

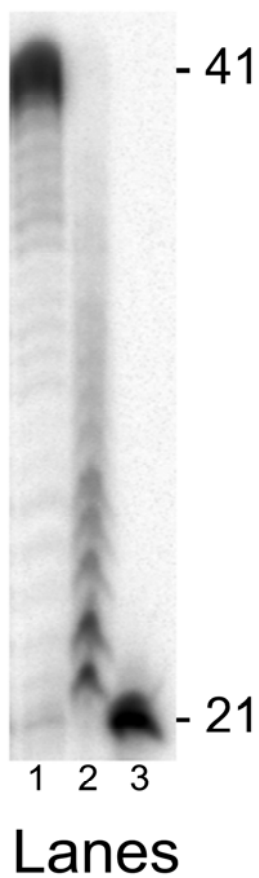
Supplementary Information

Kinetic basis of sugar selection by a Y-family DNA polymerase from *Sulfolobus solfataricus* P2

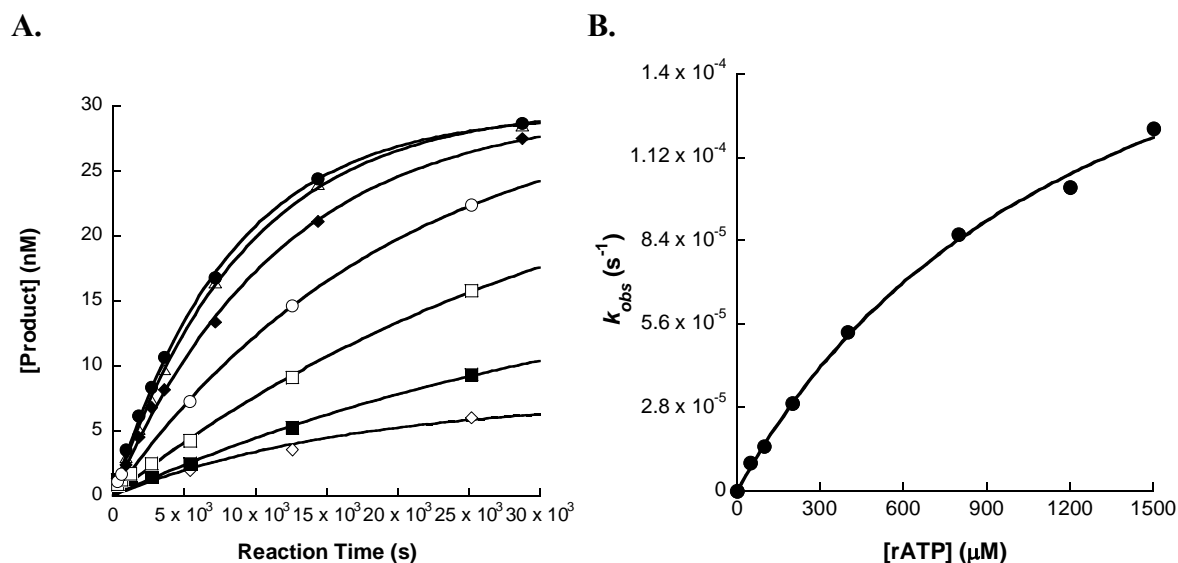
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<i>S.solf</i> Dpo4	1	MIVLFVDFDYFY Y AQVEEVLNPS
<i>S.acid</i> Dbh	1	MIVIFVDFDYF F AQVEEVLNPQ
<i>E.coli</i> UmuC	1	MFALCDVNAFY A SCETVFRPD
<i>E.coli</i> DinB	2	RKIIHVDMDCF F AAVEMRDNPA
<i>H.sapi</i> Pol κ	101	NTIVHIDMDAF Y AAVEMRDNPE
<i>H.sapi</i> Pol η	7	RVVALVDMDCF F VQVEQRQNP
<i>S.cere</i> Pol η	24	ACIAHIDMNAF F AQVEQMRCGL
<i>H.sapi</i> Pol ι	28	RVIVHVDLDCF Y AQVEMISNPE
<i>H.sapi</i> Rev1	417	SCIMHVDMDCF F VSVGIRNRPD

