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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Supplementary Data

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Figure S11. Specific activities of KlenTaq DNA polymerase variants on DNA or RNA template

Figure S12. Sanger sequencing analysis of Mut_RT amplified products

Table S1. Oligonucleotide template used in this study

Label	**Sequence**
DNA template	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 CTA CCA GTC TCA CCA AGG CC)3'
DNA template_1	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 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	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓

'✓' Active mutant included in combinatorial library construction
'+' Wild type
'×' Inactive variant

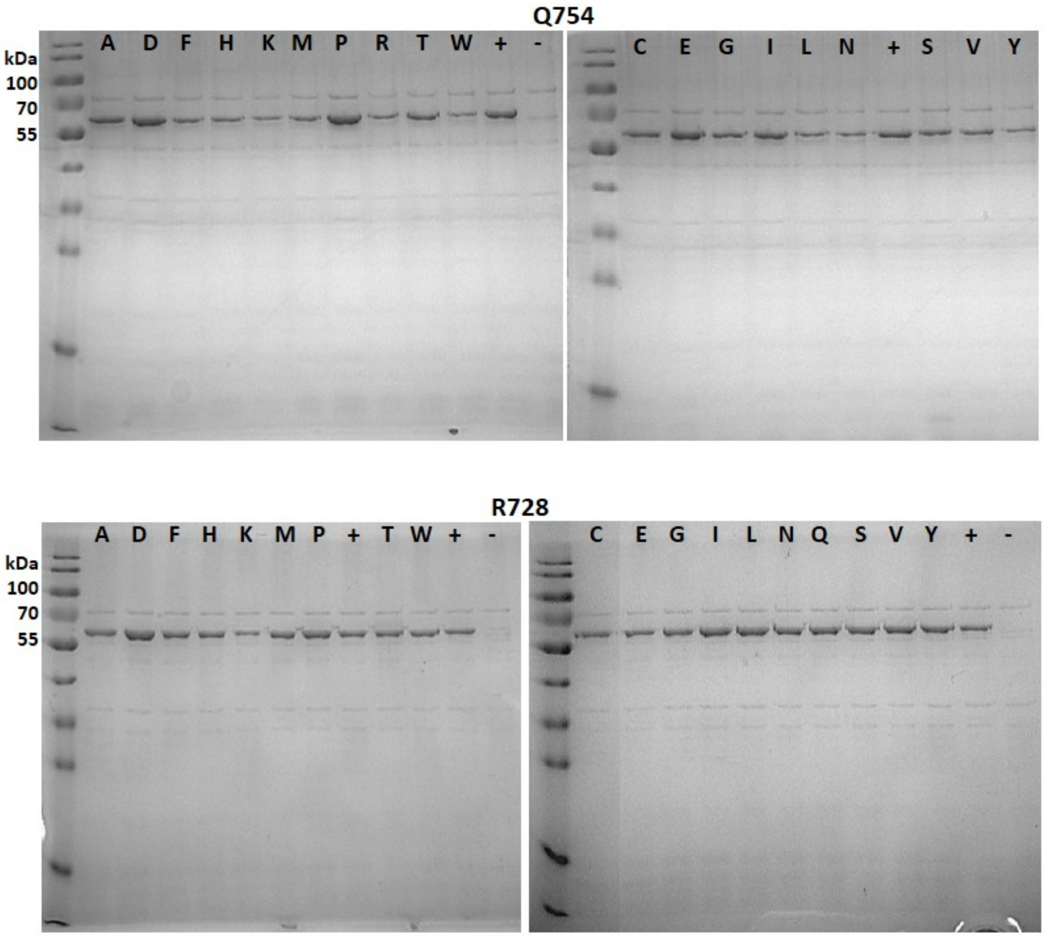
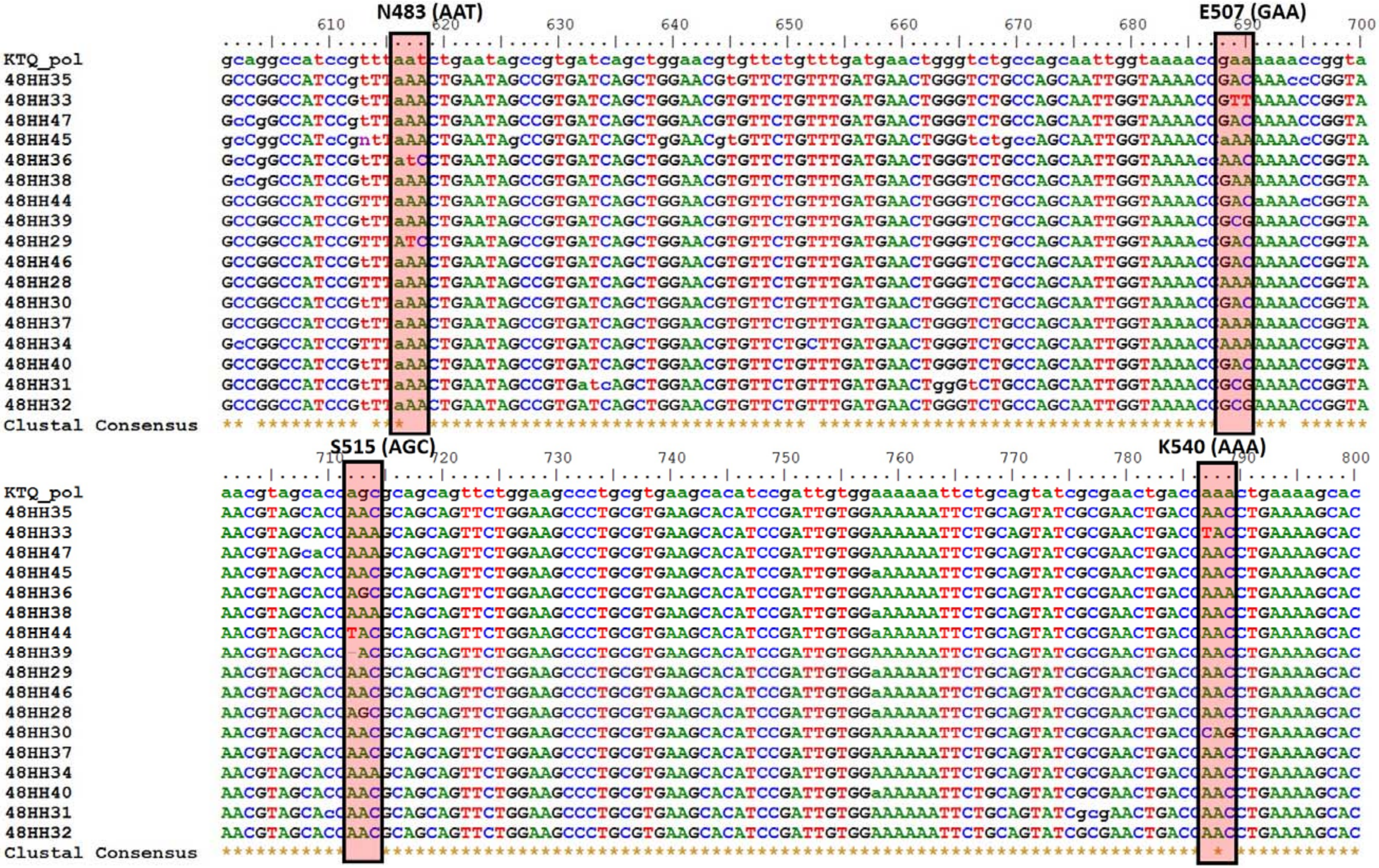


Figure S1.

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.



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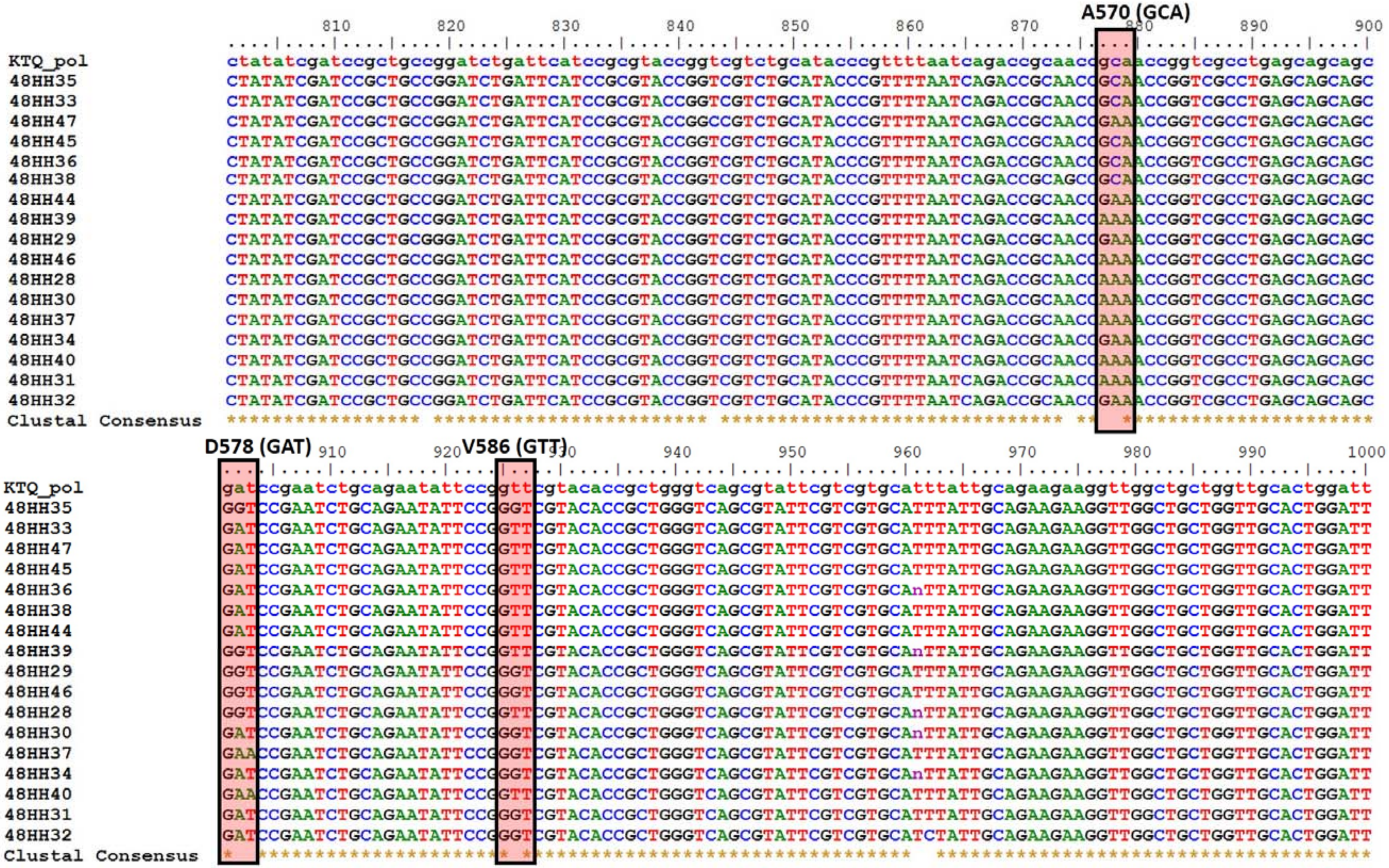

