SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Determination of complete gap-filling by human DNA polymerases. Representative agarose (0.6%) gel of Pol δ4, Pol κ, and Pol η gap-filling reactions using a $[GT]_{10}$ gapped substrate. All ratios are polymerase: gapped substrate molar ratios. N, nicked plasmid. G, unfilled gapped substrate. Complete gap-filling produces a band that runs coincident with nicked plasmid. Incomplete gap-filling produces a band that runs coincident with unfilled gap or one that runs in between the gapped and nicked substrates. Asterisks indicate reactions that were determined to be complete.

Figure S2. Mutational spectra showing (A) Pol δ4 ; (B) Pol κ ; and (C) Pol η errors within the HSV-tk coding region. For each polymerase, the spectra are a compilation of all coding mutational events from all templates examined. DNA synthesis is initiated at position 180 and proceeds right to left. MS, indicates where the microsatellites are inserted in between positions 111 and 112. The middle line of sequence is the sequence of the wild-type HSV-tk gene and the underlined bases indicate the 23 mono- and dinucleotide frameshift monitors. Base substitution mutations are shown above the sequence and frameshift mutations are shown below. Base substitution mutations shown in gray are not detectable as single mutational events. Each symbol represents one mutational event: (Δ) one base deletion; (◊) two base deletion; (▲) one base insertion with identity of the base inserted in parentheses either beside or below the symbol.

Figure S3. Determination of Pol δ4 and Pol κ activities. (A) Specific activity (nmol dTTP incorporated/nmole of enzyme*hr) for each polymerase was determined in the presence and absence of PCNA. Reaction mixtures contained 0.375 OD/mL of poly(dA)/oligo(dT) linear primer-template, 7.5 μM dTTP, 0.75μM ^{3}H-dTTP, 0.1pmol of enzyme, 0.15 μM PCNA, if appropriate, and buffer (25 mM potassium phosphate pH 7.2, 5 mM $MgCl_2$, 2.5 mM DTT, 200 μg/mL nonacetylated BSA) containing 7.5% glycerol in a final volume of 30 μL. Reactions were incubated at 37°C for 15 min, spotted onto DE81 membranes, washed, dried, and counted by liquid scintillation counter. Each bar represents the average of 2 independent reactions. (B) Primer extension reactions using primed circular $[GT]_{10}$-containing ssDNA templates in the presence and absence of PCNA. Reactions contained 100fmol of primed substrate in buffer plus

250 μM dNTPs in a final volume of 20μL, and were preincubated at 37°C for 3 min. Reactions were performed using the amounts of polymerase with addition of 500fmol PCNA as indicated. Synthesis was initiated upon addition of enzyme, and aliquots were removed at 5, 15, and 30 minutes (triangles). Reaction products were separated on an 8% denaturing polyacrylamide gel. Products corresponding to termination within the $[GT]_{10}$ microsatellite are indicated by boxed region.

Figure S4. Dual polymerase reactions using equimolar amounts of total polymerase in side-by-side reactions. Primer extension reactions were performed using 100fmol of primed $[GT]_{10}$-containing ssDNA templates in buffer (25 mM potassium phosphate pH 7.2, 5mM $MgCl_2$, 2.5 mM DTT, 200 μg/mL nonacetylated BSA, 250 μM dNTPs). Synthesis was initiated upon addition of a mixture of Pol κ and Pol δ4 (A reactions) or an equivalent molar amount of Pol δ4 alone (B reactions). Aliquots were removed at 5, 15, and 30 minutes and products were separated on an 8% denaturing polyacrylamide gel. Control lanes (C) indicate a reaction performed with Pol κ alone. Products corresponding to termination within the $[GT]_{10}$ allele are indicated by boxed region.

