Identification of *Thermus aquaticus* DNA polymerase variants with increased mismatch discrimination and reverse transcriptase activity from a smart enzyme mutant library

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Table S1. Oligonucleotide template used in this study

<u>Label</u>	Sequence
DNA	5'-d(ACC TTA GAC CCA CCC CTC CTG GCG GGC ACA CCC CCT ACT GAC CCA CCC TTG TGA ATT CTC AGT TAA TCC CGT
template	CTA CCA GTC TCA CCA AGG CC)3'
DNA	5'-d(AGC AAC AAA TAC TTC TAG GTT CAC CAC GTT TCC AAC TTT TAA ATC AGG AAT ATG GTT CAA CAG GAA TGG GAT
template_1	AAA ACT TTA ATA TTT TTA AAA ATT AAG AAA AAG GCC GGG CGC GGT GGC CCA CCC CTG TAA TCC CA) 3'

Table S2. Mutagenic oligonucleotides of active mutants

Table showing selected 12 target sites for site-specific shuffling to construct combinatorial library. Active single mutants of the respective target sites were included for shuffling and the Mutagenic oligonucleotide of 173 active mutants were pooled in shuffling reaction.

	N483	E507	S515	K540	A570	D578	V586	V783	I614	H639	F667	M747
Ala	√	✓	✓	✓	+	~	√	✓	✓	×	×	✓
Cys	✓	✓	×	×	~	~	√	~	~	×	×	✓
Asp	✓	✓	✓	×	×	+	×	✓	×	×	×	×
Glu	✓	+	✓	×	✓	✓	~	✓	~	×	×	~
Phe	×	✓	×	✓	✓	~	√	✓	✓	✓	+	~
Gly	✓	✓	✓	✓	✓	~	√	✓	✓	×	×	~
His	✓	×	✓	~	~	~	√	~	~	+	×	~
Ile	✓	✓	×	✓	✓	✓	~	✓	+	×	✓	~
Lys	✓	✓	✓	+	✓	✓	~	✓	~	×	×	~
Leu	✓	✓	×	✓	~	~	√	✓	✓	×	✓	~
Met	✓	✓	✓	~	~	~	√	~	~	×	✓	+
Asn	+	✓	✓	~	~	~	~	✓	~	✓	×	✓
Pro	✓	✓	×	×	×	×	×	×	×	×	×	~
Gln	✓	✓	✓	✓	✓	✓	~	✓	~	×	×	~
Arg	✓	✓	✓	✓	✓	✓	~	×	~	×	×	~
Ser	✓	✓	+	✓	✓	✓	~	✓	~	×	×	~
Thr	✓	✓	✓	✓	✓	✓	~	✓	~	✓	×	~
Val	✓	✓	✓	✓	✓	✓	+	+	✓	×	×	~
Trp	√	✓	×	√	√	✓	×	×	✓	×	×	~
Tyr	✓	✓	✓	✓	✓	✓	√	×	✓	✓	✓	✓
'√' A '+' W '×' Ir	Active m Vild type nactive v	utant inc	cluded in	n combir	natorial l	ibrary co	onstructi	on				



Figure S1.

Expression profile of target sites Q754 (upper panel) and R728 (lower panel). 19 single site mutants for each position were overexpressed, and heat denatured soluble fraction were analysed on SDS-PAGE gel. The mutants are represented in single letter notation of amino acids. "+" signifies wild type KlenTaq DNA polymerase, "-" plasmid control without insert.

	N483 (AAT) 630 640 650 660 670 680 E507 (GAA) 70
	·····
KTQ_pol	gcaggccatccgtttaatctgaatagccgtgatcagctggaacgtgttctgtttgatgaactgggtctgccagcaattggtaaaaccggaaaaaccggta
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48HH33	GCCGGCCATCCGTTAAAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCGTTAAAACCGGT
48HH47	GcCgGCCATCCgtT1aAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCGACAAAACCGGT
48HH45	gcCggCCATcCgntTaAACTGAATAgCCGTGATCAGCTgGAACgtGTTCTGTTTGATGAACTGGGtctgccAGCAATTGGTAAAACC <mark>aAA</mark> AAAAcCGGT
48HH36	GoCgGCCATCCGTTatCCTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAAccAAAAAACCGGT
48HH38	GeCgGCCATCCGtT1aAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCGGTA
48HH44	GCCGGCCATCCGTTTAAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCGACaAAAACCGGT
48HH39	GCCGGCCATCCGTTAAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCGGCGAAAACCGGT
48HH29	GCCGGCCATCCGTTTATCCTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAAcCGGT7
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48HH28	GCCGGCCATCCGTTTAAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCGAAAAACCGGT
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48HH34	GCCGGCCATCCGTTTAAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGCTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCAAAAACCGGT/
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48HH31	GCCGGCCATCCGtT AAACTGAATAGCCGTGatcAGCTGGAACGTGTTCTGTTTGATGAACTggGtCTGCCAGCAATTGGTAAAACCGCCAAAAACCGGT
48HH32	GCCGGCCATCCGtT AAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCGCCAAAAACCGGT
Clustal Consensus	** ******* ****************************
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	·····
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100000000	AACGTAGCACCAACGCAGCTCTGGAAGCCCTGCGTGAAGCACATCCGATTGTGGAAAAAATTCTGCAGTATCGCGAACTGACCAAACTGAAAAAGCAC
48HH40	
48HH40 48HH31	AACGTAGCACCAACGCAGCAGTTCTGGAAGCCCTGCGTGAAGCACATCCGATTGTGGAAAAAATTCTGCAGTATCgcgAACTGACCAACCTGAAAAGCAC
48HH40 48HH31 48HH32	AACGTAGCACCAACGCAGCAGTTCTGGAAGCCCTGCGTGAAGCACATCCGATTGTGGAAAAAATTCTGCAGTATCgcgAACTGACCAACCTGAAAAAGCAC AACGTAGCACCAACGCAGCTCTGGAAGCCCTGCGTGAAGCACATCCGATTGTGGAAAAAATTCTGCAGTATCGCGAACTGACCAACCTGAAAAGCAC

