Synthetic yeast 2.0

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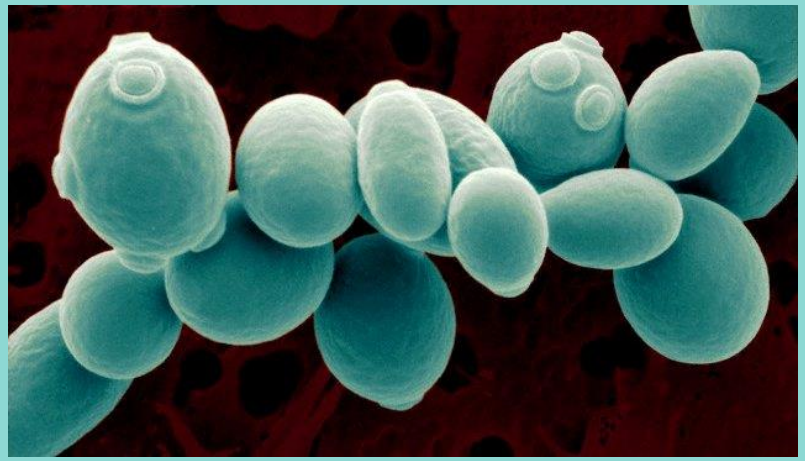
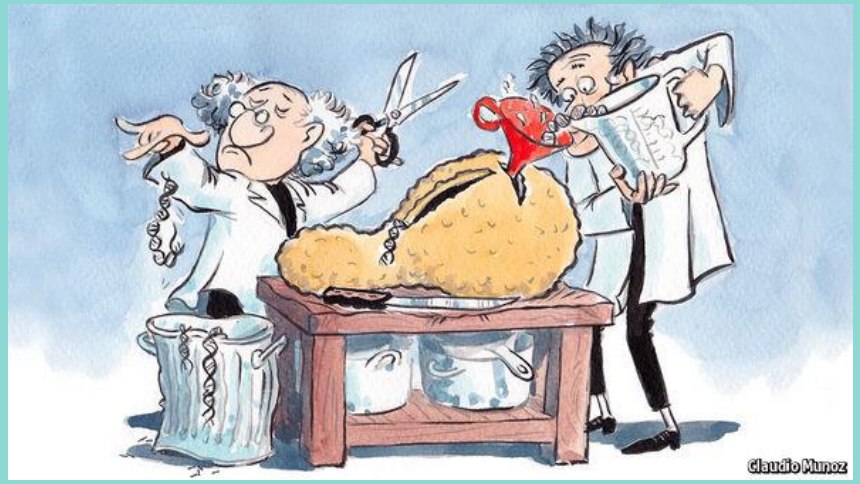
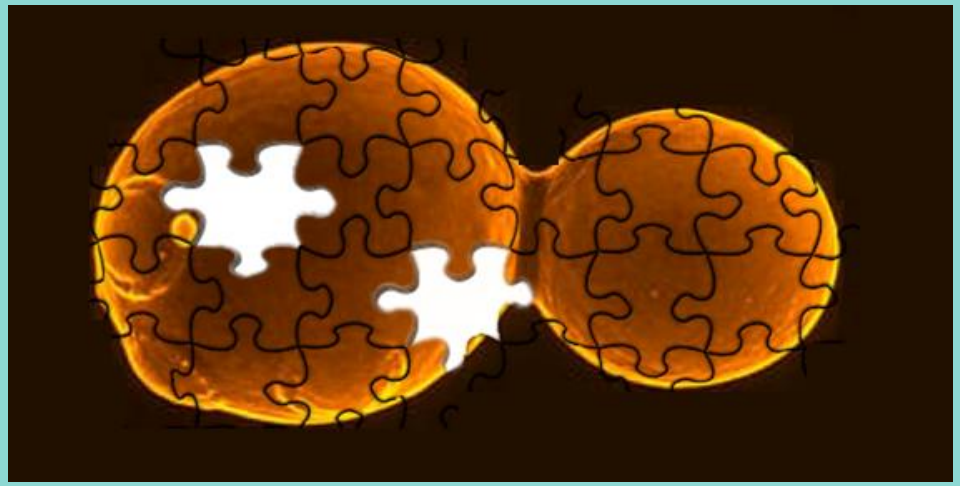


Introduction

- Yeast 2.0 is completely synthetic, carrying 16 man-made chromosomes
- Yeast 2.0 can be optimized for intended purpose
- Designing a synthetic chromosome region
- Deleting genes
- The yeast 2.0 can be harnessed to produce drugs, fuels, biomolecules etc.



Why yeast?



