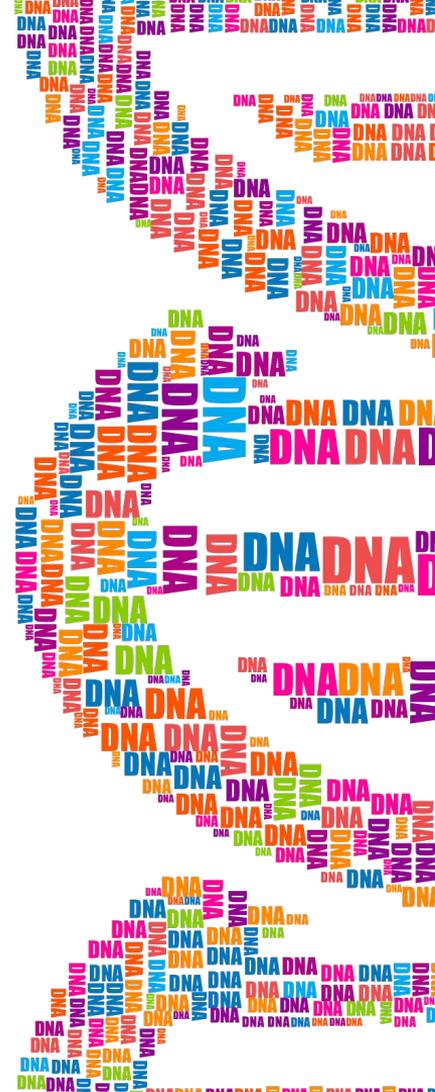


28.3.2022

Yeast 2.0

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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...



Name	YARCTy1-1
Type	LTR_retrotransposon
Description	Ty1 element, LTR retrotransposon of the Copia (Pseudoviridae) group; contains genes TYA Gag and TYB Pol, encoding proteins involved in structure and function of virus-like particles, flanked by two direct repeats; mutated in S288C
Position	chr1:160238..166162 (- strand)
Length	5,925 bp

Name	FLO1
Type	gene
Description	Lectin-like protein involved in flocculation; cell wall protein that binds mannose chains on the surface of other cells, confers flocc-forming ability that is chymotrypsin sensitive and heat resistant; important for co-flocculation with other yeasts, mediating interaction with specific species; FLO1 has a paralog, FLOS, that arose from a segmental duplication
Position	chr1:203403..208016 (+ strand)
Length	4,614 bp

Name	YAR009C
Type	transposable_element_gene
Description	Retrotransposon TYA Gag and TYB Pol genes; Gag processing produces capsid proteins, Pol is cleaved to produce protease, reverse transcriptase and integrase activities; in YARCTy1-1 TYB is mutant and probably non-functional; protein product forms cytoplasmic foci upon DNA replication stress
Position	chr1:160597..164187 (- strand)
Length	3,591 bp

Name	PHO11
Type	gene
Description	One of three repressible acid phosphatases; glycoprotein that is transported to the cell surface by the secretory pathway; induced by phosphate starvation and coordinately regulated by PHO4 and PHO2; PHO11 has a paralog, PHO12, that arose from a segmental duplication
Position	chr1:225460..226863 (+ strand)
Length	1,404 bp

