# Identifying Isolation Methods for Persistent Bacteria by Linking Transient Molecular Events to Stable Genetic Signals

**Awardee:** Kyle Allison
**Award:** Early Independence Award
**Awardee Institution:** Columbia University Health Sciences

Bacterial persistence is a particularly concerning form of population heterogeneity in which a transient fraction of bacterial cells in a population tolerates severe antibiotic treatment while the majority of the population is eliminated. These 'persisters' can contribute to chronic infection and are a major medical problem. Despite their medical and scientific importance, persisters are poorly understood. One of the greatest challenge in studying bacterial persistence results from a lack of methods to isolate persisters from the heterogeneous populations in which they occur. As a result, systems-level analysis of persistent cells has been impossible and even basic questions cannot be answered. Hence, a major challenge in the field is to develop a method to isolate persisters which allows for their analysis by systems-wide, molecular techniques. We have devised a strategy for identifying transient signals that are predictive of persistence, by linking transient molecular events to stable genetic signals that can be quantified by DNA sequencing. To this end, we have developed an approach to analyzing population heterogeneity at the single-cell level using a genome-scale library of fluorescent reporters for promoter activity in *E. coli*. We have applied this method to measure the single-cell expression distributions of 1,637 distinct promoters at high resolution, and we analyzed these distributions in order to combine the 1,637 strains into seven distinct sub-libraries using a threshold-based strategy. This strategy ensures that, within in each pooled sub-library, strains are present at equivalent abundances for a specified threshold used for flow-cytometry sorting. We can therefore sort cells in mixed populations containing hundreds of promoter-reporter strains and to select only cells with expression within the top 5% of their distribution. Sorted populations, containing mixed populations of transient phenotypic outliers, are treated with antibiotics to select for persisters. Surviving populations are enriched with strains that harbor promoters that, when highly expressed, predict persistence. Reiterating this selection approach will lead to further enrichment of predictive signals, and the abundance of strains in resulting selected populations can be determined by sequencing from DNA flanking the promoters. This work will identify highly predictive transient signals for persistence, which will then be used to isolate persistent bacteria. These methods will allow researchers to perform systems-wide characterization of persistence at the molecular level and will accelerate development of new antibiotics treatments.