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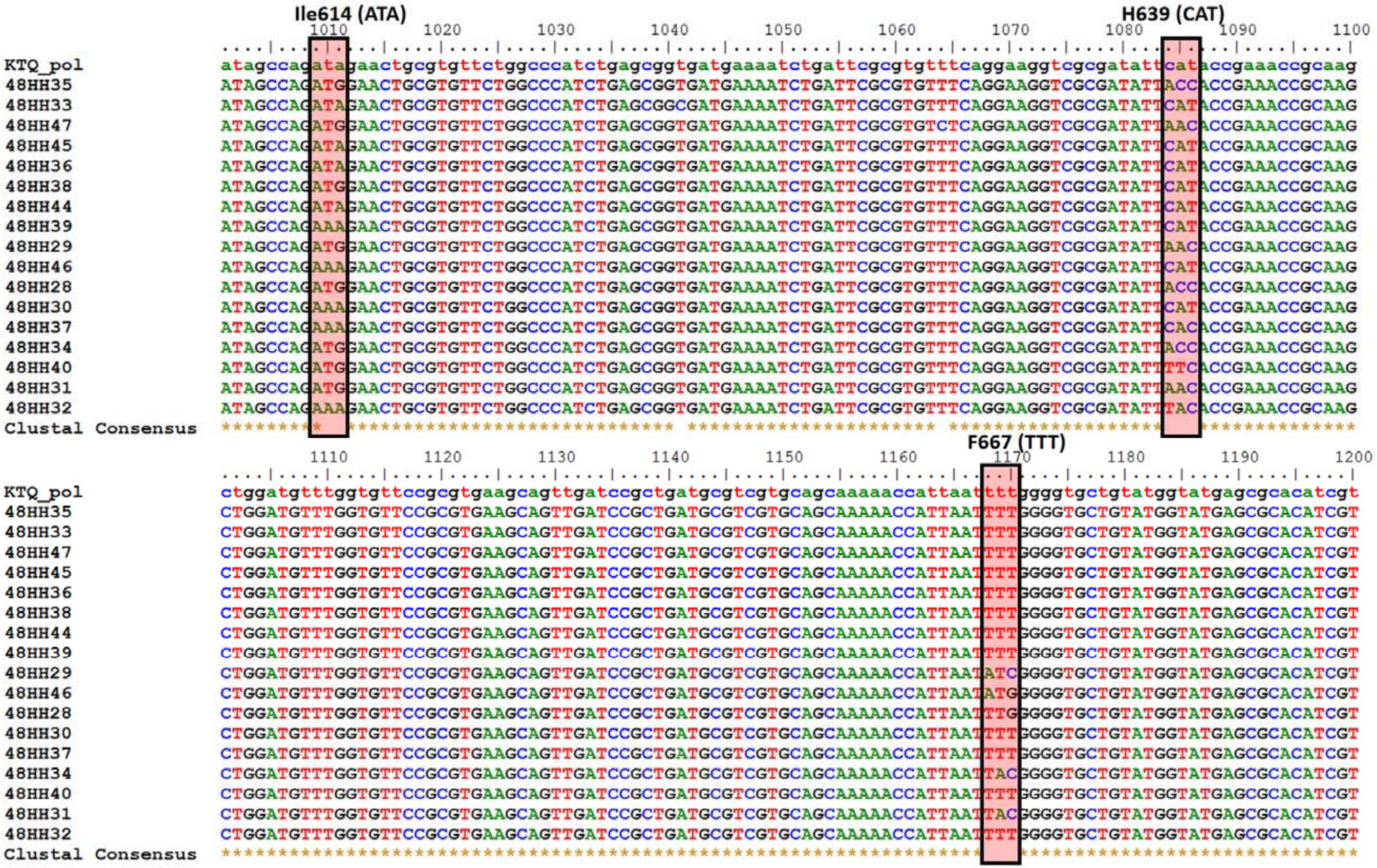

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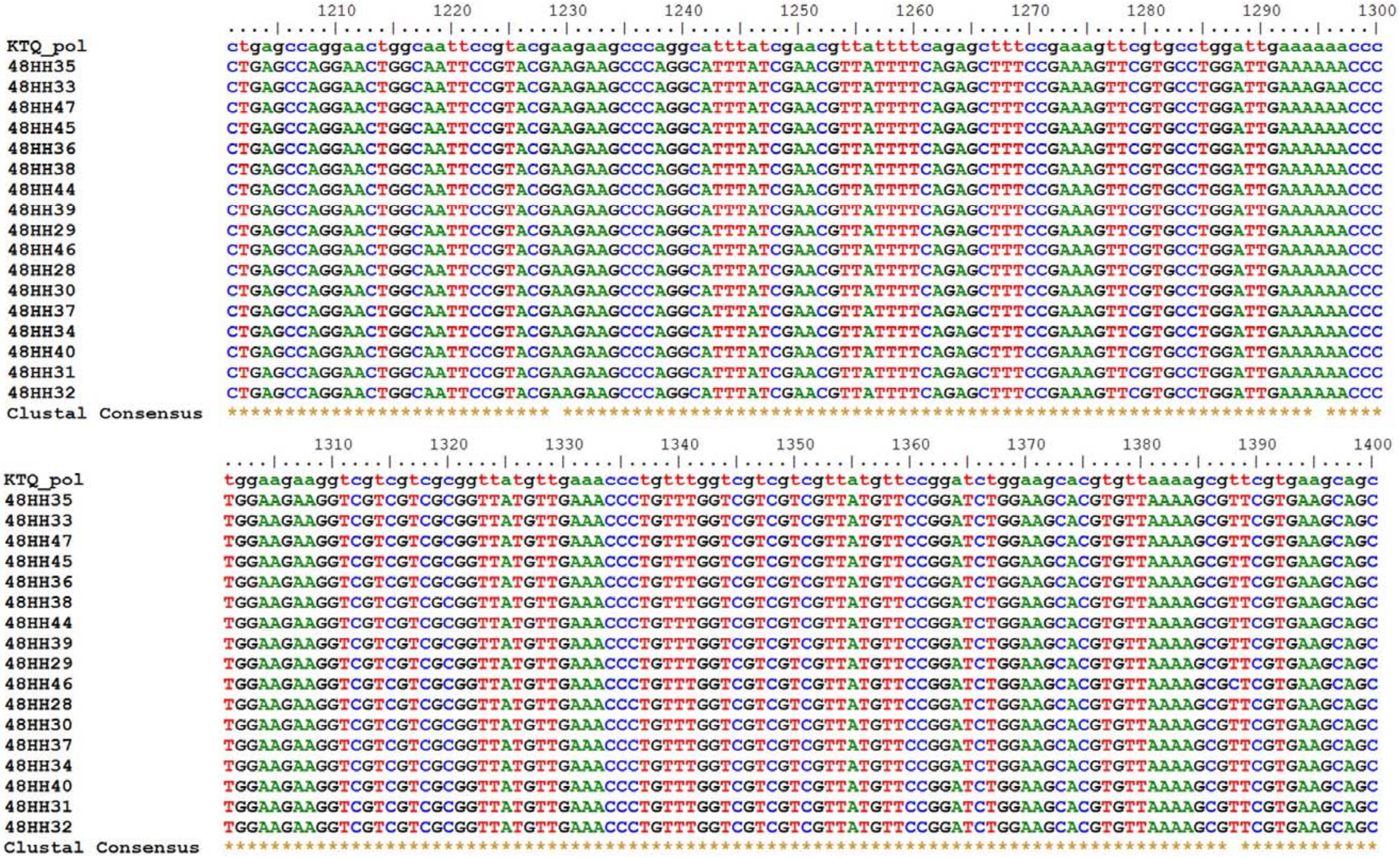

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Clustal Consensus  **************************** ***************************************************************** *****

                           1310      1320      1330      1340      1350      1360      1370      1380      1390      1400
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
KTQ_pol            tggaagaaggtcgtcgtcgcggttatgttgaaaccctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcagc
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48HH47             TGGAAGAAGGTCGTCGTCGCGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC
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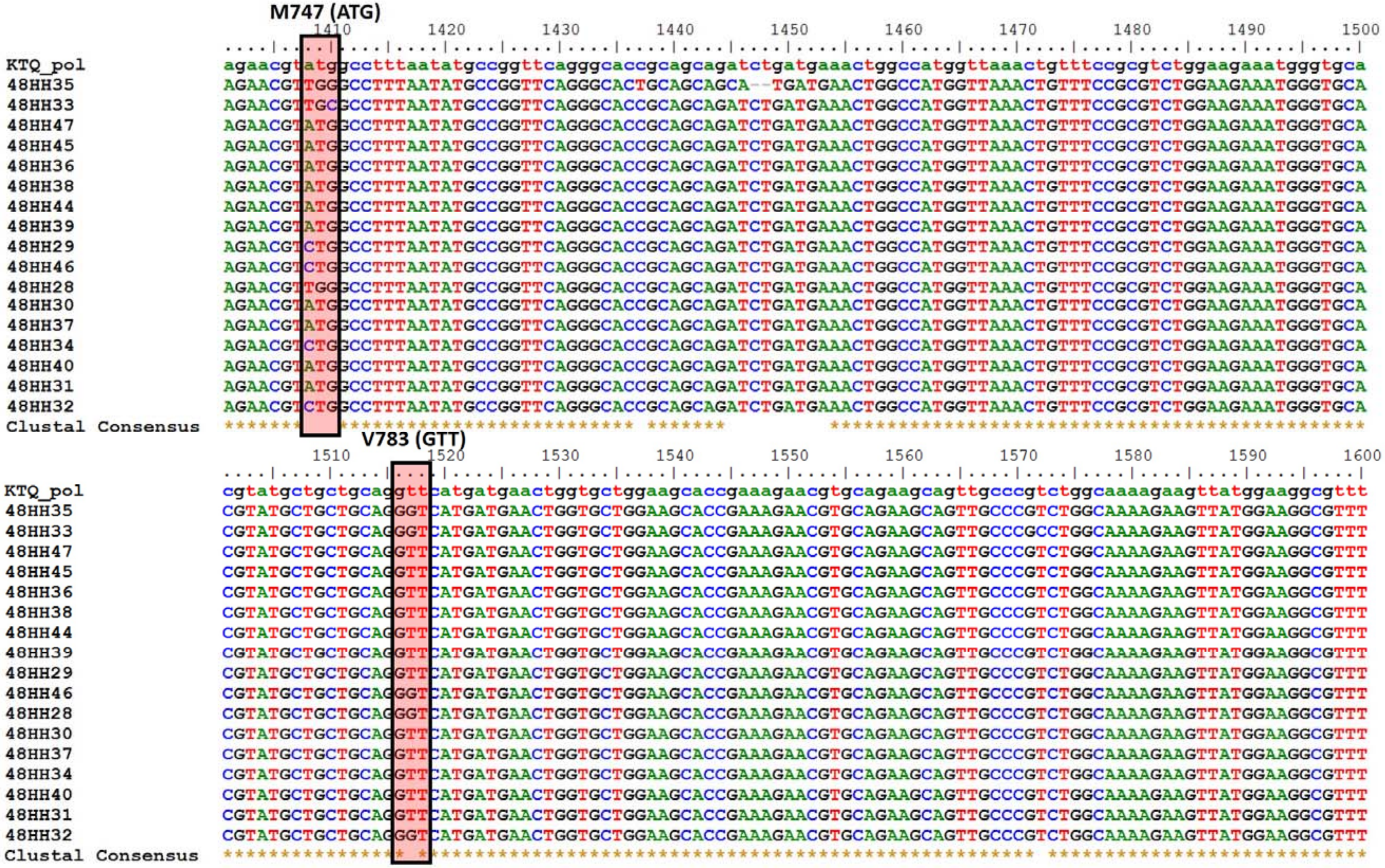

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48HH36             AGAACGTATGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
48HH38             AGAACGTATGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
48HH44             AGAACGTATGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
48HH39             AGAACGTATGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
48HH29             AGAACGTCTGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
48HH46             AGAACGTCTGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
48HH28             AGAACGTTGGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
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48HH40             AGAACGTATGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
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48HH32             AGAACGTCTGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
Clustal Consensus  *******   ************************** *******         ***********************************************

                         1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
KTQ_pol            cgtatgctgctgcaggttcatgatgaactggtgctggaagcaccgaaagaacgtgcagaagcagttgcccgtctggcaaaagaagttatggaaggcgttt
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48HH33             CGTATGCTGCTGCAGGGTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGCCTGGCAAAAGAAGTTATGGAAGGCGTTT
48HH47             CGTATGCTGCTGCAGGTTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
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48HH38             CGTATGCTGCTGCAGGTTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
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48HH39             CGTATGCTGCTGCAGGTTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
48HH29             CGTATGCTGCTGCAGGTTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
48HH46             CGTATGCTGCTGCAGGGTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
48HH28             CGTATGCTGCTGCAGGGTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
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48HH31             CGTATGCTGCTGCAGGTTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
48HH32             CGTATGCTGCTGCAGGGTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
Clustal Consensus  **************** ****************************************************** ****************************
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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.

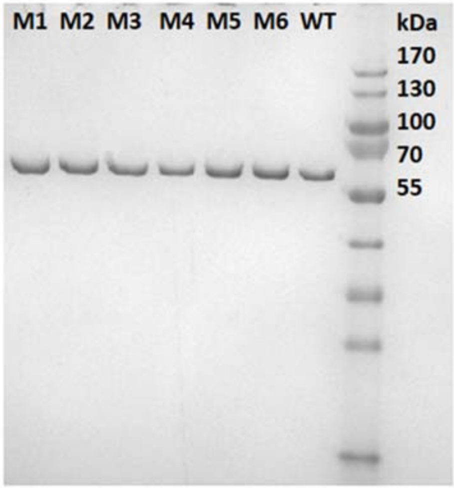


Figure S3.

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.

