**Additional information**

**Gene mining-based identification of aldo-keto reductases for highly stereoselective reduction of bulky ketones**

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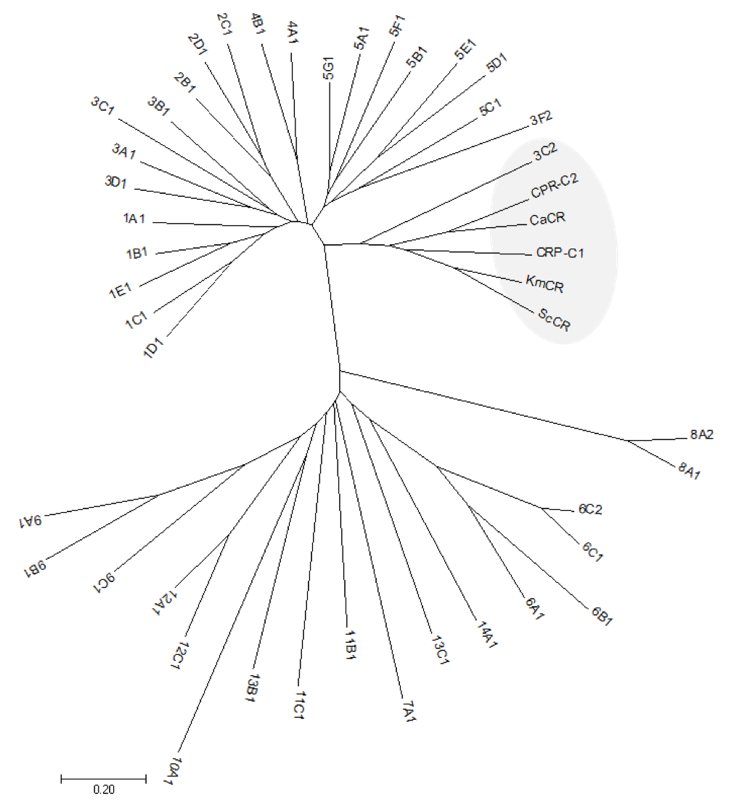
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Table S1 Oligonucleotide primers for amplification of the genes encoding AKRs involving restriction sites underlined.

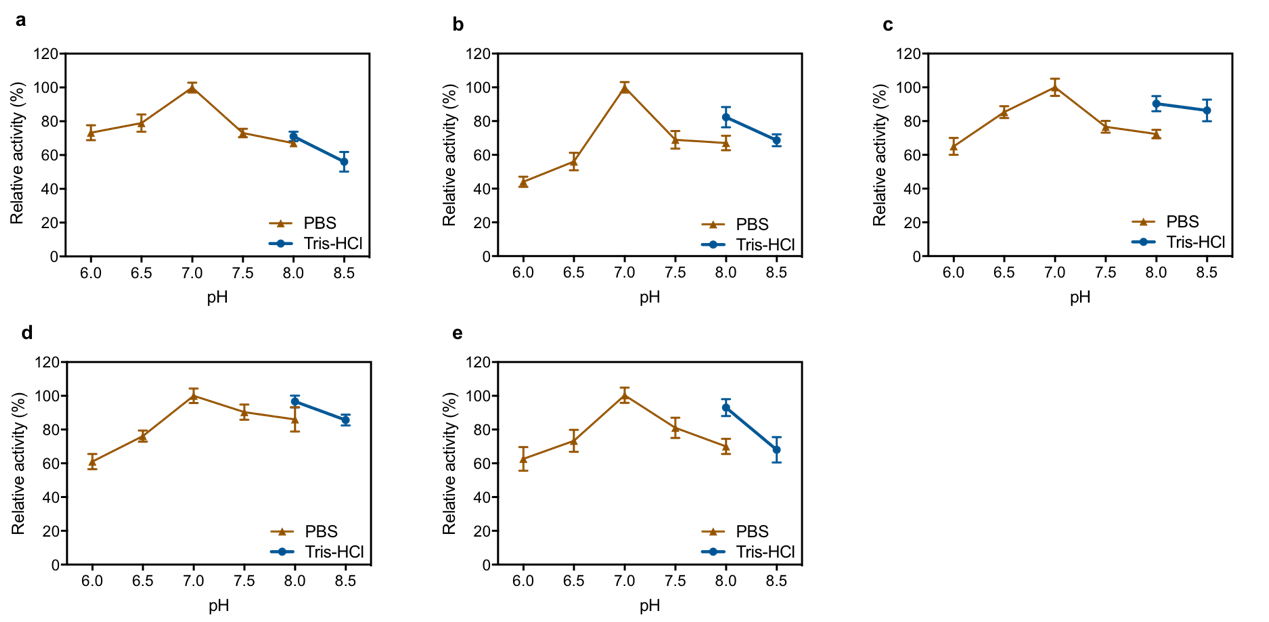
|  |  |
| --- | --- |
| Gene | Primer sequence |
| CaCR | F:5'-GCGCTCATATGATGACTTCACATACCCATCCTG-3' |
| R:5'-GGGGCTCGAGCTATAAATCTTTAAATGCTTCATGG-3' |
| ScCR | F:5'-GCCCCCCATATGATGTCATTTCACCAACAGTTC-3' |
| R:5'-AATTATGCGGCCGCTTATACTTTTTGAGCAGCGTAGTTG-3' |
| KmCR | F:5'-CCGCGGGCATATGATGACAAACCAAAAGTTCTTTAC-3' |
| R:5'-CCACCCTCGAGTCATTTTTGGGCTTCGGAGTTGTAC-3' |
| CPR-C1 | F:5'-AAGGAGATATACATATGTCACTTGCTGGAAAAGAATTT-3' |
| R:5'-GGTGGTGGTGCTCGAGATCATACTTGGAATACTCTTTCG-3' |
| CPR-C2 | F:5'-AAGGAGATATACATATGACTCAAAGTAACTTACTACC-3' |
| R:5'-GGTGGTGGTGCTCGAGCAAATCTTTAAATTGCTCATGGAAG-3' |



**Fig. S1** Phylogenetic tree of the enzymes and other AKRs generated from the web of http://www.med.upenn.edu/akr/.

paper1---/NADH%20酶活.pdf

**Fig. S2** Substrate specificities of the enzymes with NADH towards the tested carbonyl substrates, including (1a) methyl benzoylformate, (2a) ethyl benzoylformate, (3a) ethyl 2-oxo-4-phenylbutyrate, (4a) ethyl benzoylacetate, and (5a) 1-benzyl-3-pyrrolidinone. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH6.5), 0.5 mM NADH, 5 mM substrate, and the appropriate enzyme in a total volume of 100 µL. The experiments were carried out in triplicate, and the error bars represent the standard error of the mean.



**Fig. S3** The effect of pH on the enzyme activity of (a) CaCR, (b) ScCR, (c) KmCR, (d) CPR-C1, and (e) CPR-C2 in reduction of 2a. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 6.5 to 8.0) or 0.1 M Tris-HCl buffer (pH 8.0 to 8.5), 0.5 mM NADPH, 5 mM substrate, and the appropriate enzyme in a total volume of 100 µL. Maximal enzyme activity observed was set as 100% relative activity for each enzyme.