Synthetic Genome

Yeast 2.0

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Significance of Yeast 2.0

-Completely synthetic (16 man-made chromosomes)

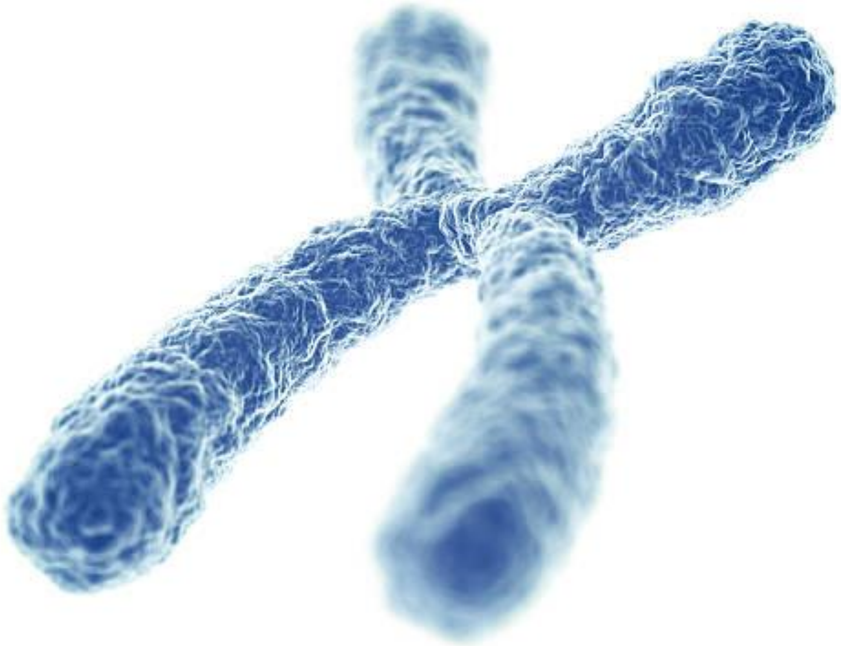
→ Can be used to design a synthetic chromosome region that produces different products
(medicine/fuel/biomolecules…)

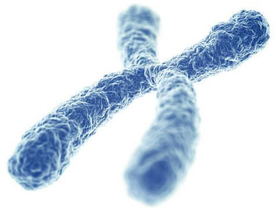
PROS:

- Genome well known
- Faster/cheaper/more simple than mammalian cell factories
- Durable to change
- Ecologically friendly: can be produced without toxic solvents/harsh conditions/generation of by-products

ADH1

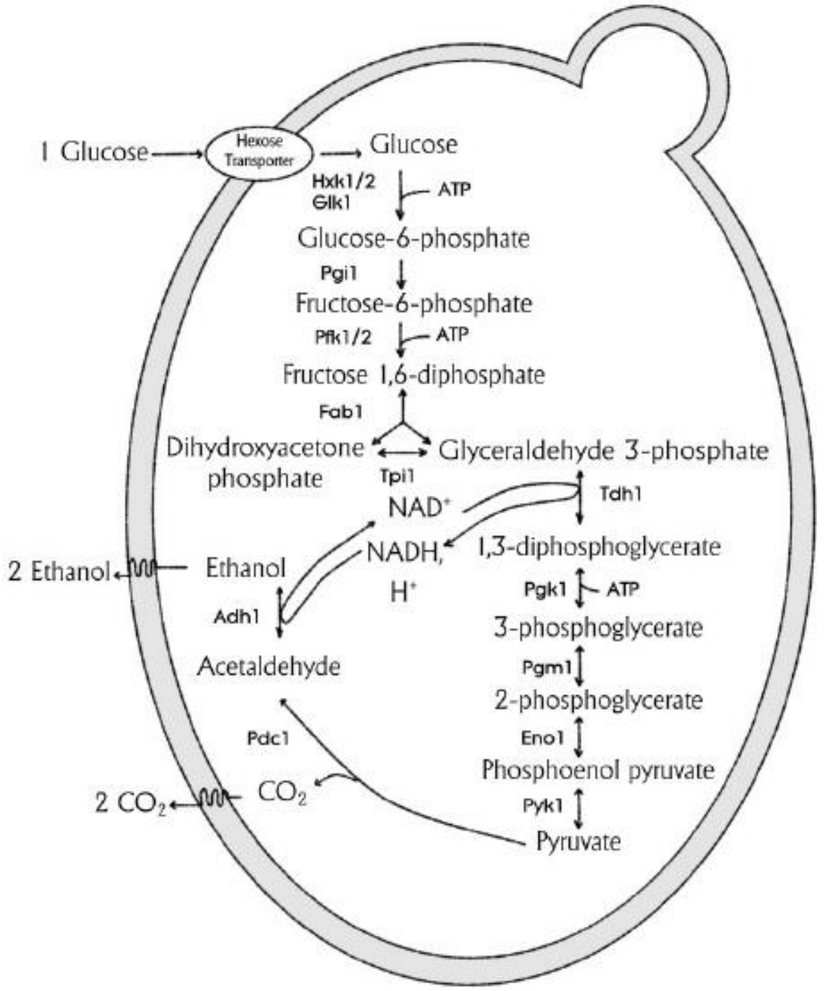
- Chromosome XV (15)
  - ~140 Kb-170 Kb
- Alcohol dehydrogenase
- Reduction of Acetaldehyde
- Sugar—Acetaldehyde—Ethanol
- Industrial applications—Bioethanol /Raw material/Chemical applications

