Directed evolution is a combinatorial method for improving the properties of proteins. Mutants created in directed evolution experiments must be screened to determine their fitness. Assay of protein variants requires their expression in a high throughput manner under conditions that are not compatible with typical expression protocols.

We have developed a novel expression vector suitable for high throughput screening in 384-well plate format. Some of the features required are protein expression without an induction step, selection for properly folded proteins, normalization of expression levels from well to well, high efficiency cloning, and low cost antibiotic selection that does not conflict with helper plasmids or cell phenotypes. In addition, the plasmid includes a method to provide variable levels of protein expression, effectively expanding the dynamic range of the assay. These features combine to create an expression vector that is both highly versatile and capable of facilitating enzyme evolution.