

Improved Multiplexed Automated Genome Engineering through Directed Evolution

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Multiplex automated genome engineering (MAGE) is a versatile tool for efficient genome-wide genetic modification. Serious limitations of MAGE's applicability include high cell death rate and low percentage of cells that are genetically modified. Using directed evolution as a tool for improving MAGE, we have shown that one particular strain of *Escherichia coli* (*E. coli*), ECN2, which evolved over 14 MAGE cycles, acquired a lower cell death rate as well as a higher percentage of genetically modified cells. However, pleiotropic antagonism was observed, at the cost of an acquired reduced growth rate for ECN2. We also observed that the increase in percentage of genetically modified cells was greater after the first eight MAGE cycles as compared to the subsequent six MAGE cycles. This suggested the presence of slower average fitness gains over successive generations of bacteria. A second *E. coli* strain, HW106, did not significantly change over the course of directed evolution, possibly due to maximum MAGE efficiency being attained. This study demonstrates how directed evolution significantly improves MAGE efficiency. Directed evolution holds great promise in paving the way for great advancements and improvements in the usage of genetically engineered bacteria for the production of alternative energy sources like biodiesel, and the improvement of the production process of critical proteins and hormones.

Awards Won:

Third Award of \$1,000