Identification of Human Butyrylcholinesterase Organophosphate-Resistant Variants through a Novel Mammalian Enzyme Functional Screen

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Supplemental Data

Table 1. Primers used in construction of libraries 0-6

Primer Name	Primer sequence	Note
KpnI_F	5' GC CGC GTC TTG GGT ACC ATG GAT AGC AAA GTC ACA ATC A 3'	Forward beginning primer
XhoI_R	5' CGC CAC TGC ATC TCG AGT TAG AGA CCC ACA CAA CTT TCT TTC TTG C 3'	Reverse ending primer
117_F	5' GG ATT TAT GGT GGT NNK TTT CAA ACT G GA ACA TC 3'[a]	For introducing site saturate mutations in 117 and 197 positions in lib 0.
117_R	5' GA TGT TCC AGT TTG AAA MNN ACC ACC ATA AAT C 3' [a]	
197_F	5' GTA ACT CTC TTT GGA NNK AGT GCA GGA GCA GC 3' [a]	
197_R	5' AGC TGC TCC TGC ACT MNN TCC AAA GAG AGT TAC A 3' [a]	
I_F	5' TAT GCA AAT TCT tgc tgt cag aac ata gat caa agt ttt cca ggc ttc cat gga tca gag atg tgg aac cca aac ACT GAC CTC 3' [b]	For introducing random mutations in 65-84 positions in lib 1.
I_R	5' TTC ACT GTC ttg tgg gtt acc cat ctc tga tcc atg gaa gcc tgg aaa act ttg atc tat gtt ctg aca gca AGA ATT TGC AT 3' [b]	
II_F	5' GCA CCT AAA CCA aaa aat gcc act gta ttg ata tgg att tat ggt ggt ggt ttt caa act gga aca tca tct tta cat gtt TAT GAT GGC AAG T 3' [b]	For introducing random mutations in 105-126 positions in lib 2.
II_R	5' GCC ATC ATA AAC atg taa aga tga tgt tcc agt ttg aaa acc acc acc ata aat cca tat caa tac agt ggc att ttt TGG TTT AGG TGC T 3' [b]	

III_F	5’ GGT GGA AAT CCT aaa agt gta act ctc ttt gga gaa agt gca gga gca gct tca gtt agc ctg cat ttg ctt tct CCT GGA AGC CAT T 3’ [b]	For introducing random mutations in 190-209 positions in lib 3.
III_R	5’ ATG GCT TCC AGG aga gaa caa atg cag gct aac tga tga agc tgc tcc act ctt tcc aaa gag tga tac act ttt AGG ATT TCC ACC A 3’ [b]	
IV_F	5’ CTT CTG AAT GAG gca ttt gtt gtc ccc tat ggg act cct ttg tca gta aac ttt ggt ccg acc gtg gat ggt GAT TTT CTC ACT 3’ [b]	For introducing random mutations in 277-296 positions in lib 4.
IV_R	5’ AGT GAG AAA ATC acc atc cac ggt cgg acc aaa gtt tac tga caa agg agt ccc ata ggg gac aac aaa tgc CTC ATT CAG A 3’ [b]	
V_F	5’ GTT AAT AAG GAT gaa ggg aca gct ttt tta gtc tat ggt gct cct ggc ttc agc aaa gat aac aat agt atc ATA ACT AGA AAA G 3’ [b]	For introducing random mutations in 325-344 positions in lib 5.
V_R	5’ TTT TCT AGT TAT gat act att gtt atc ttt gct gaa gcc agg agc acc ata gac taa aaa agc tgt ccc ttc ATC CTT ATT AAC 3’ [b]	
VI_F	5’ TCC TCC AAG CTT ccg tgg cca gaa tgg atg gga gtg atg cat ggc tat gaa att gaa ttt gtc ttt ggt TTA CCT CTG GAA 3’ [b]	For introducing random mutations in 428-448 positions in lib 6.
VI_R	5’ TTC CAG AGG TAA acc aaa gac ttc ttc ata gcc atg cat cac tcc cat cca ttc tgg cca cgg AAG CTT GGA GGA 3’ [b]	

[a]For introducing saturation mutation at target positions. N = A, T, C, or G; K = G or T; M = C or A

[b]For random mutation library construction in the target amino acid region listed, respectively. Lower case positions contain 96% indicated nucleotide and 4% N (N = A, T, C, or G). Underlined positions represent silent mutations introduced into the library to differentiate mutations introduced in the library from background original clones to facilitate the cloning.

[c]Human BChE accessin number is M16541 in the NCBI database.