

Rapid and Efficient One-Step Metabolic Pathway Integration in *E. coli*

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Project Goal:

The project aimed to develop a strategy that allows integration of heterologous constructs ranging in size from single genes to entire metabolic pathways at efficiencies high enough (>50%) to remove any requirement for further selection. With such strategies available, rationally designed pathways and genes can be rapidly tested in platform strains in an efficient and stable manner. Multiplex editing technologies could then be applied on top of such integrated constructs, establishing the basis for an efficient *in vivo* optimization strategy. The combination of these technologies can allow rapid engineering of strains for a broad range of biotechnology applications.

Abstract:

Methods for importing heterologous genes into genetically tractable hosts are among the most desired tools of synthetic biology. Many metabolic engineering applications and functional genomics studies require constructing and implementing heterologous functions from synthetic DNA. Chromosomal integration of such constructs provides better long-term stability and reduced cell-to-cell variability in copy number and expression levels, making such strains more suitable for industrial-scale processes or downstream engineering.

Genome integration methods has been available for decades in the model bacteria *Escherichia coli*, relying mostly on site-specific recombinases or the red system from the lambda phage. While gene-size constructs (~1kb) work with relative well efficiency in these systems, integration of multi-gene constructs (such as entire metabolic pathways) is a cumbersome process that requires multiple steps, selection and counter-selection strategies, and suffer from low efficiencies. By combining the genome editing tool CRISPR-Cas9 with lambda red-assisted recombination, we describe a strategy that allows highly efficient, single step integration of large pathways in *Escherichia coli*.

This strategy allows high efficiency integration in a broad range of homology arm sizes and genomic positions, with efficiencies ranging from 70 to 100% in 7 distinct loci. Using this strategy, we integrated a 10 kb construct encoding 5 genes required for production of the biofuel isobutanol in a single step, rapidly implementing production in the host strain. Further efforts from our group have demonstrated efficient integration of constructs up to 20 kb in size.

The ability to efficiently integrate entire metabolic pathways in a rapid and markerless manner will facilitate testing and engineering of novel pathways using the *E. coli* genome as a stable testing

platform. Moreover, increasingly sophisticated multiplex editing technologies could be applied on top of such integrated constructs, providing the basis for an efficient *in vivo* optimization platform.

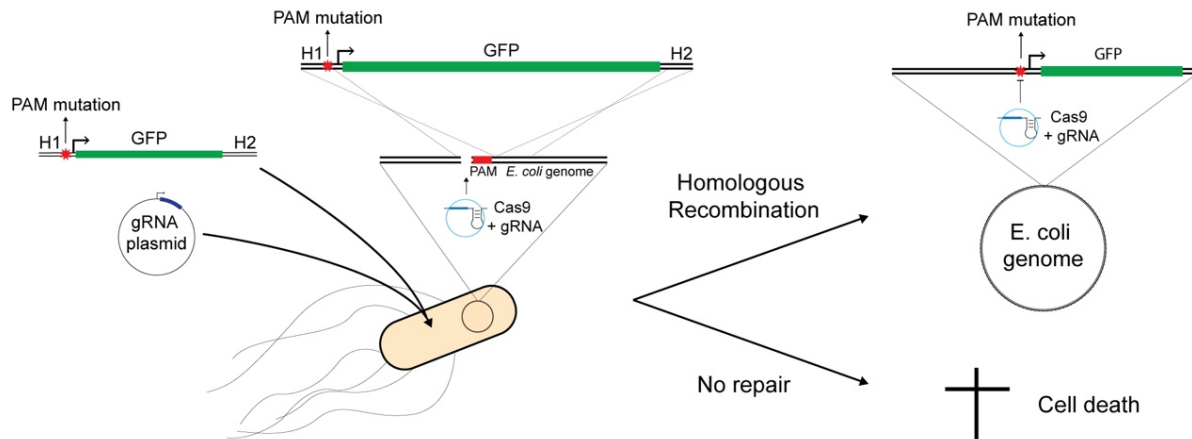


Figure 1: Overall integration strategy combining CRISPR-Cas9 and lambda red recombination for high efficiency integration of genes and pathways.

Publications:

¹Bassalo, M.C., Garst, A.D., Halweg-Edwards, A.L., Grau, W.C., Domaille, D.W., Mutalik, V.K., Arkin, A.P., Gill, R.T.. Rapid and Efficient One-Step Metabolic Pathway Integration in *E. coli*. *ACS Synth. Biol.* 2016. doi:10.1021/acssynbio.5b00187

²Garst, A.D., Bassalo, M.C., Pines, G., Lynch, S.A., Halweg-Edwards, A.L., Liu, R., Liang, L., Wang, Z., Zeitoun, R., Alexander, W.G., Gill, R.T. Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering. *Nat. Biotechnology* 2016. doi:10.1038/nbt.3718

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