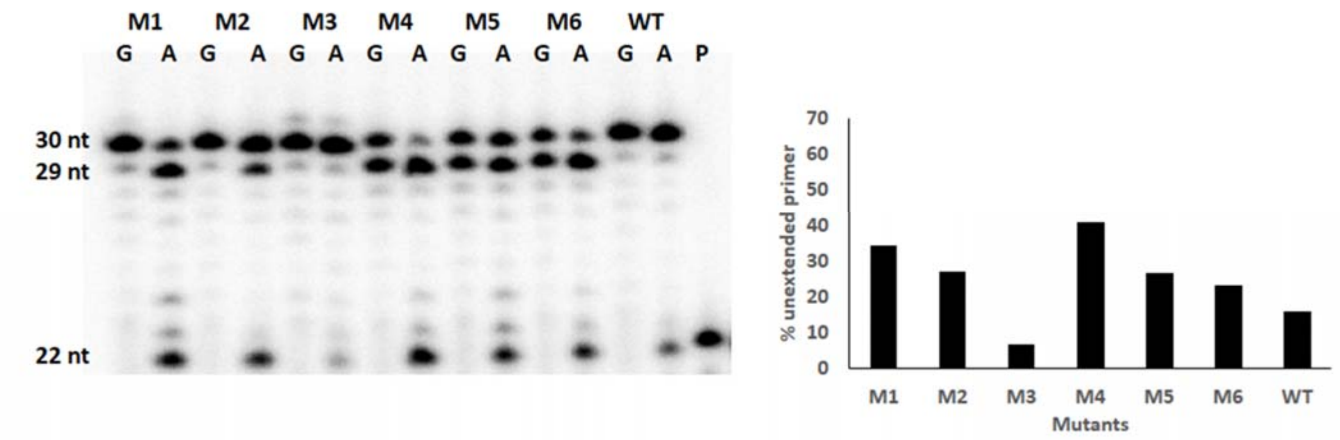
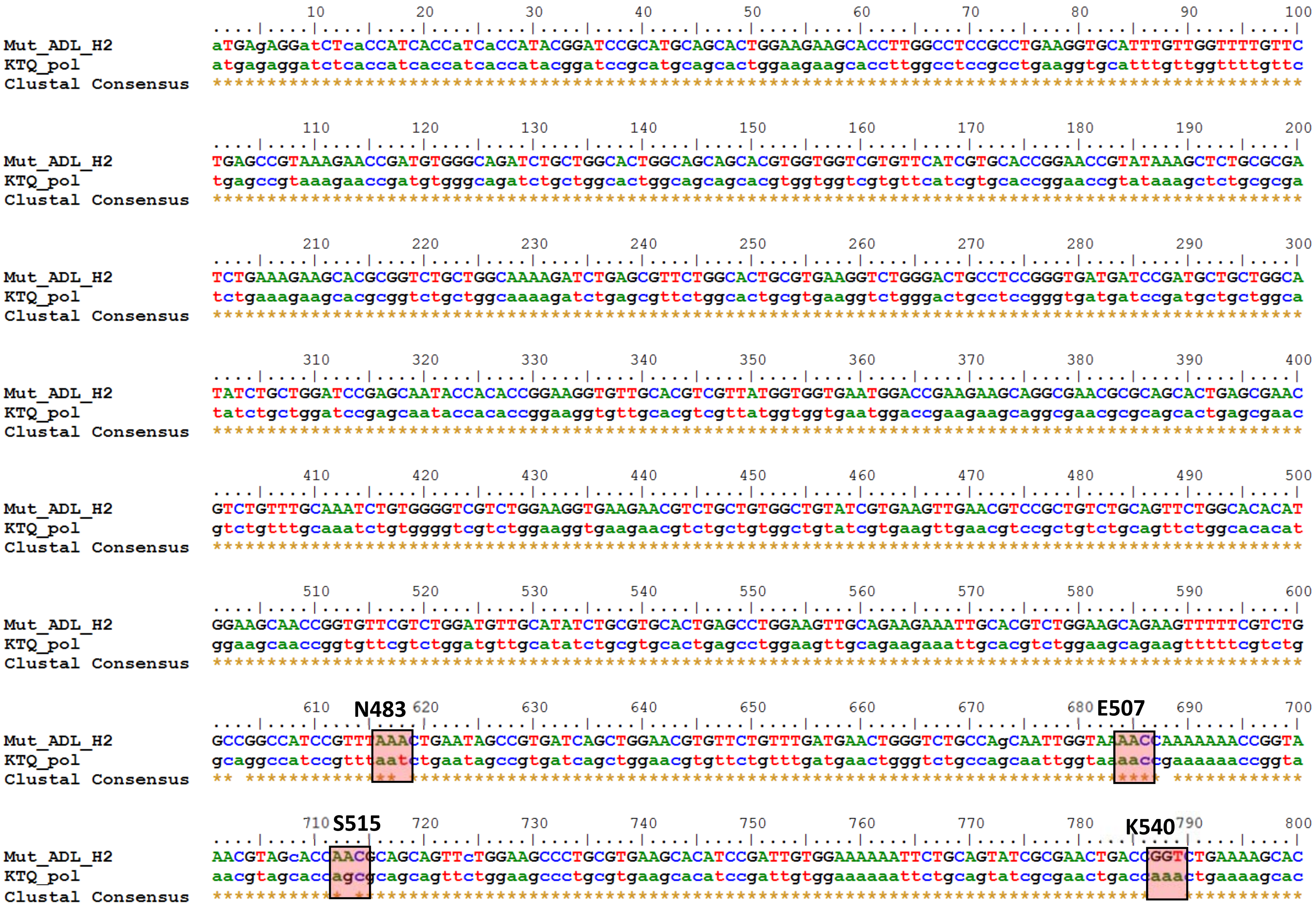


Figure S4.

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).



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                           10        20        30        40        50        60        70        80        90       100
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2         aTGAgAGGatCTcaCCATCACCaTCaCCATACGGATCCGCATGCAGCACTGGAAGAAGCACCTTGGCCTCCGCCTGAAGGTGCATTTGTTGGTTTTGTTC
KTQ_pol            atgagaggatctcaccatcaccatcaccatacggatccgcatgcagcactggaagaagcaccttggcctccgcctgaaggtgcatttgttggttttgttc
Clustal Consensus  ****************************************************************************************************

                          110       120       130       140       150       160       170       180       190       200
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2         TGAGCCGTAAAGAACCGATGTGGGCAGATCTGCTGGCACTGGCAGCAGCACGTGGTGGTCGTGTTCATCGTGCACCGGAACCGTATAAAGCTCTGCGCGA
KTQ_pol            tgagccgtaaagaaccgatgtgggcagatctgctggcactggcagcagcacgtggtggtcgtgttcatcgtgcaccggaaccgtataaagctctgcgcga
Clustal Consensus  ****************************************************************************************************

                          210       220       230       240       250       260       270       280       290       300
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2         TCTGAAAGAAGCACGCGGTCTGCTGGCAAAAGATCTGAGCGTTCTGGCACTGCGTGAAGGTCTGGGACTGCCTCCGGGTGATGATCCGATGCTGCTGGCA
KTQ_pol            tctgaaagaagcacgcggtctgctggcaaaagatctgagcgttctggcactgcgtgaaggtctgggactgcctccgggtgatgatccgatgctgctggca
Clustal Consensus  ****************************************************************************************************

                          310       320       330       340       350       360       370       380       390       400
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2         TATCTGCTGGATCCGAGCAATACCACACCGGAAGGTGTTGCACGTCGTTATGGTGGTGAATGGACCGAAGAAGCAGGCGAACGCGCAGCACTGAGCGAAC
KTQ_pol            tatctgctggatccgagcaataccacaccggaaggtgttgcacgtcgttatggtggtgaatggaccgaagaagcaggcgaacgcgcagcactgagcgaac
Clustal Consensus  ****************************************************************************************************

                          410       420       430       440       450       460       470       480       490       500
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2         GTCTGTTTGCAAATCTGTGGGGTCGTCTGGAAGGTGAAGAACGTCTGCTGTGGCTGTATCGTGAAGTTGAACGTCCGCTGTCTGCAGTTCTGGCACACAT
KTQ_pol            gtctgtttgcaaatctgtggggtcgtctggaaggtgaagaacgtctgctgtggctgtatcgtgaagttgaacgtccgctgtctgcagttctggcacacat
Clustal Consensus  ****************************************************************************************************

                          510       520       530       540       550       560       570       580       590       600
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2         GGAAGCAACCGGTGTTCGTCTGGATGTTGCATATCTGCGTGCACTGAGCCTGGAAGTTGCAGAAGAAATTGCACGTCTGGAAGCAGAAGTTTTTCGTCTG
KTQ_pol            ggaagcaaccggtgttcgtctggatgttgcatatctgcgtgcactgagcctggaagttgcagaagaaattgcacgtctggaagcagaagtttttcgtctg
Clustal Consensus  ****************************************************************************************************

                          610       620       630       640       650       660       670       680       690       700
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2         GCCGGCCATCCGTTTAAACTGAATAGCCGTGATCAGCTGGAACGTGTTCTGTTTGATGAACTGGGTCTGCCAgCAATTGGTAAAACCAAAAAAACCGGTA
KTQ_pol            gcaggccatccgtttaatctgaatagccgtgatcagctggaacgtgttctgtttgatgaactgggtctgccagcaattggtaaaaccgaaaaaaccggta
Clustal Consensus  ** ************** ********************************************************************* ************

                          710       720       730       740       750       760       770       780       790       800
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
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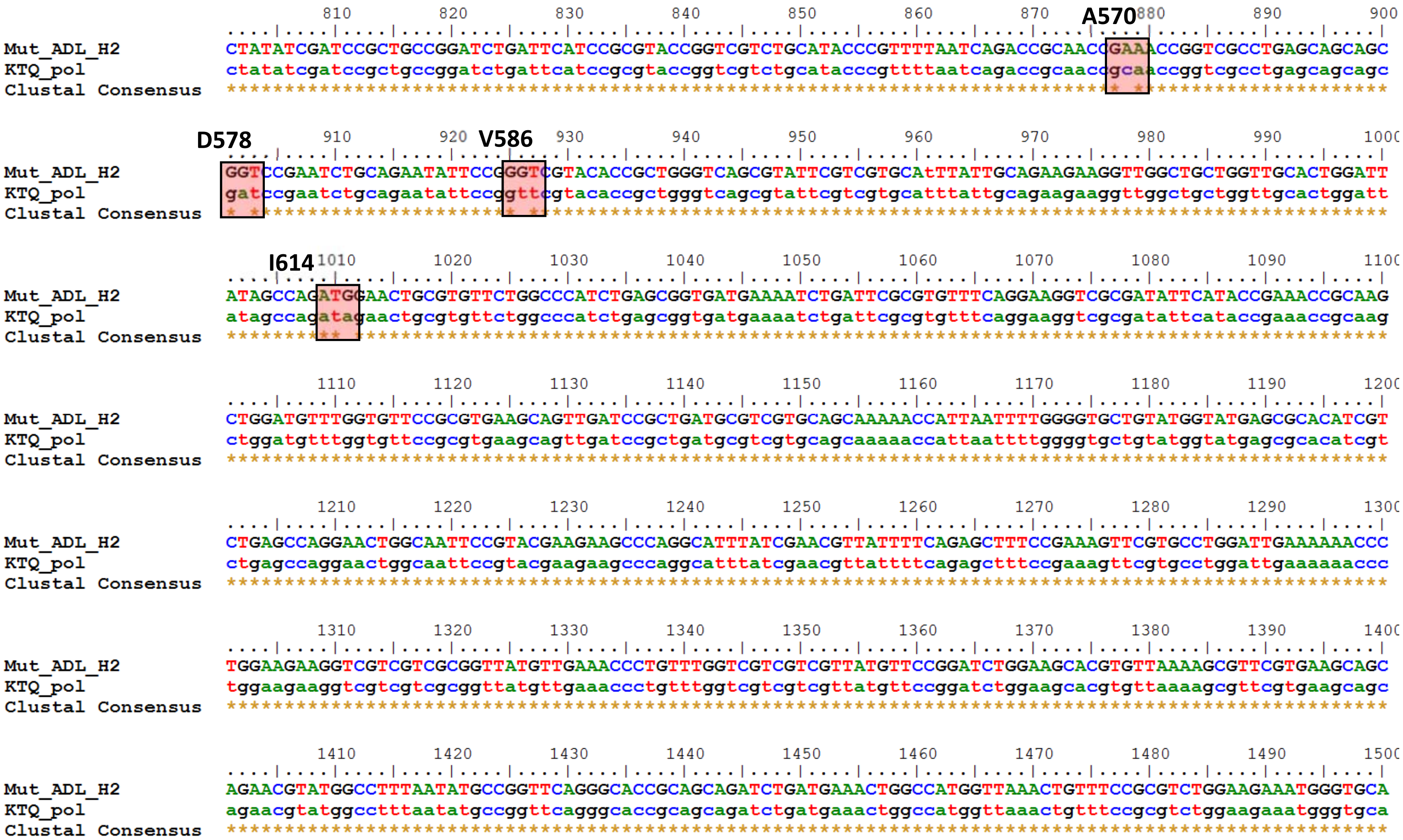

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Mut_ADL_H2        CTATATCGATCCGCTGCCGGATCTGATTCATCCGCGTACCGGTCGTCTGCATACCCGTTTTAATCAGACCGCAACCGAAACCGGTCGCCTGAGCAGCAGC
KTQ_pol           ctatatcgatccgctgccggatctgattcatccgcgtaccggtcgtctgcatacccgttttaatcagaccgcaaccgcaaccggtcgcctgagcagcagc
Clustal Consensus ***************************************************************************** **********************

                         910       920       930       940       950       960       970       980       990      1000
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2        GGTCCGAATCTGCAGAATATTCCGGGTCGTACACCGCTGGGTCAGCGTATTCGTCGTGCAtTTATTGCAGAAGAAGGTTGGCTGCTGGTTGCACTGGATT
KTQ_pol           gatccgaatctgcagaatattccggttcgtacaccgctgggtcagcgtattcgtcgtgcatttattgcagaagaaggttggctgctggttgcactggatt
Clustal Consensus * *********************** **************************************************************************