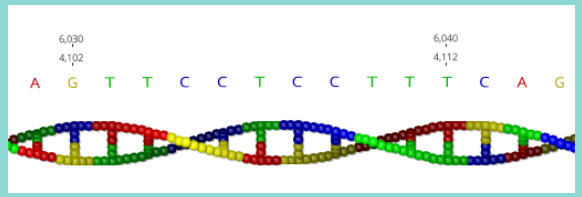
- Eukaryotic glycosylation
- Faster and cheaper and simpler than mammalian cell factories
- Robust
- Genome well known
- Non-toxic
- Ecologically friendly: Target molecule can be produced without toxic solvents, harsh conditions and the generation of by-product wastes.

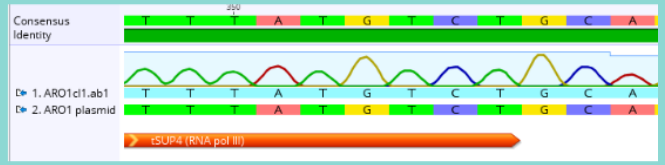
BioStudio Browser

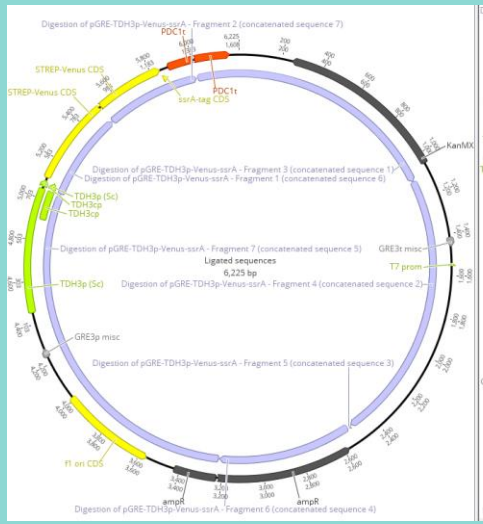
Which computer programs?