	810 820 830 840 850 860 870 A570 (GCA)
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KTQ_pol	<pre>ctatatcgatccgctgccggatctgattcatccgcgtaccggtcgtctgcatacccgttttaatcagaccgcaaccggcagccggtcgcctgagcagcagc</pre>
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Clustal Consensu	s ************************************
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18HH32	ATAGCCAGAAA BAACTGCGTGTTCTGGCCCATCTGAGCGGTGATGAAAATCTGATTCGCGTGTTTCAGGAAGGTCGCGATATTTACACCGAAACCGCAAG
Clustal Consensus	********
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ionno/	CTGGATGTTTGGTGTTCCGCGTGAAGCAGTTGATCCGCTGATGCGTGCG
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KTQ pol	${\tt ctgagccaggaactggcaattccgtacgaagaagcccaggcatttatcgaacgttattttccagagctttccgaaagttcgtgcctggattgaaaaaaccccaggcattgaaaaaaaccccaggcattgaaaaaaaccccaggcattgaaaaaaaccccaggcattgaaaaaaaccccaggcattgaaaaaaaccccaggcattgaaaaaaaccccaggcattgaaaaaaaccccaggcattgaaaaaaacccaggcattgaaaaaaaccccaggcattgaaaaaaacccaggcattgaaaaaaaccccaggcattgaaaaaaacccaggcattgaaaaaaaccccaggcattgaaaaaaacccaggcattgaaaaaaaa$
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48HH28	CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAACCC
48HH30	CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAAACCC
48HH37	CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAACCC
48HH34	CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAACCC
48HH40	CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAAACCC
48HH31	CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAACCC
48HH32	CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAACCC
Clustal Consensus	***************************************
	1210 1220 1220 1240 1250 1260 1270 1290 1290 1400
	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
KTO pol	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
KTQ_pol 48HH35	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
KTQ_pol 48HH35 48HH33	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
KTQ_pol 48HH35 48HH33 48HH47	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
KTQ_pol 48HH35 48HH33 48HH47 48HH45	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 tggaagaaggtcgtcgtcgtcgcggttatgttgaaacctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcagc TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH45	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 tggaagaaggtcgtcgtcgtcgcggttatgttgaaaccctgttggtcgtcgtcgtcgtcgtcggagcacgtgtaaaagcgttcgtgaagcagc TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTGAAGCAGCGTCGTGAAGCAGCGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH36 48HH38	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 tggaagaaggtcgtcgtcgtcgcggttatgttgaaacctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcagc TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGAGCTGTTGAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAGCACGGGTTAAAAGCGTTCGTGAAGCAGC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH36 48HH38 48HH44	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 tggaagaaggtcgtcgtcgtcgcggttatgttgaaacctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcagc TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH36 48HH38 48HH44 48HH39	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 tggaagaaggtcgtcgtcgtcgcggttatgttgaaacctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcagc TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGAGCACGGGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAGCACGGGTTAAAAGCGTTCGTGAAGCAGC
KTQ_pol 48HH35 48HH35 48HH47 48HH45 48HH36 48HH38 48HH44 48HH39 48HH29	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 tggaagaagtcgtcgtcgtcgcggttatgttgaaacctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcagc TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCATTGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH36 48HH38 48HH44 48HH39 48HH29 48HH29	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 tggaagaagtcgtcgtcgtcgcggttatgttgaaaccctgttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtggaagcacg TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGAGCACGGGTTTGGTAGGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH36 48HH38 48HH44 48HH39 48HH29 48HH29 48HH28	1310 1320 1330 1340 1350 1360 1370 1370 1380 1390 1400 tggaagaagtcgtcgtcgtcgcggttatgttgaaacctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcacg TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCTGTTGGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTGGTCGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGTCGAGCACGTGTTAAAAGCGTTCGTGAAGCAGC TGGAAGAAGGTCGTCGTCGCGCGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGCGCGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGGAGCACGGGCTCGTGGAAGCACGTGCTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH36 48HH38 48HH44 48HH39 48HH29 48HH29 48HH28 48HH30	1310132013301340135013601370138013901400tggaagaggtcgtcgtcgtcgcggttatgttgaaccctgttggtcgtcgtcgtcgtcgtcgtcgtcgaccggaccggaccggaccggttaacgcgtcgtgaagcac
KTQ_pol 48HH35 48HH35 48HH47 48HH45 48HH45 48HH36 48HH38 48HH44 48HH39 48HH29 48HH29 48HH28 48HH30 48HH30	1310 1320 1330 1340 1350 1360 1370 1380 1370 1380 1390 1400 tggaagaggtcgtcgtcgcgggttatgttgaaaccctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaagcgttcgtgaagcagc TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCATAGTTCCGGAAGCAAGC
KTQ_pol 48HH35 48HH33 48HH47 48HH45 48HH36 48HH36 48HH38 48HH44 48HH39 48HH29 48HH29 48HH28 48HH28 48HH30 48HH37 48HH37	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
KTQ_pol 48HH35 48HH35 48HH47 48HH45 48HH36 48HH36 48HH38 48HH44 48HH39 48HH29 48HH29 48HH29 48HH28 48HH30 48HH37 48HH34 48HH34	1310 1320 1330 1340 1350 1360 1370 1380 1370 1380 1390 1400 tggaagaggtcgtcgtcgtcgcggttatgttgaaccctgttggtcgtcgtcgtcgtcgtcgtcggaagcacgtgttaaaagcgtcgtgaagcacg TGGAAGAAGGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGTCGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCATGTTCCGGAACCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGGAACCAGGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGGCGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGTCGCGGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTCGTCGTCGTCGTCGTCGGAAGCACGTGTTAAAAAGCGTTCGTGAAGCACC TGGAAGAAGGTCGTCGC
KTQ_pol 48HH35 48HH35 48HH47 48HH45 48HH36 48HH38 48HH38 48HH38 48HH29 48HH29 48HH29 48HH29 48HH28 48HH28 48HH30 48HH31	1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
KTQ_pol 48HH35 48HH35 48HH47 48HH45 48HH36 48HH38 48HH38 48HH44 48HH39 48HH29 48HH29 48HH29 48HH28 48HH30 48HH37 48HH31 48HH31	1310 1320 1330 1340 1350 1360 1370 1380 1370 1380 1390 1400