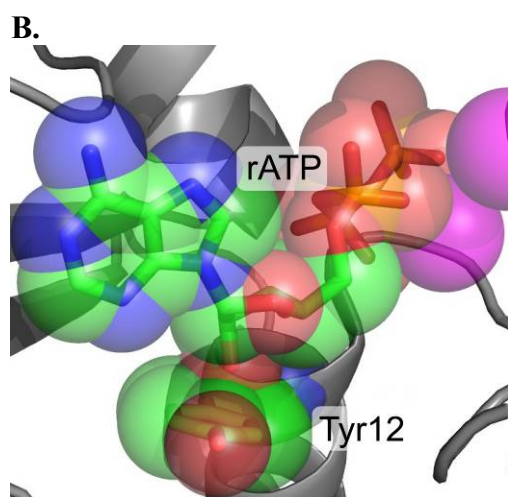
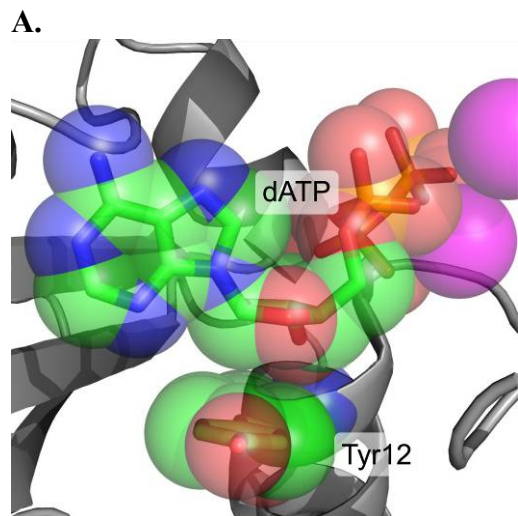
Supplementary Figure 1. Sequence alignment of the Y-family DNA polymerases. The conserved residue in bold-type is the putative ‘steric gate’ residue. Residue numbers are shown on the left side of the primary sequences.



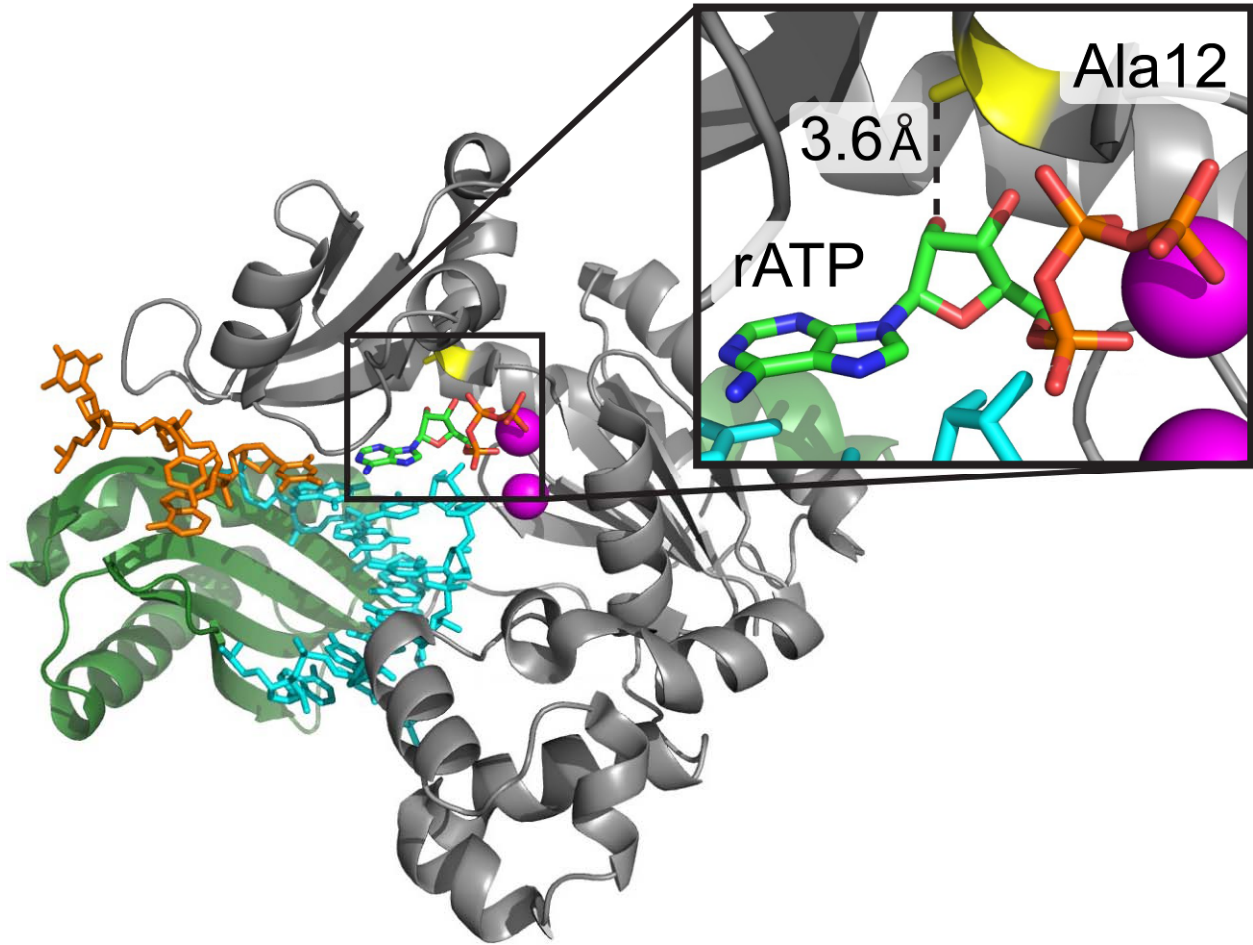
Supplementary Figure 2. Alkaline degradation of full-length extension on D-1 21/41-mer catalyzed by Y12A Dpo4 at 37 °C. Lane 1: A preincubated solution of the Y12A Dpo4 mutant (120 nM) and 5'-radiolabeled D-1 21/41-mer (30 nM) was rapidly mixed with all rNTPs (0.5 mM each). The reaction was quenched with 0.37 M EDTA after 30 min and the products are shown in lane 1. Lane 2: After the reaction in lane 1 was quenched, the solution was mixed with equal volume of 0.5 M NaOH at 60 °C for 10 min before being diluted with more 0.37 M EDTA. The resulting ladder is shown in lane 2. Lane 3: A preincubated solution of only the Y12A Dpo4 mutant (30 nM) and 5'-radiolabeled D-1 21/41-mer (30 nM) in reaction buffer (no rNTPs).