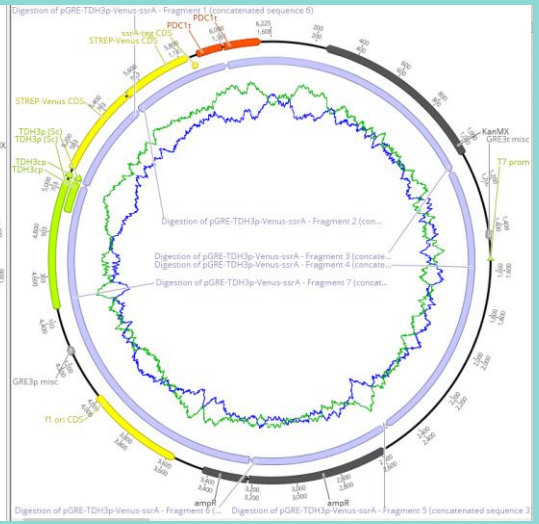
Snap gene

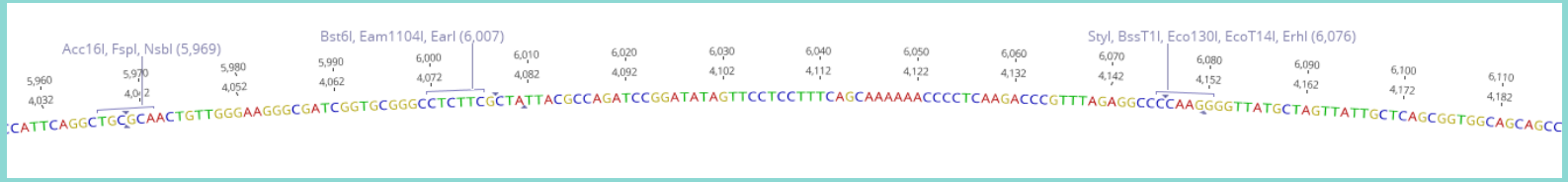
yeastgenome.org





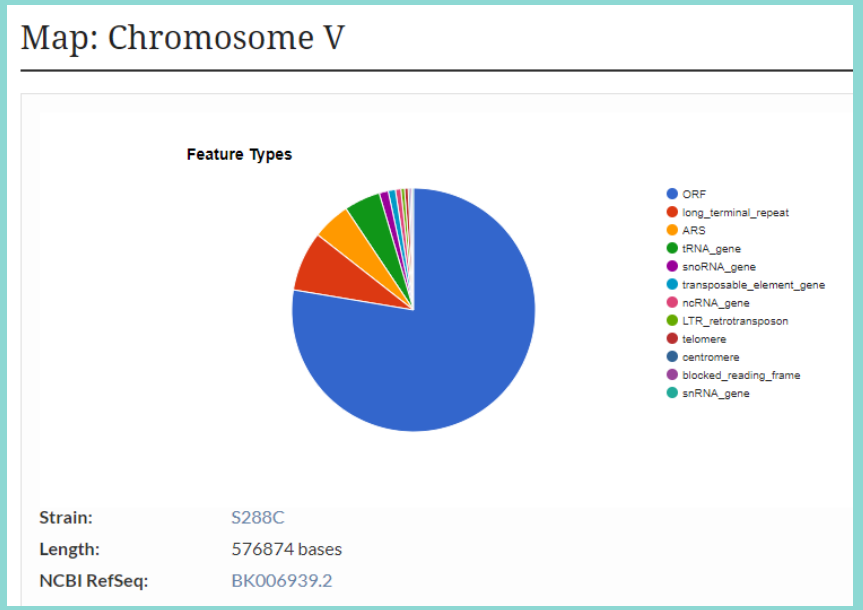


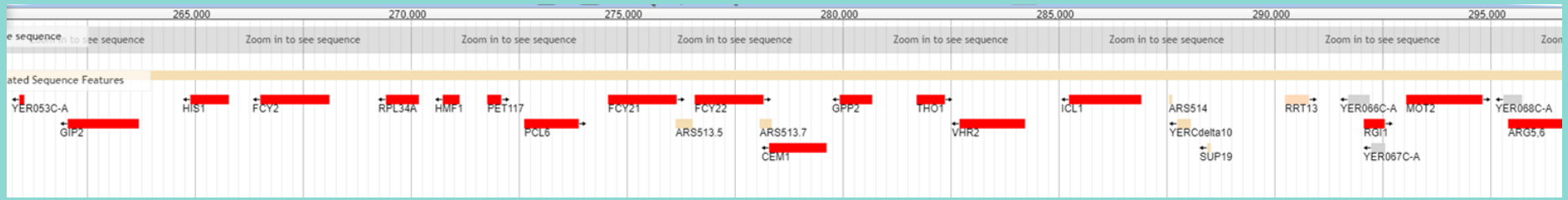




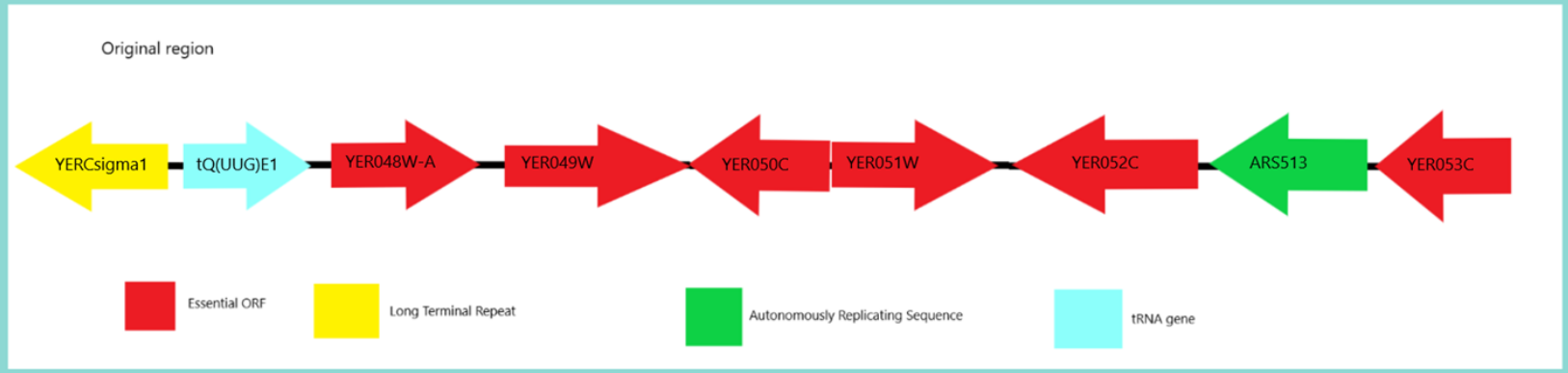
Chromosome V

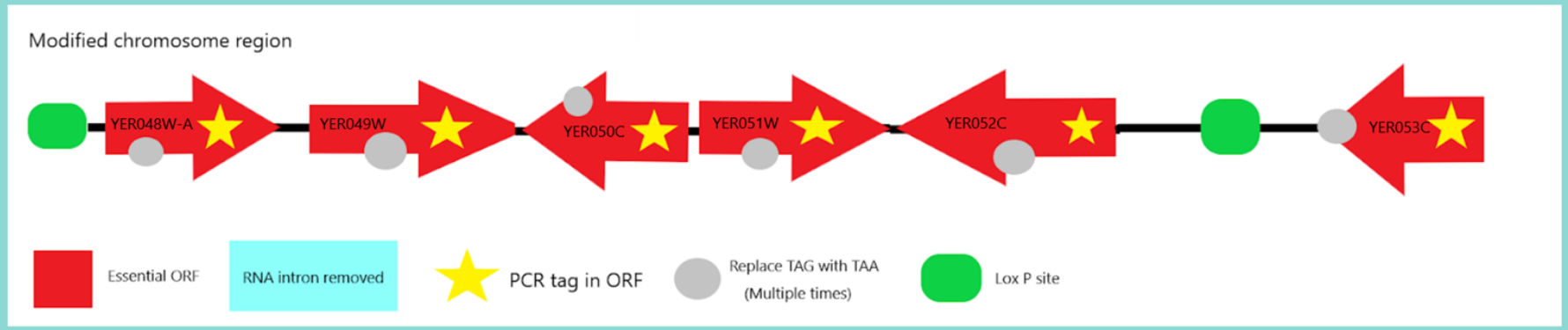
- Region: 250000-300000 of Chromosome V
- Region was chosen for interesting HOM3 gene
- HMF, GIP2 have a paralogs
- Non-essential protein: VHR2
- Dubious ORF: YER066C-A, YER067C-A, YER068C-A
- Protein of unknown function: YER053C-A & RGI1
- Molecular function unknown: PET117 & HMF1 & RRT13
- Autonomously Replicating Sequence: ARS513, ARS513.5, ARS513.7, ARS514
- tRNA gene: tQ(UUG)E1, tS(UGA)E
- Long terminal repeat: YERCsigma1, YERCdelta10



