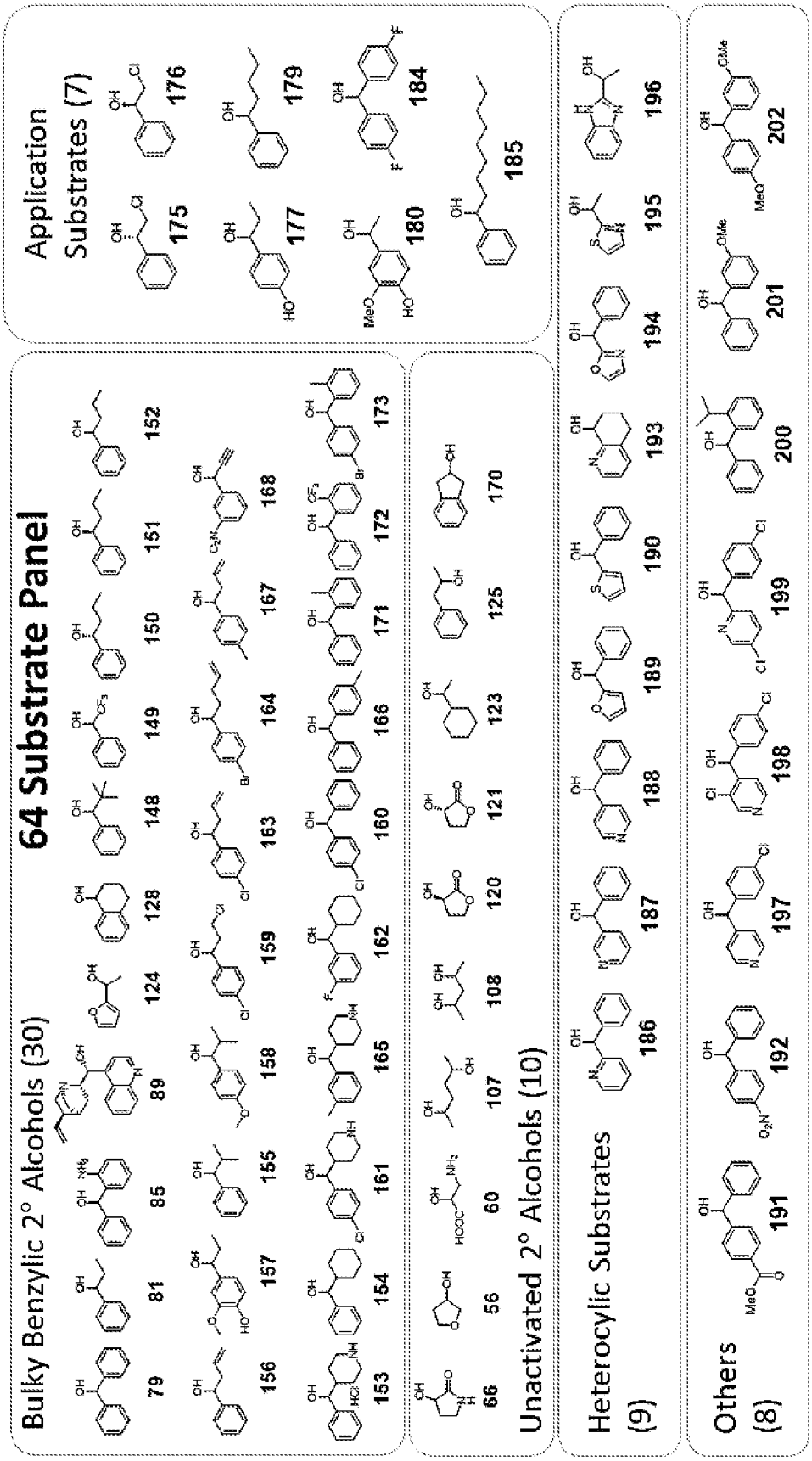
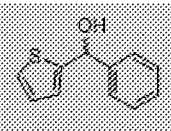
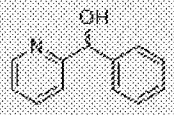
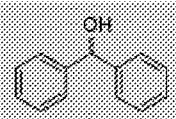
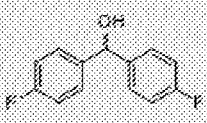
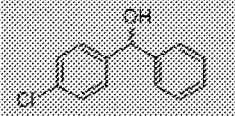