                        1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2        ATAGCCAGATGGAACTGCGTGTTCTGGCCCATCTGAGCGGTGATGAAAATCTGATTCGCGTGTTTCAGGAAGGTCGCGATATTCATACCGAAACCGCAAG
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Clustal Consensus ********** *****************************************************************************************

                        1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2        CTGGATGTTTGGTGTTCCGCGTGAAGCAGTTGATCCGCTGATGCGTCGTGCAGCAAAAACCATTAATTTTGGGGTGCTGTATGGTATGAGCGCACATCGT
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Clustal Consensus ****************************************************************************************************

                        1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2        CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAACCC
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Clustal Consensus ****************************************************************************************************

                        1310      1320      1330      1340      1350      1360      1370      1380      1390      1400
                  ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2        TGGAAGAAGGTCGTCGTCGCGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTTATGTTCCGGATCTGGAAGCACGTGTTAAAAGCGTTCGTGAAGCAGC
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Clustal Consensus ****************************************************************************************************

                        1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
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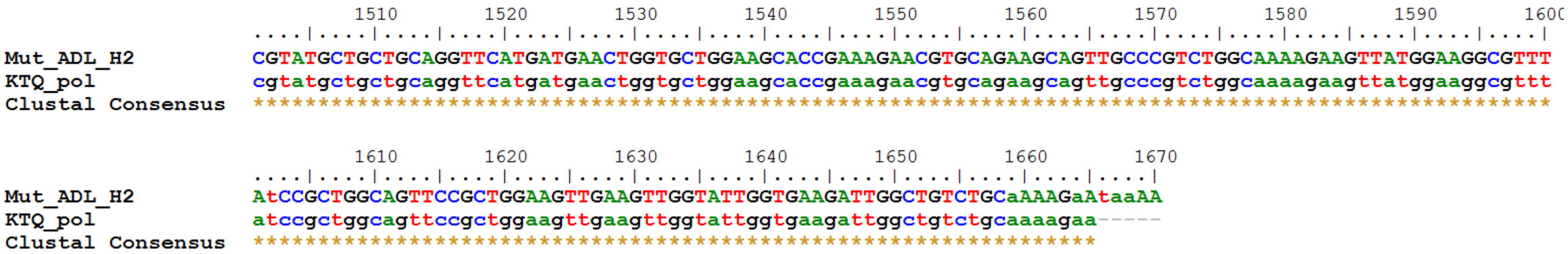

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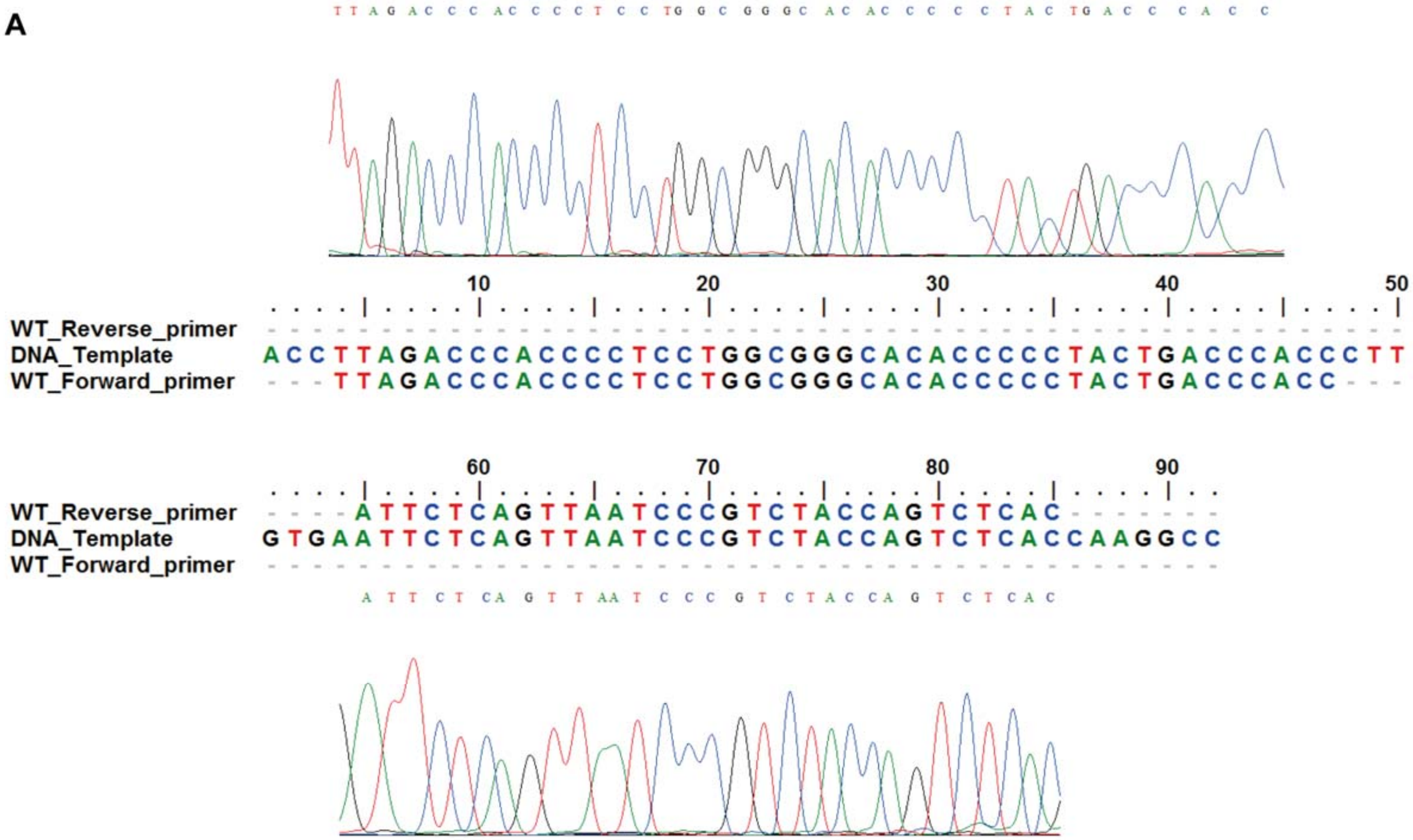
Clustal Consensus   ****************************************************************************************************

                            1610      1620      1630      1640      1650      1660      1670
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_ADL_H2          AtCCGCTGGCAGTTCCGCTGGAAGTTGAAGTTGGTATTGGTGAAGATTGGCTGTCTGCaAAAGaAtaaAA
KTQ_pol             atccgctggcagttccgctggaagttgaagttggtattggtgaagattggctgtctgcaaaagaa-----
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 arbitary.


A
TTAGACCCACCCCTCCTGGCGGGCACACCCCCTACTGACCCACC
10
20
30
40
50
WT_Reverse_primer
DNA_Template ACCTTAGACCCACCCCTCCTGGCGGGCACACCCCCTACTGACCCACCCTT
WT_Forward_primer ---TTAGACCCACCCCTCCTGGCGGGCACACCCCCTACTGACCCACC---
60
70
80
90
WT_Reverse_primer ----ATTCTCAGTTAATCCCGTCTACCAGTCTCAC-------
DNA_Template GTGAATTCTCAGTTAATCCCGTCTACCAGTCTCACCAAGGCC
WT_Forward_primer
ATTCTCAGTTAATCCCGTCTACCAGTCTCAC

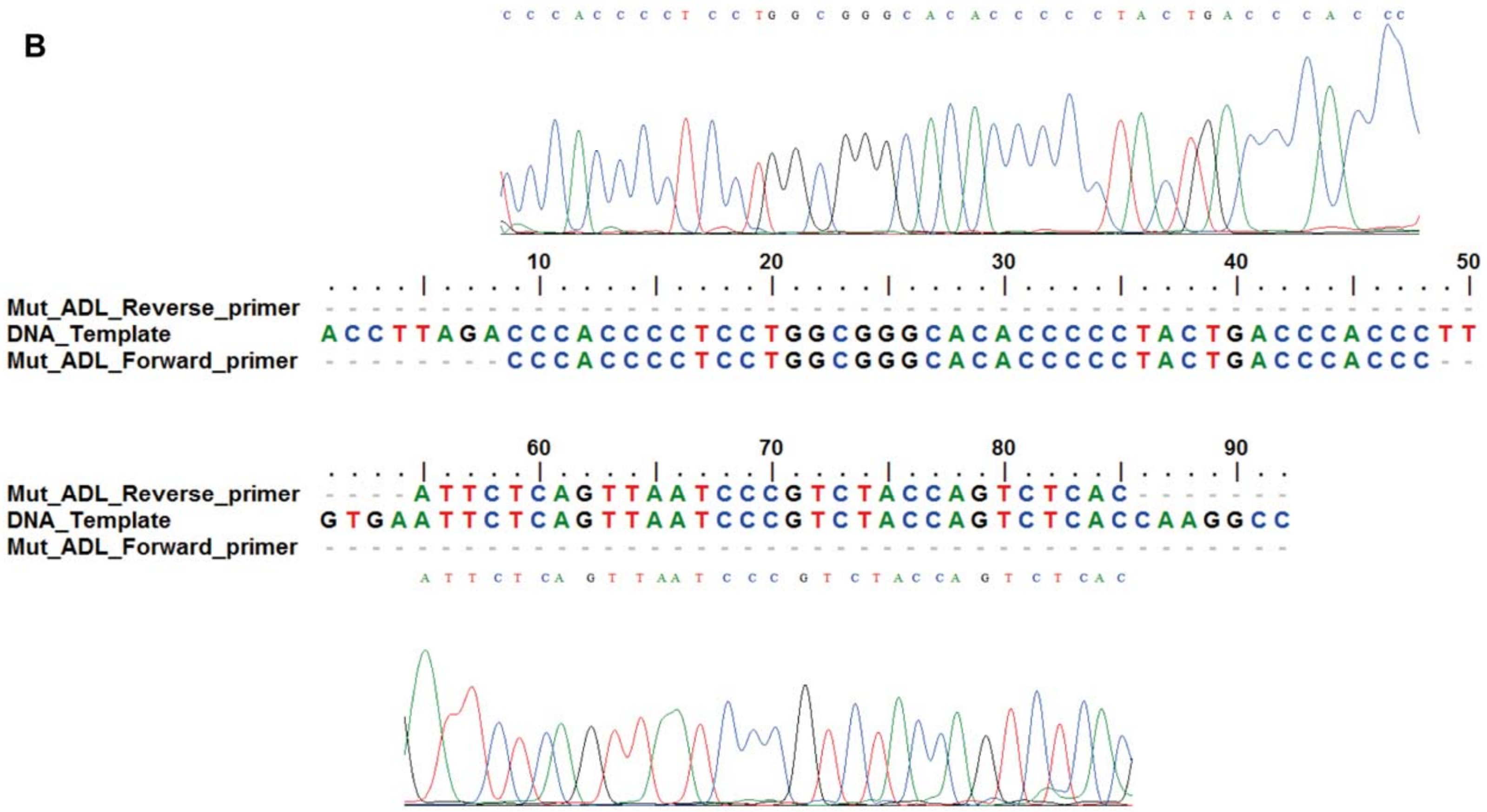


Figure S6. Sanger sequencing analysis of amplified products. The products that were amplified by respective enzymes (WT, Mut_ADL and Mut_RT) were presented in sequence alignment. Section of electropherogram of sequencing reactions with forward primer [5'-d(CTT GGT GAG ACT GGT AGA CG)-3'] (above sequence 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.

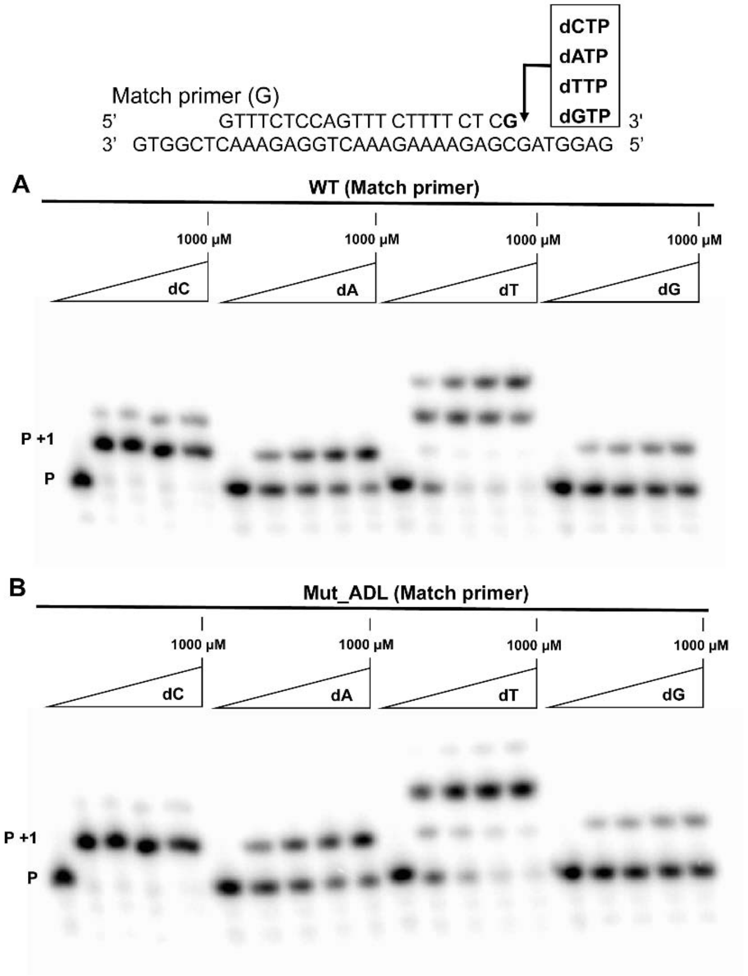


Figure S7. Single nucleotide misinsertion assay for wild type KlenTaq DNA polymerase and Mut_ADL. The reaction (20 µl) contained 1X KlenTaq reaction buffer, 250 nM template (5'-d(GAG GTA GCG AGA AAA GAA ACT GGA GAA ACT CGG TG)3'), 150 nM of 5' radioactively labelled Match primer (5'-d(GTT TCT CCA GTT 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 µM). 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.