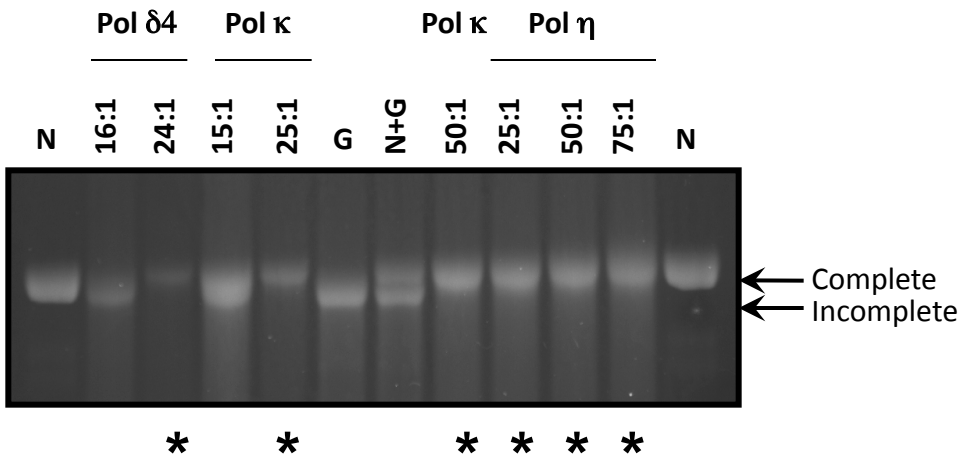
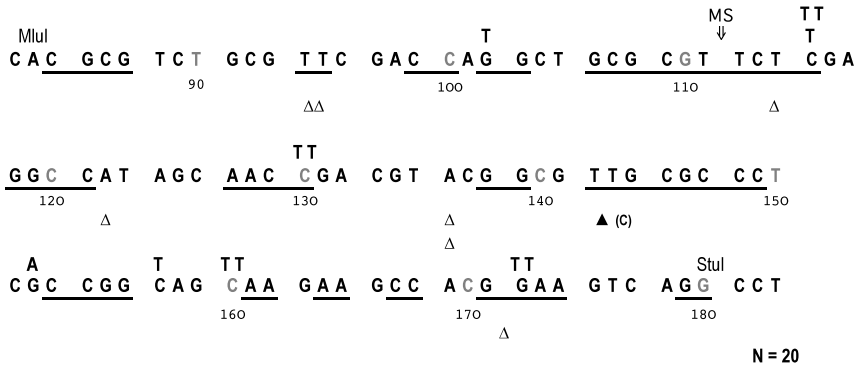
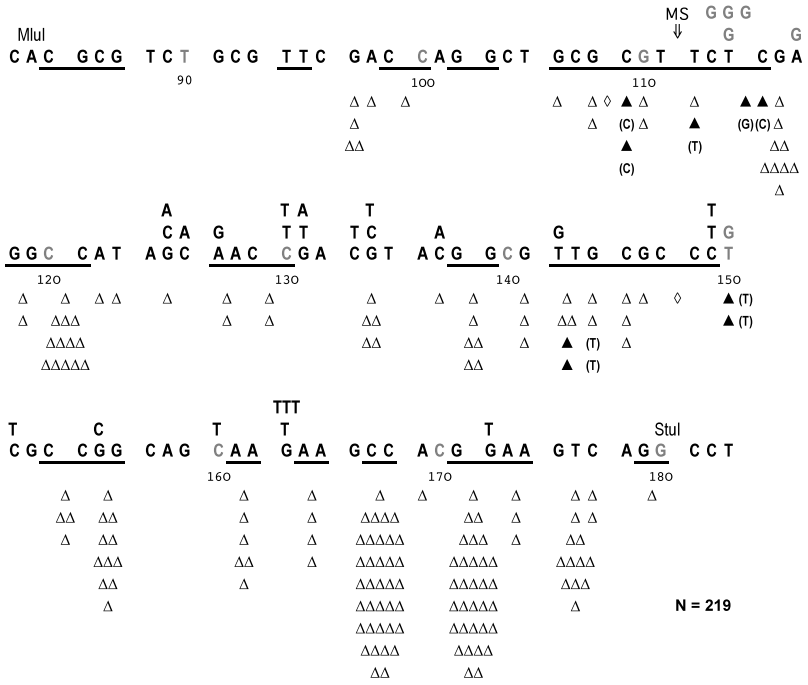

Pol δ4
Pol κ
Pol κ
Pol η
N
16:1
24:1
15:1
25:1
G
N+G
50:1
25:1
50:1
75:1
N
Complete
Incomplete
*
*
*
*
*
*

