

Supplementary Materials

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Boosting the Efficiency of Site-Saturation Mutagenesis for a Difficult-to-Randomize Gene by a Two-Step PCR Strategy

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Fig. S1. The plasmid pRSFduet-1 harboring P450-BM3 gene with a total length of 3222 bp. The heme domain sequence of P450-BM3 is underlined, the target positions are bold-marked.

Fig. S2. Sequencing chromatograms for NNK-based library Y51X generated from one-step PCR or two-step PCR processes.

Fig. S3. Sequencing chromatograms for NNK-based library S72X generated from one-step PCR or two-step PCR processes.

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Fig. S7. Comparison of number of colonies developed on the agar plates for different NNK-based SSM libraries. (A) SSM libraries constructed by partially over-lapped primer in one-step PCR process; (B) SSM libraries constructed by non-overlapping primer in two-step PCR process.

Fig. S8. Comparison of amino acid distribution for different positions (Y51, S72, L75, L437 and T438) for both one-step PCR and two-step PCR methods.

Table S1. Sequence of primers used in this study.

Table S2. Screening results of one-step PCR approach with partially overlapped primer for NNK-based site-saturation mutagenesis.

Table S3. Screening results of two-step PCR approach with non-overlapping primers for NNK-based site-saturation mutagenesis.

ATGAAACATCACCATCACCATCACCCCATGAGCGATTACGACATCCCCACTACTGAGAAT
CTTTATTTTCAGGGCGCAATTAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAA
AATTTACCGTTATTAACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAA
TTAGGAGAAATCTTTAAATTCGAGGCGCCTGGTTGGGTAACGCGCT**ACTTATCAAGTCAGC**
GTCTAATTAAGAAGCATGCGATGAATCACGCTTTGATAAAAACTT**ATCGCAAGCGCTTA**
AATACCTAAGAGAATTCCTAGGAGACGGGTTAGGATTAAGCTGGACGCATGAAAAAATT
GGAAAAAGCGCATAATATCTTACTTCCAAGCTTCAGTCAGCAGGCAATGAAAGGCTATC
ATGCGATGATGGTTCGATATCGCCGTGCAGCTTGTTCAAAAAGTGGGAGCGTCTAAATGCAG
ATGAGCATATTGAAGTACCGGAAGACATGACACGTTTAAACGCTTGATACAATTGGTCTTTG
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TTGCCACTCGTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTTCAAGAT
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GACGTTACCAAGTGAGTGAAGCAGACGCTCGCTTATGGCTGCAGCAGCTAGAAGAAAA
GGCCGATACGCAAAAAGACGTGTGGGCTGGGTAA

Fig. S1. The plasmid pRSFduet-1 harboring P450-BM3 gene (GenBank accession number: NZ_CP009920.1) with a total length of 3222 bp. The heme domain sequence of P450-BM3 is underlined, the target positions are bold-marked.

