Development of a Humanized and Genomically SCRaMbLe-able Synthetic Neochromosome Platform for Coenzyme A Biosynthesis Study

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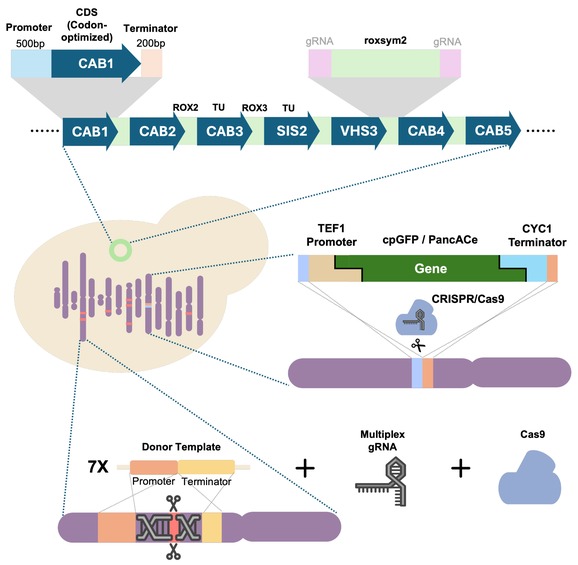
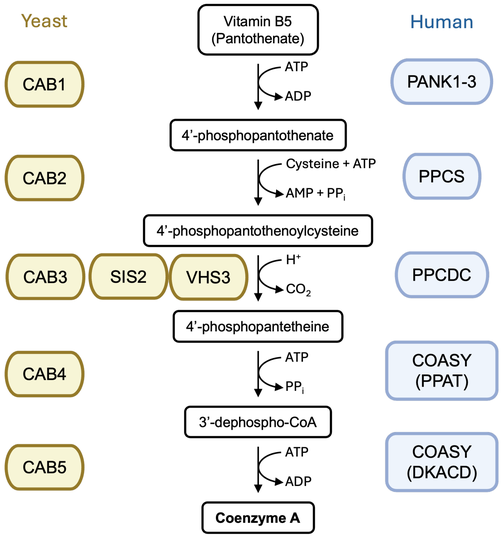
Graphical Abstract



Introduction

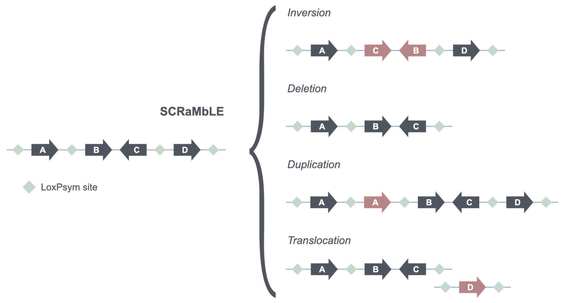
Coenzyme A as a core Metabolic Hub

- Universal Metabolic Hub
- Conserved Pathway



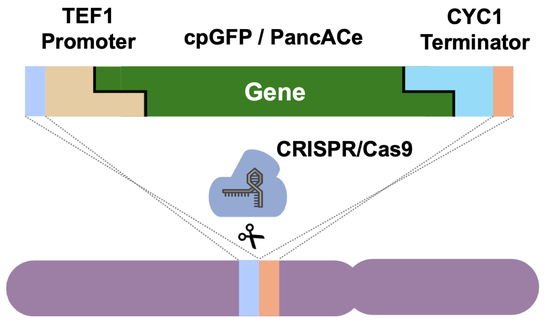
Methodology

SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution)



Results

Integration of PancACe Biosensor

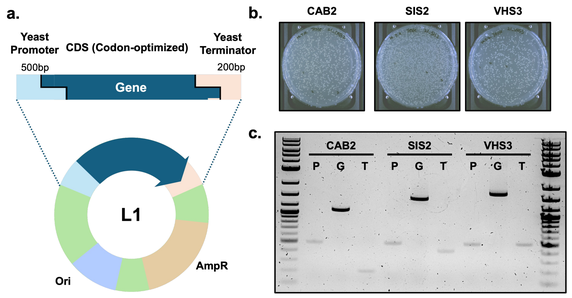


- **Non-Destructive Metabolic Monitoring:** Resolves the limitations of cell-lysing mass spectrometry and high-burden colorimetric systems to enable real-time, non-disruptive acetyl-CoA quantification in *S. cerevisiae*.
- **Yeast Adaptation of the PancACe System:** Employs a codon-optimized ratiometric fluorescent architecture (cpGFP combined with a CoA-detecting PanZ domain) driven by a strong constitutive TEF1 promoter.
- **Successful Level 1 Strain Engineering (L1):** Achieved precise *in vivo* platform integration via CRISPR-Cas9-mediated homologous recombination into the stable XI-5 genomic safe harbor, validated by colony PCR and sequencing.
- **Level 2 Functional Validation (L2):** Full functional fluorescent characterization remains uncompleted within the project's strict two-month timeframe, establishing a clear bottleneck for ongoing laboratory optimization.