Figure S1

A. hpolδ



B. hpolκ



C. hpolη

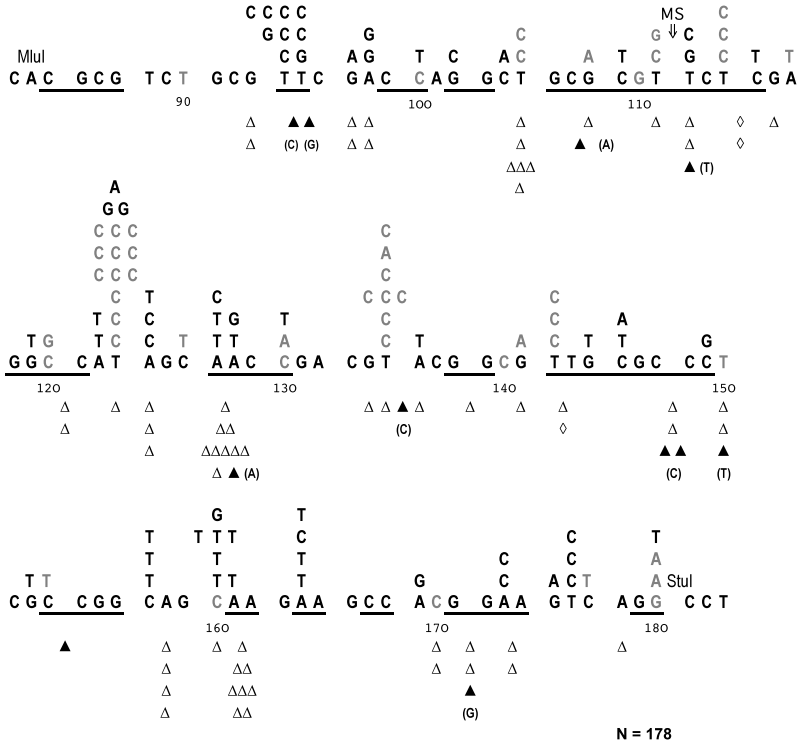


Figure S2

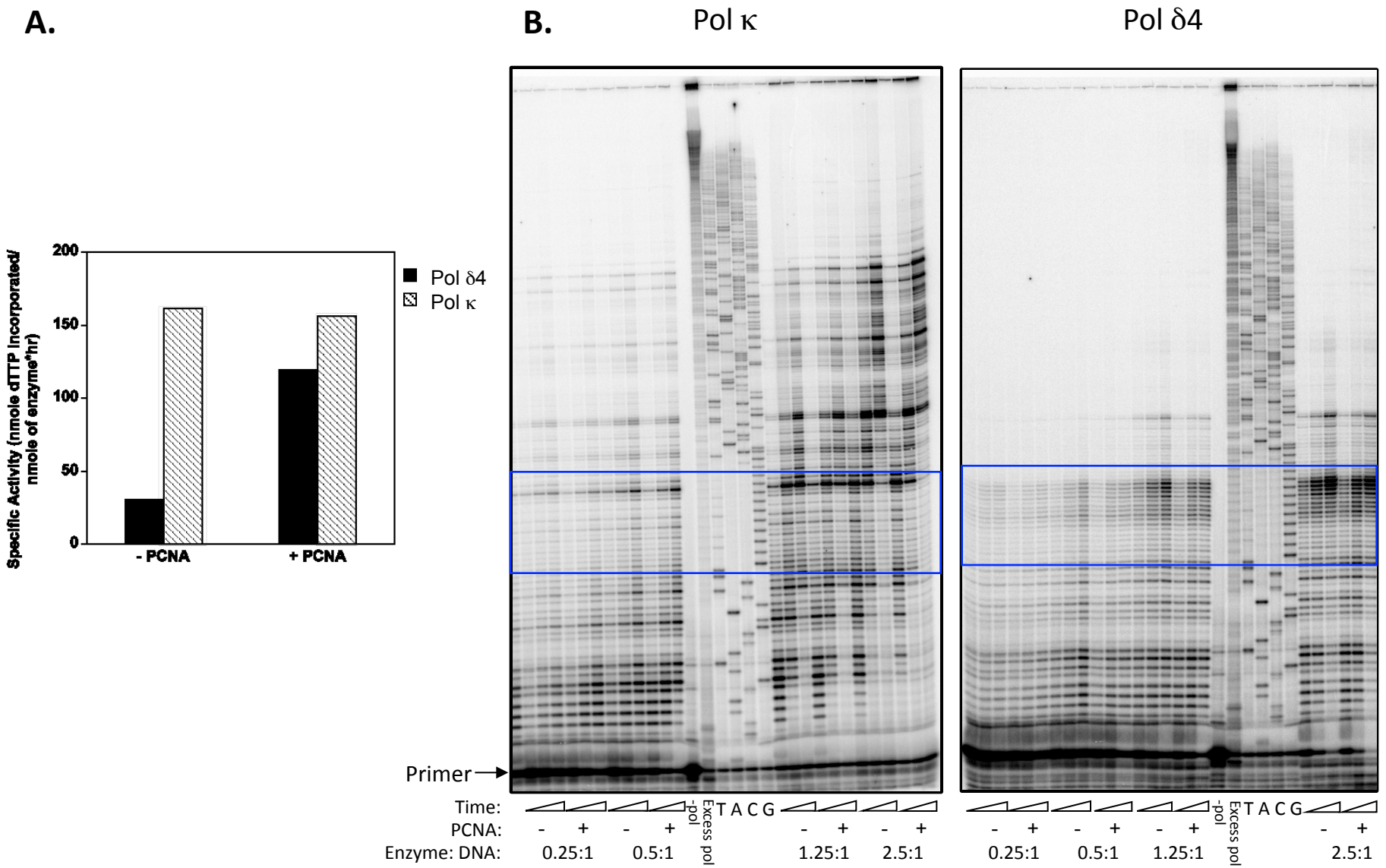

A.
B.
Pol κ
Pol δ4
Specific Activity (nmole dTTP Incorporated/ nmole of enzyme*hr)
200
150
100
50
0
- PCNA
+ PCNA
Pol δ4
Pol κ
Primer
Time:
PCNA:
Enzyme: DNA:
- + - + -pol Excess pol T A C G - + - + - + - + -pol Excess pol T A C G - +
0.25:1
0.5:1
1.25:1
2.5:1
0.25:1
0.5:1
1.25:1
2.5:1

Figure S3

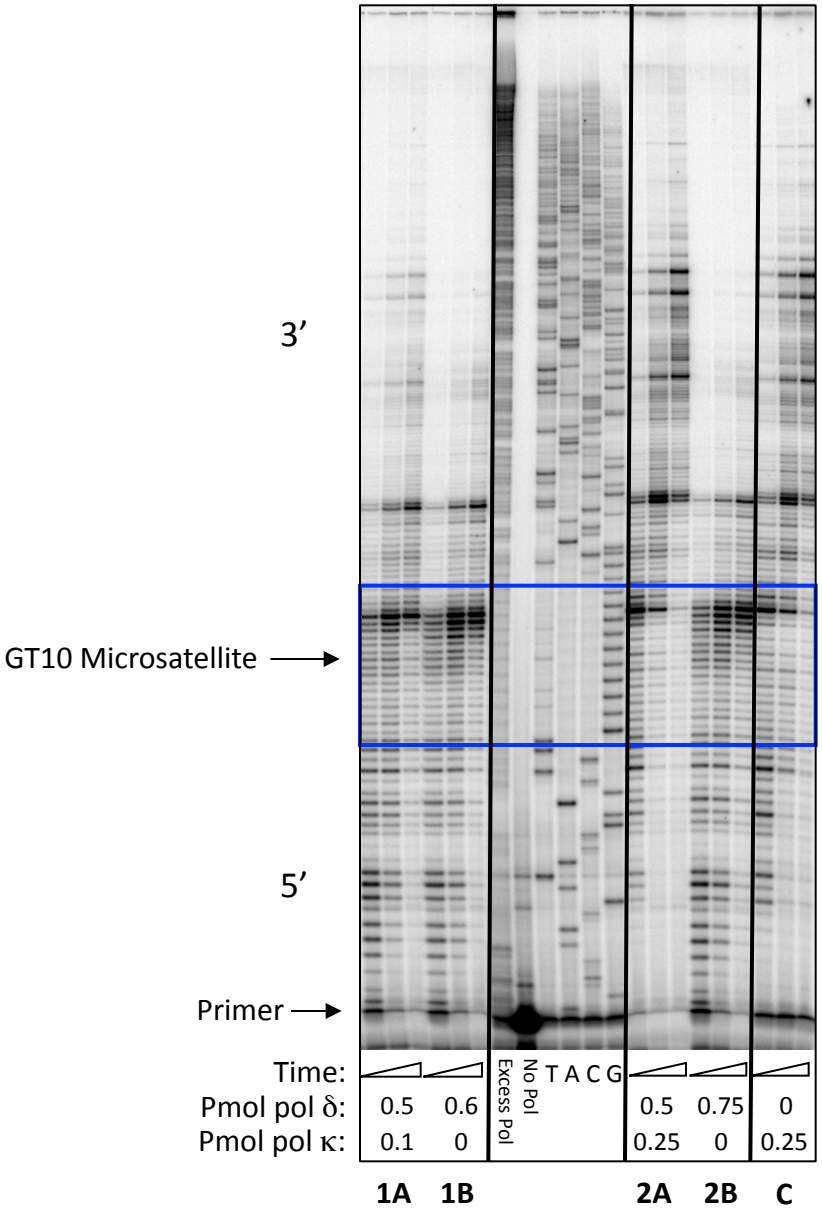

3’
GT10 Microsatellite
5’
Primer
Time:
Pmol pol δ:
Pmol pol κ:
No Pol
Excess Pol
T A C G
0.5
0.6
0.1
0
0.5
0.75
0.25
0
0
0.25
1A
1B
2A
2B
C

Figure S4