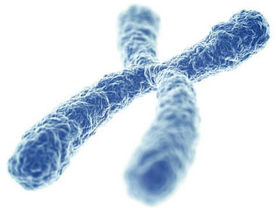




ADH1
Significance

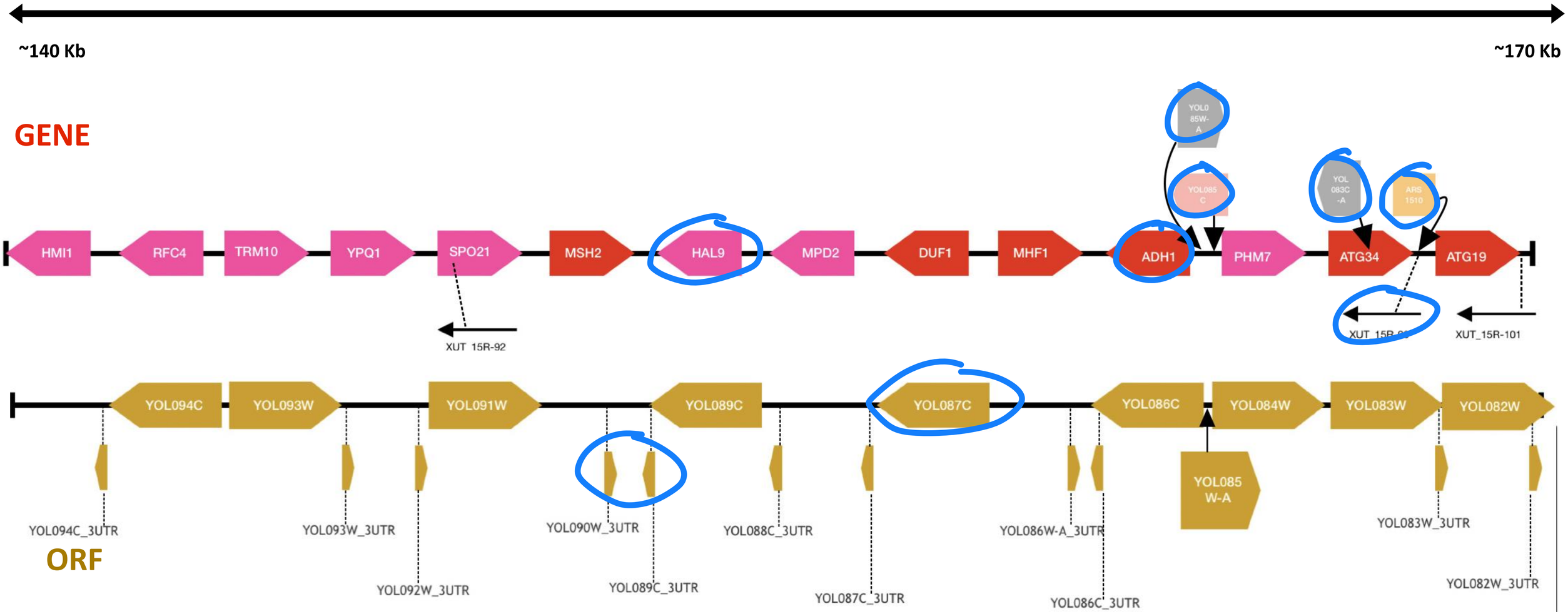
- Alcohol dehydrogenase
- Alcoholic fermentation
- Reduction of Acetaldehyde
- Last step in glycolytic pathway
- Sugar—Acetaldehyde—Ethanol