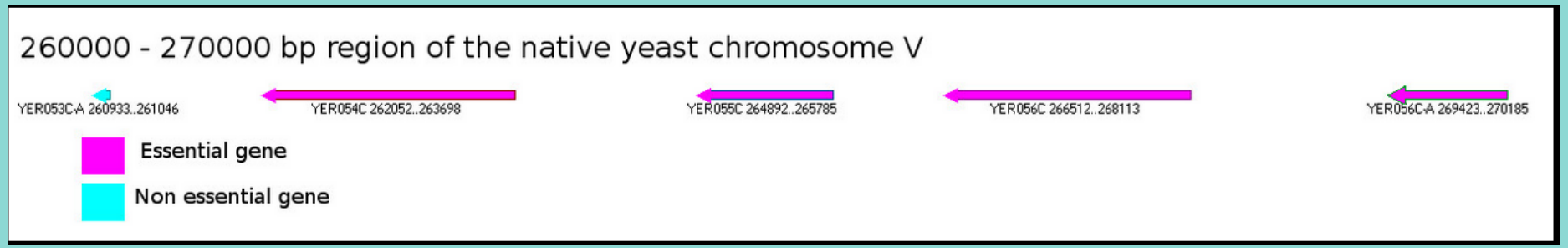


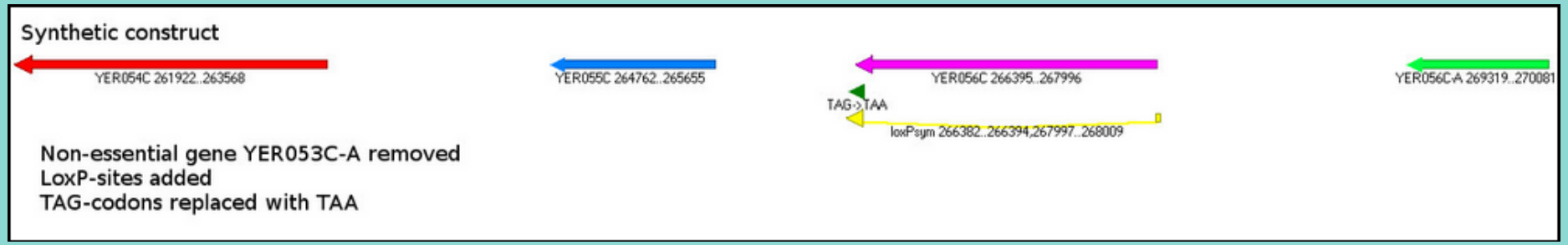
Region: 250000-260000



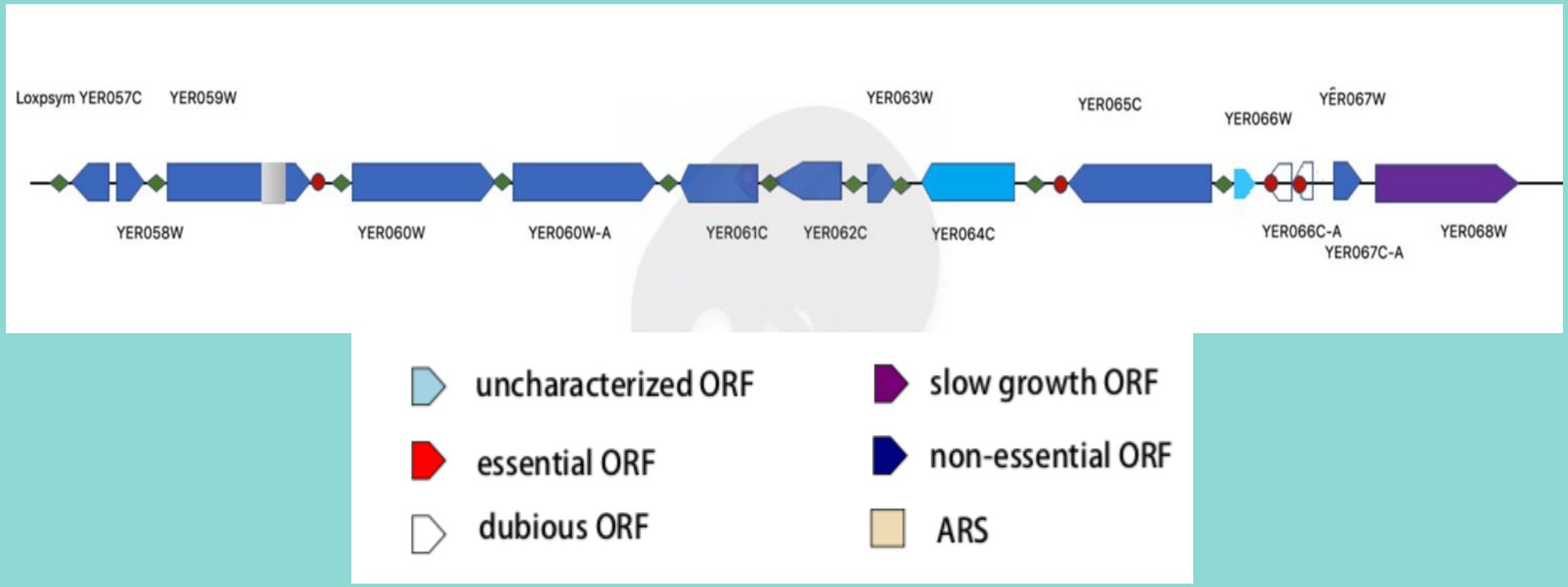


Region: 260000-270000





Region: 270000-30000



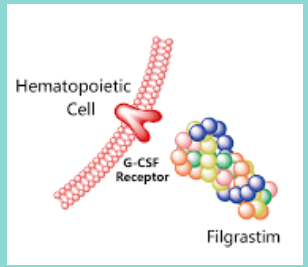
What would you use the yeast for?

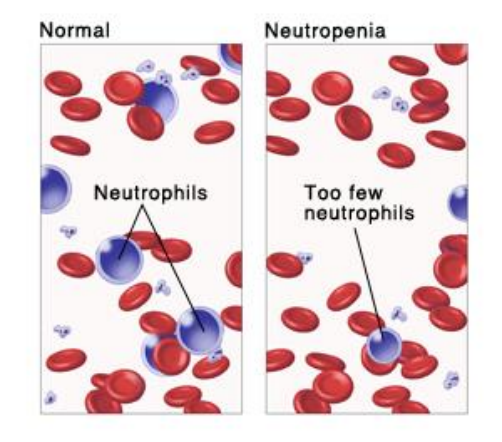
- Use: produce G-CSF biosimilar
- Deleting MNN1 improves glycan homogeneity by eliminating interfering mannosyltransferase activities
- GlcNAc2Man3GlcNAc2 glycan abundance increased by enhancing UDP-GlcNAc transport into Golgi apparatus via expression of transporter

Granulocyte colony-stimulating factor

- Human G-CSF is a single polypeptide chain protein of 174 amino acids with O-glycosylation at one threonine residue
- Filgrastim is a G-CSF analogue used for treatment of neutropenia
- Bone marrow donation was the earlier option
- Lenograstim is the glycosylated form (CHO cells)
- Other filgrastim products non-glycosylated (*E. coli*)
- 3% helical (3 helices; 10 residues)