	M747 (A	AIG)	101212	10.00	1012-10123	0.000	000000	100000	101222421 10		2010.0
	141	0	1420	1430	1440	1450	1460	1470	1480	1490	1500
WMO = -1											• 1
KIQ_POI	agaacguatg	Jeetttaa	itatgeegg	ttcagggcac	cgcagcagat	ctgatgaaa	etggeeatggt	taaactgtttt	egegtetggaa	gaaatgggtg	ICa
488835	AGAACGITGG	SCCTTTAA	ATATGCCGG	TTCAGGGCAC	TGCAGCAGCA	ATGATGAA	CTGGCCATGGI	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH33	AGAACGITGC	SCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAI	CTGATGAAA	CTGGCCATGGI	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH47	AGAACGIATG	SCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGI	TAAACTGTTTC	CCGCGTCTGGAA	GAAATGGGTG	CA
48HH45	AGAACGIATG	GCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGI	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH36	AGAACGIATG	SCCTTTA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGI	TAAACTGTTTC	CCGCGTCTGGAA	GAAATGGGTG	CA
48HH38	AGAACGIATG	SCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGI	TAAACTGTTTC	CCGCGTCTGGAA	GAAATGGGTG	CA
48HH44	AGAACGIATG	SCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGT	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH39	AGAACGTATG	GCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGI	TAAACTGTTTC	CCGCGTCTGGAA	GAAATGGGTG	CA
48HH29	AGAACGICTG	GCCTTTA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAAA	CTGGCCATGGT	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH46	AGAACGICTG	SCCTTTAA	TATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGT	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH28	AGAACGTTGG	SCCTTTAA	TATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGT	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH30	AGAACGTATG	GCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAAA	CTGGCCATGGI	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH37	AGAACGIATG	SCCTTTAA	ATATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAAA	CTGGCCATGGT	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH34	AGAACGICTG	SCCTTTAA	TATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAAA	CTGGCCATGGT	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH40	AGAACGTATG	GCCTTTAA	TATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAAA	CTGGCCATGGI	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH31	AGAACGTATG	SCCTTTA	TATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAAA	CTGGCCATGGI	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
48HH32	AGAACGICTG	SCCTTTA	TATGCCGG	TTCAGGGCAC	CGCAGCAGAT	CTGATGAAA	CTGGCCATGGI	TAAACTGTTTC	CGCGTCTGGAA	GAAATGGGTG	CA
Clustal Consensus	******	******	*******	********	******	**:	*********	********	*********	********	**
		V783 (GTT)	1500	1510		15.00	4550	1500		
	151	° .	1520	1530	1540	1550	1560	1570	1580	1590	1600
KTO pol									· · · · · · · · · ·	+	
A80035	CGTATCCTCC		CATGATGA		GAAGCACCGA	AAGAACGTC	CAGAAGCAGU			TCCAACCCT	
4000033	COTATOCIGO	ICCACCO I	CARCARCA		CAAGCACCGA	AAGAACGIG	CAGAAGCAGII				
400033	CGIAIGCIGC.	I GCAGGGI	CATGATGA		GAAGCACCGA	AAGAACGIG	CAGAAGCAGII	GCCCGCCTGGC		TGGAAGGCGI	
408847	CGTATGCTGC	I GCAGGT1	CATGATGA	ACTGGTGCTG	GAAGCACCGA	AAGAACGTG		GCCCGTCTGG	AAAAGAAGTTA	TGGAAGGCGT	
488845	CGTATGCTGC	rgCAGGT1	CATGATGA	ACTGGTGCTG	GAAGCACCGA	AAGAACGTG		GCCCGTCTGG	AAAAGAAGTTA	TGGAAGGCGT	
488836	CGTATGCTGC	rgCAGGTI	CATGATGA	ACTGGTGCTG	GAAGCACCGA	AAGAACGTG	CAGAAGCAGT	GCCCGTCTGG	AAAAGAAGTTA	TGGAAGGCGI	
48HH38	CGTATGCTGC	rgCAGGTI	CATGATGA	ACTGGTGCTG			~~ ~~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~				
48HH44					GAAGCACCGA	AAGAACGTG	CAGAAGCAGTI	GCCCGTCTGG	CAAAAGAAGTTA	TGGAAGGCGI	
1000 10	CGIAIGCIGC	IGCACGTI	CATGATGA	ACTGGTGCTG	GAAGCACCGA	AAGAACGTG AAAGAACGTG	CAGAAGCAGTI CAGAAGCAGTI	GCCCGTCTGG	CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGI	TTT
40HHS9	CGTATGCTGC	IGCACGTI IGCACGTI	CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA	AAGAACGTG AAAGAACGTG AAAGAACGTG	CAGAAGCAGTI CAGAAGCAGTI CAGAAGCAGTI	GCCCGTCTGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGT	TTT
48HH29	CGTATGCTGC CGTATGCTGC	rgcaggt1 rgcaggt1 rgcaggt1	CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA	AAGAACGTG AAAGAACGTG AAAGAACGTG AAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI	TTT TTT TTT
48HH29 48HH46	CGTATGCTGC CGTATGCTGC CGTATGCTGC	rgcaggt1 rgcaggt1 rgcaggt1 rgcaggt1 rgcaggg1	CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	AAGAACGTG AAAGAACGTG AAAGAACGTG AAAGAACGTG AAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG(GCCCGTCTGG(GCCCGTCTGG(GCCCGTCTGG(GCCCGTCTGG(CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI	TTT TTT TTT TTT
48HH29 48HH46 48HH28	CGTATGCTGC CGTATGCTGC CGTATGCTGC CGTATGCTGC	rgcaggtt Fgcaggtt Fgcaggtt Fgcagggt Fgcagggt	CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	AAAGAACGTG AAAGAACGTG AAAGAACGTG AAAGAACGTG AAAGAACGTG AAAGAACGTG	CAGAAGCAGTI CAGAAGCAGTI CAGAAGCAGTI CAGAAGCAGTI CAGAAGCAGTI CAGAAGCAGTI	GCCCGTCTGG(GCCCGTCTGG(GCCCGTCTGG(GCCCGTCTGG(GCCCGTCTGG(GCCCGTCTGG(CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI	TTT TTT TTT TTT TTT
48HH29 48HH46 48HH28 48HH30	CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC'	FGCAGGTT FGCAGGTT FGCAGGTT FGCAGGGT FGCAGGGT	CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI	TTT TTT TTT TTT TTT TTT
48HH29 48HH46 48HH28 48HH30 48HH37	CGTATGCTGC CGTATGCTGC CGTATGCTGC CGTATGCTGC CGTATGCTGC CGTATGCTGC	FGCAGGTT FGCAGGTT FGCAGGTT FGCAGGGT FGCAGGGT FGCAGGTT FGCAGGTT	CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG LAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG GCCCGTCTGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI TGGAAGGCGI	TTT TTT TTT TTT TTT TTT
48HH29 48HH46 48HH28 48HH30 48HH37 48HH34	CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC'	FGCAG GTT FGCAG GTT FGCAG GTT FGCAG GGT FGCAG GGT FGCAG GTT FGCAG GTT	CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	NAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG GCCCGTCTGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT	TTT TTT TTT TTT TTT TTT TTT
48HH29 48HH46 48HH28 48HH30 48HH37 48HH34 48HH40	CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC'	IGCAG GTI IGCAG GTI IGCAG GTI IGCAG GGI IGCAG GTI IGCAG GTI IGCAG GTI IGCAG GTI	CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	NAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG NAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG GCCCGTCTGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT	TTT TTT TTT TTT TTT TTT TTT TTT
48HH29 48HH26 48HH28 48HH30 48HH30 48HH37 48HH34 48HH40 48HH31	CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC'	IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGGT IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI	CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG GCCCGTCTGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG GCCCGTCTGGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT	TTT TTT TTT TTT TTT TTT TTT TTT TTT
48HH29 48HH46 48HH28 48HH30 48HH37 48HH37 48HH34 48HH40 48HH31 48HH32	CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC' CGTATGCTGC'	IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI IGCAGGTI	CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA CATGATGA	ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG ACTGGTGCTG	GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA GAAGCACCGA	IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG IAAGAACGTG	CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT CAGAAGCAGTT	GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG GCCCGTCTGG	CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA CAAAAGAAGTTA	TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT TGGAAGGCGT	TTT TTT TTT TTT TTT TTT TTT TTT TTT

