Yeast 2.0

CHEM-E8125 Synthetic Biology

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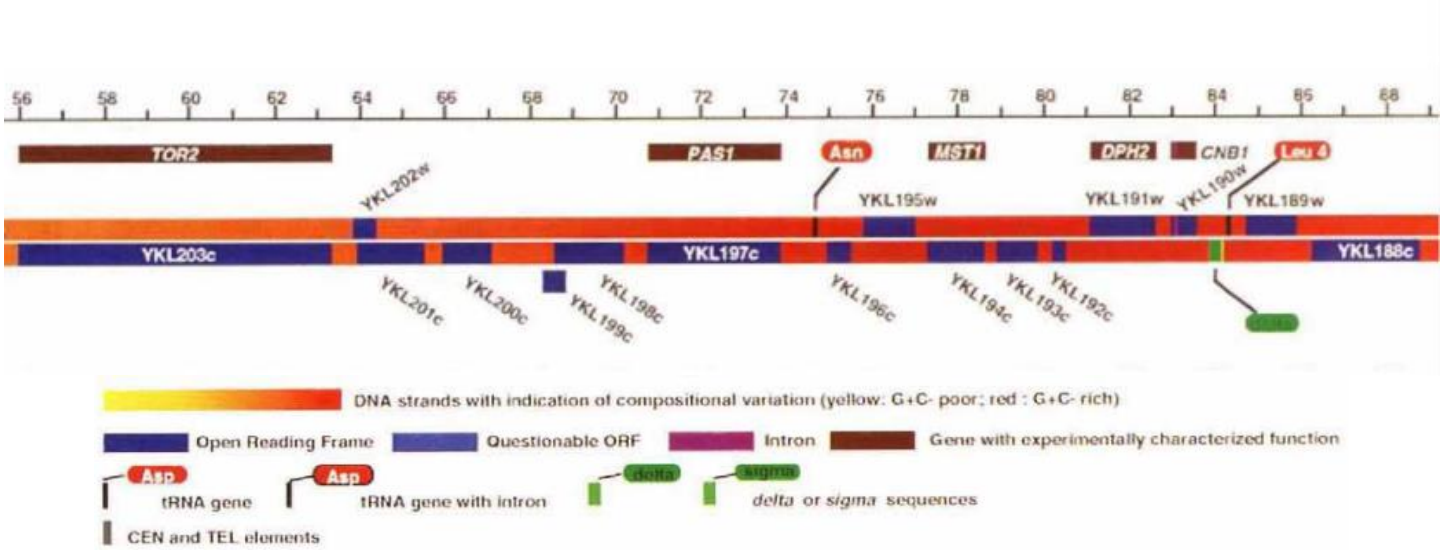
The chosen chromosome and region

- The chosen chromosome is chromosome 11, chrXI, with 666,448 bp in total
- The range to be synthesized is 56kb to 89kb



Dujon, B., Alexandraki, D., André, B. *et al.*

List of ORFs and encoded proteins

- YKL203c/TOR2. Kinases involved in nutrient-related growth control. An essential gene.
- YKL202w: Dubious open reading frame, unlikely to encode a functional protein. Obviously non-essential.
- YKL201c: Mannosylphosphate transferase Mnn6p, which is involved in mannosylphosphorylation of N-linked oligosaccharides. Important, but not essential.
- YKL200c: Merged open reading frame, with YKL201c, does not encode a discrete protein.
- YKL199c: Merged open reading frame with YKL198c.
- YKL198c: Putative serine/threonine protein kinase which regulates spermine uptake and is involved in polyamine transport. Essential to the sperminide transport.
- YKL197c/PAS1: AAA-peroxin, which heterodimerizes with AAA-peroxin Pex6p and participates in the recycling of peroxisomal signal receptor Pex5p.
- YKL196c: Vesicle membrane protein (v-SNARE) with acyltransferase activity, involved in trafficking to and within the Golgi. Essential gene.
- YKL195w: Import and assembly protein in mitochondrial intermembrane space, plays a role in mediating the import and oxidative folding of substrates including some small proteins. Essential in its functions.
- YKL194c/MST1: Mitochondrial threonyl-tRNA synthetase which aminoacylates some tRNA-molecules. Essential for translation.
- YKL193c: Regulatory subunit of the type 1 protein phosphatase (PP1) Glc7p. Essential gene.
- YKL192c: Mitochondrial matrix acyl carrier protein which is involved in biosynthesis of octanoate (a precursor to lipoic acid). A non-essential gene in some yeasts, but essential in the S288c-strain.
- YKL191w/DPH2: Protein required for synthesis of diphthamide, which is involved in the process of translation.A non-essential gene.
- YKL190w/CNB1: Calcineurin B, a regulatory subunit of calcineurin, which regulates a molecule associated with transcription. A non-essential gene.
- YKL189w: Component of the RAM signaling network, is involved in regulation of Ace2p activity and cellular morphogenesis.