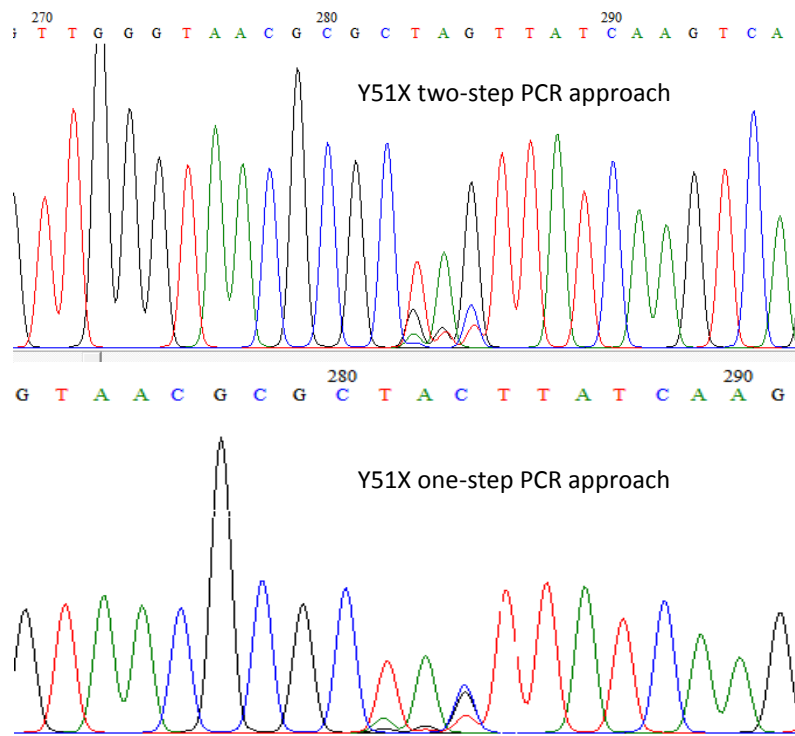


Fig. S2. Sequencing chromatograms for NNK-based library Y51X generated from one-step PCR or two-step PCR processes.

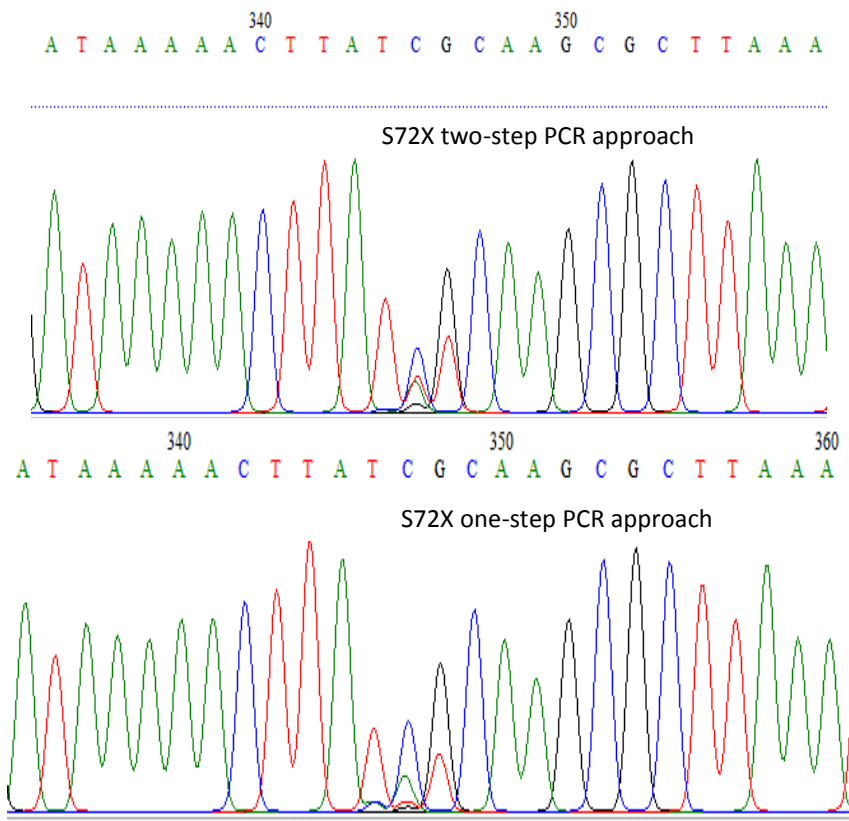


Fig. S3. Sequencing chromatograms for NNK-based library S72X generated from one-step PCR or two-step PCR processes.