Figure S2.

Multiple sequence alignment of random 20 clones from the combinatorial library constructed through shuffling of 173 PCR active mutants. Wild type KlenTaq DNA polymerase nucleotide sequence was also included in alignment. The mutational positions are labelled in single letter notation of amino acids with their respective wild type codons in brackets. Black solid line boxes with red shade highlights the change of codons in respective clones. Note all the positions got shuffled even for positions H639 and F667 with less number of active mutants. Ruler in the alignment is arbitrary number.



Figure S3.

SDS-PAGE gel of affinity purified wild type KlenTaq DNA polymerase and selected positive hits from allelic discrimination screening. Mutants were labelled as M1, M2, M3, M4, M5, M6 and wild type KlenTaq DNA polymerase as WT.



Figure S4.

Primer extension assay of selected mutants purified to homogenity along with wild type KlenTaq DNA polymerase. Mutants were labelled as M1, M2, M3, M4, M5, M6 and wild type KlenTaq DNA polymerase as WT. 30nM purified enzymes were applied in matched (G) and mismatched (A) reaction containing 100µM dNTPs in 1X KlenTaq reaction buffer and the primer extension was stopped after 10min (Left). Plot showing the percentage of mismatched (A) primer remain unextended in mismatch primer template reaction. Among mutants tested, mutant M4 had 40% of mismatch primer remained unextended whereas WT had only 16% primer unextended. M4 was characterized further and named as Mut_ADL (Right).