64 Substrate Panel
Bulky Benzylic 2° Alcohols (30)
79 81 85 89 124 128 148 149 150 151 152
156 157 155 158 159 163 164 167 168
153 154 161 165 162 160 166 171 172 173
Unactivated 2° Alcohols (10)
66 56 60 107 108 120 121 123 125 170
Application Substrates (7)
175 176 177 179 180 184 185
Heterocylic Substrates (9)
186 187 188 189 190 193 194 195 196
Others (8)
191 192 197 198 199 200 201 202

Fig. 1

Selected Top Variant Examples

Substrate	Name	Mutation vs M3-5	Activity Fold vs M3-5*
 **190**	GOh2044	F217A, N268W, A24H	14,700
 **186**	GOh2012	F217A, N268W, A195T	14,400
 **79**	GOh2012	F217A, N268W, A195T	9,700
 **184**	GOh2044	F217A, N268W, A24H	8,600
 **160**	GOh2070	F217A, N268W, D239E	7,400

*rounded to the nearest 100

Fig. 2

Mutant List

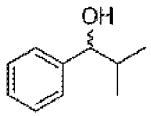
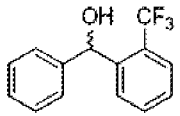
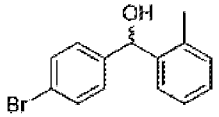
Backbone = M3-5

- GOh1052 – F217A + N268W

Backbone = GOh1052

- GOh2012 – A195T
- GOh2027 – Y352W
- GOh2037 – F487R
- GOh2039 – E218K
- GOh3001 – A195T, Y352W

HPLC Yields

 **155**	 **172**	 **173**
GOh3001 – 10%	GOh3001 – 35%	GOh3001 – 95%
GOh2012 – 5%	GOh2037 – 25%	GOh2039 – 90%
GOh2027 – 5%	GOh2039 – 19%	GOh2027 – 87%
GOh2039 – 5%	GOh2012 – 18%	
	GOh2027 – 18%	
GOh1052 – 4%	GOh1052 – 14%	GOh1052 – 75%
	Benchmark	

Conditions: Purified protein (50 µL, 400 µg/mL), 5 µL HRP (1 mg/mL), 5% DMSO v/v, 1 mM substrate, 25 °C, 220 rpm, topped up to 200 µL with 100 mM sodium phosphate (pH 7.0) buffer (in 2 mL HPLC vials), 24 hours.

Fig. 3

List of Best Mutants

Variant	Mutation	Comment	Activity Fold Change*	Residual Activity*	Relative Expression	Soluble/ Insoluble
GOh1052	Backbone	F217A, N268W from M3-5	1.00	56%	1.00	0.43
GOh2001	A195S	Generalist activity, Best relaxed enantioselectivity	1.24	39%	0.68	0.45
GOh2008	N259T	Generalist activity	1.12	84%	0.68	0.41
GOh2012	A195T	Generalist activity	1.80	3%	0.95	0.50
GOh2044	A24H	Good activity	2.00	74%	0.69	0.25
GOh2081	A195V	Good activity	2.04	19%	1.15	0.80
GOh2082	E218F	Best 25 °C activity	2.68	78%	1.03	1.24
GOh3001	A195T, Y352W	Good activity, stability, solubility	2.00	3%	0.66	0.62
GOh3002	N341W, Y352T	Best expression	1.21	66%	10.09	0.43
GOh3006	K389I, I28R	Best 40 °C activity, Best thermostability	2.00	92%	0.93	0.34
GOh3013	I28R, A195T, N259T, K389I	Good activity, solubility	2.36	57%	0.97	0.99
GOh3023	I28R, A195T, N341W, K389I	Good activity, Best solubility	2.16	52%	0.45	2.01
GOh3026	I28R, A195T, N259T, T447A	Good activity, solubility	2.28	58%	0.35	0.61
GOh3038	I28R, A195T, N259T, N341W, K389I	Good activity	2.24	62%	0.59	0.42

**Measured with substrate 152 (1-phenyl-1-butanol). Activity measured by high performance liquid chromatography (HPLC) conversions.*

Fig. 4

Low Activity High Activity*

*Activity = (reaction rate) x (maximum absorbance) x 100. Where activity = measure of enzymatic activity. Reaction rate = initial rate of reaction measured via change in absorbance over the first 8 mins. Maximum absorbance = maximum absorbance value recorded over the 24 hour reaction period. For details on colorimetric assay conditions please see paragraph [0110] Focused secondary alcohol substrate panel colorimetric assay.