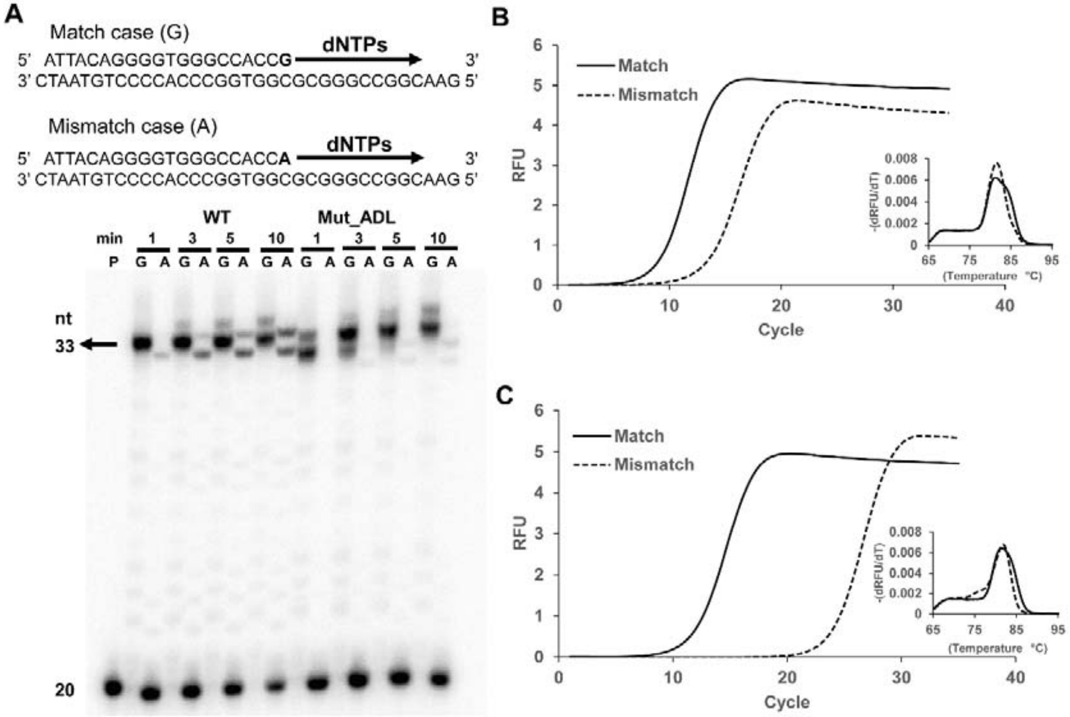
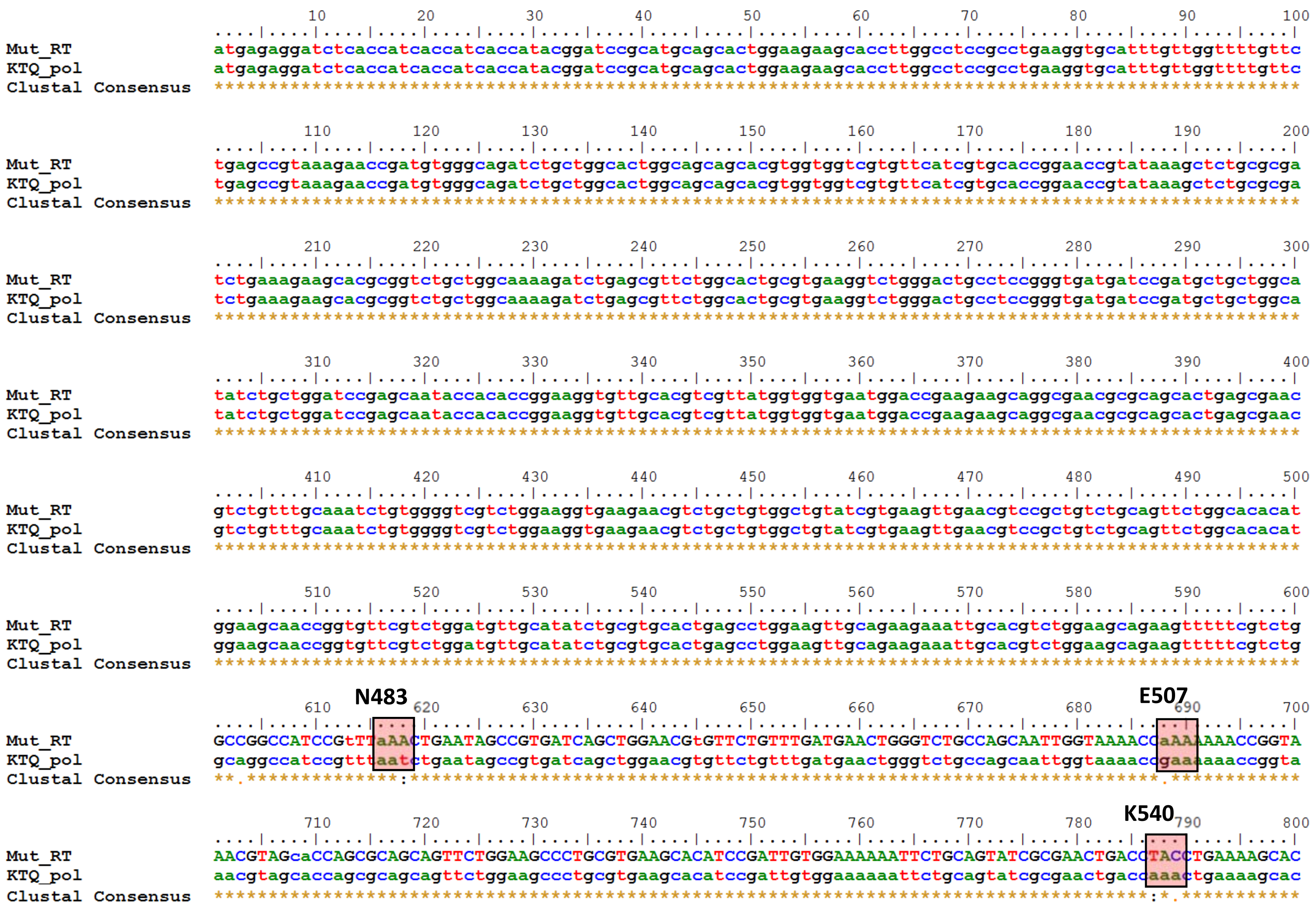


Figure S8. Improved allelic discrimination on different sequence context. (**A**) Sequence of employed primer-template pair. The 3' end of primer either terminates with a matched (G) or mismatched (A) nucleotide. Primer extension reaction was performed as indicated in material section for allelic discrimination, 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 µl) 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 CA) 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
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             atgagaggatctcaccatcaccatcaccatacggatccgcatgcagcactggaagaagcaccttggcctccgcctgaaggtgcatttgttggttttgttc
KTQ_pol            atgagaggatctcaccatcaccatcaccatacggatccgcatgcagcactggaagaagcaccttggcctccgcctgaaggtgcatttgttggttttgttc
Clustal Consensus  ****************************************************************************************************

                          110       120       130       140       150       160       170       180       190       200
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             tgagccgtaaagaaccgatgtgggcagatctgctggcactggcagcagcacgtggtggtcgtgttcatcgtgcaccggaaccgtataaagctctgcgcga
KTQ_pol            tgagccgtaaagaaccgatgtgggcagatctgctggcactggcagcagcacgtggtggtcgtgttcatcgtgcaccggaaccgtataaagctctgcgcga
Clustal Consensus  ****************************************************************************************************

                          210       220       230       240       250       260       270       280       290       300
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             tctgaaagaagcacgcggtctgctggcaaaagatctgagcgttctggcactgcgtgaaggtctgggactgcctccgggtgatgatccgatgctgctggca
KTQ_pol            tctgaaagaagcacgcggtctgctggcaaaagatctgagcgttctggcactgcgtgaaggtctgggactgcctccgggtgatgatccgatgctgctggca
Clustal Consensus  ****************************************************************************************************

                          310       320       330       340       350       360       370       380       390       400
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             tatctgctggatccgagcaataccacaccggaaggtgttgcacgtcgttatggtggtgaatggaccgaagaagcaggcgaacgcgcagcactgagcgaac
KTQ_pol            tatctgctggatccgagcaataccacaccggaaggtgttgcacgtcgttatggtggtgaatggaccgaagaagcaggcgaacgcgcagcactgagcgaac
Clustal Consensus  ****************************************************************************************************

                          410       420       430       440       450       460       470       480       490       500
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             gtctgtttgcaaatctgtggggtcgtctggaaggtgaagaacgtctgctgtggctgtatcgtgaagttgaacgtccgctgtctgcagttctggcacacat
KTQ_pol            gtctgtttgcaaatctgtggggtcgtctggaaggtgaagaacgtctgctgtggctgtatcgtgaagttgaacgtccgctgtctgcagttctggcacacat
Clustal Consensus  ****************************************************************************************************

                          510       520       530       540       550       560       570       580       590       600
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             ggaagcaaccggtgttcgtctggatgttgcatatctgcgtgcactgagcctggaagttgcagaagaaattgcacgtctggaagcagaagtttttcgtctg
KTQ_pol            ggaagcaaccggtgttcgtctggatgttgcatatctgcgtgcactgagcctggaagttgcagaagaaattgcacgtctggaagcagaagtttttcgtctg
Clustal Consensus  ****************************************************************************************************

                          610       620       630       640       650       660       670       680       690       700
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             GCCGGCCATCCGtTTaAACTGAATAGCCGTGATCAGCTGGAACGtGTTCTGTTTGATGAACTGGGTCTGCCAGCAATTGGTAAAACCaAAAAAACCGGTA
KTQ_pol            gcaggccatccgtttaatctgaatagccgtgatcagctggaacgtgttctgtttgatgaactgggtctgccagcaattggtaaaaccgaaaaaaccggta
Clustal Consensus  **.**************:*********************************************************************.************

                          710       720       730       740       750       760       770       780       790       800
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             AACGTAGcaCCAGCGCAGCAGTTCTGGAAGCCCTGCGTGAAGCACATCCGATTGTGGAAAAAATTCTGCAGTATCGCGAACTGACCTACCTGAAAAGCAC
KTQ_pol            aacgtagcaccagcgcagcagttctggaagccctgcgtgaagcacatccgattgtggaaaaaattctgcagtatcgcgaactgaccaaactgaaaagcac
Clustal Consensus  **************************************************************************************:*.***********
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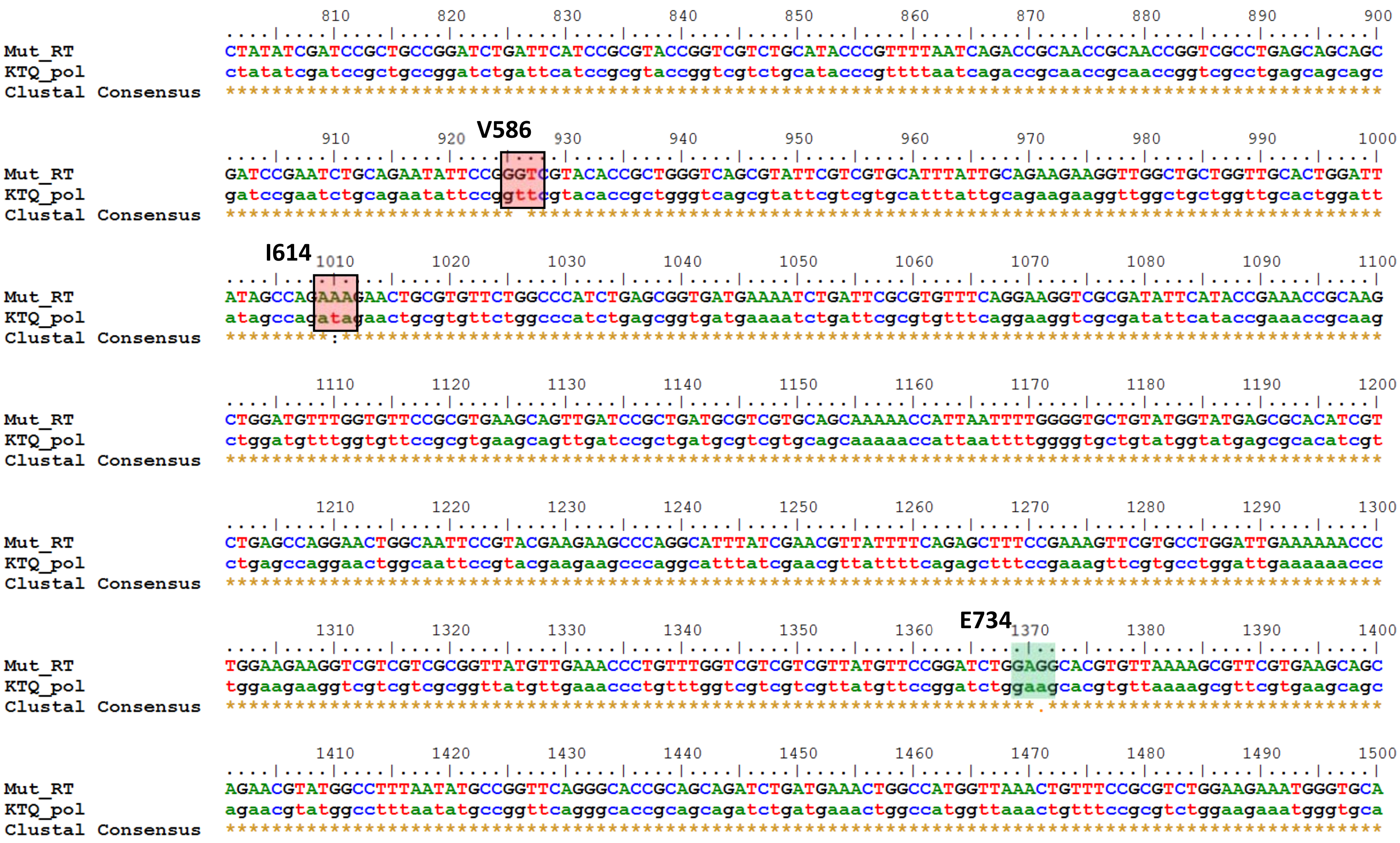

