



Directed Enzyme Evolution: Screening and Selection Methods (Methods in Molecular Biology)

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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.

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