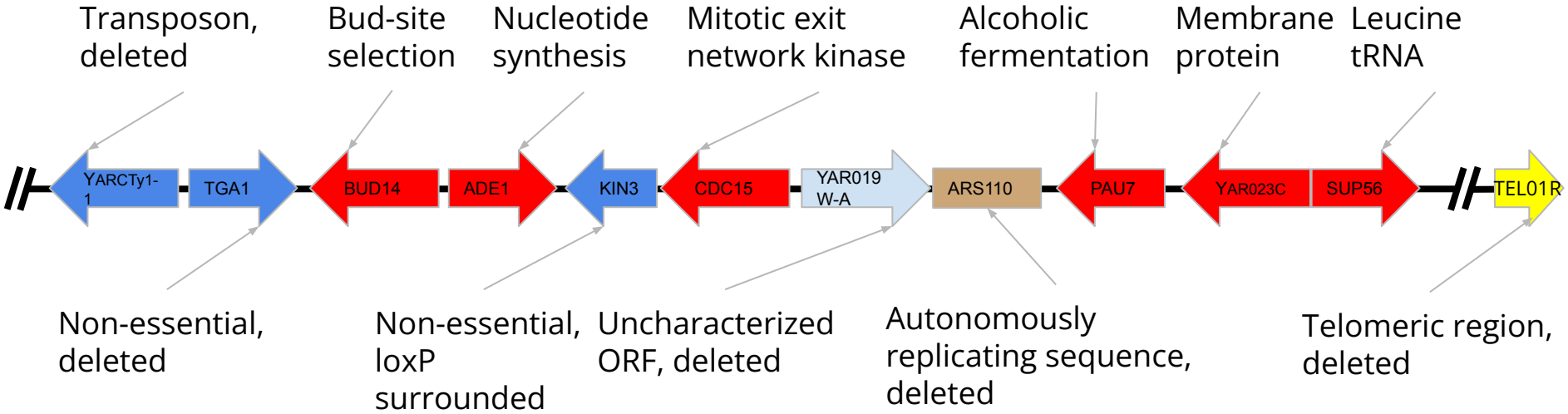


Illustration of the design - the yeast chr 1, 160 000 ->



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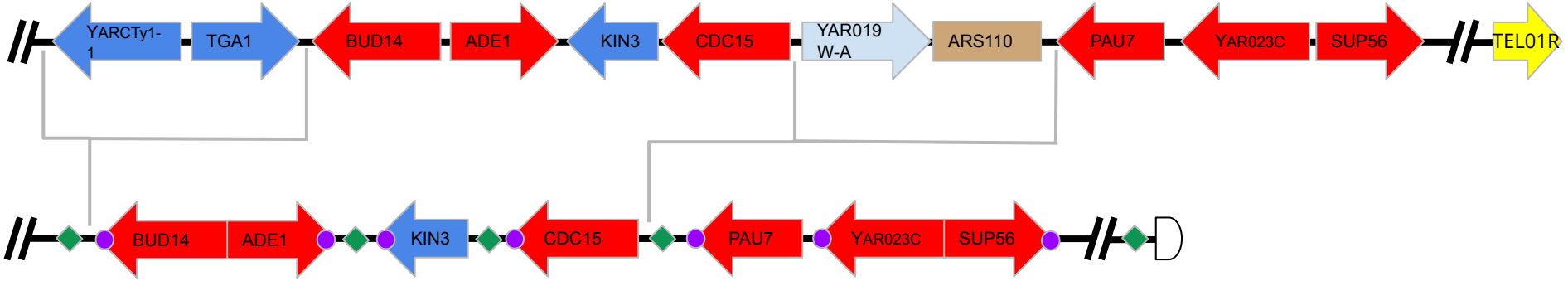


