Exploring the Adaptive Mutational Spectrum using Massively Tagged Populations of Experimentally Evolving Yeast. **Sherlock, G.**[1], Levy, S.[1,2], Blundell, J.[1], Venkataram, S.[1], Dunn, B.[1], Chang, J.[1], Petrov, D.[1], Fisher, D.[1] [1]Stanford University, Stanford, CA 94305; corresponding author gsherloc@stanford.edu, [2]Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, New York 11794–5252, USA.

Population sequencing has allowed the identification of 10s-100s of beneficial mutations, but typically only identifies alleles that reach a frequency of at least 1%. Identifying adaptive events at lower frequencies will allow us to gain a better understanding of the adaptive process, the mapping of genotype to fitness, and the genes and pathways that are adaptive targets under the evolutionary conditions. We have used a lineage tracking system to follow the dynamics of adaptive evolution of 500,000 isogenic lineages of *Saccharomyces cerevisiae* under a glucose limited regime. By tracking the lineage tags over time, we have been able to determine that ~20,000 of the lineages gain a beneficial mutation during the experiment, which occurs over only 200 generation. Furthermore, we have also been able to generate a distribution of fitness effects for these lineages [1]. This system allows us to identify clones belonging to specific lineages, which have an associated fitness, and to target for whole genome sequencing clones that gained beneficial mutations and that are from independent lineages. We have isolated over 4,000 clones from the evolution, at a point when most adaptive clones are likely to contain only a single adaptive mutation; we sequenced each clone's lineage tag, and used its known trajectory to determine whether the clone was adaptive during the evolution. We have whole genome sequenced several hundred such adaptive clones and identified many of the putative adaptive mutation(s) that have independently arisen in these evolutions. The RAS/cAMP/PKA pathway is frequently a target for adaptation under our conditions, with other possible adaptive mutations arising from genome duplications or copy number variations. Furthermore, in several cases where a gene has a paralog, beneficial mutations are recovered in one paralog significantly more frequently than that other. Finally, we have found that even when mutations affect the same pathway, that the fitness conferred by mutations in a given gene tends to be specific for that gene, and distinct from the fitness effect of mutations in other genes in the pathway.

References:

1. Levy, S.F., Blundell, J., Venkataram, S., Petrov, D. Fisher, D. and Sherlock, G. (2015). Quantitative evolutionary dynamics using high-resolution lineage tracking. *Nature, in press*.