Retron Library Recombineering: Construction and Functional Interrogation of Trackable Genomic Edits

Max G. Schubert[1,2]* (mschubert@g.harvard.edu), Daniel Goodman,[2,3] **George M. Church**[1,2,3]

[1]Harvard University, Cambridge, MA; [2]Wyss Institute of Harvard, Boston, MA; [3]Massachussetts Institute of Technology, Cambridge, MA

http://arep.med.harvard.edu

Project Goals: Develop methods for synthesis of specific DNAs *in vivo* using Retron constructs. Use the DNAs produced to conduct efficient, multiplexed genome-engineering, and develop a tracking methodology wherein mutants are uniquely barcoded for phenotype measurement.

Advances in DNA synthesis and DNA sequencing enable powerful new systematic surveys of genomes, but precise genome editing has yet to be applied to genome-wide studies. Transposon-insertion sequencing and CRISPR knockout screens both efficiently alter genomes, and rapidly evaluate thousands of mutants as a pooled library using Next-Generation Sequencing (NGS)[1]. However these methods non-specifically ablate a locus using marker or scar sequences, and cannot create specific edits/alleles of interest. Here we present Retron Library Recombineering, a method that efficiently makes precise edits across the *E. coli* genome, and enables NGS tracking of the resulting edited cells. Single-stranded DNA (ssDNA) are generated *in vivo* using natural retro-elements called retrons, and these ssDNA serve as donors for lambda-RED recombineering[2]. This technique is more efficient at producing desired edits than existing methods, but more importantly, it enables the abundance of all mutant cells in a library to be measured by NGS of the cassette. We demonstrate efficient genome editing and accurate fitness measurement using this technique, and explore this technique for "de-bugging" the construction of genetically recoded organisms, and "de-convoluting" large sets of mutations observed in evolution experiments. Thus, the fitness contribution of thousands of individual mutations, and their epistatic relationships, can be defined all in one growth vessel. Unlike current high-throughput genome-wide screens, this method can be used to explore natural sequence variation or new genotypes of interest. Retrons are a simple, potent mechanism for producing ssDNA *in vivo* and will have diverse applications for precise genome engineering.

References

1. A. N. Gray *et al.*, High-throughput bacterial functional genomics in the sequencing era. *Curr. Opin. Microbiol.* **27**, 86–95 (2015).
2. F. Farzadfard, T. K. Lu, Genomically encoded analog memory with precise in vivo DNA writing in living cell populations. *Science*. **346**, 1256272 (2014).

This project has been funded by DOE grant DE-FG02-02ER63445.