Universal telomere cap

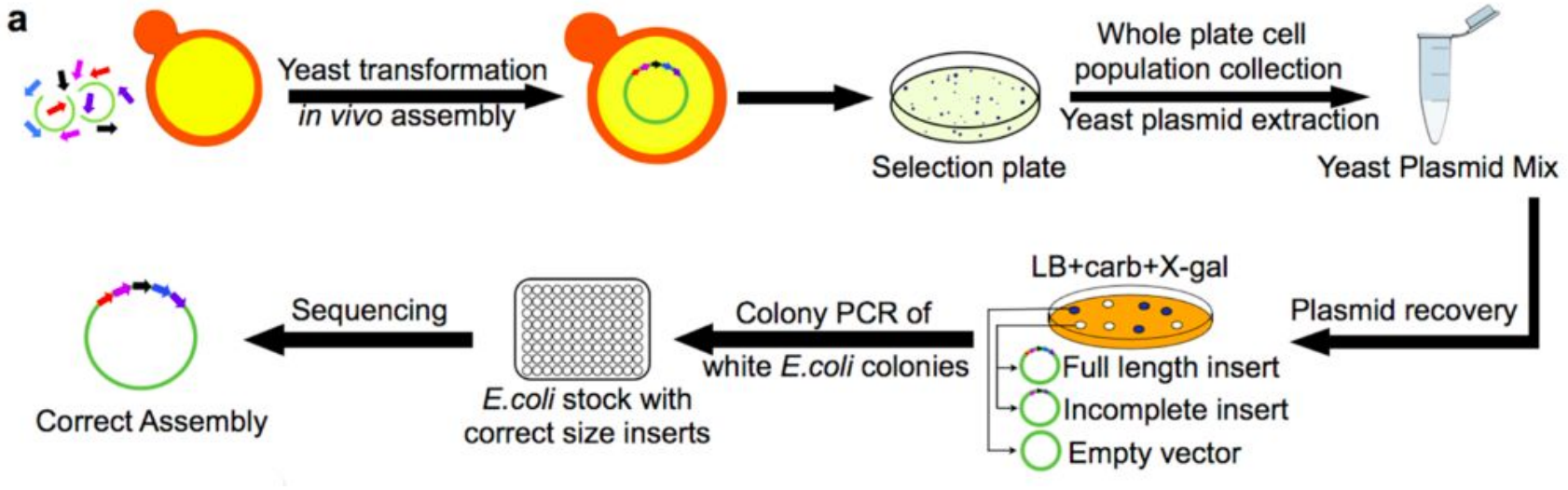
Telomere

TAG -> TAA codon replacement

loxPsym

Wet lab construction procedure

For example the rapid assembly of DNA overlapping multi-fragment (RADOM):



Lin, Q. et al. 2015, RADOM, an Efficient In Vivo Method for Assembling Designed DNA Fragments up to 10 kb Long in Saccharomyces cerevisiae, *ACS synthetic biology*, *4*:3, 213–220, doi:10.1021/sb500241e.

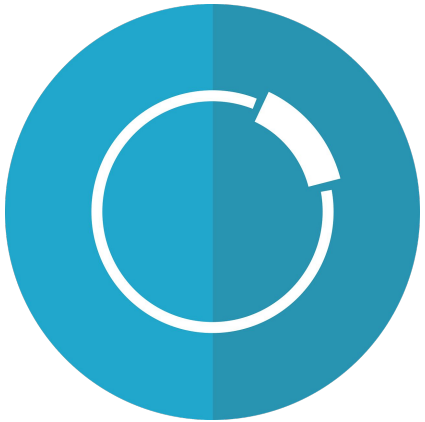
Computer programs

SGD - Saccharomyces genome database

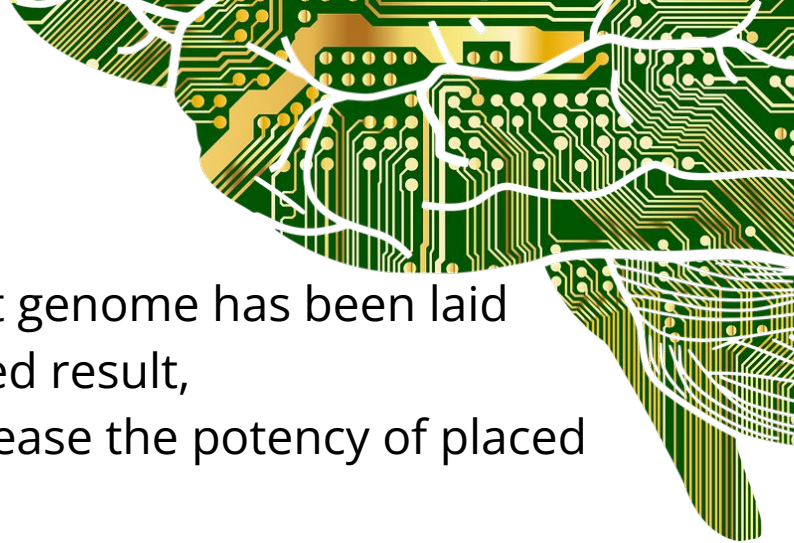
BioStudio - design of synthetic DNA on chromosomal scale

Sequence Polishing Library - optimisation of codon usage

SnapGene viewer - design and construction of plasmids



Future considerations



- Basis for further modifications of the yeast genome has been laid
- Provided that the alterations lead to desired result, further modification could be done to increase the potency of placed traits or to introduce new elements:
  - Duplication, multiplication, overexpression of certain genetic elements
  - Introduction of more specific promoter regions
  - Foreign genetic elements
  - Modify phenotypic characteristics (e.g., cell wall, proteome, metabolome...)

