Characterizing the portability of RecT-mediated oligonucleotide recombination

Gabriel T. Filsinger[a,b] (filsinger@g.harvard.edu), Timothy M. Wannier[b,c,1], Felix B. Pedersen[d,1], Isaac D. Lutz[e,1], Julie Zhang[f,1], Devon A. Stork[b,g], Kevin Gozzi[h], Helene Kuchwara[c], Verena Volf[b,i], Stan Wang[b,c], Seth L. Shipman[j,k], John Aach[c], Michael T. Laub[h], **George M. Church**[b, c, 2].

[a]Department of Systems Biology, Harvard Medical School, Boston, Massachusetts, USA.

[b]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, Massachusetts, USA.

[c]Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA.

[d]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark.

[e]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

[f]Department of Mathematics, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

[g]Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, USA.

[h]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

[i]Harvard University John A. Paulson School of Engineering and Applied Sciences, Cambridge, Massachusetts, USA.

[j]Gladstone Institutes, San Francisco, CA

[k]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA

1. These authors contributed equally

Project Goals: Characterize the requirements for the portability of recombineering methods

Abstract: Improved methods for genome editing in bacteria would enhance our ability to engineer human commensals, pathogens, and chassis for biosynthesis. Currently, methods of homologous recombination are limited in microbes since Cas9 is lethal in a majority of species. Phage encoded RecT proteins improve the efficiency of homologous recombination, but these proteins have been established in few species and are not broadly portable. Here, we reveal that this host-limitation is minimally defined by a requirement for compatibility between phage RecTs and the host's single-stranded DNA-binding protein (SSB). We characterize the RecT-SSB interaction, finding that it is mediated by 7 amino acids on the SSB C-terminal tail, and provide evidence that RecTs are portable between species where a host SSB interaction is maintained. Co-expressing cognate RecT-SSB pairs broadens recombineering activity, and in certain species improves recombination efficiency up to ~1000 fold even when the RecT proteins alone are non-functional. We use both rational selection of RecT proteins, and the screening of RecT-SSB pairs to establish

oligonucleotide recombination in *L. rhamnosus* and *C. crescentus*. We then show how dominant negative versions of the host MutL protein can enable efficient single-nucleotide mutagenesis in species beyond *E. coli*. In *L. lactis* we use an optimized recombineering method with dominant negative mismatch repair to generate libraries of variants at selected genomic loci, and show that the method far surpasses the capabilities of error-prone or mutagenic methods. Specifically, we make millions of mutations within the spectinomycin binding pocket, and characterize a unidentified landscape of epistatic effects that include 2-5 mutations. Due to the requirement for many simultaneous mutations, these variants are inaccessible through error-prone genomic diversification methods, but have the highest fitness in the presence and absence of selection. This work elucidates requirements for the portability of recombineering methods and emphasizes recombineering as the pre-eminent technique for generating genomic variants beyond what is accessible using evolution.

This project has been funded by DOE grant DE-FG02-02ER63445. Dr. Church is a founder of companies in which he has related financial interests: ReadCoor; EnEvolv; and 64-x. For a complete list of Dr. Church's financial interests, see also arep.med.harvard.edu/gmc/tech.html