

P023 Developing amine racemase enzymes with the aid of a novel high-throughput screen

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Chiral amines are valuable intermediates in many synthetic processes, however classic kinetic resolution of racemates can only achieve a maximum yield of 50 %. The aim of this work is to develop a family of amine racemase enzymes for use in combination with enantioselective lipases in dynamic kinetic resolutions to prepare a range of optically pure amines in high yield. As no amine racemases have been previously reported, the approach chosen here was to use directed evolution to evolve amino acid racemases to accept amines as substrates. Three amino acid racemases were selected from the literature and sequence alignments and were cloned as candidates for directed evolution. A variety of techniques were employed to produce variant libraries and these were screened for novel activity towards amino acids and amines. To identify novel racemase activity from large libraries of variant enzymes a high-throughput screen was required. A novel racemase screen was developed based on an existing screen for amine oxidase activity and was shown to be effective in both solution and solid phase. The potential for this screen to be adapted to detect many different classes of enzymes was investigated. The main criteria was that the first enzyme can produce an enantiospecific substrate for a β -reporter oxidase. To demonstrate possible alternate uses of the screen, *w*-transaminase activity was screened for from libraries of *Vibrio fluvialis* and *Pseudomonas putida* genomic fragments using an (*S*)-selective amine oxidase as the reporter oxidase.