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                          810       820       830       840       850       860       870       880       890       900
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             CTATATCGATCCGCTGCCGGATCTGATTCATCCGCGTACCGGTCGTCTGCATACCCGTTTTAATCAGACCGCAACCGCAACCGGTCGCCTGAGCAGCAGC
KTQ_pol            ctatatcgatccgctgccggatctgattcatccgcgtaccggtcgtctgcatacccgttttaatcagaccgcaaccgcaaccggtcgcctgagcagcagc
Clustal Consensus  ****************************************************************************************************

                          910       920       930       940       950       960       970       980       990      1000
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             GATCCGAATCTGCAGAATATTCCGGGTCGTACACCGCTGGGTCAGCGTATTCGTCGTGCATTTATTGCAGAAGAAGGTTGGCTGCTGGTTGCACTGGATT
KTQ_pol            gatccgaatctgcagaatattccggttcgtacaccgctgggtcagcgtattcgtcgtgcatttattgcagaagaaggttggctgctggttgcactggatt
Clustal Consensus  ************************* **************************************************************************

                         1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             ATAGCCAGAAAGAACTGCGTGTTCTGGCCCATCTGAGCGGTGATGAAAATCTGATTCGCGTGTTTCAGGAAGGTCGCGATATTCATACCGAAACCGCAAG
KTQ_pol            atagccagatagaactgcgtgttctggcccatctgagcggtgatgaaaatctgattcgcgtgtttcaggaaggtcgcgatattcataccgaaaccgcaag
Clustal Consensus  *********:******************************************************************************************

                         1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             CTGGATGTTTGGTGTTCCGCGTGAAGCAGTTGATCCGCTGATGCGTCGTGCAGCAAAAACCATTAATTTTGGGGTGCTGTATGGTATGAGCGCACATCGT
KTQ_pol            ctggatgtttggtgttccgcgtgaagcagttgatccgctgatgcgtcgtgcagcaaaaaccattaattttggggtgctgtatggtatgagcgcacatcgt
Clustal Consensus  ****************************************************************************************************

                         1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             CTGAGCCAGGAACTGGCAATTCCGTACGAAGAAGCCCAGGCATTTATCGAACGTTATTTTCAGAGCTTTCCGAAAGTTCGTGCCTGGATTGAAAAAACCC
KTQ_pol            ctgagccaggaactggcaattccgtacgaagaagcccaggcatttatcgaacgttattttcagagctttccgaaagttcgtgcctggattgaaaaaaccc
Clustal Consensus  ****************************************************************************************************

                         1310      1320      1330      1340      1350      1360      1370      1380      1390      1400
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             TGGAAGAAGGTCGTCGTCGCGGTTATGTTGAAACCCTGTTTGGTCGTCGTCGTTATGTTCCGGATCTGGAGGCACGTGTTAAAAGCGTTCGTGAAGCAGC
KTQ_pol            tggaagaaggtcgtcgtcgcggttatgttgaaaccctgtttggtcgtcgtcgttatgttccggatctggaagcacgtgttaaaagcgttcgtgaagcagc
Clustal Consensus  **********************************************************************.*****************************

                         1410      1420      1430      1440      1450      1460      1470      1480      1490      1500
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT             AGAACGTATGGCCTTTAATATGCCGGTTCAGGGCACCGCAGCAGATCTGATGAAACTGGCCATGGTTAAACTGTTTCCGCGTCTGGAAGAAATGGGTGCA
KTQ_pol            agaacgtatggcctttaatatgccggttcagggcaccgcagcagatctgatgaaactggccatggttaaactgtttccgcgtctggaagaaatgggtgca
Clustal Consensus  ****************************************************************************************************
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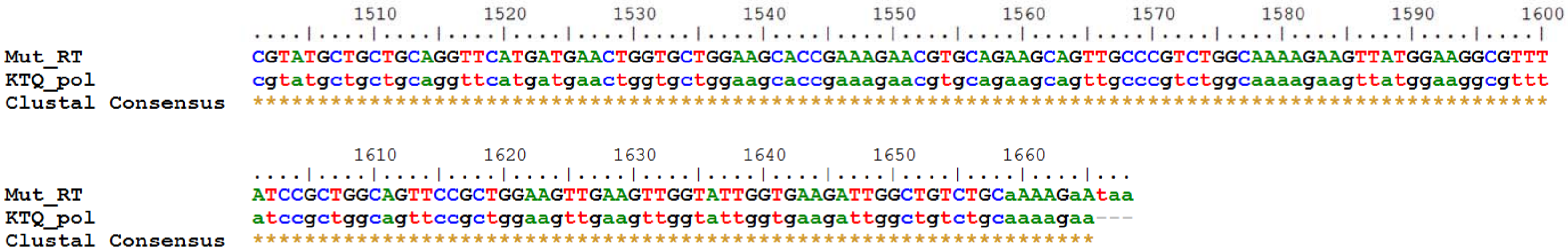

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                                  1510      1520      1530      1540      1550      1560      1570      1580      1590      1600
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Mut_RT                   CGTATGCTGCTGCAGGTTCATGATGAACTGGTGCTGGAAGCACCGAAAGAACGTGCAGAAGCAGTTGCCCGTCTGGCAAAAGAAGTTATGGAAGGCGTTT
KTQ_pol                  cgtatgctgctgcaggttcatgatgaactggtgctggaagcaccgaaagaacgtgcagaagcagttgcccgtctggcaaaagaagttatggaaggcgttt
Clustal Consensus        ****************************************************************************************************

                                  1610      1620      1630      1640      1650      1660
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|...
Mut_RT                   ATCCGCTGGCAGTTCCGCTGGAAGTTGAAGTTGGTATTGGTGAAGATTGGCTGTCTGCaAAAGaAtaa
KTQ_pol                  atccgctggcagttccgctggaagttgaagttggtattggtgaagattggctgtctgcaaaagaa---
Clustal Consensus        *****************************************************************
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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 arbitary. Position E734 marked in green box without border line, was not a target site for mutagenesis and a silent mutation (A→G) had occurred during PCR amplifcation.

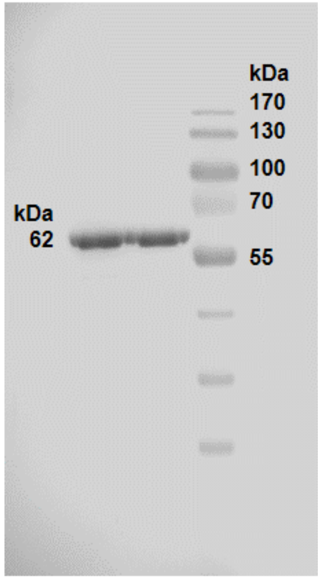


Figure S10 **.**

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

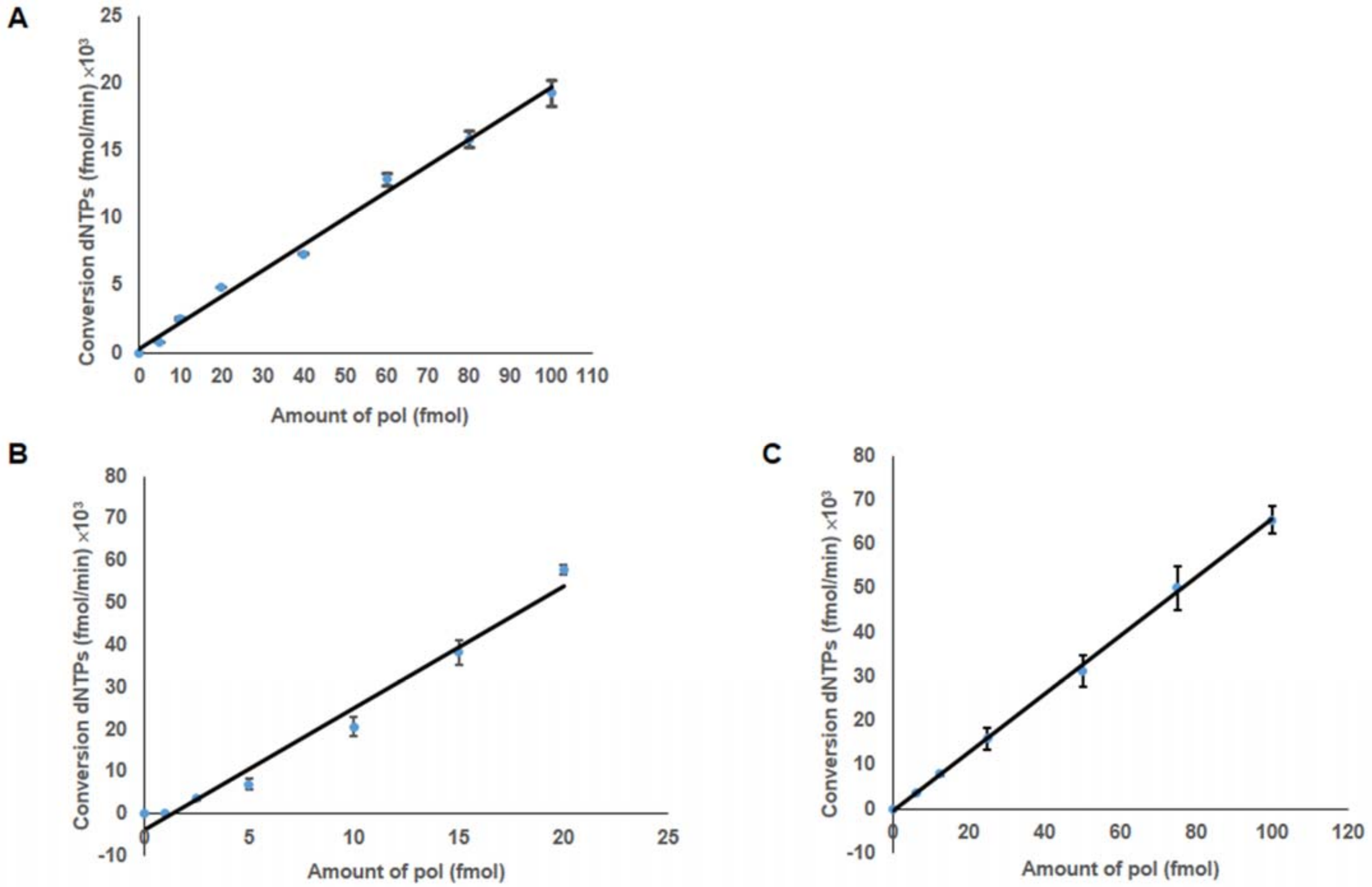
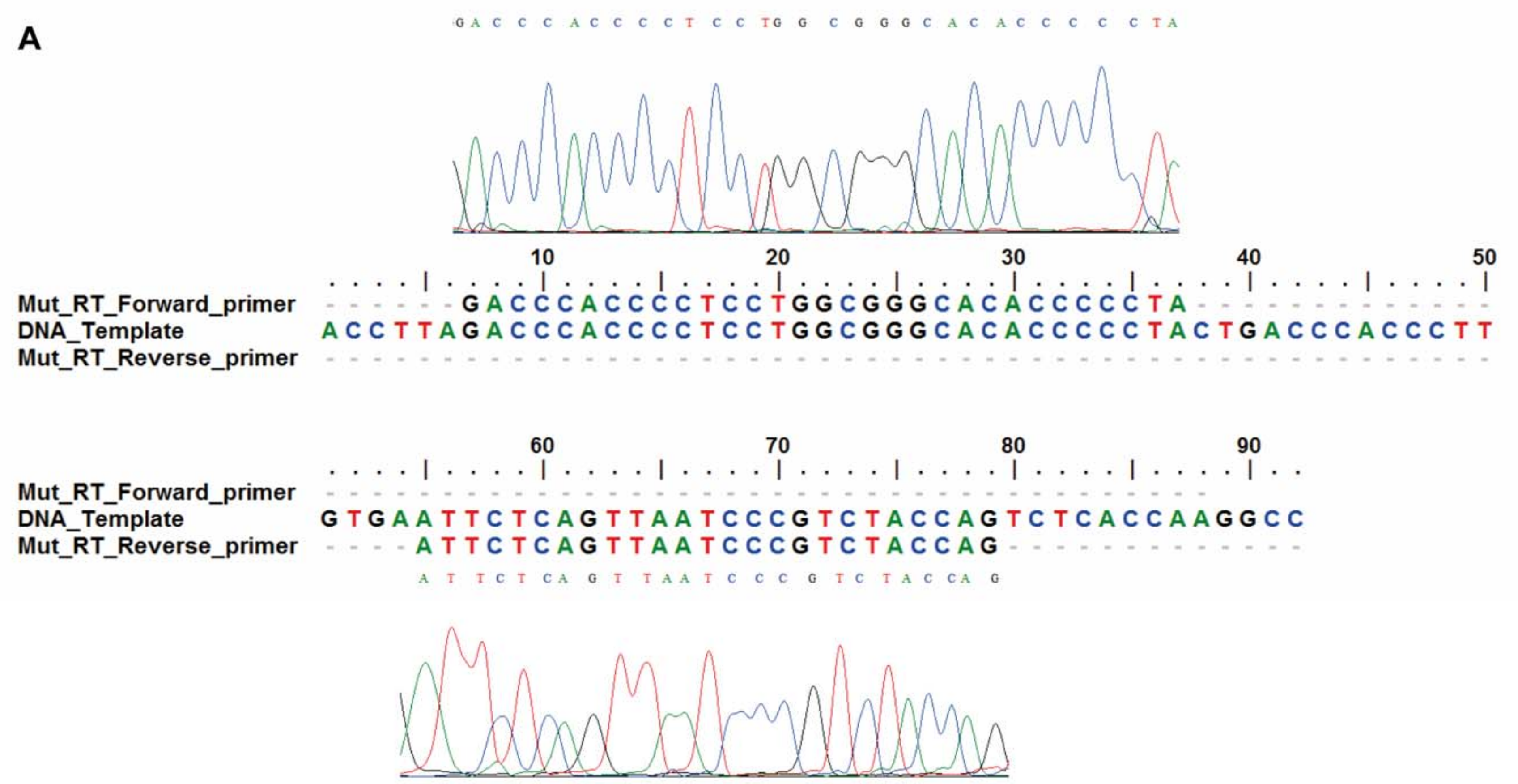