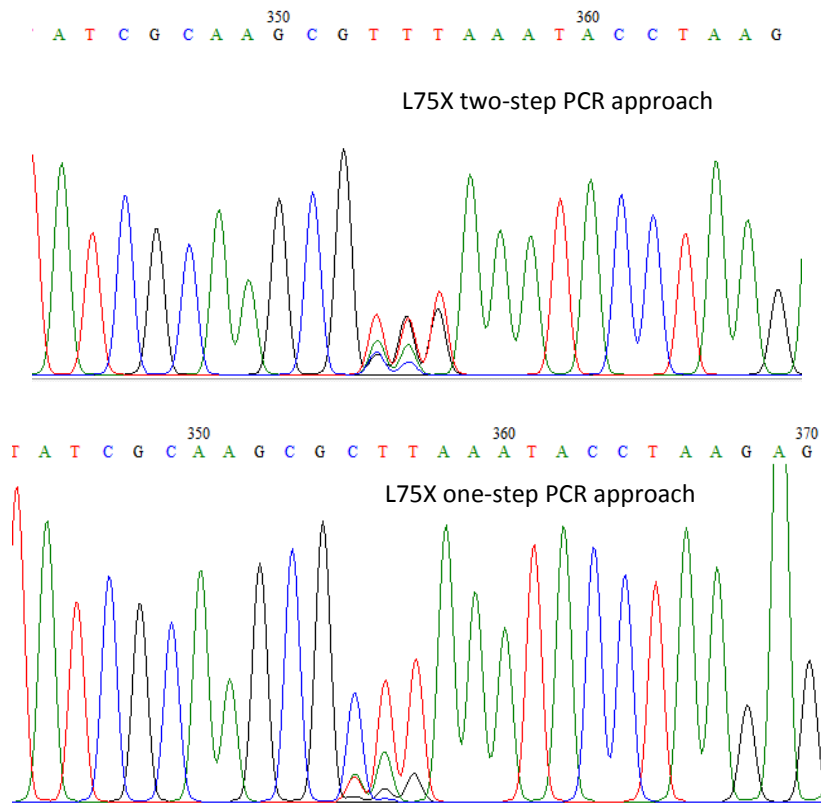


Fig. S4. Sequencing chromatograms for NNK-based library L75X generated from one-step PCR or two-step PCR processes.

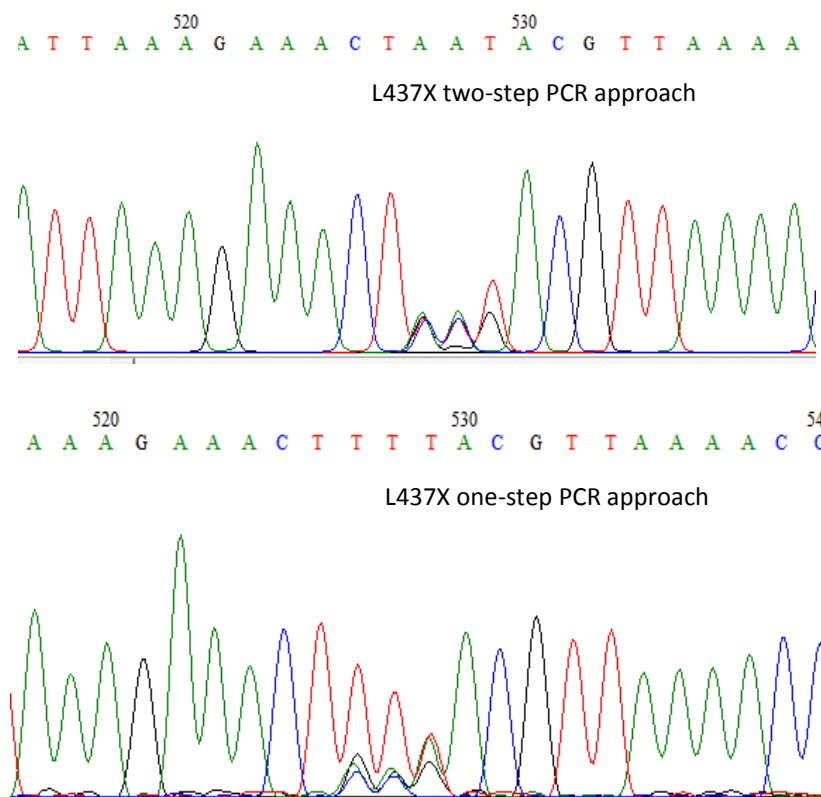


Fig. S5. Sequencing chromatograms for NNK-based library L437X generated from one-step PCR or two-step PCR processes.