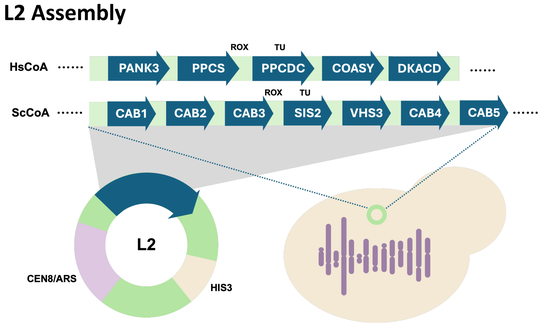
Results

L1 & L2 Design & Assembly of Neochromosome

L1 Assembly



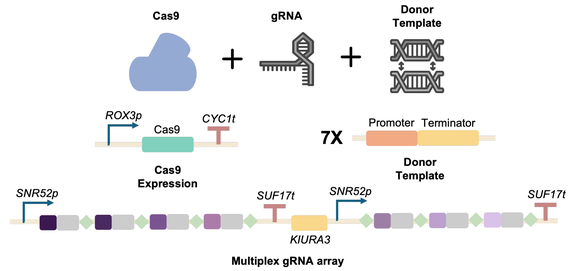
L2 Assembly



- **Orthologous Pathway Re-Engineering**: Establishes a cross-species evaluation framework by designing human orthologs (PANK3, PPCS, PPCDC, COASY, DCAKD) under native *S. cerevisiae* regulatory sequences.
- **Strategic Codon Optimization**: Engineered synthetic coding sequences (CDS) to isolate the neochromosomes from future CRISPR-Cas9 deletions aimed at removing native genomic copies.
- **Orthogonal SCRaMbLE System Design**: Integrates a symmetrical Dre-ROX framework (roxsym2) flanking each expression cassette, decoupling neochromosome diversification from the host strain's native Cre-LoxP system to maximize gene-swapping efficiency.
- **Successful Level 1 (L1) Transcription Unit Construction**: Achieved robust, sequence-verified assembly of all twelve individual human and yeast expression cassettes within E. coli cloning vectors.
- **Level 2 (L2) Framework Status**: Devised the multi-gene hierarchical workflows for the 5-gene human (HsCoA) and 7-gene yeast (ScCoA) neochromosomes, with physical in vivo homologous recombination and strain testing remaining an active laboratory objective.

Results

Multiplex CRISPR-Cas9 Genomic Deletion Strategy



- **Endogenous Evacuation**: Designed a 7-locus CRISPR framework (CAB1–5, SIS2, VHS3) to force phenotypic reliance on synthetic neochromosomes.
- **Sequence Safeguarding**: Leveraged codon optimization to ensure gRNAs exclusively target native loci while leaving synthetic genes intact.
- **Polycistronic Array**: Utilized a tRNA-gRNA array exploiting host RNase P/Z enzymes for simultaneous multi-locus cleavage.

Key Takeaways

This study establishes a foundational **in vivo synthetic platform** for metabolic engineering by designing cross-species CoA neochromosomes alongside a living-cell *PancACe* biosensor framework. Serving as a **pioneering blueprint for replacing an entire endogenous pathway**, this work represents the **first framework to SCRaMbLE a humanized genome structure** in yeast. By decoupling neochromosome *Dre-ROX* diversification from host *Cre-loxP* shuffling, this architecture enables unbiased, genome-wide co-evolution to uncover novel regulatory nodes. Coupled with an aerobic **glycerol/biosensor screening sieve** and **Nanopore long-read sequencing**, this iterative pipeline opens unprecedented possibilities for human biomedical and genomic research within a tunable yeast model.

Acknowledgements

Thank you to Warren Ho (direct mentor), Prof. Patrick Cai (faculty supervisor), all members of the Cai Lab, and the Laidlaw Foundation for their invaluable support and funding.

Key Citations

Wang, J.-Y., Cao, Y.-Y., Chen, Y.-N., Wu, X.-L., He, B.-T., Zhu, S.-Y., Zhou, X., Wu, Y., Li, B.-Z., & Yuan, Y.-J. (2022). Artificial nondirectional site-specific recombination systems. *iScience*, *25*(1), 103716. https://doi.org/10.1016/j.isci.2021.103716

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