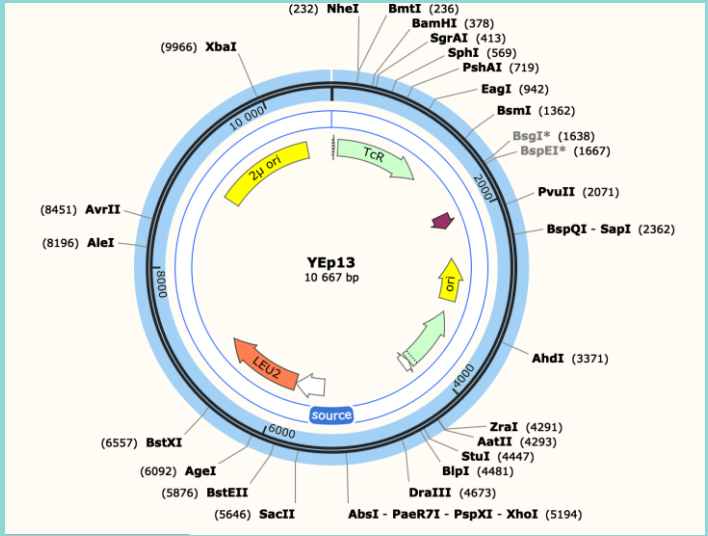
- 42% beta sheet (25 strands; 134 residues)





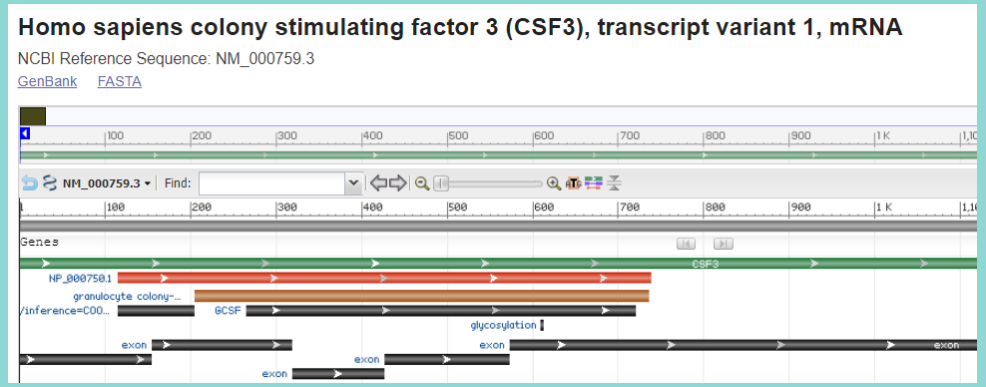
Yep 13

- Cloning can be done in 1 step instead of 2
- One-step gene replacement in yeast by cotransformation (Hans Rudolph et al.)

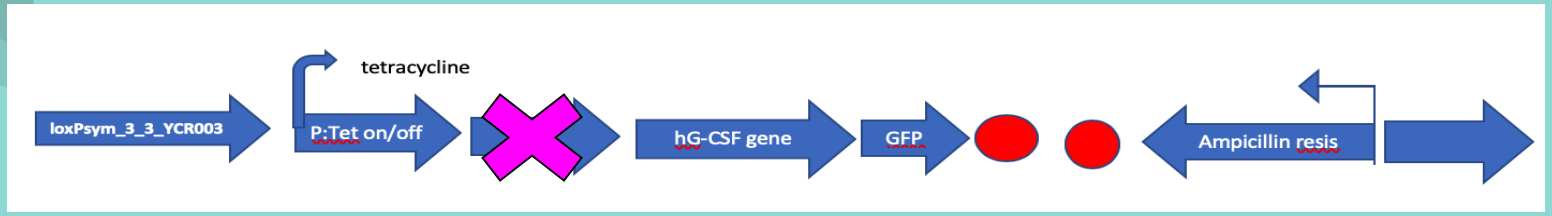


G-CSF gene

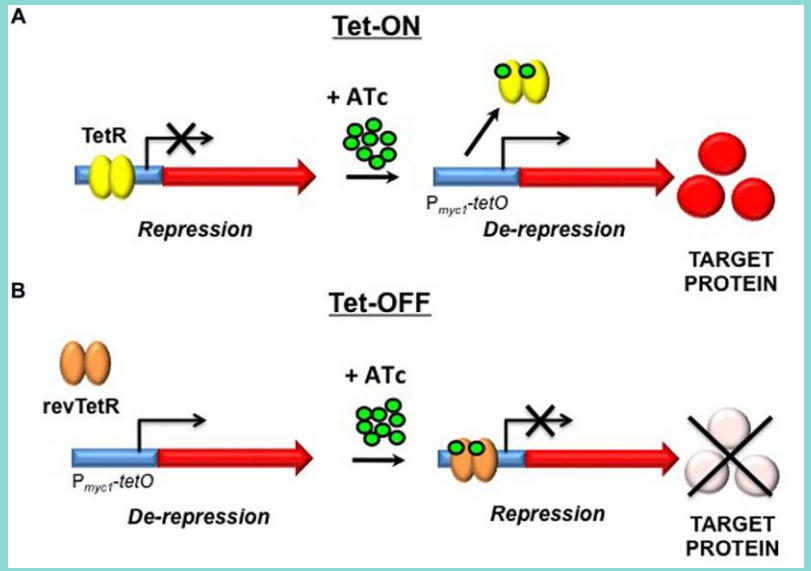
- The G-CSF gene is found in human chromosome="17