Supplementary Figure 3. Mismatched rATP incorporation into DNA substrate D-1 (Table 1) catalyzed by the Y12A Dpo4 mutant at 37 °C. **(A)** A preincubated solution of the Y12A Dpo4 mutant (120 nM) and 5'-radiolabeled DNA D-1 (30 nM) was rapidly mixed with increasing concentrations of rATP (50 μM, \diamond ; 100 μM, \blacksquare ; 200 μM, \square ; 400 μM, \circ ; 800 μM, \blacklozenge ; 1200 μM, \triangle ; 1500 μM, \bullet) before being quenched by 0.37 M EDTA after various reaction times. Each time course of product formation was fit to Eq 1 (Materials and Methods) to yield k_{obs} values. **(B)** Plot of k_{obs} values versus rATP concentrations was fit to Eq 2 (Materials and Methods) to obtain a k_p of $(2.2 \pm 0.1) \times 10^{-4} \text{ s}^{-1}$ and a $K_{d, rATP}$ of $1,292 \pm 154 \text{ } \mu\text{M}$ (Table 3).



Supplementary Figure 4. Magnification of the active site of the wild-type Dpo4. The Dpo4-bound incoming nucleotide was dATP (**A**) or rATP (**B**). The ternary crystal structure of the wild-type Dpo4 (grey with Tyr12 in multiple colors), and dATP (multiple colors) in (**A**) is from PDB 2AGQ. The ribose ring in (**B**) is from the rATP in PDB 3ETH while the rest of the structure is identical to (**A**). The catalytic metal ions are in magenta.



Supplementary Figure 5. Model of an RNA/DNA primer/temple duplex into the DNA binding cleft of the Y12A Dpo4 mutant. The 5-mer RNA/DNA duplex (cyan) was taken from the coordinates given in PDB 2QK9. The single-strand portion of the DNA template (orange) was taken from the coordinates given in PDB 2AGQ. The Y12A Dpo4 mutant (the polymerase core in grey, the little finger domain in green) was based on the coordinates from PDB 2AGQ. The incoming rATP (multiple colors) was modeled into the polymerase active site as in Supplementary Figure 3B. The catalytic metal ions are in magenta. Residue Ala12 is shown in yellow. The distance from the 2'-OH of the rATP ribose ring to the side chain of Ala12 is 3.6 Å.