Re-write the Yeast Genome for Customised Biosynthesis Control

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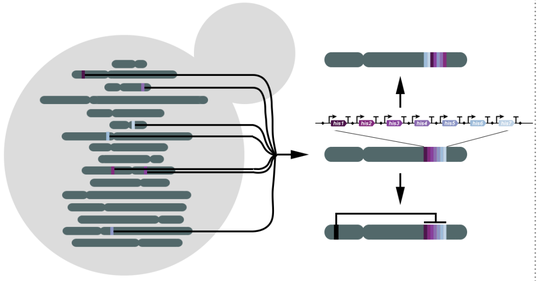
OVERVIEW

Synthetic genomics is a field in synthetic biology aiming to assemble whole genomic DNAs while manipulating the genome content into a customised way. *Saccharomyces cerevisiae* yeast, a model organism with its whole genome sequence determined and well understood, is a good candidate for genome de novo redesign and synthesis.

Here, we demonstrate the feasibility of defragmenting a yeast genome and the benefits of co-regulating a synthetic cluster that can be exploited for genome optimisation. We generated two functional synthetic chromosome clusters by genetically re-locating the genes associated with histidine and tryptophan biosynthesis. A master regulation switch was engineered to achieve the targeted and independent co-regulation of the gene expression. We introduced an inducible recombination system to create dynamic changes within the clusters for studying the rules that underly the natural eukaryotic genome organisation. These investigations would improve our ability to design and build custom synthetic genomes in the future.



ACKNOWLEDGEMENTS

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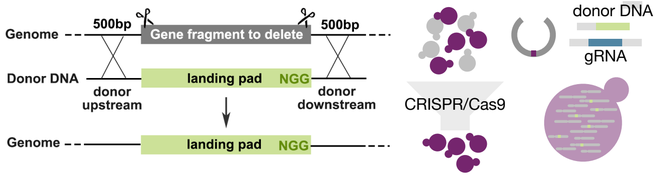
REFERENCES

[1] Michael L *et al*. ACS Synth. Biol, 2015
[2] Keung A *et al*. Cell, 2014

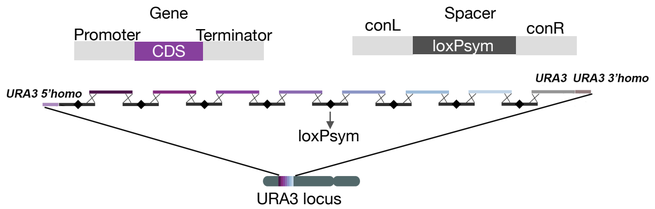
DEFRAGMENTATION

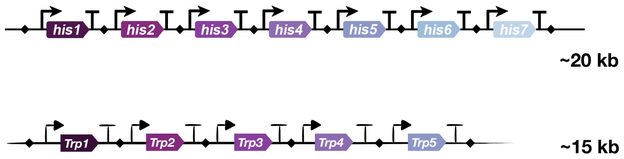
All the genes in the specific pathway were deleted from the BY4741 genomic loci. A landing pad encoding the Cas9 targetable sequence was inserted within the deletion donor for future genome editing. Based on the clean deletion strain, gene fragments were then reassembled with their flanking regulatory sequences into synthetic clusters at the *ura3* locus. Clusters have synthetic spacers (184 bp) between genes, each has a loxPsym site to drive SCRaMbLE rearrangements.

Gene Deletion



Genome Integration

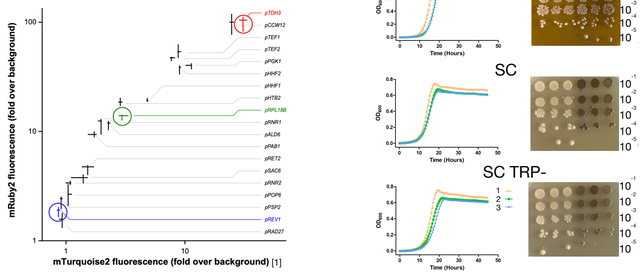




REFACTORING

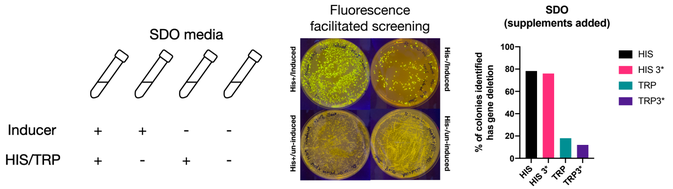
To better understand gene regulation within the cluster, and how expression levels affect TRP pathway function, we refactored the TRP genes by replacing native promoters and 3'UTRs with synthetic modular versions [1]. Fitness assay showed subtle difference in growth but significant difference in metabolic production by both defragmentation and refactoring, especially tryptophan was not provided.

Synthetic Modular Parts



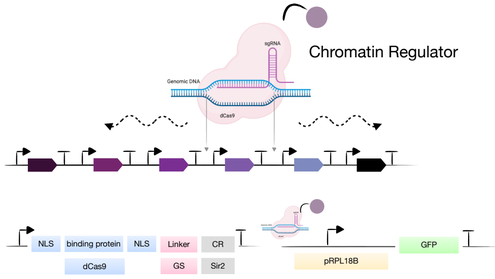
1 - wildtype; 2 - TRP genes defragmented; 3 - TRP genes refactored; 4 - wildtype with the tryptophan reporter;
5 - TRP genes defragmented with the tryptophan reporter; 6 - TRP genes refactored with the tryptophan reporter.

SCRaMbLE



Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution (SCRaMbLE) can induce the CRE recombinase expression which generate either deletion, inversion or duplication between loxPsym sites. We use SCRaMbLE system to test cluster stability under different selection pressure. A fluorescence reporter was developed to screen the SCRaMbLEd colonies by identifying GFP expression. When there is no selection pressure of the end product (histidine or tryptophan), the defragmented HIS cluster tend to lose genes within the cluster compared with the defragmented TRP cluster.

REGULATION



Repositioning may leads to dysregulation of the genes due to the loss of native regulatory context and control. Synthetically introducing epigenetic control could rescue fitness by coordinating expression across the pathway. To achieve this, a reporter cassette was constructed to test the silencing performance of the chromatin regulator (CR) by targeting upstream of the synthetic promoters [1, 2].

CONCLUSIONS

We generated two functional synthetic chromosome clusters by relocating and clustering of genes involved in histidine and tryptophan biosynthesis and showed no significant defect in cell growth.

Defragmentation and refactoring of TRP genes effect a change in tryptophan biosynthesis.

SCRaMbLE showed that TRP genes are more essential to the cells than HIS genes without selection pressure.

FUTURE WORK

Future efforts will aim to determine if the changes in tryptophan biosynthesis is due to altered expression or regulation of the genes.

We will test the performance of the synthetic epigenetic switch and determine the advantage of silencing a cluster.