ADH1
in anaerobic conditions

- ADH1 activity helps yeast (*S. cerevisiae*) cells grow in anaerobic conditions
- Overexpression of ADH1 -> enhances the formaldehyde resistance of yeast cells
- ADH1 transcription repressed if cells are grown on non-fermentable carbon source



YOL085 W-A =Dubious ORF

ADH1 =Non-Essential Gene

YOL086C =Essential ORF

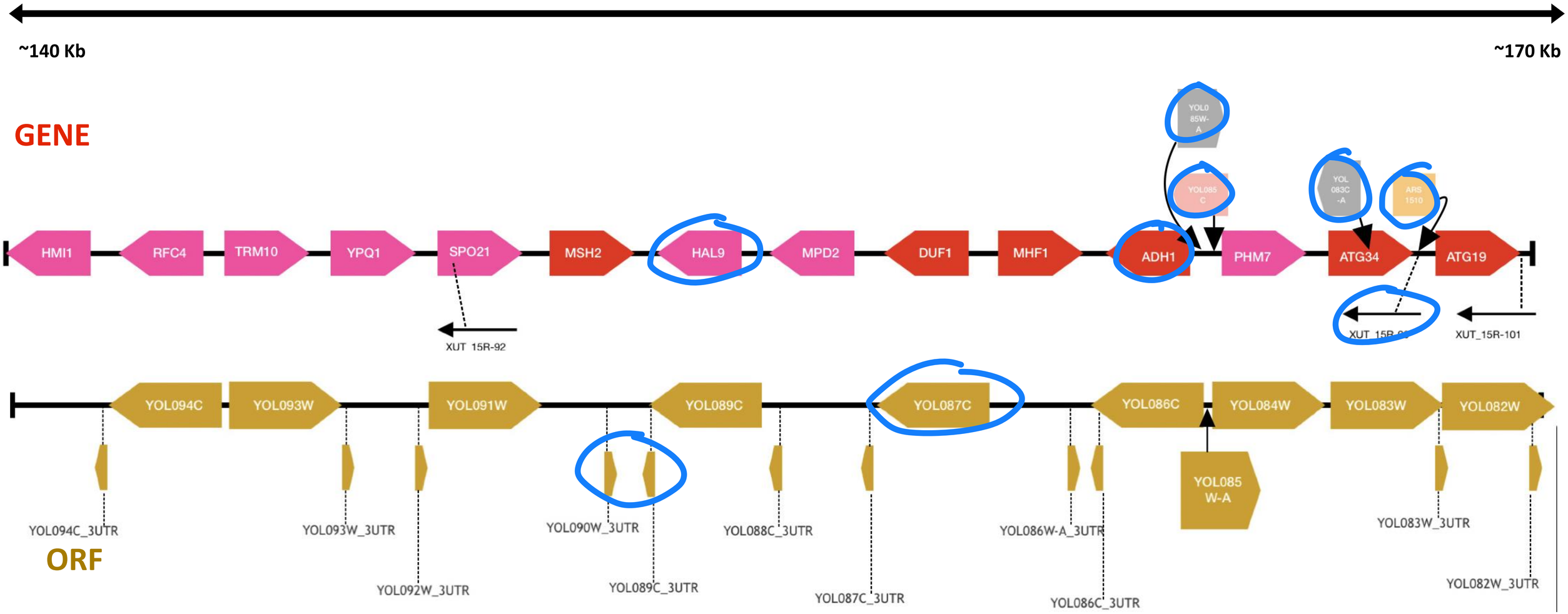
=ncRNA

HMI1 =Unclassified Gene

YOL085C =Putative Protein