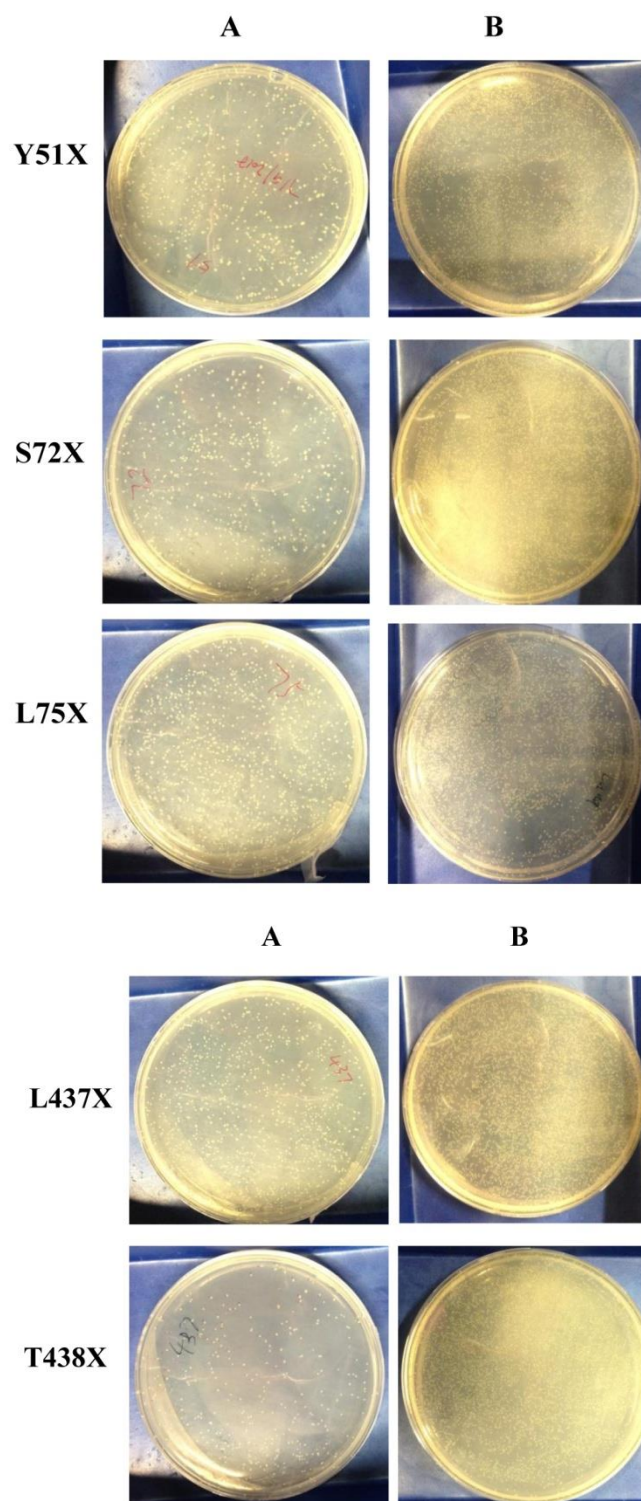


Fig. S7. Comparison of number of colonies developed on the agar plates for different NNK-based SSM libraries. (A) SSM libraries constructed by partially over-lapped primer in one-step PCR process; (B) SSM libraries constructed by non-overlapping primer in two-step PCR process.

