An assay to resolve dynamic fitness costs of gene-expression at single cell level

Dr. Somenath Bakshi, University Lecturer, Department of Engineering

Email: somenath.bakshi@eng.cam.ac.uk Website: https://www.bakshilab.net/

Daily supervisor: Charlie Wedd (cdw42@cam.ac.uk), PhD student

What is fitness cost? Bacterial cells are masters of tuning their gene expression to adapt to their circumstances. They can make transporters to import food or to pump drugs out in order to survive and thrive. They can also make antibiotics to inhibit their competitors or produce compounds to degrade antibiotics to improve population survival. However, all of these need to be finely tuned to match their circumstances to avoid costs of futile expression. The 'cost' of expressing a gene required for adaptation is dictated by the required amount of cellular resources, which are otherwise invested in cellular growth and maintenance, often termed as metabolic burden. Fitness costs of gene-expression are of fundamental interest, since these dictate the ecology and evolution of the species. Cells have intricate regulatory strategies to ensure timely expression of these genes, such that their benefits always exceed their costs. Even in the time-of-need, cells often divide the labor across their population and even timeshare the burden to reduce the costs on individuals.

Why do we need a new assay to quantify fitness cost? Since gene-expression can be transient, and can vary across the population, it has been difficult to estimate what the associated 'costs of expression' are. In order to quantify the true costs of expression, we need to estimate the growth-rates of individual cells and accurately identify 'active' and 'inactive' states to estimate the cost from the difference in their growth-rates. Population level measurements of fitness-cost, such as bulk growth comparisons or the state-of-the-art competition assays, can't resolve these, since they both time-average and population average the costs of expression, and therefore underestimate the true costs of expression. To address these limitations, we are developing an assay to simultaneously monitor the gene-expression and growth-rates of individual cells, such that we can compute the fitness of cells in 'active' and 'inactive' states. In this project, we plan to run the pilot experiments, develop an analysis pipeline, and design an orthogonal assay for control.

The proposed assay for resolving fitness costs of dynamic gene-expression: Our lab specialises on using a microfluidic device that enables us to monitor the gene-expression and growth-physiology of $>10^3$ cells in parallel. Since we can track these cells over many division events, we can compute detailed distribution of growth-rates for the population. Therefore, we can achieve a very fine resolution (~500 ms) to estimate the mean division rate of bacteria which divides every ~20 mins. We can use this platform to estimate the fitness costs of gene-expression by labeling the gene-of-interest with a fluorescent marker. Using timelapse fluorescence imaging, we can identify when cells are actively expressing the gene and when they are not. We can pull the data of division times from these two classes and compare them to estimate fitness costs of expression. In this project, we plan to run pilot experiments with an inducible gene to switch between 'on' and 'off' states and develop an analysis pipeline to compute the fitness cost of the 'on' state. In addition to this, as an orthogonal control, we will build a platform to image and count colonies from cells from a bulk culture to estimate the fitness differences from the changes in their relative abundance over time.

Specific aims: (1) Compute the fitness costs of expression of a gene by turning it 'on' and 'off' using an inducer and estimating relative growth-rates of individual cells in the microfluidic device. (2) Construct a plate imaging platform to count colonies from a mixed culture containing cells with and without this gene (constantly 'on' or 'off'). (3) Compute the relative fitness costs for each pair using the relative colony counts and compare with results from aim 1.

- **Useful knowledge, skills and attributes:** Microbiology, Microscopy, Instrumentation, Image analysis, and Statistics
- **Access and supervision:** The project is primarily lab based (synthetic biology and imaging labs in the Bakshi Lab, Department of Engineering). However a significant part of the task is to develop the analysis pipeline for the two assays, which can be done remotely. It would be helpful if the student gets in touch with the supervisor (Somenath Bakshi) and the daily supervisor (Charlie Wedd) in advance, so that we can provide necessary instructions for the student to get a quick start. Also, we need to make sure all the risk assessments to carry out the work and training required to access the labs are done in advance.
- **Duration:** 8 weeks
- **Links to relevant supporting information on the web:**
    a. Fitness costs :
        - https://schaechter.asmblog.org/schaechter/2019/03/of-terms-in-biology-bacterial-fitness.html
        - https://evolutionnews.org/2010/03/thank_goodness_the_ncse_is_wro/
    b. Competition assays: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4427439/
    c. Macroscope for imaging colonies: https://openwetware.org/wiki/Macroscope
    d. Our recent paper: https://www.nature.com/articles/s41564-021-00900-4