Designed DNA insert



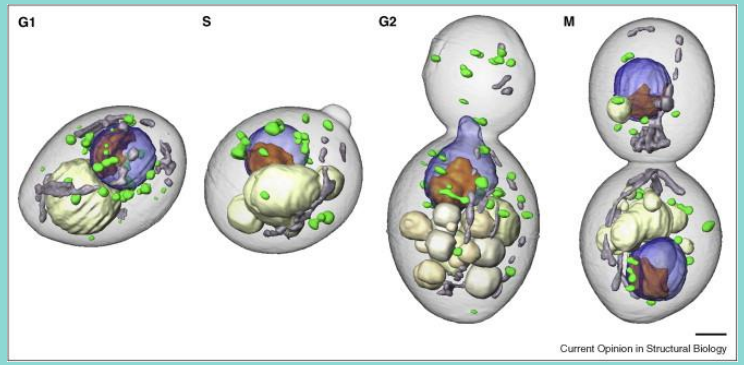
Tet operator regulation



Include and omit

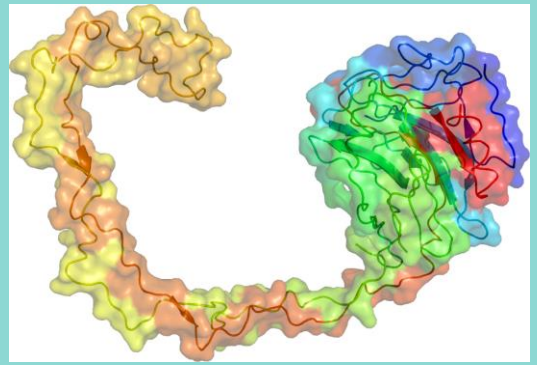
Omit

- Yblo16W = cell fusion
- YLC067C = silenced gene
- Proteasomes
- Introns
- YGL038C = characteristic of yeast glycosylation



Include

- Yer008C = post translational modification
- Ybl040C = vesicle mediated protein transport
- ER chaperone calnexin
- Selection marker (NAT & G418 or URA3 & LEU2)
- Tet-ON (DOX inducible)



Why did you choose this place to insert designed DNA?

chrIII	S000000581	YCL076W	ORF	NO VALUE	NO VALUE	NO VALUE	S. cerevisiae	Dubious	1392	2135	1
chrIII	S000000580	YCL075W	pseudogene	NO VALUE	NO VALUE	NO VALUE	S. cerevisiae	NO VALUE	2126	2566	1
chrIII	S000000579	YCL074W	pseudogene	NO VALUE	NO VALUE	NO VALUE	S. cerevisiae	NO VALUE	2824	3750	1
chrIII	S000000575	YCL073C	ORF	GEX1	Glutathione EXchanger	glutathione exchanger	S. cerevisiae	Verified	6479	8326	-1
chrIII	S000000574	YCL069W	ORF	VBA3	Vacuolar Basic Amino acid transporter	basic amino acid transporter	S. cerevisiae	Verified	9706	11082	1
chrIII	S000000573	YCL068C	ORF	NO VALUE	NO VALUE	NO VALUE	S. cerevisiae	Uncharacterized	11503	12285	-1
chrIII	S000000572	YCL067C	ORF	HMLALPHA2	Hidden Mat Left ALPHA	ALPHA2 homeodomain mating type protein alpha2	S. cerevisiae	Verified	12386	13018	-1
chrIII	S000000571	YCL066W	ORF	HMLALPHA1	Hidden Mat Left ALPHA	ALPHA1 transcriptional co-activator mating type protein alpha	S. cerevisiae	Verified	13262	13809	1
chrIII	S000000570	YCL065W	ORF	NO VALUE	NO VALUE	NO VALUE	S. cerevisiae	Dubious	13751	14119	1
chrIII	S000000569	YCL064C	ORF	CHA1	Catabolism of Hydroxy Amino acids	L-serine/L-threonine ammonia-lyase CHA1	S. cerevisiae	Verified	15798	16880	-1
chrIII	S000000568	YCL063W	ORF	VAC17	VACuole related	YCL062W	S. cerevisiae	Verified	17290	18561	1
chrIII	S000000566	YCL061C	ORF	MRC1	Mediator of the Replication Checkpoint	chromatin-modulating protein MRC1 YCL060C	S. cerevisiae	Verified	18816	22106	-1
chrIII	S000000564	YCL059C	ORF	KRR1	contains KRR-R motif	ribosome biosynthesis protein KRR1	S. cerevisiae	Verified	22429	23379	-1
chrIII	S000000563	YCL058C	ORF	FYV5	Function required for Yeast Viability	MDF1	S. cerevisiae	Verified	23523	23981	-1
chrIII	S000028518	YCL058W-A	ORF	ADF1	Antisense of Depressing Factor	NO VALUE	S. cerevisiae	Verified	23584	23925	1
chrIII	S000007547	YCL057C-A	ORF	MIC10	Mitochondrial contact site and Cristae organizing system	MCS10 MIO10 MOS1	S. cerevisiae	Verified	24032	24325	-1
chrIII	S000000562	YCL057W	ORF	PRD1	PRoteinase yscD	metalloendopeptidase	S. cerevisiae	Verified	24768	26906	1
chrIII	S000000561	YCL056C	ORF	PEX34	PEroXin	NO VALUE	S. cerevisiae	Verified	26925	27359	-1
chrIII	S000000560	YCL055W	ORF	KAR4	KARyogamy	NO VALUE	S. cerevisiae	Verified	27929	28936	1