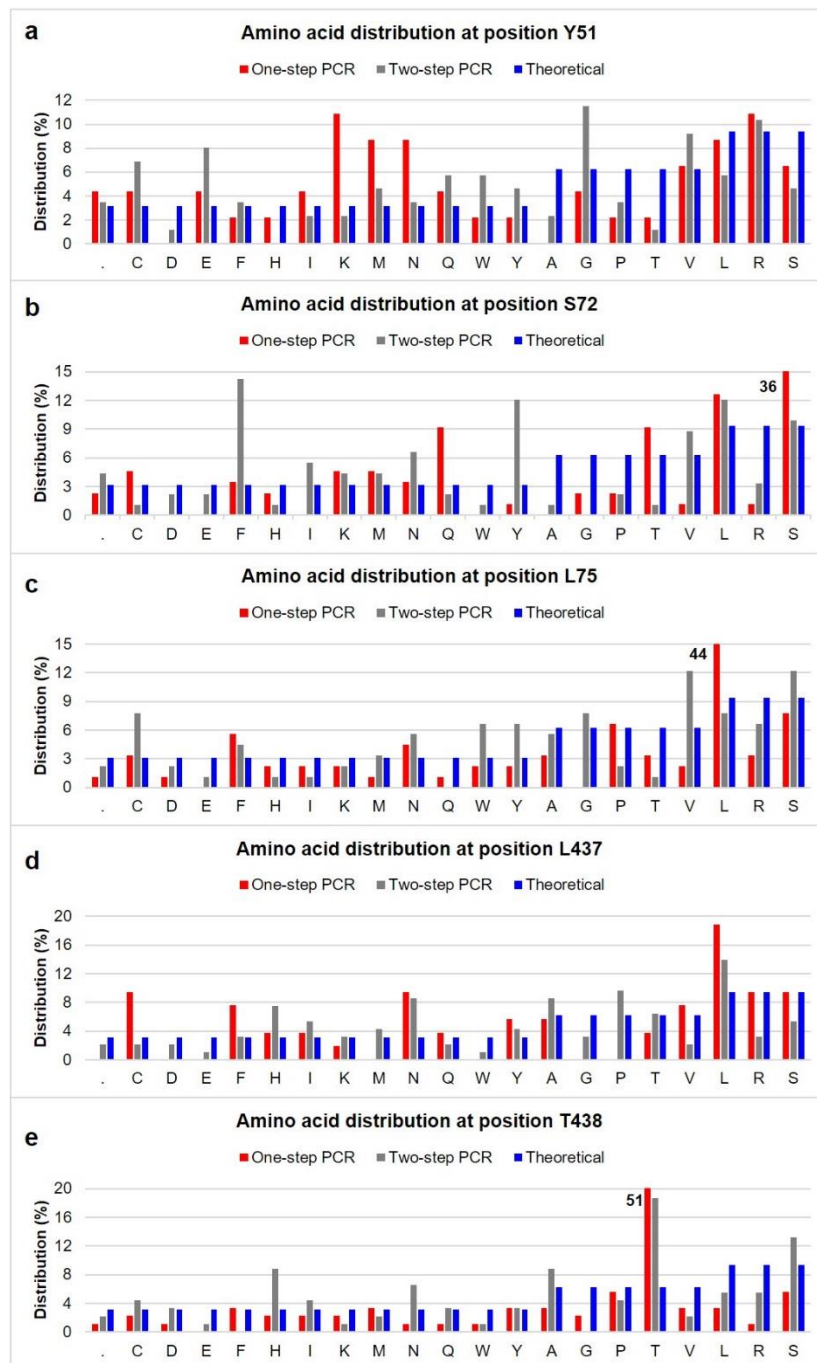


Fig. S8. Comparison of amino acid distribution for different positions (Y51, S72, L75, L437 and T438) for both one-step PCR and two-step PCR methods.

Table S1. Sequence of primers used in this study.

libraries	Wild-type code	Primer	Primer sequence from 5' to 3'	
One-step PCR	Y51X	Forward	GGGTAACGCGC N KTATCAAGTCAGCGTCTAATTAAG	
		Reverse	TGACTTGATAAM N NGCGGTTACCCAACCAGGCGCCTC	
	S72X	Forward	GATAAAAACTTAN N KCAAGCGCTTAAATACCTAAGAGAATTC	
		Reverse	TTAAGCGCTTGM N NTAAGTTTTATCAAAGCGTGATTCATC	
	L75X	Forward	CTTATCGCAAGCG N KAAATACCTAAGAGAATTCCTAGG	
		Reverse	CTTAGGTATTTM N NCGCTTGCATAAGTTTTATCAAAAGCG	
	L437X	Forward	TTAAAGAAACT N KACGTTAAAACCTGAAGGCTTTGTGG	
		Reverse	CAGGTTTTAACG M NNAGTTTCTTTAATATCCAGCTCGTAG	
	T438X	Forward	AAGAACTTTAN N KTTAAAACCTGAAGGCTTTGTGGTAAAG	
		Reverse	CAGGTTTTAAM N NTAAAGTTTCTTTAATATCCAGCTCGTAG	
	Two-step PCR	Y51X	Forward	GGGTAACGCGC N KTATCAAGTCAGCGTCTAATTAAG
			Reverse ^b	CATCGCTTTGTTCCACCGCTTGCTTTGCGATCTGC
S72X		Forward	GATAAAAACTTAN N KCAAGCGCTTAAATACCTAAGAGAATTC	
		Reverse ^b	CATCGCTTTGTTCCACCGCTTGCTTTGCGATCTGC	
L75X		Forward	CTTATCGCAAGCG N KAAATACCTAAGAGAATTCCTAGG	
		Reverse ^b	CATCGCTTTGTTCCACCGCTTGCTTTGCGATCTGC	
L437X		Forward ^c	GCAGATCGCAAAGCAAGCGGTGAACAAAGCGATG	
		Reverse	CAGGTTTTAACG M NNAGTTTCTTTAATATCCAGCTCGTAG	
T438X		Forward ^c	GCAGATCGCAAAGCAAGCGGTGAACAAAGCGATG	
		Reverse	CAGGTTTTAAM N NTAAAGTTTCTTTAATATCCAGCTCGTAG	