	M3-5	**F217A**	**F217A, N268W**	**F217A, N268W, A195S**	**F217A, N268W, N259T**	**F217A, N268W, A195T**	**F217A, N268W, A24H**	**F217A, N268W, I28R**	**F217A, N268W, A195V**	**F217A, N268W, E218F**	**F217A, N268W, A195T, Y352W**	**F217A, N268W, I28R, A195T, N259T, K389I**	**F217A, N268W, I28R, A195T, N259T, N341W, K389I**
	1001b	*1036*	*1052*	*2001*	*2008*	*2012*	*2044*	*2045*	*2081*	*2082*	*3001*	*3013*	*3038*
79	0.6	2929.2	4126.7	4584.6	4513.5	5483.3	4754.4	4684.4	4193.2	7823.6	3945.6	7036.3	8332.6
81	0.5	156.7	205.2	150.9	310.2	242.3	230.1	327.3	261.1	744.9	282.6	485.9	601.7
85	0.1	390.1	153.8	206.4	286.4	275.4	334.1	369.2	297	617	608	602.9	772.3
89	0.4	0.8	1.8	0.1	0.1	0.1	0.2	0.2	0.5	0.9	0.1	0.6	0.8
124	118	144.8	407.6	703.8	717.4	192.3	492.5	591.9	441.4	1283.6	612.6	695.7	818.5
148	0.3	0.1	0.3	0.8	0.6	0.3	0.1	0.1	0.3	0.7	0.1	0.1	1
149	1	19.4	39.8	130.4	64.7	12.1	77.2	129	92.3	460	65.7	190	239.8
150	0.7	191.9	114.3	1326.9	686.9	337.9	1005	1180.8	1266.9	1994.1	1316.1	1537.7	1762
151	0.7	131.8	56.5	966.7	567.2	277.3	785.3	1091.1	1019.1	1312.9	1096	1801.9	2095.4
152	0.8	147.5	66.4	1834.6	3380.6	2242.1	1042.8	1276.2	1209.5	1918.2	1423.9	1779.1	2122.4
156	7.8	63.2	364.2	491.2	904.3	333.7	532.6	618.9	493.7	835.7	658.7	798.2	906.9

