

# Supplementary Data

## Probing Conformational Changes of Human DNA Polymerase $\lambda$ Using Mass Spectrometry-Based Protein Footprinting

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**Supplementary Table 1.** Positively-charged residues that potentially stabilize the triphosphate moiety of an incoming nucleotide and/or pyrophosphate product.

DNA polymerase (PDB)	Family	$\alpha$ -phosphate	$\beta$ -phosphate	$\gamma$ -phosphate	Notes	Reference
Human Pol $\lambda$ (1XSN)	X	None	R420 (3.00 and 3.10 Å) S417 (3.06 Å)	R386 (2.90 and 3.04 Å)	Backbone of S417	[1]
Human Pol $\lambda$ (1XSP)	X	Linked to primer	R420 (2.82 and 3.74 Å) S417 (2.65 Å)	R386 (2.77, 3.39, 3.73 and 3.77 Å) G426 (3.08 Å)	PPi bound Backbone for S417 and G426	[1]
Human Pol $\beta$ (1BPY)	X	None	R183 (2.84 and 2.85 Å)	None	Some R nearby but >4.0 Å	[2]
Pol mu (2IHM)	X	None	R323 (3.23 Å)	K325 (2.83 Å) H329 (2.93 and 3.05 Å)	Side chain and backbone for H329	[3]
Mouse TdT (1KEJ)	X	R454 (2.57 Å)	R336 (3.57 Å)	H342 (2.84 Å)	Backbone for H342	[4]
Dpo4 (1JX4)	Y	R51 (3.13 Å) Y10 (3.40 Å)	R51 (2.82 Å) K159 (2.93 Å) Y48 (3.71 Å)	N/A	dADP, backbone of Y10	[5]
Dpo4	Y	K159 (3.64 Å)	F11 (3.03 and 3.03 Å)	R51 (2.89 and 3.53 Å)	dCTP,	[6]

(2ATL)			K159 (2.93 Å)	Y10 (3.09 Å) K159 (3.12 and 3.27 Å) Y48 (2.55 Å)	backbone of Y10 and F11	
Dpo4 (2AGO)	Y	None when linked to primer	K159 (3.58, 3.65 and 3.82 Å)	R51 (3.04 and 3.94 Å) Y10 (3.13 and 3.28 Å)	dGMP-PPi structure backbone of Y10	[7]
Dbh (3BQ1)	Y	None	F11 (2.93 Å) Y10 (3.34 Å)	K160 (3.08, 3.17, and 3.59 Å) R50 (3.17 and 3.22 Å)	Backbone of Y10 and F11	[8]
Yeast Pol eta (2R8J)	Y	R73 (3.24 and 3.35 Å)	R73 (3.02 and 3.15 Å)	R67 (3.64 and 4.01 Å) K289 (2.89 Å)	dCTP, 3'-Cisplatin structure, AA numbering is according to PDB file	[9]
Human Pol iota (2FLL)	Y	None	C37 (3.63 Å)	R71 (2.92 and 3.02 Å) K77 (3.60 Å) K214 (2.60 Å) F38 (3.34 Å)	Backbone for C37 and F38	[10]
Human Pol kappa (2OH2)	Y	None	F111 (2.95 Å)	R144 (2.74, 3.64 and 3.69 Å) K328 (2.81 Å) A110 (3.28 Å)	Backbone for A110 and F111	[11]
Rev1 (2AQ4)	Y	None	N414 (2.94 Å)	R408 (2.69 and 2.77 Å) K525 (3.02 Å) C365 (2.62 Å)	Backbone for C365	[12]
T7 (1SKR)	A	K522 (2.85 Å)	H506 (2.75 Å) G478 (3.40 Å)	R518 (2.83 and 3.33 Å) K522 (3.60 Å) G478 (2.71 Å)	Backbone for G478	[13]
<i>Bacillus stearothermo philus</i> DNA polymerase I Fragment (1LV5)	A	K706 (3.10 Å)	H682 (3.59 Å) I657 (3.79 and 3.90 Å)	R702 (2.91 and 3.18 Å) Q656 (3.21 Å)	Backbone for Q656	[14]
<i>Tag</i>	A	None within 4.0	None within 4.0 Å	None within 4.0 Å	open ternary	[15]

(2KTQ)		Å				
<i>Taq</i> (3KTQ)	A	K663 (3.01 and 3.94 Å)	H639 (3.10 Å)	R659 (2.97 and 2.87 Å)	active ternary	[15]
RB69 (2OZS)	B	K560 (3.19 Å)	N564 (3.72 Å)	R482 (2.80 and 2.94 Å) K560 (2.80 Å)		[16]
Phi29 (2PYL)	B	None	N387 (3.50 Å) K383 (3.60 and 3.89 Å)	K371 (3.90 Å)		[17]
HIV-1 RT (2IAJ)	RT	None	None	K219 (3.07 Å)	Mutant, no DNA RNA/DNA substrate, ATP is bound	[18]

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