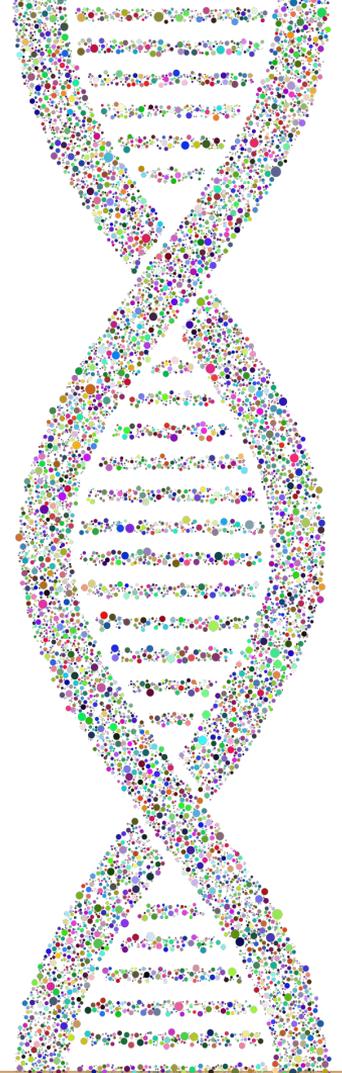
Name	CBC15
Type	gene
Description	Hippo-like kinase of the Mitotic Exit Network; promotes exit by activating the Dbp2 kinases; component of a non-canonical Hippo pathway with Sps1p required for prospore membrane closure, spindle disassembly and sustained release of Cdc14p during meiotic anaphase II; complexes with Sps1p and contributes to its phosphorylation; phosphorylates the RNP411 CTD during mitosis; localizes to the bud neck and SPB during anaphase and telophase; relocalizes to the cytoplasm upon DNA replication stress
Position	chr1:172211..175135 (- strand)
Length	2,925 bp

Name	YAT1
Type	gene
Description	Outer mitochondrial carnitine acetyltransferase; minor ethanol-inducible enzyme involved in transport of activated acyl groups from the cytoplasm into the mitochondrial matrix; phosphorylated
Position	chr1:190193..192256 (+ strand)
Length	2,064 bp

Name	YAR010C
Type	transposable_element_gene
Description	Retrotransposon TYA Gag gene co-transcribed with TYB Pol; Gag processing produces capsid proteins; in YARCTy1-1 TYB is mutant and probably non-functional
Position	chr1:164544..165866 (- strand)
Length	1,323 bp