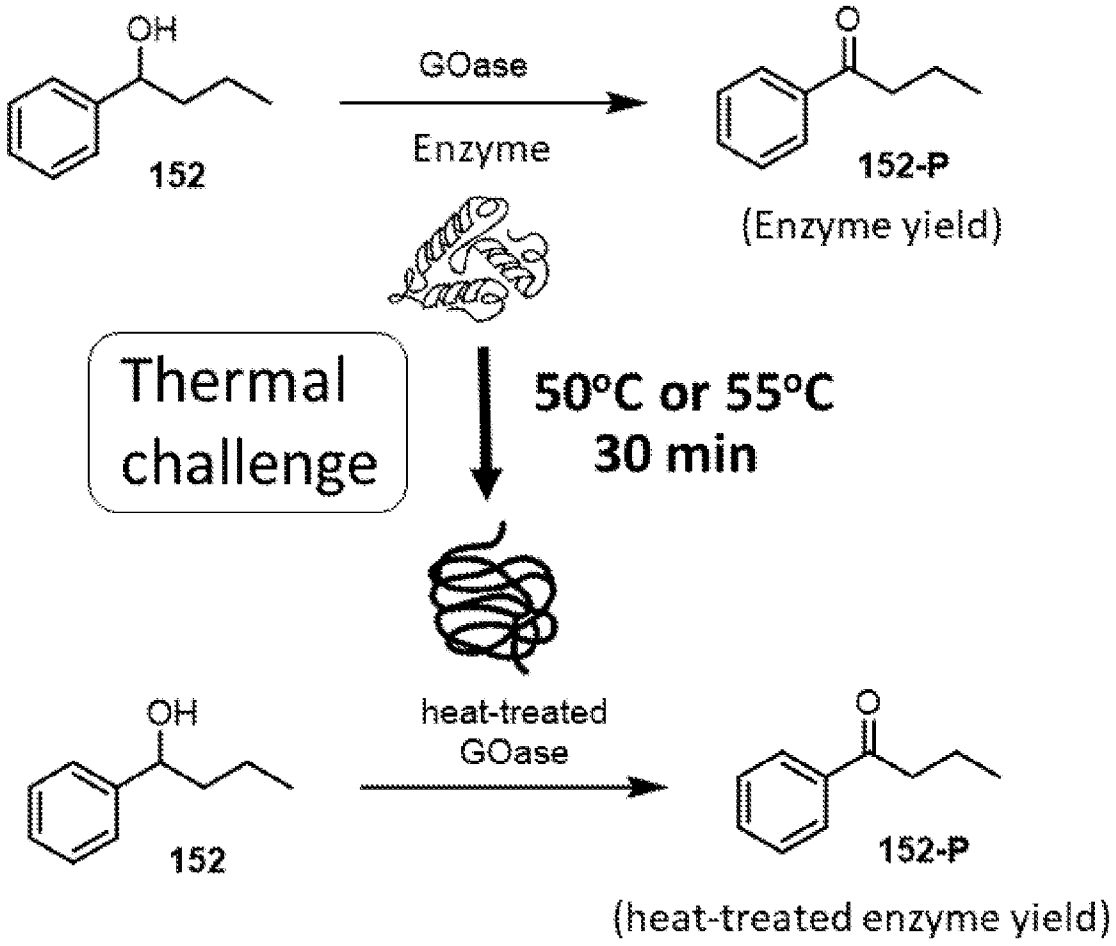
Fig. 5

158	0.5	0.2	0.5	2	3	1.1	0.7	1.3	1.6	4.2	1	1.6	3.9
159	5.5	793.8	2550.6	3613.4	3550	1802.9	4000.3	3872.4	4093.6	6913.9	3910.2	7088.9	7541.2
164	268.2	996.4	2993.4	3360.9	2560.3	3810.9	4089.3	3865.2	4140	5015.2	4459	5081.8	5406.2
167	63.6	37.5	352	499.7	1135.1	343.1	783.9	846.8	657.4	1194.4	834.3	1267.1	1301
168	6306.8	124.1	5769.1	2230.2	142.6	1159.9	36271.7	33071.3	39774.4	1465.7	1856.2	42874.7	1042.1
153	0.4	3.1	1.7	3.3	14	4.4	3.1	4.8	3.8	2.7	4.9	6.7	7.6
154	0.4	356.3	203.8	411.5	719.4	564.2	253.9	333.9	312.6	564.3	473.4	635.9	690
160	1.5	6921.1	5884.1	5996.2	6529.3	6393.3	8582.4	6469.7	10517	14582.4	3621.3	11659.1	11204.9
161	0.5	0	0	0.7	0.5	1.3	0	0	2.4	1.5	5.4	0.9	1.1
165	0.4	0.1	0.2	0.1	0.2	0.1	0	0	0	0	0.1	0.1	0.1
166	0.8	2451.3	2773.5	3860.5	4051.5	4768.5	4569.2	4065.2	4038.3	5766	4555.5	6200.7	6414.2
155	0.7	2	1.8	4.5	16.2	2.8	0.9	1.4	0.4	4.7	0.8	1.5	1.3
162	0.5	0.1	0.3	1.3	1.1	2	0.7	0.5	0.2	0.3	0.4	0.1	0.4
163	48.6	57.2	102.7	1387.8	695.1	2524.1	2611.6	2473.1	2847.8	4059	1522.8	5024.6	5292.5
173	0.7	267.7	127.5	198.7	158.4	214.8	169.7	198.6	256.5	300.6	361.1	351.8	360
171	0.4	872.3	593.3	726.4	813.8	700.1	705.4	811.1	735.7	1337.3	1422.9	1319	1362.9
172	0.4	6.4	15	18.3	17.5	27.1	21.6	29.4	15.5	49.4	13.1	60.5	76.6
128	2.8	287.3	43.8	899.6	569.5	307.7	570.7	679.9	718.6	1038.3	936.8	1209.4	1220.4
66	2.6	3.4	0.7	4.5	10.1	5	9	12.4	4.2	2.6	3.9	21.7	24.7
56	26.3	1.2	1.3	0.8	0.7	0.4	0.6	1.1	1.2	1.5	0.6	1.2	1.5
60	3.1	0.2	0.1	0.1	0.1	0	0	0	0	0.1	0.1	0.3	0.1
107	28	3	3.2	3.9	5.9	2.8	5.2	7.2	7.2	16.5	5.6	11.7	8.5
108	4	0.5	0.5	1	1.5	0.6	1.7	2.1	2	3.7	1.8	4.7	3.5
120	134.8	4.5	2.9	4.5	8.3	5.9	24.7	29.8	8.9	4.8	14.5	45.8	47.7
121	8.9	25.7	1.3	2	5.1	1.6	13	14.5	3.7	3.7	2.9	14.7	14.7
123	1.2	0.1	0.1	1.4	0.7	0.5	0.4	0.6	0.7	3.9	0.6	1.5	1.1
125	4	0.2	0.3	4.5	3.6	1.3	3	3.2	2.7	2.6	5.7	6	6.3
175	65.6	1132.1	1405.4	909.5	1172.9	1779.9	3190.3	2859.9	2738.6	6201.9	2054.4	3628.2	3975.9