Genes

- Most of the ORFs contain either essential or at the least important genes
- One exception:
  - The ORF YKL202w most likely only encodes a protein that has no function
- YKL201c and YKL200c are merged ORFs, marked as YKL201c.
- YKL199c and YKL198c are also merged ORFs, marked as YKL199c.

Megachunk design

Why chromosome 11?

- Chromosome 11 has not been synthesized yet

Why this region?

- We wanted to have a design that enables SCRaMbLE recombination
- Chosen region contains tRNA and an unnecessary ORF (YKL202w) → can be replaced with loxP sites for SCRaMbLE
- PCRTags added to most ORFs → changes are easily detected after SCRaMbLE
- Stop codons are swapped from TAG to TAA to enable later introduction of new coding schemes with unnatural amino acids

Megachunk design

How?

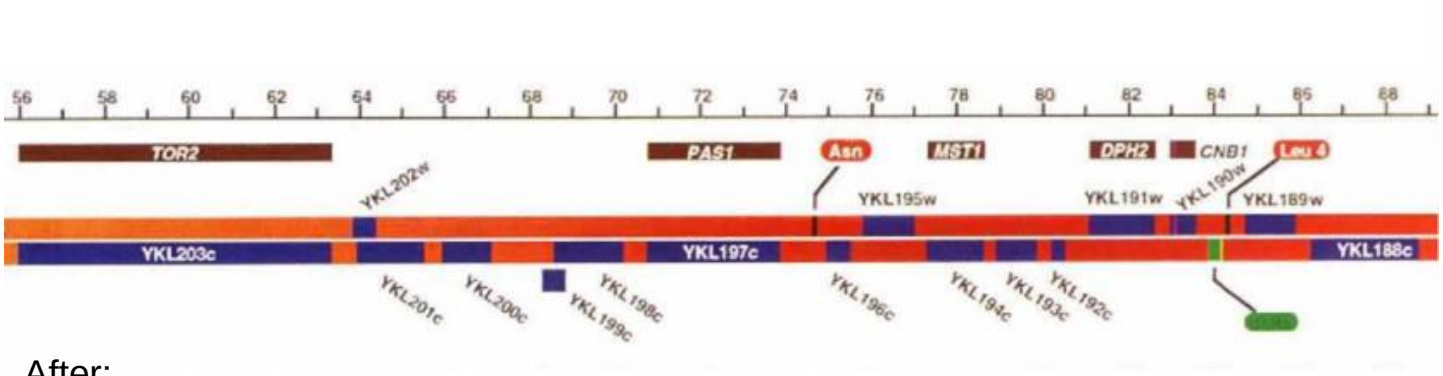
- Yeastgenome.org & browse.yeastgenome.org
  - For investigating the chromosome and the genes
- Genome design software BioStudio:
  - Adding PCRTags (BioStudio PCR tagger)
  - Swapping stop codons (BioStudio codon juggler)
  - Intermediate editing steps such as deletions (BioStudio graphical user interface)
  - Inserting loxP sites (BioStudio chromosome splicer)
- Database of Essential Genes
  - For the survival of an organism
- GeneDesign
  - Designing genes or building blocks through manipulation of codons, designing restriction sites and oligonucleotides