Bold-marked bases in the primers indicate positions that contain the mutagenic bases in the primer sequence.

^a N = A, C, G, T (equimolar amounts); K = G, T (equimolar amounts); M = A, C (equimolar amounts)

^b The same non-mutagenic reverse primer, which was shared for construction of libraries of Y51X, S72X and L75X.

^c The same non-mutagenic forward primer (reverse and complementary form of the primer mentioned in footnote b), which was shared for libraries construction of L72X and T438X.

Table S2. Screening results of one-step PCR approach with partially overlapped primer for NNK-based site-saturation mutagenesis.

	Y51X		S72X		L75X		L437X		T438X
1	AAG	1	AAG	1	AAG	1	AAG	1	AAG
2	AAG	2	AAG	2	AAG	2	AAT	2	AAG
3	AAG	3	AAG	3	AAT	3	AAT	3	AAT
4	AAG	4	AAG	4	AAT	4	AAT	4	ACG
5	AAG	5	AAT	5	AAT	5	AAT	5	ACG
6	AAT	6	AAT	6	AAT	6	AAT	6	ACG
7	AAT	7	AAT	7	ACG	7	ACG	7	ACG
8	AAT	8	ACG	8	ACG	8	ACG	8	ACG
9	AAT	9	ACG	9	ACT	9	AGG	9	ACG
10	ACG	10	ACG	10	AGG	10	AGG	10	ACG
11	AGG	11	ACG	11	AGT	11	AGT	11	ACG
12	AGG	12	ACT	12	ATG	12	AGT	12	ACG
13	AGG	13	ACT	13	ATT	13	ATT	13	ACG
14	AGT	14	ACT	14	ATT	14	ATT	14	ACG
15	ATG	15	ACT	15	CAG	15	CAG	15	ACG
16	ATG	16	AGG	16	CAT	16	CAG	16	ACG
17	ATG	17	AGT	17	CAT	17	CAT	17	ACG
18	ATG	18	AGT	18	CCG	18	CAT	18	ACG
19	ATT	19	ATG	19	CCG	19	CGG	19	ACG
20	ATT	20	ATG	20	CCG	20	CGG	20	ACG
21	ATT	21	ATG	21	CCG	21	CGT	21	ACG
22	CAG	22	ATG	22	CCT	22	CTG	22	ACG
23	CAG	23	CAG	23	CCT	23	CTT	23	ACG
24	CAT	24	CAG	24	CGG	24	CTT	24	ACG
25	CCT	25	CAG	25	CGT	25	GCT	25	ACG
26	CGT	26	CAG	26	CTG	26	GCT	26	ACG
27	CGT	27	CAG	27	CTG	27	GCT	27	ACG
28	CTT	28	CAG	28	CTT	28	GTG	28	ACG
29	GAG	29	CAG	29	CTT	29	GTT	29	ACG
30	GAG	30	CAG	30	CTT	30	GTT	30	ACG
31	GGT	31	CAT	31	CTT	31	GTT	31	ACG
32	GGT	32	CAT	32	CTT	32	TAT	32	ACG
33	GTG	33	CCG	33	CTT	33	TAT	33	ACG
34	GTG	34	CCT	34	CTT	34	TAT	34	ACG
35	GTT	35	CTG	35	CTT	35	TCG	35	ACG
36	TAC	36	CTG	36	CTT	36	TCT	36	ACG
37	TAC	37	CTT	37	CTT	37	TCT	37	ACG
38	TAC	38	CTT	38	CTT	38	TGT	38	ACG
39	TAC	39	CTT	39	CTT	39	TGT	39	ACG
40	TAC	40	GAG	40	CTT	40	TGT	40	ACG
41	TAC	41	GGG	41	CTT	41	TGT	41	ACG
42	TAC	42	GGT	42	CTT	42	TGT	42	ACG
43	TAC	43	TAG	43	CTT	43	TTA	43	ACG
44	TAC	44	TAG	44	CTT	44	TTA	44	ACG
45	TAC	45	TAT	45	CTT	45	TTA	45	ACG