Fig. 5 (cont.)

176	4.2	461.7	451.5	462.7	648	582.3	1468.5	1328.2	1008.2	2028.4	1355	1680.3	1811.6
179	1.7	624.6	1024.1	1073.4	1364	1546.4	1786.4	1724.1	1770.4	2607.9	2673.2	2460.3	2669.2
184	4.3	5196.8	4896.5	5121.9	5486.3	3679.6	36824.6	33113.1	13104.4	37389.4	24662.9	42443.9	42171.6
185	1.6	4.8	14.5	3.2	21.5	4.9	33.7	33	50.2	74.2	56.9	73.8	71.4
186	0.5	5194.2	1611.7	765.6	2709.3	7372.3	5344.8	4562	4651.3	5750.4	3704.2	6055.4	6334.4
187	2.7	5328	3966.2	1610	5294.5	6529.8	7967.2	6943.9	7118.5	14773.3	3666.3	11405.2	11418.3
188	5.6	6390.3	3460	1502	4641	4427.5	7234.5	6234.8	6145.6	13542	3592.1	10572.9	10923.1
189	6.2	7609.5	5197.9	2204.1	6187.6	3579.5	35879.3	31598.7	13079.7	37008.2	2760	39615.5	40537.4
190	2.5	10880.4	4862.3	2125.4	5394.2	2874.3	36573.1	9346.6	12773	34572.6	3285.6	41026	40887.3
191	2.3	4892.3	1997.9	1142.6	2662.7	2075.7	3560.9	3567.3	3066.3	6841.2	5178.7	5490.7	5708.4
192	6.9	9990	4507.4	4446.5	5393.4	3860.9	11662.5	10369.5	10102.2	14326.5	4104.4	12296.6	11545.4
193	121.2	2438.6	1287.4	585	2052	629.1	5473.4	6044.4	5979.5	6157	2120.6	8513.1	7666
194	58.8	8628.4	4889.1	1168.2	6404	5651.1	37926.7	35941.4	12265.6	38840.3	4135.3	41919.5	41775.2
195	562.4	243.7	762.8	114.9	639.4	966.1	1228.8	1240.2	1205.9	2639.1	1740.4	1940.1	2001.9
196	1.6	6.4	68.7	31.8	90	69.1	102.8	85.8	110.1	90.2	117.6	156.2	169.8
197	22.1	4368.8	4339	3389.6	4535	4484.1	10884.4	10398.9	10435.3	33967.2	2193.3	11129.9	10837.7
198	195.1	12524.7	5827.3	3983.6	4587	3742.1	37136.6	35636	11565.5	38413.9	2437.5	42362.3	42404.5
199	10.9	5764.6	4551	3627.8	4396.3	3551.8	34491.3	9491.2	12537.6	37792.8	2546.8	39605.4	40834.8
200	0.1	114.7	8.9	4.9	6	6.3	11.7	17.3	10.2	31.1	35.9	29.8	30.5
201	1.3	6527.6	2450.6	2110.2	2468.3	2617.8	5377.8	5102.6	4278.5	8880.2	4594.8	8114.1	8282.8
202	8	5243.8	3493.1	3560.2	3792.2	3755.9	9088.7	7836	6174.9	12697.9	5104.8	11532	11552.6

Fig. 5 (cont.)



$$\textbf{Residual Activity} = \frac{(heat-treated\ enzyme\ yield)}{(enzyme\ yield)}$$

Fig. 6