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



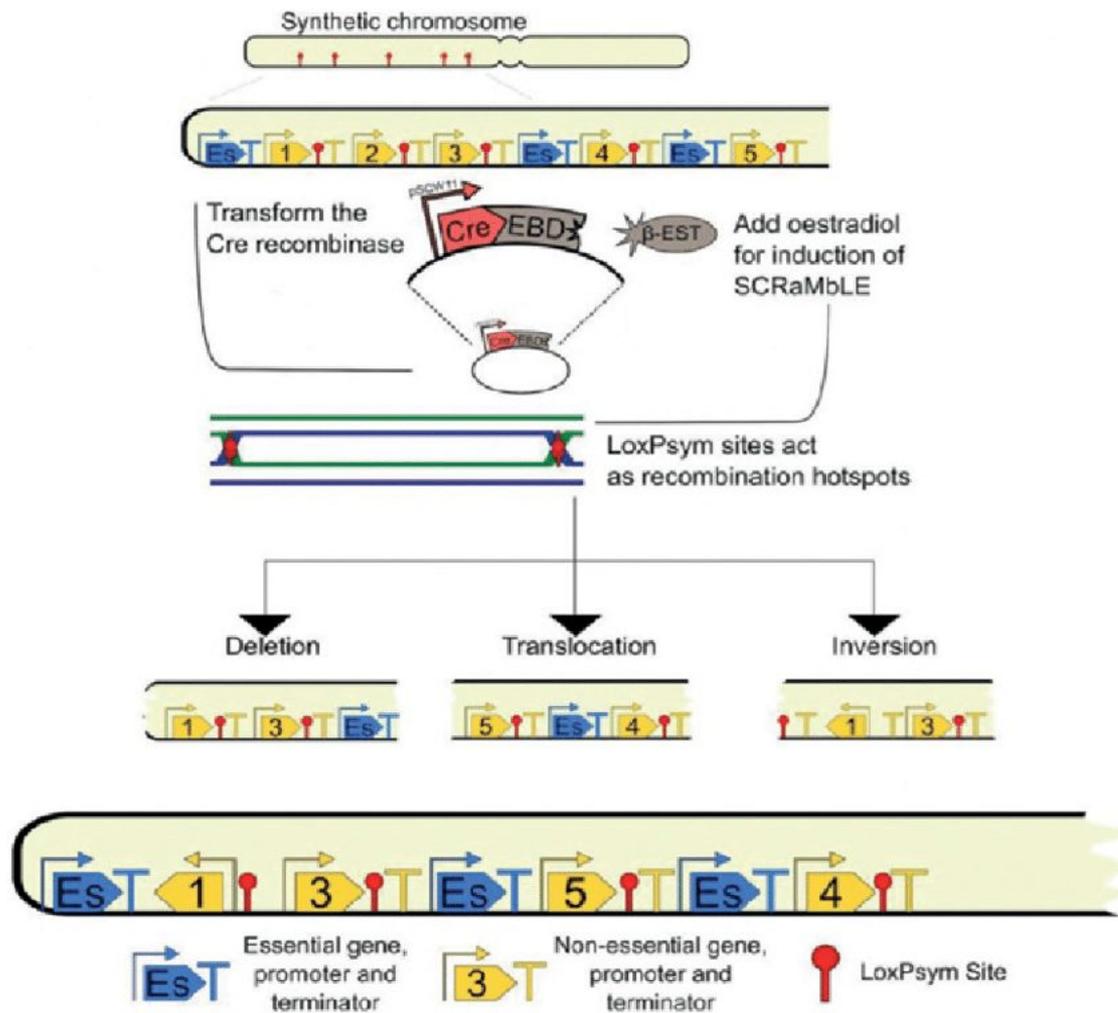
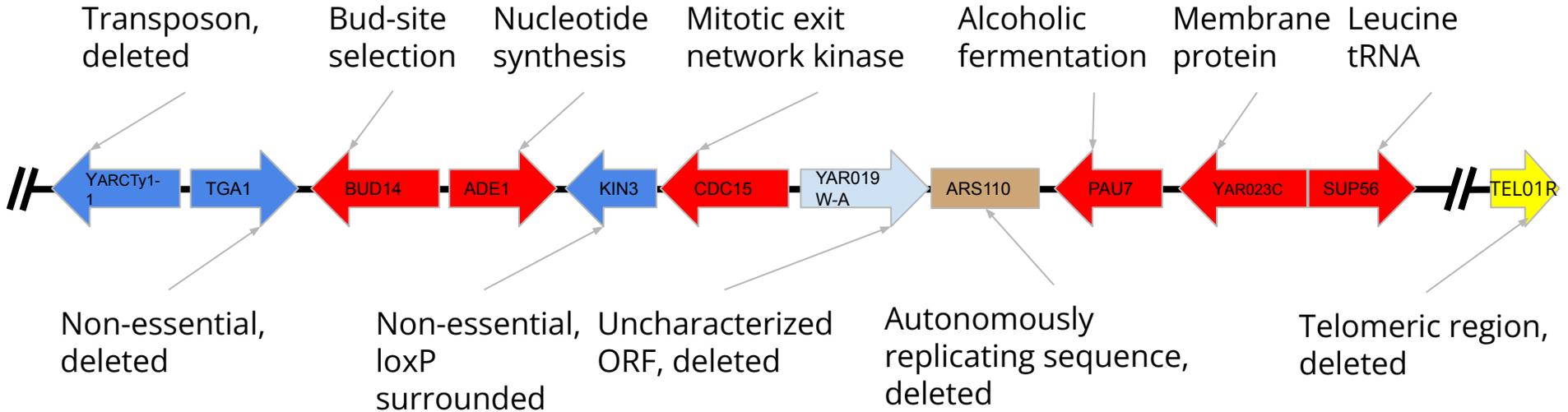