		10	20	30	40	50	60	70	80	90	100
Mat ADI NO		.									•
MUT_ADL_H2	arGAGAGGa	atereacca	TCACCATCAC	CATACGGATC			GCACCTTGGCC		AGGTGCATTT	STTGGTTTTGT	TC .
Clustal Consensus	********	*********	**********	*********	***********	*********	**********	*********	*********	*********	**
		110	120	130	140	150	160	170	180	190	200
				••••••							•
Mut_ADL_H2	TGAGCCGT	AAAGAACCG	ATGTGGGCAG	ATCTGCTGGC	ACTGGCAGCA(GCACGTGGTG	GTCGTGTTCA:	CGTGCACCG	GAACCGTATA	AAGCTCTGCGC	GA
KTQ_pol	tgagccgta	aaagaaccg	atgtgggcaga	atctgctggc	actggcagcag	gcacgtggtgg	gtcgtgttcat	cgtgcaccg	gaaccgtata	aagetetgege	:ga
Clustal Consensus	*******	******	*******	*******	********	*******	*******	*******	*******	*******	**
		210	220	230	240	250	260	270	280	290	300
Mut ADL H2	TCTGAAAG	AGCACGCG	GTCTGCTGGC	AAAAGATCTG		CACTGCGTGA	AGGTCTGGGA	TGCCTCCGG	TGATGATCC	GATGCTGCTGG	CA
KTO pol	tetgaaaga	agcacgcg	atctactaac	aaaagatctg	agcattetag	actgegtga	aggtetggga	tacctccaa	rtgatgatcc	atgetgetgg	rca
Clustal Consensus	*******	*******	********	*******	********	********	*******	*******	********	*******	**
		310	320	330	340	350	360	370	380	390	400
			•••••••••••	••••••			•• •••	• • • • • • •	••••••	•••••••	• [
Mut_ADL_H2	TATCTGCTC	GGATCCGAG	CAATACCACA	CCGGAAGGTG	TTGCACGTCG	TATGGTGGT	GAATGGACCG	AGAAGCAGG	CGAACGCGCA	G <mark>CACT</mark> GAGCGA	AC
KTQ_pol	tatetgete	ggat <mark>cc</mark> gag	caataccaca	ccggaaggtg	ttgcacgtcg	tatggtggt	gaatggaccga	agaagcagg	cgaacgcgca	g <mark>cactgagc</mark> ga	ac
Clustal Consensus	*******	******	*******	******	*******	******	******	*******	******	******	**
		410	420	430	440	450	460	470	480	490	500
Mut ADL H2	GTCTGTTT	CAAATCTG	TGGGGTCGTC'	TGGAAGGTGA	AGAACGTCTG	TGTGGCTGT	ATCGTGAAGT	GAACGTCCG	CTGTCTGCAG	TTCTGGCACAC	
KTO pol	atetattta	rcaaatcto	tagaatcatc	tggaaggtga	agaacgtctg	tataactat	atcotoaaot	gaacgtccg	tgtctgcag	ttctggcacac	at
Clustal Consensus	*******	******	*******	*******	******	*******	******	*******	*******	*******	**
		510	520	530	540	550	560	570	580	590	600
			•••••••••••	•• •••• ••			•• •••	• • • • • • •	••••••	•••••••••••••••••••••••••••••••••••••••	•
Mut_ADL_H2	GGAAGCAA	CGGTGTTC	GTCTGGATGT	IGCATATCTG	CGTGCACTGAC	SCCTGGAAGT	FGCAGAAGAA	ATTGCACGTC	FGGAAGCAGA	AGTTTTTCGTC	TG
KTQ_pol	ggaagcaad	ccggtgttc	gtctggatgt	tgcatatctg	cgtgcactgag	geetggaagt	tgcagaagaaa	attgcacgtc	tggaagcaga	agtttttcgtc	:tg
Clustal Consensus	*******	******	******	******	*******	*******	******	********	******	******	**
	1	⁶¹⁰ N483	3 620	630	640	650	660	670	680 E507	690	700
Mut ADI, H2	GCCGGCCA	CCCTTTAA	ACTGAATAGC	CGTGATCAGC	rggaacgtgt	CTGTTTGAT	TAACTGGGTC	GCCAGCAAT	TGGTAZAACC		TA
KTO pol	gcaggccat	ccgtttaa	tetgaatage	cotoatcage	tggaacgtgt	ctotttoat	gaactgggtc	gccagcaat	togtaaaacc	raaaaaaccoo	rta
Clustal Consensus	** *****	******	*******	*******	******	*******	******	*******	*****	******	**
		710 \$515	720	730	740	750	760	770	780 VEA	790	800
		· · <u>· · · </u> · ·		•• ••• ••							•
Mut_ADL_H2	AACGTAGC	ACCAACGCA	GCAGTT CTGG	AAGCCCTGCG	FGAAGCACAT	CGATTGTGG	AAAAAATTCTO	CAGTATCGC	GAACTGACCG	GTCTGAAAAGC	:AC
KTQ_pol	aacgtagca	accagegea	g <mark>cagttct</mark> gga	aagccctgcg	tgaagcacato	cgattgtgga	aaaaattcto	gcagtatcgc	gaactgacca	aactgaaaago	ac
Clustal Consensus	*******	******	********	********	********	********	*******	********	*******	******	:**

	810	820	830	840	850	860	870	4570 ⁸⁸⁰	890	900
Mut ADI US								CCCAR CCCC		
MUC_ADL_HZ	CTATATCGATCC	GCTGCCGGATCT	GATTCATCCG		TUTGCATAC			CUGAAACCGG		
Clustal Consensus	***********	**********	**********	***********	*********	***********	********	*** *****	***********	*****
Ciustai Consensus										
	- 70 010		00 000	0.4.0	050	0.00	070	0.00	000	1000
D	5/8 ⁹¹⁰	920 V5	86 930	940	950	960	970	980	990	
Mut ADL H2	GGTCCGAATCTG	CAGAATATTCCC	GGTCGTACAC	GCTGGGTCAG	CGTATTCGT	GTGCAtTTA	TGCAGAAGA	AGGTTGGCTG	CTGGTTGCACT	GGATT
KTQ pol	gatecgaatetg	cagaatattccd	gttcgtacaco	getgggtead	cgtattcgt	gtgcatttat	tgcagaaga	aggttggctg	ctggttgcact	ggatt
Clustal Consensus	* *******	*****	* *******	*********	******	*********	*******	*******	*********	****
	1614 1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
	· · · · · · · · · · · · · · · · · · ·	•••	• • • • • • • •	• • • • • • • •			••••	• • • • • • • •	• • • • • • • •	••••
Mut_ADL_H2	ATAGCCAGATGG	AACTGCGTGTTC	TGGCCCATCT	GAGCGGTGAT	AAAATCTGA	TTCGCGTGTTT	CAGGAAGGT	CGCGATATTC	ATACCGAAACO	GCAAG
KTQ_pol	atagccagatag	aactgcgtgttc	tggcccatcto	gagcggtgatg	Jaaaatctgal	ttcgcgtgttt	caggaaggt	cgcgatattc	ataccgaaaco	gcaag
Clustal Consensus	*******	*****	*******	* * * * * * * * * * * *	*******	*******	******	******	*********	*****
	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
Mut ADL H2	CTGGATGTTTGG	TGTTCCGCGTGA	AGCAGTTGAT		TCGTGCAGC	AAAAACCATT/	ATTTTGGGG	TGCTGTATGG	TATGAGCGCAC	ATCGT
KTO pol	ctggatgtttgg	tgttccgcgtga	agcagttgat	coctgatoc	tcgtgcage	aaaaccatta	atttgggg	toctotatoo	tatgagegeag	atcot
Clustal Consensus	*****	*****	*******	*********	*******	*******	*******	******	*********	****
	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
			• • • • • • • •						• • • • • • • •	••••
Mut_ADL_H2	CTGAGCCAGGAA	CTGGCAATTCCG	TACGAAGAAG	CCAGGCATTI	ATCGAACGT	TATTTTCAGAC	CTTTCCGAA	AGTTCGTGCC	TGGATTGAAAA	AACCC
KTQ_pol	ctgagccaggaa	ctggcaattccg	tacgaagaago	cccaggcattt	atcgaacgt	tattttcagag	gettteegaa	agttcgtgcc	tggattgaaaa	aaccc
Clustal Consensus	******	*****	******	*********	********	******	******	******	**********	****
	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400
Mut ADL H2	TGGAAGAAGGTC	GTCGTCGCGGTT	ATGTTGAAAC	CTGTTTGGT	GTCGTCGTT	ATGTTCCGGA	CTGGAAGCA	CGTGTTAAAA	GCGTTCGTGAZ	GCAGC
KTO pol	tggaagaaggtc	atcatcacaatt	atottoaaaco	ctatttaat	atcatcatt	atgttccggat	ctogaagca	cotottaaaa	gcgttcgtgaa	acage
Clustal Consensus	*****	*****	*******	*********	*******	*******	*******	******	*********	****
	1410	1420	1430	1440	1450	1460	1470	1480	1490	1500
				• • • • • • • •		• • • • • • • •			• • • • • • • •	••••
Mut_ADL_H2	AGAACGTATGGC	CTTTAATATGCC	GGTTCAGGGC	ACCGCAGCAG	TCTGATGAA	ACTGGCCATGO	TTAAACTGT	TTCCGCGTCT	GGAAGAAA <mark>T</mark> GO	GTGCA
KTQ_pol	agaacgtatggc	ctttaatatgcc	ggttcagggca	accgcagcaga	atctgatgaaa	actggccatgg	gttaaactgt	tteegegtet	ggaagaaatgo	gtgca
Clustal Consensus	******	*******	*******	*********	******	*********	******	******	********	*****

