
Preface

Directed evolution comprises two distinct steps that are typically applied in an iterative fashion: (1) generating molecular diversity and (2) finding among the ensemble of mutant sequences those proteins that perform the desired function according to the specified criteria. In many ways, the second step is the most challenging. No matter how cleverly designed or diverse the starting library, without an effective screening strategy the ability to isolate useful clones is severely diminished. The best screens are (1) high throughput, to increase the likelihood that useful clones will be found; (2) sufficiently sensitive (i.e., good signal to noise) to allow the isolation of lower activity clones early in evolution; (3) sufficiently reproducible to allow one to find small improvements; (4) robust, which means that the signal afforded by active clones is not dependent on difficult-to-control environmental variables; and, most importantly, (5) sensitive to the desired function. Regarding this last point, almost anyone who has attempted a directed evolution experiment has learned firsthand the truth of the dictum “you get what you screen for.”

The protocols in *Directed Enzyme Evolution* describe a series of detailed procedures of proven utility for directed evolution purposes. The volume begins with several selection strategies for enzyme evolution and continues with assay methods that can be used to screen enzyme libraries. Genetic selections offer the advantage that functional proteins can be isolated from very large libraries simply by growing a population of cells under selective conditions. In genetic complementation assays, an extraneous gene serves to provide an essential function that is missing from the host cell owing to a chromosomal lesion.

Screening strategies involve individual characterization of clones that are arrayed spatially in microtiter plates, on Petri dishes, or by other means. The majority of chapters in *Directed Enzyme Evolution* describe microtiter plate assays for several important classes of enzymes and strategies for adapting those to engineer physical properties such as thermostability. Although relatively time-consuming and often low-throughput, such assays represent the most effective means for evolving enzymes of industrial interest. Solid-phase screens using colorimetric substrates are inherently easier than liquid-phase assays, and their ability to provide quantitative, as opposed to qualitative, performance information can be enhanced using digital imaging systems. Nevertheless, there is a practical limit on the number of colonies that can be screened this way, usually tens of thousands for liquid assays, and perhaps hundreds of thousands for solid-phase screens. A significantly higher throughput can be realized with single-cell fluorescent assays coupled with

the isolation of desired cells by fluorescence-activated cell sorting. Such assays have been developed for several enzymatic activities including proteases, recombinases, and t-RNA synthetases. The cost of the higher throughput, however, is acquiring less information on each clone.

The upper limit for flow cytometric screens is realistically about 10^8 – 10^9 clones. In principle at least, even larger libraries can be screened using filamentous phage display. Representative examples of how phage libraries can be interrogated for enzymatic activity have been included in this volume. However, it is important to note that the implementation of phage- or flow cytometry-based assays requires considerably more molecular biology expertise compared to simpler solid-phase or microtiter plate-based assays. In addition, such assays can take the evolutionist dangerously far away from screening for the desired function, which might well include such things as ability to be expressed in a particular host cell or enzymatic activity under a specific set of conditions.

Clearly, the chapters in *Directed Enzyme Evolution* represent only a small fraction of the screening systems that are employed in directed evolution studies. We apologize in advance to the readers who could not find a screen for their favorite enzyme in this volume. We hope however, that the detailed protocols and advice included in these chapters will provide a helpful framework for designing new assays suitable for specific applications.

Frances H. Arnold
George Georgiou



<http://www.springer.com/978-1-58829-286-5>

Directed Enzyme Evolution
Screening and Selection Methods

Arnold, F.H.; Georgiou, G. (Eds.)

2003, XV, 383 p., Hardcover

ISBN: 978-1-58829-286-5

A product of Humana Press