Illustration of the design - the yeast chr 1, 160 000 ->



Essential gene

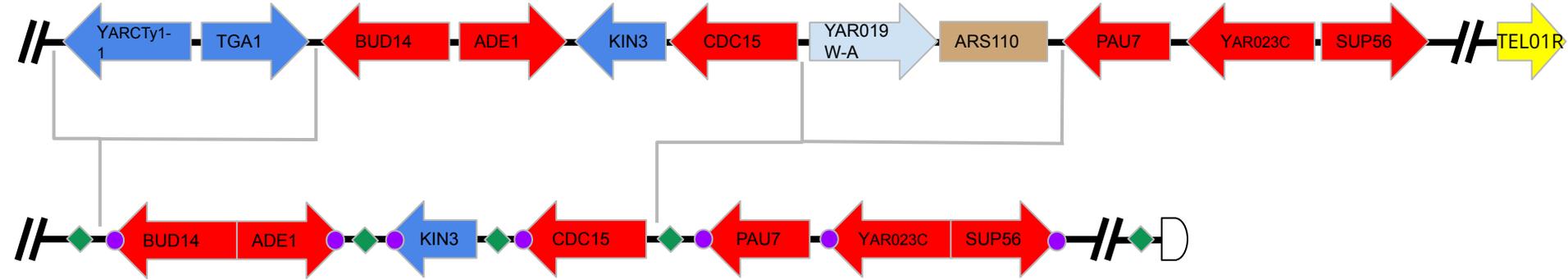
Non-essential gene

Uncharacterized ORF

Telomere



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



Universal
telomere cap

D

Telomere



TAG -> TAA codon
replacement

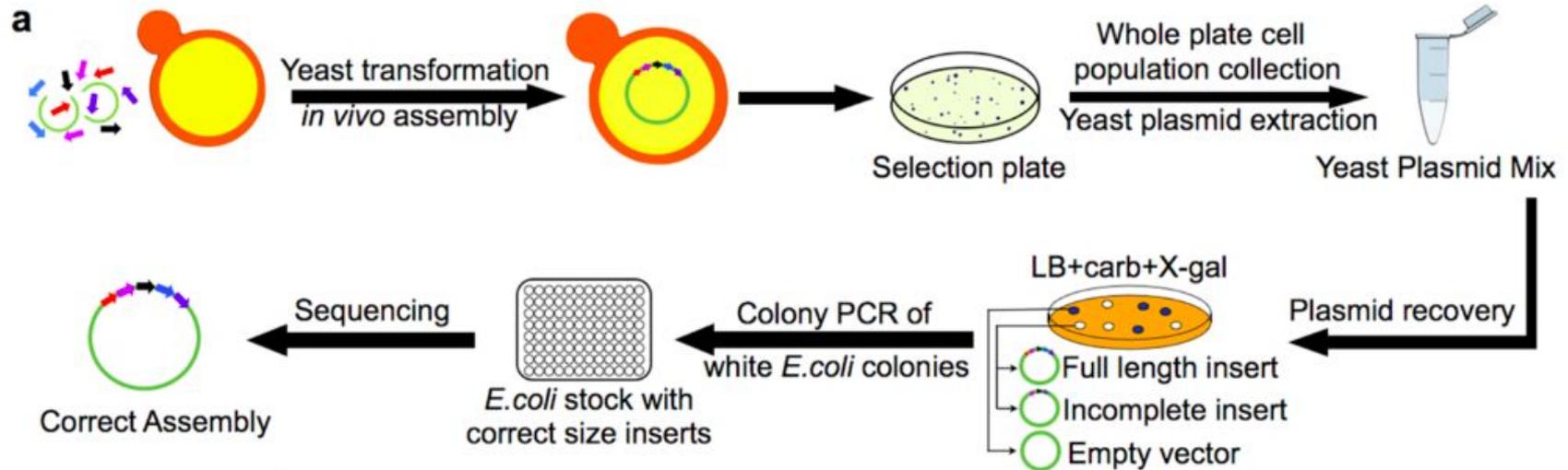


loxPsym



Wet lab construction procedure

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



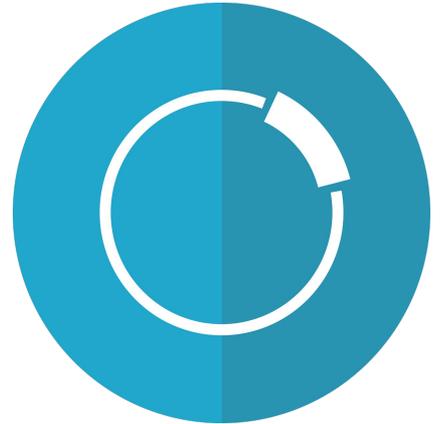
Computer programs

SGD - Saccharomyces genome database

BioStudio - design of synthetic DNA on chromosomal scale

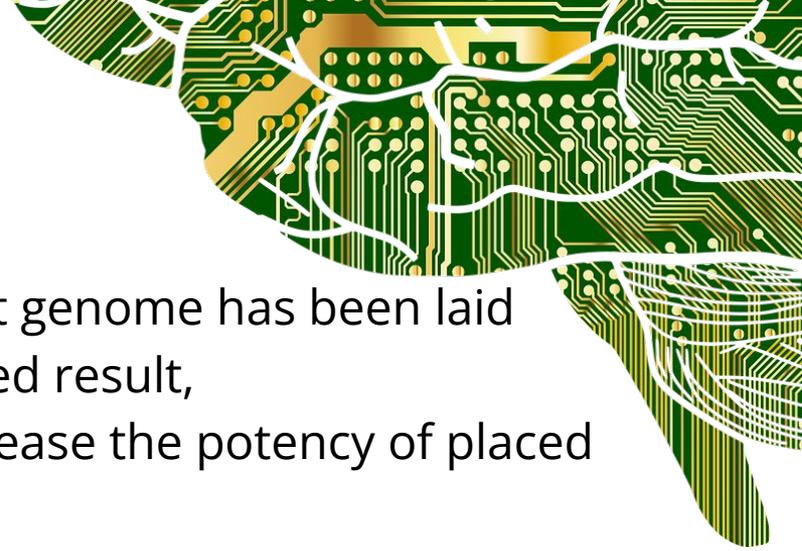
Sequence Polishing Library - optimisation of codon usage

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](#)

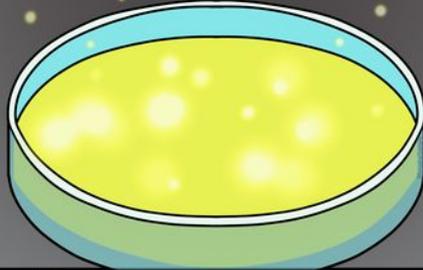
Annotations 157 entries for 61 genes

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:17218312
DUR3	nutrient uptake: increased	classical genetics	overexpression	S288C	spermidine		Uemura T, et al. (2007) PMID:17218312
SAM3	nutrient uptake: increased	classical genetics	overexpression	S288C	putrescine		Uemura T, et al. (2007) PMID:17218312
SAM3	nutrient uptake: increased	classical genetics	overexpression	S288C	spermidine		Uemura T, et al. (2007) PMID:17218312
TAT1	nutrient uptake: decreased	homozygous diploid	overexpression	W303	tryptophan	Temperature: reduced temperature, 10 °C	Vicent I, et al. (2015) PMID:25728022
TPO1	nutrient uptake: increased	classical genetics	overexpression	S288C	spermine		Tomitori H, et al. (1999) PMID:9928844
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:10272489

Thank you!

Any questions?

IN A
WORLD...



@AmoebaSisters



with no oxygen,

My love!
How will we make
ATP?!



One little
yeast cell...



will do
fermentation.



It's about time...
for a lot of
glycolysis.

@AmoebaSisters