ARS 1510 =Autonomously Replicating Sequence

=3’ UTR



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~140 Kb

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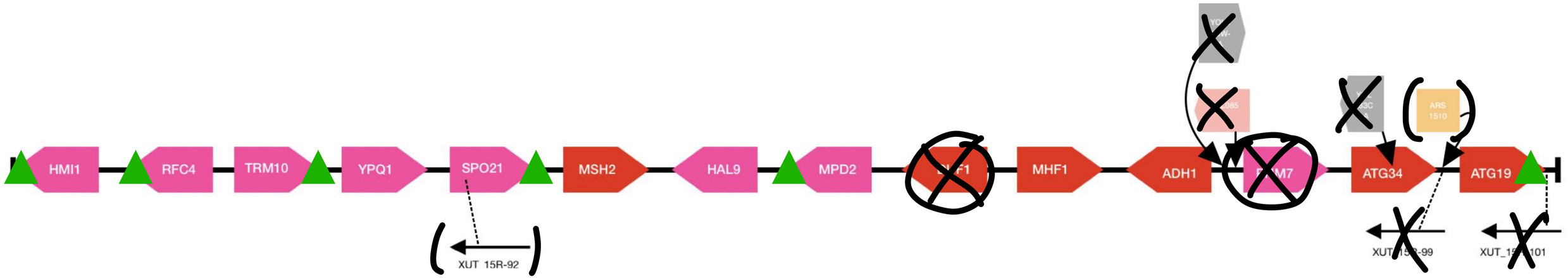
Modifications

Deletions

TAG/TGA—>TAA

PCR tags into ORF

Potential ADH1 modifications



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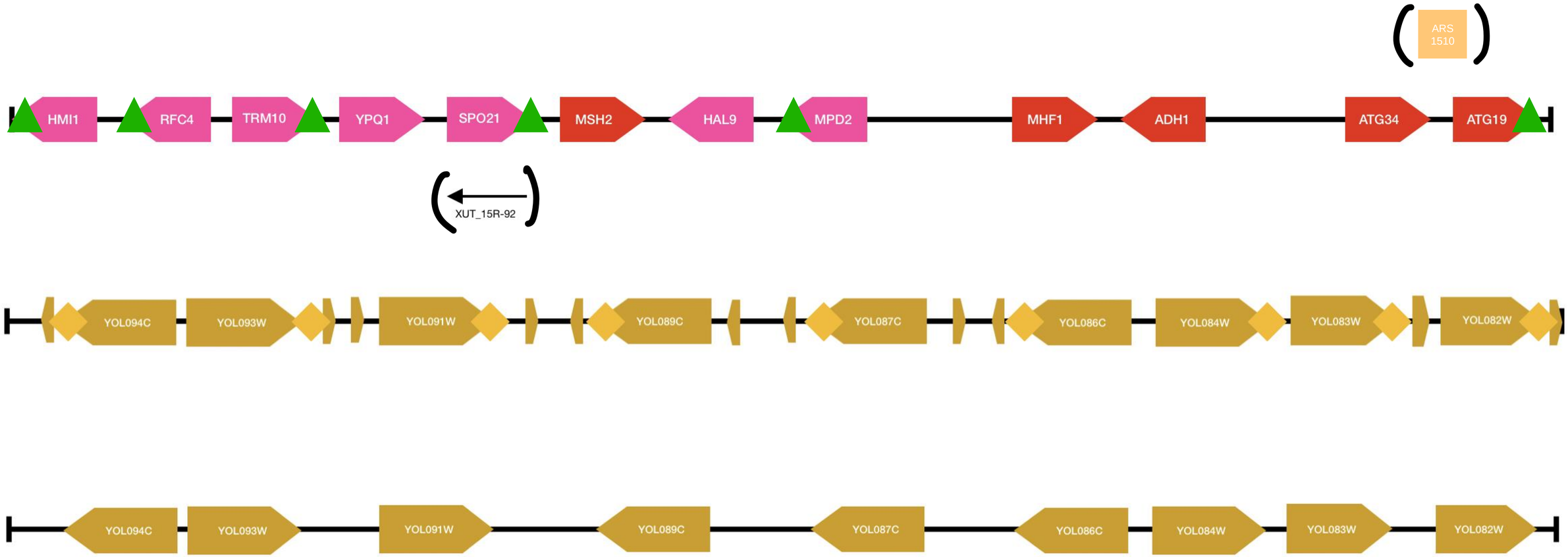
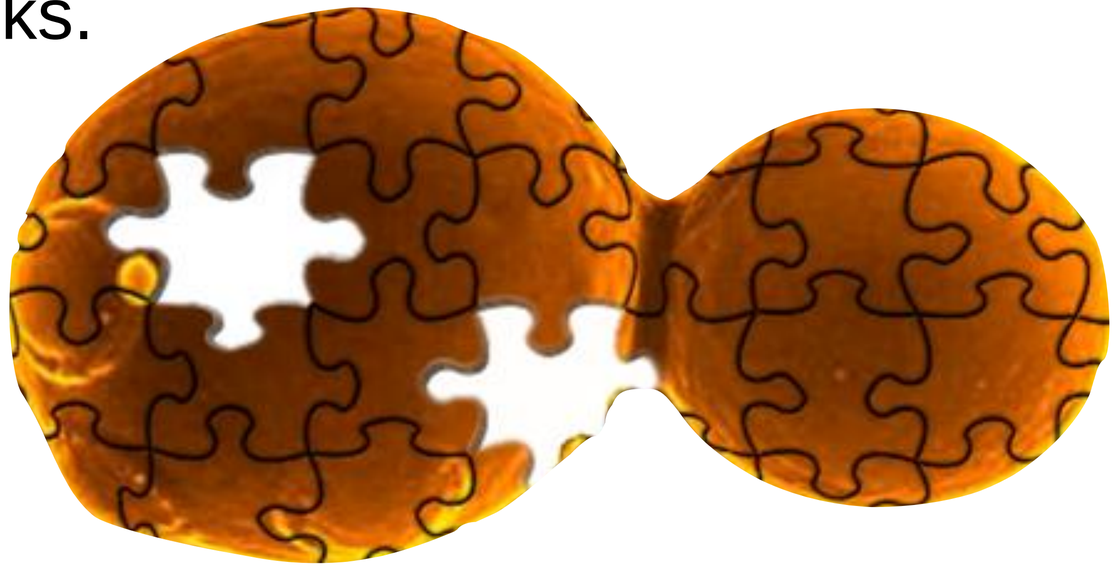

