

A High-Throughput Flow Cytometry-Based Approach to High-Resolution Genetic Interaction Measurements

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Abstract

A comprehensive understanding of cellular physiology is a fundamental goal of systems biology. In *S. cerevisiae*, the systematic measurement of genetic interactions has recently emerged as a powerful tool to identify biological pathways, to functionally dissect physical complexes, to assign functions to poorly characterized genes, and to gain insights into the organization of cellular processes and networks. However, current approaches for the measurement of genetic interactions have limited sensitivity, primarily due to the inaccuracy of current high-throughput methods used to measure strain fitness. This problem is further exacerbated by the low frequency of gene pairs that exhibit a genetic interaction (compared to the vast majority that do not) and the fact that even strong *bona fide* interactions may correspond to only small differences in growth rate. Here, we describe the development of a highly accurate approach to measure fitness based on competitive growth (Figure 1). In this strategy, wildtype and mutant strains are marked by different fluorescent tags and grown as co-cultures in a high-throughput format. Flow cytometry is then used to monitor the relative abundance of the competing mutant and wildtype strains over time, from which relative growth rates can be derived. We find that this technique significantly improves the quality of genetic interaction data (Figure 2) and allows for the detection of novel genetic interactions not identifiable by current approaches.

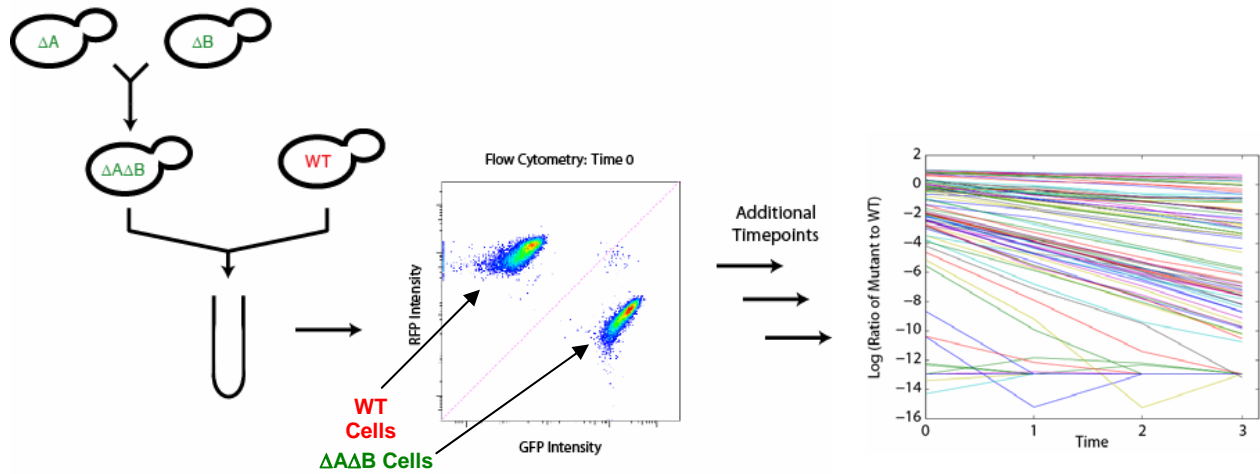


Figure 1. Schematic of the flow cytometry-based approach to fitness measurements. GFP-marked mutant cells are competed with RFP-marked wildtype cells and measured by flow cytometry at four time-points. At the right, the relative ratio of mutant to wildtype cells at each time point is plotted for many different mutant strains. Here, the slope of the line corresponds to the relative growth defect of the mutant strain.

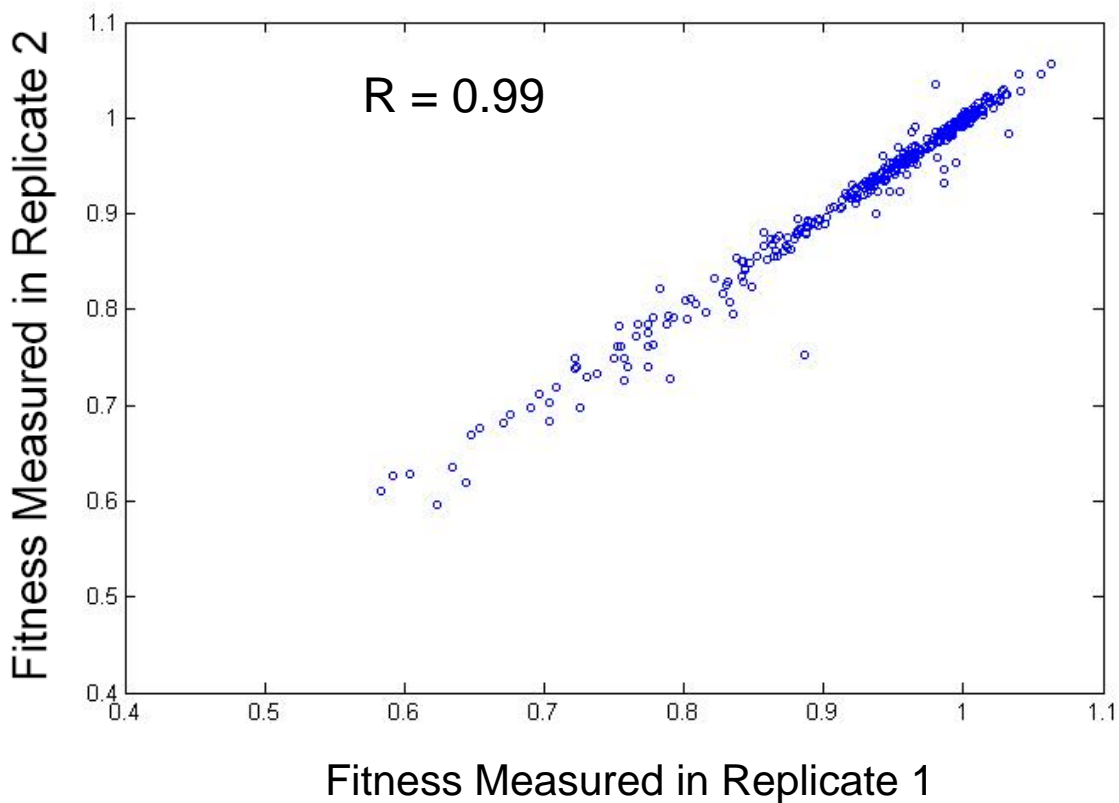


Figure 2. Replicate fitness measurements show very high agreement for a set of ~300 yeast deletion strains measured with the flow cytometry method.