A Model for Designing Adaptive Laboratory Evolution Experiments

The occurrence of mutations is a cornerstone of the evolutionary theory of adaptation, capitalizing on the rare chance that a mutation confers a fitness benefit. Natural selection is increasingly being leveraged in laboratory settings for industrial and basic science applications. Despite increasing deployment, there are no standardized procedures available for designing and performing adaptive laboratory evolution (ALE) experiments. Thus, there is a need to optimize the experimental design, specifically for determining when to consider an experiment complete and for balancing outcomes with available resources (i.e., laboratory supplies, personnel, and time). To design and to better understand ALE experiments, a simulator, ALEsim, was developed, validated, and applied to the optimization of ALE experiments. The effects of various passage sizes were experimentally determined and subsequently evaluated with ALEsim, to explain differences in experimental outcomes. Furthermore, a beneficial mutation rate of $10^{-6.9}$ to $10^{-8.4}$ mutations per cell division was derived. A retrospective analysis of ALE experiments revealed that passage sizes typically employed in serial passage batch culture ALE experiments led to inefficient production and fixation of beneficial mutations. ALEsim and the results described here will aid in the design of ALE experiments to fit the exact needs of a project while taking into account the resources required and will lower the barriers to entry for this experimental technique.

**Importance**
ALE is a widely used scientific technique to increase scientific understanding, as well as to create industrially relevant organisms. The manner in which ALE experiments are conducted is highly manual and uniform, with little optimization for efficiency. Such inefficiencies result in suboptimal experiments that can take multiple months to complete. With the availability of automation and computer simulations, we can now perform these experiments in an optimized fashion and can design experiments to generate greater fitness in an accelerated time frame, thereby pushing the limits of what adaptive laboratory evolution can achieve.