Figure S11.

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).

A
G A C C C A C C C C T C C T G G C G G G C A C A C C C C C T A
10
20
30
40
50
Mut_RT_Forward_primer ------GACCCACCCCTCCTGGCGGGCACACCCCCTA-------------
DNA_Template ACCTTAGACCCACCCCTCCTGGCGGGCACACCCCCTACTGACCCACCCTT
Mut_RT_Reverse_primer --
60
70
80
90
Mut_RT_Forward_primer ---
DNA_Template GTGAATTCTCAGTTAATCCCGTCTACCAGTCTCACCAAGGCC
Mut_RT_Reverse_primer ----ATTCTCAGTTAATCCCGTCTACCAG-------------
A T T C T C A G T T A A T C C C G T C T A C C A G

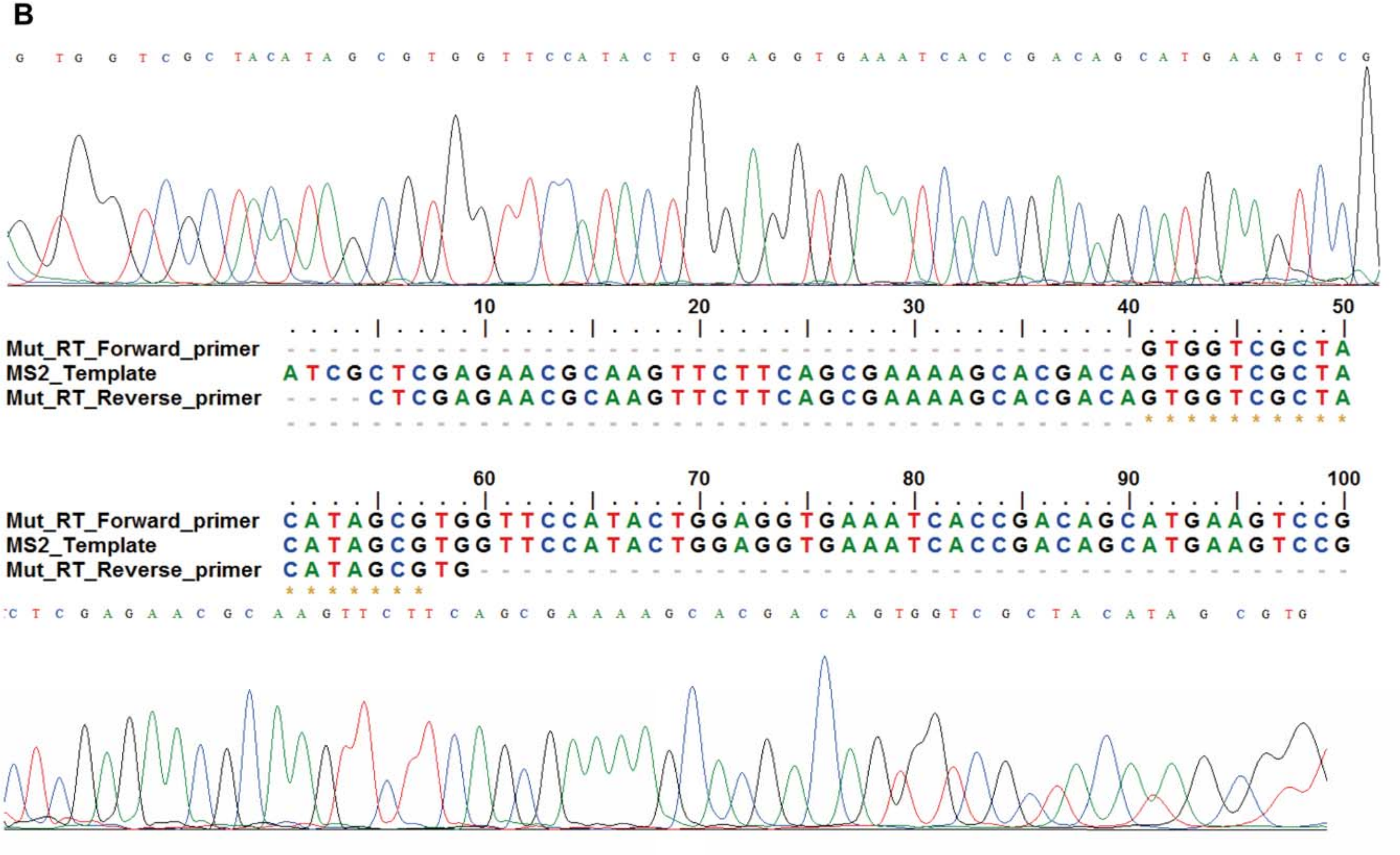


Figure S12. Sanger sequencing analysis of Mut_RT amplified products. A) Amplificate from DNA dependent DNA synthesis was presented in sequence alignment. Section of electropherogram of sequencing reactions with forward primer [5'-d(CTT GGT GAG ACT GGT AGA CG)-3'] (above sequence alignment) and reverse 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)

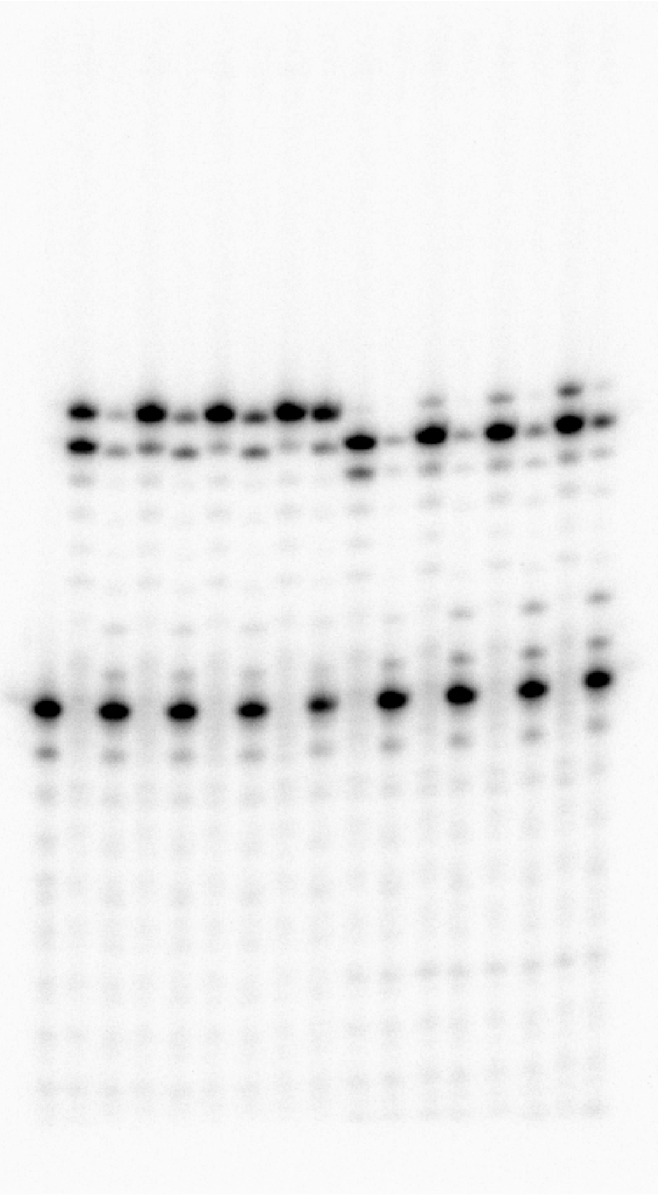


Figure S14 (Full image of 5C)

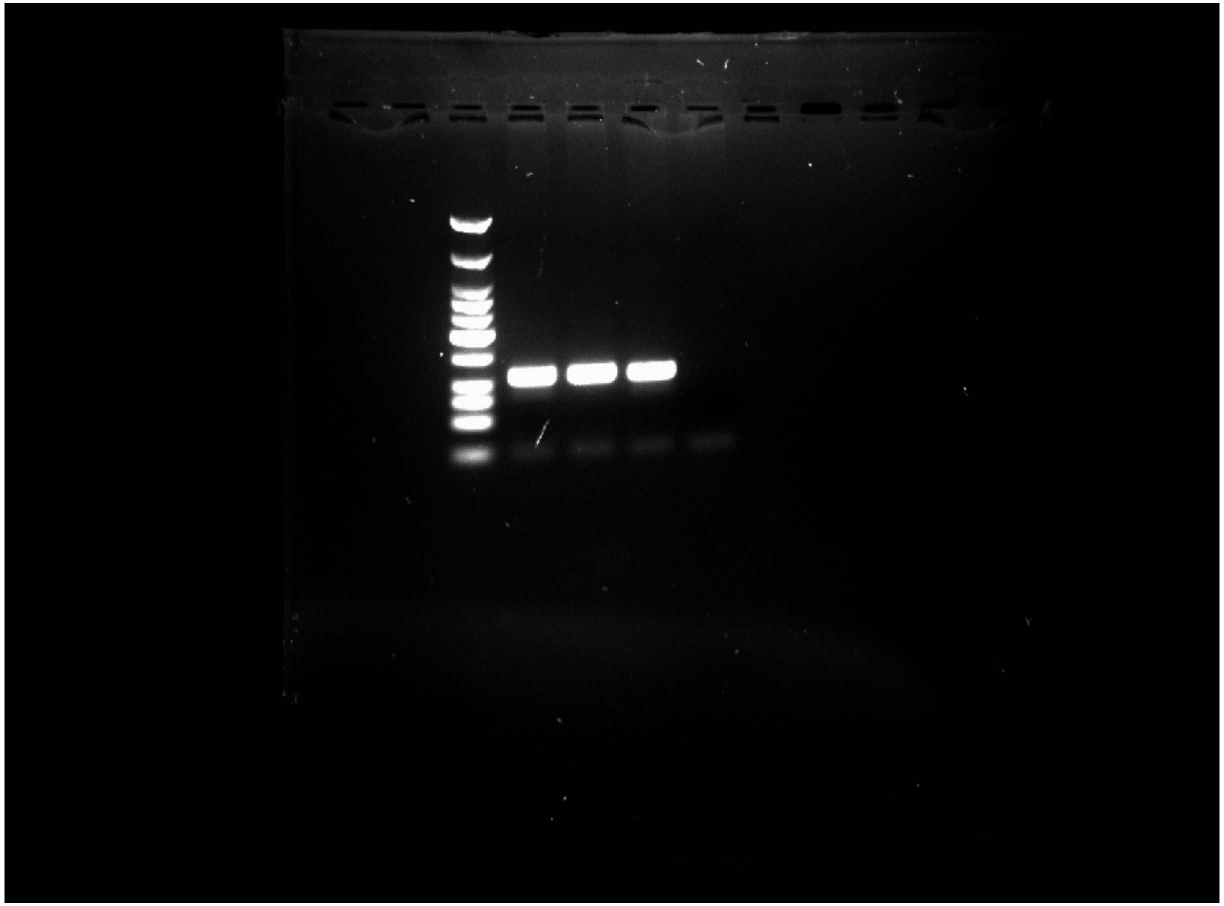


Figure S15 (Full image of 5E)

