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Application of Molecular Breeding to Biocatalysis

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Background

Maxygen is a leader in the application of synthetic biology to directed evolution. The Company's molecular breeding technology represents an enhancement over other methods of diversity creation for the successful deployment of laboratory-based evolution. This technology has been used for the improvement and optimization of protein function in many areas of biotechnology [1-5].

Engineering of enzymes to the needs of industrial biocatalysis is a fruitful area for the application of directed evolution. Enzymes can catalyze a wide variety of chemical reactions, including those that occur in nature and some that have been invented by organic chemists. They can be adapted as environmentally friendly and cost-effective alternatives to conventional catalysts for a multitude of purposes [6, 7].

Although much academic work has focused on the use of rational design for modification of enzyme properties, directed evolution can be employed even when lacking structural or other detailed information about the enzymes.

A critical feature of any directed evolution program is the quality of the diversity that forms the starting material for a campaign of screening for improved function. Use of random or directed mutational strategies, while often very clever, will typically produce libraries with limited diversification and low functional content [8].

Molecular breeding draws upon naturally occurring genetic diversity and creates libraries of chimeric genes through homologous recombination. A clear advantage of this approach is the fact that the majority of the variants encoded by such libraries are functional, thus reducing the burden of screening large numbers. Even when a truly high-throughput assay system is available, allowing tens of thousands of variants to be tested, libraries made by molecular breeding provide a richer opportunity to identify optimized proteins compared to traditional methods.

Applications

Directed evolution makes it possible to engineer proteins for changes in activity, allostery, binding affinity, expression, folding, fluorescence, solubility, substrate scope, selectivity (enantio-, stereo-, and regioselectivity), stability (temperature, organic solvents, pH), and more [9]. This approach is particularly useful for the engineering of complex biosynthetic traits in microbial systems. For either individual enzymes or metabolic pathways, no prior knowledge of the genetic basis of the targeted trait is needed [10]. The molecular breeding approach owes its success in part to the phenomenon of epistasis whereby the 'best' amino acid at one site depends on that or those at others [11].

Some specific examples of enzyme improvements can be highlighted. Transaminases are important biocatalysts in organic synthesis for preparation of chiral amino compounds and the industrial production of high-value chemical products. They have been engineered to accept spatially bulky substrates, perform stereoselective synthesis, selected specifically for practical applications in a manufacturing setting [7, 12-14]. Artificial metalloenzymes have been developed that combine the versatile reaction scope of transition metals with the beneficial catalytic features of enzymes [15]. P450 monooxygenases identified using directed evolution can catalyze the stereo- and regiospecific hydroxylation of chemically inert C-H bonds [16].

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