- Insert designed DNA into Chromosome III
- Already synthetic
- Easy, simple, a lot of restriction sites and ORFs
- Just add/replace non-essential gene with filgrastim gene
- Yep13 for transformation

How would you make the strain a better chassis?

- Adding useful target genes
- Use metabolic modelling to redirect fluxes and optimise production
- Using genetic switches to enhance production and yield (by diverting metabolic fluxes to desired pathways)
- Use different promoters for different induction
- Creating even more durable/tolerant strains
- Gene deletion for glycosylation

Wet lab construction procedure

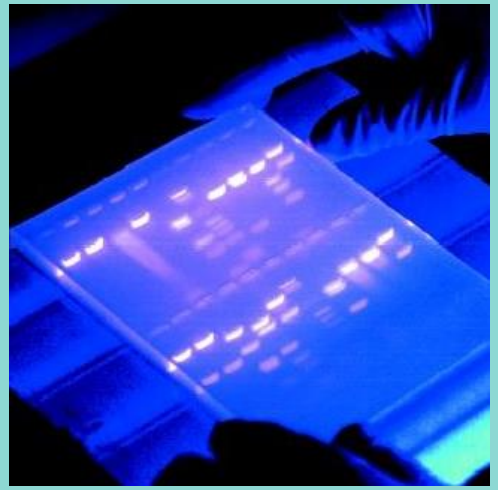
Gene deletion for glycosylation:

- Clone ordered oligos into *E. coli*
- UDPGlcNAc transporter gene from *K. lactis*
- Digested and ligated into plasmid
- Transformed into yeast
- Target genes replaced with kanMX4 or natNT2 cassette and 50 bp flanks

Wet lab construction procedure

Homologous recombination with URA3 and LEU2 auxotrophy:

- Order sequence as plasmid
- Digest and linearise plasmid
- Electrophoresis selection
- Cut correct length band
- Transform DNA into yeast
- Auxotrophic marker plates
- Use PCR and sequencing to check that correct “chunk” was transformed
- Repeat until all “chunks” have been transformed



Why is the Yeast 2.0 synthetic?

- Sc2.0, a highly modified *Saccharomyces cerevisiae* genome reduced in size by nearly 8%, with 1.1 megabases of the synthetic genome deleted, inserted, or altered.
- Non-native genes
- Non-native expression systems
- Differs from native organism, doesn't exist in nature
- Genome has been modified for better controlling and robustness

What is the significance and impact of the yeast 2.0?

- Profound and fundamental questions and answers of the properties of chromosomes function of RNA splicing
- Availability of a fully synthetic genome allows direct testing of evolutionary questions that are not otherwise approachable
- Can help to explain the function of certain fragments of DNA in genome
- Can help to optimize yeast as production host organism of cell factory

The goal of the Sc2.0 project is the complete synthesis of a custom-designed genome for a eukaryotic model organism to serve as a platform for systematic studies of eukaryotic chromosomes.

Thank You!

Sources

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https://bioinformatics.org/firstglance/fgij/where.htm PST modification etc.

http://syntheticyeast.org/sc2-0/goals/

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One-step gene replacement in yeast by cotransformation