- Randomised mutagenesis combined with GWAS

Possible genes to overexpress

Nutrient uptake increasing genes

- Degradation of URea
  - DUR3
- S-AdenosylMethionine metabolism
  - SAM3
- Transporter of POlyamines
  - TPO1
  - TPO2
  - TPO3
  - TPO4
- Vitamin H Transporter
  - VHT1

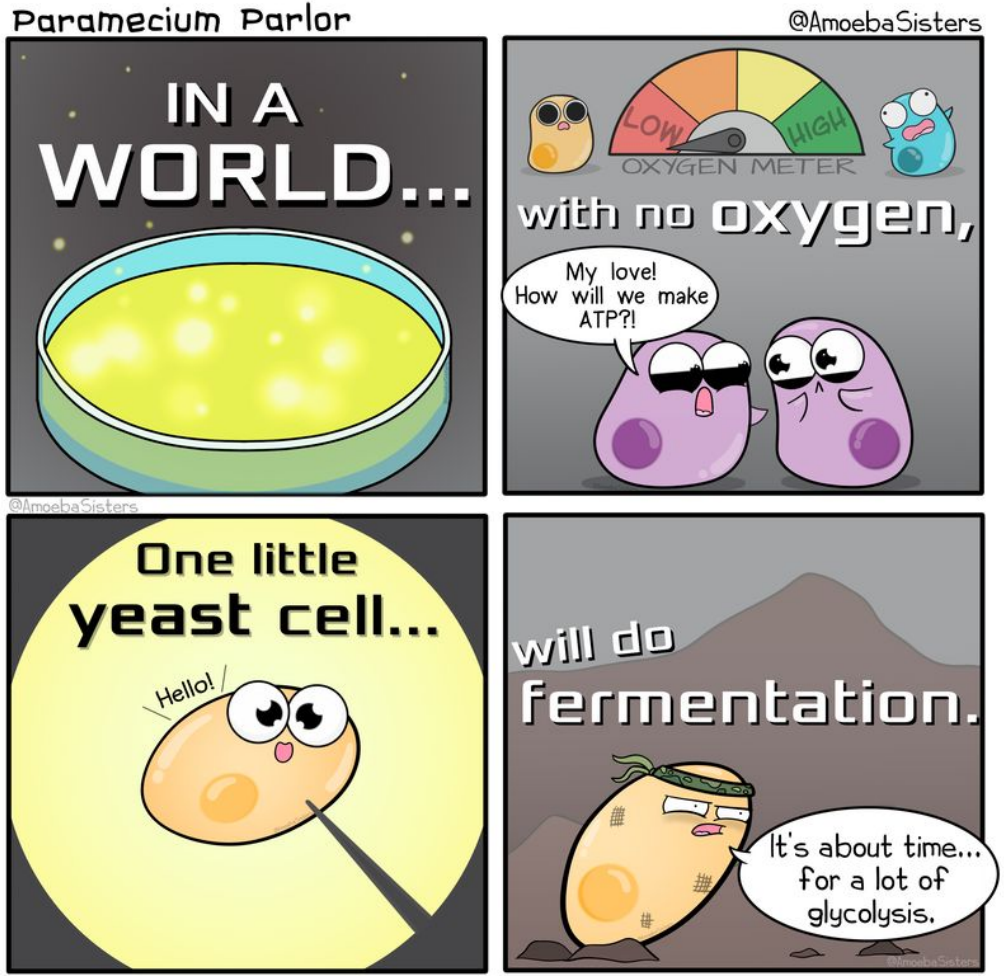
Annotations 157 entries for 61 genes

Filter table

Gene	Phenotype	Experiment Type	Mutant Information	Strain Background	Chemical	Details	Reference
DUR3	nutrient uptake: increased	classical genetics	overexpression	S288C	putrescine		Uemura T, et al. (2007) PMID:17218213
DUR3	nutrient uptake: increased	classical genetics	overexpression	S288C	spermidine		Uemura T, et al. (2007) PMID:17218213
SAM3	nutrient uptake: increased	classical genetics	overexpression	S288C	putrescine		Uemura T, et al. (2007) PMID:17218213
SAM3	nutrient uptake: increased	classical genetics	overexpression	S288C	spermidine		Uemura T, et al. (2007) PMID:17218213
TAT1	nutrient uptake: decreased	homozygous diploid	overexpression	W303	tryptophan	**Temperature:** reduced temperature, 10 °C	Vicent I, et al. (2015) PMID:25725023
TPO1	nutrient uptake: increased	classical genetics	overexpression	S288C	spermine		Tomitori H, et al. (1999) PMID:9920864
TPO1	nutrient uptake: increased	classical genetics	overexpression	X2180-1A	spermine		Tomitori H, et al. (2001) PMID:11171066
TPO2	nutrient uptake: increased	classical genetics	overexpression	X2180-1A	spermine		Tomitori H, et al. (2001) PMID:11171066
TPO3	nutrient uptake: increased	classical genetics	overexpression	X2180-1A	spermine		Tomitori H, et al. (2001) PMID:11171066
TPO4	nutrient uptake: increased	classical genetics	overexpression	X2180-1A	spermine		Tomitori H, et al. (2001) PMID:11171066
VHT1	nutrient uptake: increased rate	classical genetics	overexpression	Other	biotin	**Details:** biotin transport rates are increased and independent of extracellular biotin concentration	Stolz J, et al. (1999) PMID:10373489

Thank you!

Any questions?


Paramecium Parlor
@AmoebaSisters
IN A WORLD...
LOW
HIGH
OXYGEN METER
with no oxygen,
My love! How will we make ATP?!
@AmoebaSisters
One little yeast cell...
Hello!
will do fermentation.
It's about time... for a lot of glycolysis.
@AmoebaSisters