→ short sequences made according the design by DNA synthesis machine

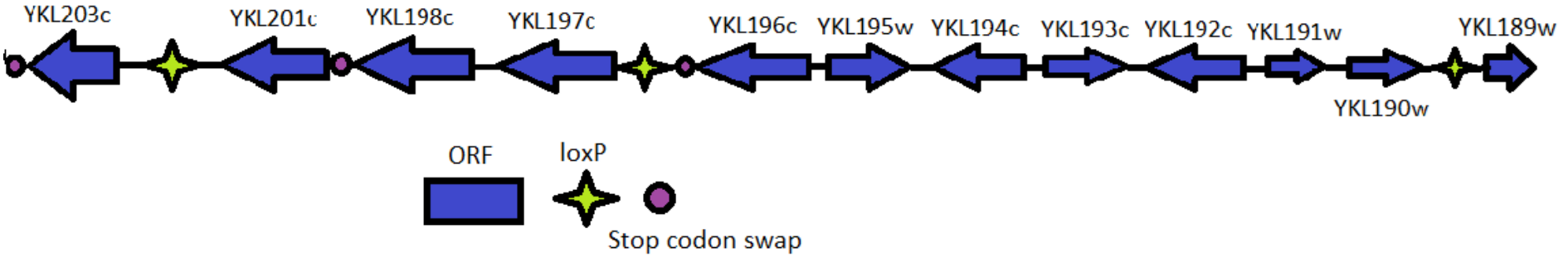
- Wet lab construction procedure
  - PCR to ligate short sequences into larger strands
  - Insertion in a yeast cell → strands weaved into chunks
  - Recombination of chunk with original yeast chromosome

Megachunk design

Before:



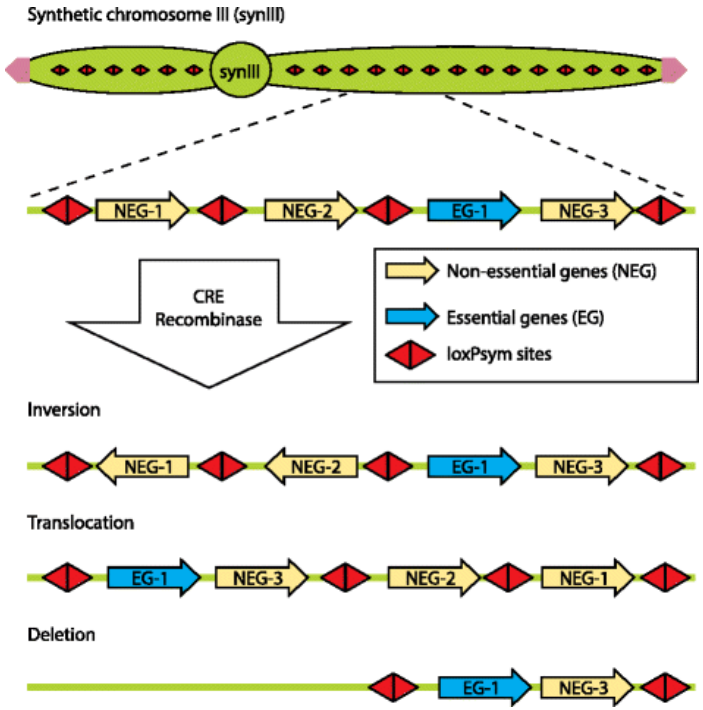
After:



SCRaMbLE mechanism

= Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution

- LoxP = Site-specific recombination sequences
- LoxP specific recombinase (CRE) is expressed through an inducible promoter
- Rearrangements at designed recombinase sites → mutations such as translocations, inversions, deletions and insertions → chromosomal rearrangements, combinatorial genomic diversity



Examples of SCRaMbLE recombination

Annaluru, N., Ramalingam, S. and Chandrasegaran, S. (2015)

SCRaMbLE mechanism

- Applications:
  - Analysis of genome structure, content and function
  - Acceleration of evolution
  - Creation of conditional minimal genome
  - Creation of phenotypic diversity
- Can be implemented in Sc2.0 genome
  - loxP sites introduced in all non-essential genes at 3′ UTRs and points of deletions

Significance and impact of yeast 2.0

- To completely understand the functioning of an organism, one should be able to design and redesign one
  - Deep understanding of the genes, genome organisation, chromosomes
- Successful Yeast 2.0 would work as a template for engineering other purposes into yeast
  - application specific synthetic chromosomes could be marketed

Use and further development

Use

- Replacing nonessential genes with genes of interest facilitates the production of biofuels and biomolecules
- Aids in research
  - Answers to evolutive questions
  - Platform for studies of eukaryotic chromosomes

Further development

- Yeast 3.0
  - Relocation of repetitive genes in 2.0 had only minuscule effect on cell growth → bigger changes might be viable
  - Shortening the genome
  - Relocation of essential genes to a centromeric plasmid (eArray)
  - More insight into how much of the yeast genome is still redundant, and in general what is the minimal genome to still yield viable cells in specific circumstances

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The data about the genes was searched from https://www.yeastgenome.org/ using the name of the ORF as a search word (e.g. YKL202w).