Mutagenesis of the Yeast Reductase Gene, YDL124w
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Background
The Aldo-Keto Reductase (AKR) superfamily of enzymes has been used to asymmetrically synthesize chiral molecules. In particular, we have been using yeast aldose reductases to reduce α/β-keto substrates into chiral alcohols. While there has been a lot of attention given to the screening of substrates by these enzymes, the regulation of stereoselectivity is not clearly understood. It appears that AKR stereoselectivity is determined by at least in part by the number of amino acid residues found in their Substrate Specificity Loop A (A Loop) region. Yeast AKRs with an A Loop comprised of 5-10 amino acids were found to yield one enantiomer, while yeast AKRs with an A Loop of 20-30 amino acids produced the opposite enantiomer [1]. Computer modeling of these different sized specificity loops found that they occupied mirror image positions with respect to the substrate binding pocket, and presumably accounting for the formation of the opposite enantiomers [1].

Abstract
However, the size of the substrate loop is not the entire story. In order to garner a greater understanding of how amino acid composition of the A Loop impacts stereoselectivity, we employed Alanine-scanning mutagenesis to systematically change the amino acids found in YDL124w's A Loop. Changes to the YDL124w reductase gene were introduced using circular mutagenesis. Positive clones were identified by restriction digest and verified by DNA sequencing. Once verified, GST chimeric proteins of each mutant were expressed in E. coli and screened against keto substrates. The preliminary results from the β-keto substrate, Ethyl 2-chlorophenylacetate, is reported here.

Figure 5. Reduction of Ethyl 2-chlorophenylacetate. The reduction of Ethyl 2-chlorophenylacetate can generate four different stereoisomeric alcohols (see Figure 4). The resulting product profiles generated by the reduction of Ethyl 2-chlorophenylacetate by the wildtype YDL124w reductase and the indicated mutants are shown above. The data show that the mutations in YDL124W's Loop A region can affect the either the ratio of stereospecific alcohols produced or the activity of the enzyme. In both cases it suggests that the enzyme's ability to interact with this specific substrate has been altered.

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Reference