HT-CRISPRi studies of gene regulation and function in *E. Coli*

Harneet S. Rishi[1,2] (HSRishi@lbl.gov), Esteban Toro,[2,3] David Chen,[3,4] Guillaume Cambray,[5] Honglei Liu,[6] Xiaowo Wang,[6,7] Lei S. Qi,[8,9] and **Adam P. Arkin**[2,3]

[1]Biophysics Graduate Program, University of California Berkeley, Berkeley, CA; [2]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA; [3]Department of Bioengineering, University of California Berkeley, Berkeley, CA; [4]UC Berkeley-UCSF Graduate Program in Bioengineering, Berkeley, CA; [5]California Institute for Quantitative Biosciences, University of California Berkeley, Berkeley, CA; [6]Bioinformatics Division, Center for Synthetic and Systems Biology, Tsinghua National Laboratory for Information Science and Technology, Beijing, China; [7]Department of Automation, Tsinghua University, Beijing, China; [8]Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA; and [9]California Institute for Quantitative Biomedical Research, San Francisco, CA

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Project Goals: Synthetic biology aims to leverage the engineering principles of modularity, standardization, and reliability with the design-build-test-learn cycle to rapidly engineer novel biological functions. One of the key hurdles in adopting this strategy is that the innate complexity of biological systems (ex: unmapped interaction networks, context dependence, temporal & spatial population variations) makes it difficult to understand first principles, which in turn makes it difficult to predictably build scalable systems. High-throughput technologies to quantitatively characterize function-phenotype landscapes can help overcome such barriers. As such, we aim to (1) develop CRISPRi into a scalable, high-throughput platform for rapid profiling of genomic features in *E. coli* across a number of conditions and (2) extend the functionality of the HT-CRISPRi platform to perform double transcriptional knockdowns for investigating the epistatic landscape of *E. coli* and revealing the basis of complex traits.

The CRISPR-associated protein Cas9 has been adapted as a versatile tool for transcriptional regulation, genome editing, and imaging in a number of organisms. Here, we apply the catalytically inactive dCas9 to conduct high-throughput transcriptional and regulatory studies in *E. coli*. Using an Agilent OLS library of 32992 unique sgRNAs, we targeted 4500 genes, 5400 promoters, 640 transcription factor binding sites, and 106 sRNAs in the *E. coli* genome. These genomic targets cover a wide range of functionalities such as metabolism, stress response, transport, and cell division. By leveraging CRISPRi with next-generation sequencing, we were able to interrogate the fitness effect of transcriptional knockdown for each of the aforementioned genomic features in a single-pot experiment both aerobically and anaerobically. Our fitness results agreed well with current knockout databases, and our ability to induce transcriptional knockdown at any point during an experiment has allowed us to explore target essentiality under different conditions (aerobic, anaerobic, minimal media, etc.) with great ease. We demonstrated this by showing that although *nrdA* and *nrdB* are essential under aerobic conditions – and are annotated as such in databases – they are dispensable anaerobically. We also show that CRISPRi can recapitulate genomic features with redundant functions. Furthermore, our genome-wide

library has allowed us to explore the robustness of CRISPR interference within bacterial operons. We are currently extending the HT-CRISPRi platform to scalably perform double transcriptional knockdowns in *E*. coli in order to interrogate libraries of knockdown pairs for epistatic genetic interactions. Overall, HT-CRISPRi enables single-pot, precise measurements of fitness for a large set of genomic features and will prove useful in genomic studies of model and non-model organisms.