

P017 High throughput FACS screening for directed evolution of alcohol oxidase

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The synthesis of chiral compounds with high enantiomeric excess has emerged into one of the most important fields of organic chemistry. The use of enzymes to prepare enantiomerically pure compounds can provide elegant and efficient strategies. Biocatalytic deracemisation is increasingly seen as an attractive method for obtaining a single enantiomer in high yield.

Our proposed method of deracemisation combines an irreversible, enantioselective enzyme-catalysed oxidation of a chiral alcohol with a non-selective reduction of the prochiral ketone to generate the racemic substrate for the enzyme.

We have recently cloned aryl alcohol oxidase (AAO) from *Pleurotus eryngii* and the enzyme shows very low activity against secondary alcohols. In order to evolve this enzyme we have developed a novel screening system using an engineered biosensor. The system is currently being optimised using the AAO gene from *Pleurotus eryngii*. The hydrogen peroxide resulting from the action of this enzyme upon addition of veratryl alcohol is sufficient to induce expression of the green fluorescent protein (GFP) so that cells expressing a functional oxidase gene are distinguishable by Fluorescence Activated Cell Sorting (FACS). With our biosensor, single cells can be sorted from a large mixed population by FACS and grown for further analysis.