High Throughput Functional Variant Screens *via* In-vivo Production of Single-stranded DNA

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Project Goals: Explore recombineering using substrates produced *in-vivo*, via specialized bacterial retro-elements. Construct a pooled Functional Genomics system, combining efficient editing and NGS-based tracking of mutants. Deploy this tool toward Energy-related goals, and work toward expanding its functionality to diverse bacteria.

Tremendous genetic variation exists in nature, but our ability to create and characterize individual genetic variants remains far more limited in scale. Likewise, synthetic variants aid our understanding of gene and genome function, but computational design of variants outpaces experimental measurement of their effect. Here, we show *in-vivo* production of single-stranded DNA via the targeted reverse-transcription of Retrons enables efficient and continuous generation of precise genomic edits in *Escherichia coli* at greater than 90% efficiency. This tool also effectively couples phenotypes to a targeted sequencing output, enabling pooled high-throughput screens of genetic variants, a process we call Retron Library Recombineering (RLR). We measure antibiotic resistance resulting from synthetic variants using both qualitative and quantitative RLR protocols for pooled phenotypic measurement. RLR can also be performed using natural variants as input, and we demonstrate this by using sheared genomic DNA of an evolved bacterium as an input substrate for RLR. In this way, we identify causal variants leading to antibiotic resistance, and demonstrate a saturating genomic RLR library, in which tens of millions of barcoded experiments are performed within each single pool, and all genetic variants in a strain are exhaustively tested. Pooled genomic editing using ssDNA produced *in vivo* thus presents new avenues for creating and exploring variation at the whole genome scale. We also introduce our future directions, in which RLR could enable unique applications in Bio-Energy and Negative Carbon Emissions, if adapted for use outside of *Escherichia coli.*

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