46	TAC	46	TCG	46	CTT	46	TTA	46	ACG
47	TAC	47	TCG	47	CTT	47	TTA	47	ACT
48	TAC	48	TCG	48	CTT	48	TTA	48	ACT
49	TAC	49	TCG	49	CTT	49	TTA	49	ACT
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51	TAC	51	TCG	51	CTT	51	TTA	51	AGT
52	TAC	52	TCG	52	CTT	52	TTA	52	AGT
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54	TAC	54	TCG	54	CTT	54	TTA	54	ATG
55	TAC	55	TCG	55	CTT	55	TTA	55	ATG
56	TAC	56	TCG	56	CTT	56	TTA	56	ATT
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59	TAC	59	TCG	59	CTT	59	TTA	59	CAT
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62	TAC	62	TCG	62	GCT	62	TTA	62	CCG
63	TAC	63	TCG	63	GCT	63	TTA	63	CCG
64	TAC	64	TCG	64	GTG	64	TTA	64	CCG
65	TAC	65	TCG	65	GTT	65	TTA	65	CCG
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67	TAC	67	TCG	67	TAT	67	TTA	67	CTG
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69	TAC	69	TCG	69	TCT	69	TTA	69	GCG
70	TAC	70	TCG	70	TCT	70	TTA	70	GCG
71	TAC	71	TCG	71	TCT	71	TTA	71	GCT
72	TAC	72	TCG	72	TCT	72	TTA	72	GGG
73	TAC	73	TCG	73	TCT	73	TTA	73	GGG
74	TAC	74	TCG	74	TCT	74	TTA	74	GTG
75	TAC	75	TCG	75	TGG	75	TTA	75	GTT
76	TAC	76	TCG	76	TGG	76	TTA	76	GTT
77	TAC	77	TCT	77	TGT	77	TTA	77	TAG
78	TAC	78	TCT	78	TGT	78	TTA	78	TAT
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		92	TTT			92	TTT		
						93	TTT		

Table S3. Screening results of two-step PCR approach with non-overlapping primers for NNK-based site-saturation mutagenesis.

	Y51X		S72X		L75X		L437X		T438X
1	AAG	1	AAG	1	AAG	1	AAG	1	AAG
2	AAG	2	AAG	2	AAG	2	AAG	2	AAT
3	AAT	3	AAG	3	AAT	3	AAG	3	AAT
4	AAT	4	AAG	4	AAT	4	AAT	4	AAT
5	AAT	5	AAT	5	AAT	5	AAT	5	AAT
6	ACG	6	AAT	6	AAT	6	AAT	6	AAT
7	AGG	7	AAT	7	AAT	7	AAT	7	ACG
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80	TGT	80	TTT	80	TGT	80	TCT	80	TCT
81	TGT	81	TTT	81	TGT	81	TCT	81	TCT
82	TGT	82	TTT	82	TGT	82	TGG	82	TCT
83	TGT	83	TTT	83	TGT	83	TGT	83	TCT
84	TGT	84	TTT	84	TTG	84	TGT	84	TCT
85	TTG	85	TTT	85	TTG	85	TTA	85	TGG
86	TTG	86	TTT	86	TTG	86	TTA	86	TGT
87	TTG	87	TTT	87	TTG	87	TTA	87	TGT
88	TTG	88	TTT	88	TTG	88	TTA	88	TGT
89	TTT	89	TTT	89	TTT	89	TTA	89	TGT
90	TTT	90	TTT	90	TTT	90	TTG	90	TTG
91	TTT	91	TTT	91	TTT	91	TTT	91	TTG
		92	TTT	92	TTT	92	TTT		
					93	TTT			