Figure S16 (Full image of 6B)

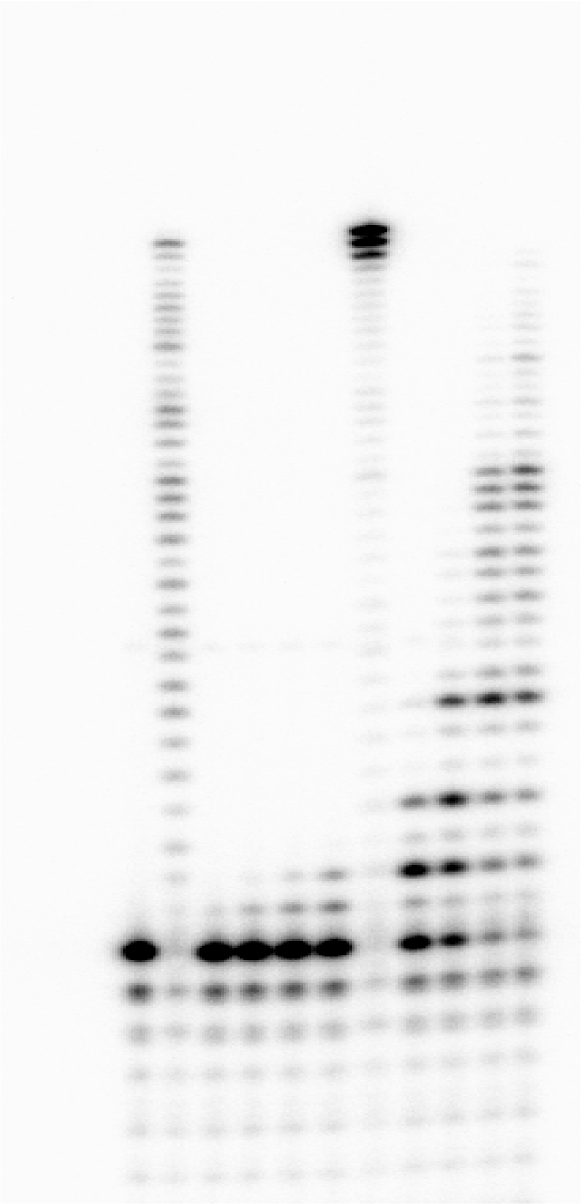


Figure S17 (Full image of 6D)

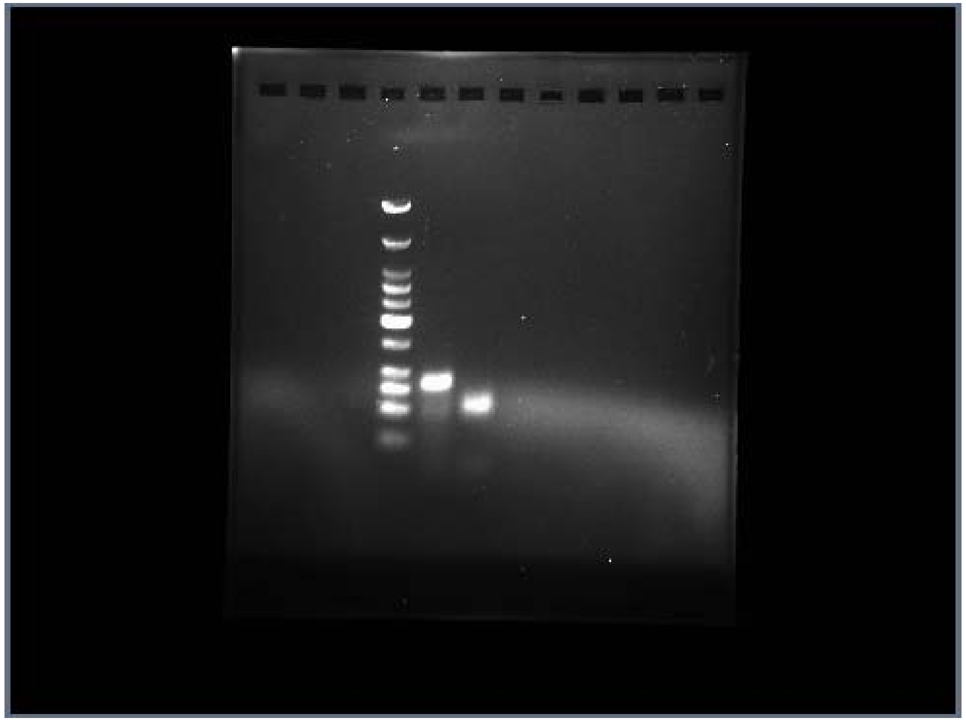


Figure S18 (Full image of S7)

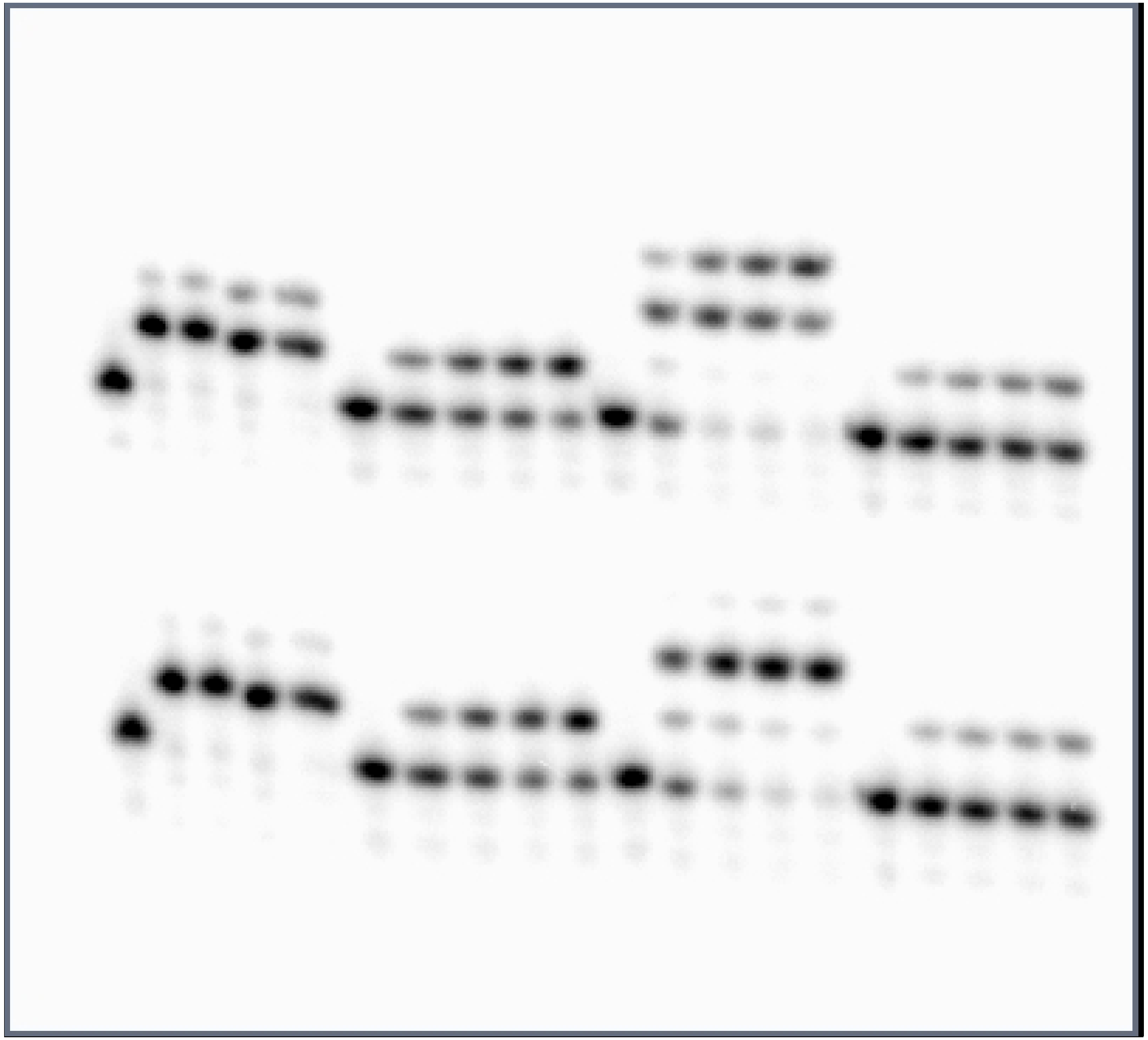
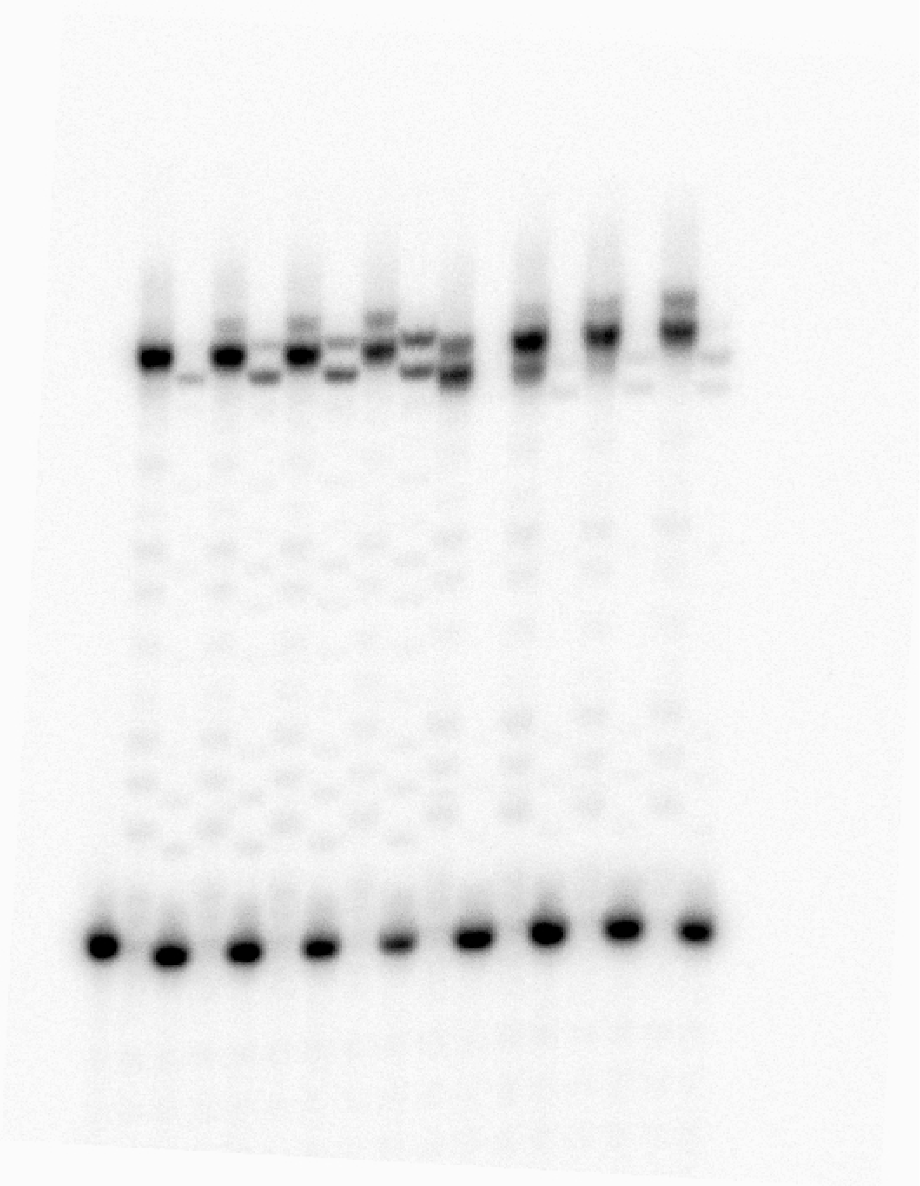


Figure S19 (Full image of S8)



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