	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600
										· · · ·
Mut ADL H2	CGTATGCTGCTGCAG	GTTCATGAT	GAACTGGTGC	FGGAAGCACC	GAAAGAACGT(GCAGAAGCAG	TTGCCCGTCTO	GCAAAAGAAG	TTATGGAAGG	CGTTT
KTQ pol	cgtatgctgctgcag	gttcatgat	gaactggtgc	tggaagcacc	gaaagaacgto	gcagaagcag	ttgeeegtete	gcaaaagaad	uttatggaagg	cqttt
Clustal Consensus	****	*******	******	********	*******	*******	*********	******	*******	****
	1610	1620	1630	1640	1650	1660	1670			
Mut ADL H2	Atccgctggcagttc	CGCTGGAAG	TGAAGTTGG	TATTGGTGAA	GATTGGCTGT	CTGCaAAAGa	AtaaAA			
KTO pol	atccgctggcagttc	cgctggaag	ttgaagttgg	tattggtgaa	gattggctgt	ctgcaaaagaa	a			
Clustal Consensus	*****	********	*******	*******	*******	*******	*			

Figure S5.

Multiple sequence alignment of wild type KlenTaq DNA polymerase and Mut_ADL. The difference in nucleotide sequence at the target sites are marked in red box with solid black line. The position of target residues that got mutated were marked above the red boxes. The numbering in alignment ruler are arbitrary.



Α



Figure S6. Sanger sequencing analysis of amplified products. The products that were amplified by respective enzymes (WT, Mut_ADL and Mut_RT) were presented in sequence alignment. Section of electropherogram of sequencing reactions with forward primer [5'-d(CTT GGT GAG ACT GGT AGA CG)-3'] (above sequence alignment) and reverse primer [5'-d(TTA GAC CCA CCC CTC CTG GCG)-3'] (below sequence alignment) respectively were shown. A) WT – Amplificate from DNA dependent DNA synthesis B) Mut_ADL - Amplificate from DNA dependent DNA synthesis.



Figure S7. Single nucleotide misinsertion assay for wild type KlenTaq DNA polymerase and Mut_ADL. The reaction (20 μ l) contained 1X KlenTaq reaction buffer, 250 nM template (5'-d(GAG GTA GCG AGA AAA GAA ACT GGA GAA ACT CGG TG)3'), 150 nM of 5' radioactively labelled Match primer (5'-d(GTT TCT CCA GTT TCT TTT CTC G)3'). After pre-incubation at 95°C for 2 min, the reaction mixture was cooled to 55°C. The reaction was performed in presence of 10 nM of enzyme and individual all four nucleotides (0, 200, 400, 600, 1000 μ M). The reactions were allowed to proceed for 10 min and terminated with 2 volumes of stopping solution (80% [v/v] formamide, 20 mM EDTA, 0.25% [w/v] bromophenol blue, 0.25% [w/v] xylene cyanol). 'P' indicates the unextended primer and 'P + 1' denotes the primer extended by one nucleotide, and so forth. Full-length gel is given in Supplementary Fig. S18.



Figure S8. Improved allelic discrimination on different sequence context. (**A**) Sequence of employed primer-template pair. The 3' end of primer either terminates with a matched (G) or mismatched (A) nucleotide. Primer extension reaction was performed as indicated in material section for allelic discrimination, by employing the indicated DNA substrates along with wild-type (WT) and mutant DNA polymerase Mut_ADL, respectively. Reactions were stopped at different time points, as indicated. Full-length gel is given in Supplementary Fig. S19. (**B**, **C**) Real time PCR reaction (20 µI) contained 1X KlenTaq reaction buffer, 200 µM dNTPs,50 mM betaine (Sigma), 50 nM Taq DNA polymerase aptamer [5'-d(CGA TCA TCT CAG AAC ATT CTT AGC GTT TTG TTC TTG TGT ATG ATC G)3'] and 0.6X SYBR[®] Green I (Sigma). As template, 139nt [sequence of promoter region of Homo sapiens Nanog homeodomain transcription factor (NANOG) gene]¹ 100 pM DNA template_1 (sequence in Supplementary Table S1) was used with 200 nM primer either Match_case [5'-d(ATT ACA GGG GTG GGC CAC CG) 3'] or Mismatch_case [5'-d(ATT ACA GGG GTG GGC CAC CG) 3'] and 200 nM Rev_primer [5'-d(GGT TCA CCA CGT TTC CAA CTT TTA AAT CAG G) 3'] in two separate reactions. After an initial denaturation at 95°C for 1 min, two step thermal cycling was repeated for 35 cycles (95°C for 10 s and 68°C for 60 s) and the reaction was analysed by real-time PCR curves and melting curve measurement within the Light Cycler[®] 96 instrument (Roche). Real time PCR experiments with purified enzymes (200 nM), showing the discrimination property of (**B**) wild-type KlenTaq DNA polymerase and (**C**) mutant DNA polymerase Mut_ADL respectively. Insert plot showing their corresponding melting peaks.