ARS
1510
HMI1
RFC4
TRM10
YPQ1
SPO21
MSH2
HAL9
MPD2
MHF1
ADH1
ATG34
ATG19
XUT_15R-92
YOL094C
YOL093W
YOL091W
YOL089C
YOL087C
YOL086C
YOL084W
YOL083W
YOL082W
YOL094C
YOL093W
YOL091W
YOL089C
YOL087C
YOL086C
YOL084W
YOL083W
YOL082W

Computer programs that could be used

- Computer program that could be used for this is for example SnapGene
- SnapGene is a licensed software widely used
- Yeastgenome.org for the genome database the biological information

Lab construction procedure

- Procedure starts by synthesizing small building blocks (750bp) with PCR.
- The yeast cell weaves building blocks into minichunks (2-4kb). Assembled by homologous recombination.
- Minichunks recombined into original chromosome until completely made of the synthetic chunks.
- ⟶ Synthetic yeast



SCRaMbLE

- Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution (SCRaMbLE) can be implemented in the Sc2.0 genome by introducing thousands of symmetrical loxP sites.

- The loxP sites are site-specific recombination sequences, where recombination occurs.

- They are introduced in all non-essential genes at the 3′ UTRs and points where deletions are made.

- → Massive chromosome rearrangements (~5000 sites genome-wide) to produce large genotypic diversity.

- The loxP specific recombinase can be expressed as following:

1. galactose induces a promoter
2. the promoter induces the recombinase
3. recombinase binds to the sequences and scrambling of the genome occur.

Why SCRaMbLE?

- Recombination between the loxP sites can lead to a large number of genome rearrangements (i.e, strains with with massive structural variations/different properties)

- →A fast tool for evolution as it creates a yeast library, which potentially drives phenotypic evolution (i.e., tolerance for temperature or a substance)

- A powerful strategy for numerous applications in analyzing genome structure/content/function to accelerate evolution with the use of conditional minimal genomes.

In The Future

- Synthetic Yeast Genome project (Yeast 2.0) is a global project that uses biodesign concepts and synthetic biology to advance science.
- Their goal is to improve quality of life in a sustainable environment.
- Using synthetic biology in yeast 2.0 could be a future-defining scientific frontier.
- The genome-engineering tools will help develop advanced biomanufacturing of beneficial products.
- → Drugs can be produced cheaper/faster and even new discoveries can be made with SCRaMbLE, which can improve the quality of life.