	10	20	30	40	50	60	70	80	90	100
Mart DW			•••• ••••				•••• ••••			
MUT_RT	atgagaggatetea	ccatcaccatc	accatacgga	tecgeatgea	agcactggaad	gaagcacctto	geeteegeet	gaaggtgcat	ttgttggtt	ttgtte
KTQ_pol	atgagaggatetea	ccatcaccatc	accatacgga	teegeatgea	agcactggaag	gaagcacctto	geeteegeet	gaaggtgcat	ttgttggtt	ttgttc
Ciustai Consensus										
	110	120	130	140	150	160	170	180	190	200
M11+ DT	+gaggggtaaagaa					· · · · · · · ·			taaagatata	
KTO pol	tgageegtaaagaa		agatetgetg	gcactggcag	reageacgtg	ataataatat	catogtgcac	oggaaccyta	taaagetet	acacaa
Clustal Consensus	**********	*********	*********	*********	*********	******	*********	********	********	******
Crustar Consensus										
	21.0	220	220	240	250	260	270	200	200	200
	210	220	230	240	250	260 	270	280	290	300
Mut RT	tetgaaagaagcac	acaatetacta	gcaaaagatc	tgagcgttct	ggcactgcgt	tgaaggtetge	gactgectec	gggtgatgat	ccgatgctg	ctooca
KTO pol	tctgaaagaagcac	acaatetacta	gcaaaagato	tgagcgttct	ggcactgcgt	tgaaggtctg	gactgcctcc	gggtgatgat	ccgatgetg	ctooca
Clustal Consensus	******	*******	******	********	*******	*********	******	******	******	*****
	310	320	330	340	350	360	370	380	390	400
		1				1 • • • • 1 • • • • 1				1
Mut RT	tatctgctggatcc	gagcaatacca	caccggaagg	tgttgcacgt	cgttatggt	ggtgaatggad	cgaagaagca	ggcgaacgcg	cagcactgad	gcgaac
KTQ pol	tatctgctggatcc	gagcaatacca	caccggaagg	tgttgcacgt	cgttatggt	ggtgaatggag	cqaaqaaqca	ggcgaacgcg	cagcactgad	gcgaac
Clustal Consensus	******	*******	*******	*******	*********	*******	******	*******	******	*****
	410	420	430	440	450	460	470	480	490	500
										• • • •
Mut_RT	gtctgtttgcaaat	<mark>ctgtggggtc</mark> g	r <mark>tctggaaggt</mark>	gaagaacgto	etgetgtgget	tgtatcgtgaa	gttgaacgto	egetgtetge	agttetggea	acacat
KTQ_pol	gtctgtttgcaaat	<mark>ctgtggggtc</mark> g	r <mark>tet</mark> ggaaggt	gaagaacgto	etgetgtgget	tgtatcgtgaa	gttgaacgto	egetgtetge	agttetggea	acacat
Clustal Consensus	*****	******	*****	*******	*********	*******	******	*****	*****	*****
	510	520	530	540	550	560	570	580	590	600
	••••	• • • • • • • •								• • • •
Mut_RT	ggaagcaaccggtg	ttcgtctggat	gttgcatatc	tgcgtgcact	gagcctggaa	agttgcagaag	Jaaattgcacg	tctggaagca	gaagttttt	cgtctg
KTQ_pol	ggaagcaaccggtg	ttcgtctggat	gttgcatatc	tgcgtgcact	gageetggaa	agttgcagaag	Jaaattgcacg	tetggaagea	gaagttttt	cgtctg
Clustal Consensus	*****	******	******	******	*********	*******	*******	******	*****	*****
	N	183							F507	
	610	620	630	640	650	660	670	680	690	700
	••••	••••								• • • •
Mut_RT	GCCGGCCATCCGtT	TAAACTGAATA	GCCGTGATCA	GCTGGAACGt	GTTCTGTTTC	GATGAACTGGG	TCTGCCAGCA	ATTGGTAAAA	COAAAAAAA	CCGGTA
KTQ_pol	gcaggccatccgtt	taatetgaata	gccgtgatca	.g <mark>ct</mark> ggaacgt	gttetgttte	gatgaactggg	rtctgccagca	attggtaaaa	cogaaaaaaa	ccggta
Clustal Consensus	**.********	***:******	*****	********	********	*******	******	******	**.*****	*****
								ĸ	540	
	710	720	730	740	750	760	770	780	790	800
Mut_RT	AACGTAGCaCCAGC	GCAGCAGTTCT	GGAAGCCCTG	CGTGAAGCAC	CATCCGATTG	IGGAAAAAATT	CTGCAGTATC	GCGAACTGAC	GTACCTGAA	AAGCAC
KTQ_pol	aacgtagcaccagc	gcagcagttct	ggaagccctg	cgtgaagcad	catecgattg	tggaaaaaatt	ctgcagtato	gcgaactgac	qaaactgaaa	aagcac
Clustal Consensus	*********	********	*******	********	**********	*********	*******	*******	* :* . *****	*****

	810	820	830	840	850	860	870	880	890	900
MUL_RT	CTATATCGATCCGCT	GCCGGATCTG	ATTCATCCGC	GTACCGGTCG	TCTGCATACO	CGTTTTAAT	CAGACCGCAAC	CGCAACCGGT	CGCCTGAGCA	GCAGC
Clustal Consensus	*************	*********	*********	**********	*********	*******	***********	********	*******	*****
Ciustai Consensus										
	010	000 V58	36 • • • •	0.4.0	0.5.0	0.00	070	000	000	1000
	910	920	930	940	950	960	970 	980		
Mut RT	GATCCGAATCTGCAG	AATATTCCCG	GTCGTACAC	GCTGGGTCA	CGTATTCGT	CGTGCATTTA	TGCAGAAGA	GGTTGGCTGC	TGGTTGCACT	GGATT
KTQ pol	gatecgaatetgeag	aatattccgg	ttegtacaco	getgggtead	gcgtattcgto	cgtgcattta	ttgcagaagaa	agttggctgc	tggttgcact	ggatt
Clustal Consensus	*****	*****	*******	*********	*******	********	*********	*******	******	****
	I614 1010	1020	1030	1040	1050	1060	1070	1080	1090	1100
	· · · · · · · • • • • •									
Mut_RT	ATAGCCAGAAAGAAC	TGCGTGTTCT	GGCCCATCTO	GAGCGGTGAT (GAAAATCTGA	TTCGCGTGTT	CAGGAAGGT	GCGATATTCA	TACCGAAACC	GCAAG
KTQ_pol	atagccagatagaac	tgcgtgttct	ggcccatcto	gagcggtgatg	jaaaatctga	ttcgcgtgtt	tcaggaaggto	.gcgatattca	taccgaaacc	gcaag
Clustal Consensus	*****	******	********	*********	*******	******	*********	******	******	****
	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
MUE_RT	CTGGATGTTTGGTGT	TCCGCGTGAA	GCAGTTGATC	CGCTGATGCG	FCGTGCAGC	AAAAACCATT	AATTTTGGGGG	GCTGTATGGT	ATGAGCGCAC	ATCGT
Clustel Conconcus		teegegtgaa	gcagttgatt	egetgatge	Jtegtgeagea	44444CC4LL	***********	.getgtatggt	atgagegeae	ategt
Ciustai consensus			~~~~~~~						~~~~~~~	
	1010	1000	1000	1040	1050	1000	1070	1000	1000	1000
	1210	1220	1230	1240	1250	1260	1270 	1280	1290	1300
Mut RT	CTGAGCCAGGAACTG	GCAATTCCGT	ACGAAGAAG	CCAGGCATT	ATCGAACGT	TATTTTCAGA	CTTTCCGAAZ	GTTCGTGCCT	GGATTGAAAA	AACCC
KTO pol	ctgagccaggaactg	gcaattccgt	acgaagaag	ccaggcattt	atcgaacgt	tattttcaga	gettteegaaa	attcatacct	ggattgaaaa	aaccc
Clustal Consensus	*******	******	********	*********	********	*******	*********	******	******	****
						_				
	1310	1320	1330	1340	1350	1360 E	734 ₁₃₇₀	1380	1390	1400
Mut_RT	TGGAAGAAGGTCGTC	GTCGCGGTTA	TGTTGAAAC	CTGTTTGGT	GTCGTCGTT	ATGTTCCGGA	FCTGGAGGCAG	GTGTTAAAAG	CGTTCGTGAA	GCAGC
KTQ_pol	tggaagaaggtcgtc	gtcgcggtta	tgttgaaaco	ctgtttggto	gtcgtcgtta	atgttccgga	tetggaagead	gtgttaaaag	cgttcgtgaa	gcagc
Clustal Consensus	******	******	********	*********	********	*******	*****	*******	******	****
	1410	1420	1430	1440	1450	1460	1470	1480	1490	1500
MUT_RT	AGAACGTATGGCCTT	TAATATGCCG	GTTCAGGGC	ACCGCAGCAG	ATCTGATGAA	ACTGGCCATG	JTTAAACTGT1	TCCGCGTCTG	GAAGAAATGG	GTGCA
KTQ_pol	agaacgtatggcctt	taatatgccg	gttcagggca	accgcagcaga	atctgatgaaa	actggccatg	gttaaactgtt	teegegtetg	gaagaaatgg	gtgca
Ciustal Consensus	*******	*******	********	***********	*********	********	***********	********	********	****

	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600
Mut RT	CGTATGCTGCTGCAGG	TTCATGATG	GAACTGGTGCI	GGAAGCACC	GAAAGAACGTG	CAGAAGCAG	TGCCCGTCTC		TTATGGAAGG	CGTTT
KTQ pol	cgtatgctgctgcage	ttcatgato	aactggtgct	ggaagcacco	gaaagaacgtg	cagaagcagt	tgcccgtctc	gcaaaagaag	ttatggaagg	cqttt
Clustal Consensus	****	*******	*******	********	*******	********	******	******	******	****
	1610	1620	1630	1640	1650	1660				
Mut RT	ATCCGCTGGCAGTTCCGCTGGAAGTTGAAGTTGGTGAAGATTGGCTGTCTGCaAAAGaAtaa									
KTQ pol	atecgctggcagttccgctggaagttgaagttggtgtgtgtggtgtctgcaaaagaa									
Clustal Consensus	***************************************									

Figure S9.

Nucleotide sequence alignment of wild type KlenTaq DNA polymerase and Mut_RT. Red boxes with solid black line marks the target sites of mutations and the change in codon. The numbering in alignment ruler are arbitrary. Position E734 marked in green box without border line, was not a target site for mutagenesis and a silent mutation ($A \rightarrow G$) had occurred during PCR amplification.



Figure S10

SDS-PAGE of Ni-NTA purified 6X-His tagged WT (left) and Mut_RT (middle).





Specific activities. (A) dNTP conversion rate plotted against the amount of Mut_RT applied on RNA template. dNTP conversion rate of Mut_RT (B) and wild type KlenTaq DNA polymerase (C) plotted against the amount of enzyme applied on DNA template. Error bars describe SD (n = 3).





Figure S12. Sanger sequencing analysis of Mut_RT amplified products. A) Amplificate from DNA dependent DNA synthesis was presented in sequence alignment. Section of electropherogram of sequencing reactions with forward primer [5'-d(CTT GGT GAG ACT GGT AGA CG)-3'] (above sequence alignment) and reverse primer [5'-d(TTA GAC CCA CCC CTC CTG GCG)-3'] (below sequence alignment) respectively were shown. B) Amplificate from RNA dependent DNA synthesis. Sequenced with primers RT_For (forward primer) and RT_Rev (Reverse primer) of MS2 template.

Figure S13 (Full image of 4A)





Figure S14 (Full image of 5C)

Figure S15 (Full image of 5E)



Figure S16 (Full image of 6B)



Figure S17 (Full image of 6D)



Figure S18 (Full image of S7)



Figure S19 (Full image of S8)



Reference:

1. Li,J., Han,C., Deng,J. and Wang,H. Cloning and Preliminary Characterization of Human Nanog Promoter, Unpublished, GenBank: GU433186.1 GI: 289547169, Animal Biotechnology, Northwest A&F University, Taicheng Road No. 3, Yangling, Shaanxi 712100, China