SUPPLEMENTARY DATA

Biochemical analysis of active site mutations of human polymerase η

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Supplementary Table 1 – Primer sequences used in site-directed mutagenesis reactions to generate single amino acid mutants in the catalytic core of human DNA polymerase η (amino acids 1-511). Sequence differences compared to wild type are underlined.

Mutant	Forward Primer	Reverse Primer
M14V	5′-GTGGTTGCTCTCGTGGACGTGGACTGTTTTTTTGTTC	5′-GAACAAAAAAACAGTCCACGTCCACGAGAGCAACCAC
F17L	5′-GTGGACATGGACTGTTTGTTTGTTCAAGTGGAG	5′-CTCCACTTGAACAAACAAACAGTCCATGTCCAC
Q38A	5′-CCTTGTGCAGTTGTAGCGTACAAATCATGG	5′-CCATGATTTGTAGGCTACAACTGCACAAGG
Q38V	5′-CCTTGTGCAGTTGTAGTGTACAAATCATGG	5′-CCATGATTTGTACACTACAACTGCACAAGG
Y52E	5′-GGAATAATTGCAGTGAGTGAGGAAGCTCGTGCATTTGG	5′-CCAAATGCACGAGCTTCCTCACTCACTGCAATTATTCC
R55A	5′-AGTTATGAAGCTGCTGCATTTGGAGTC	5′-GACTCCAAATGCAGCAGCTTCATAACT
R61A	5′-GCATTTGGAGTCACTGCAAGTATGTGGGC	5′-GCCCACATACTTGCAGTGACTCCAAATGC
S62A	5′-GGAGTCACTAGAGCTATGTGGGCAGAT	5′-ATCTGCCCACATAGCTCTAGTGACTCC
S62G	5′-GGAGTCACTAGAGGTATGTGGGCAGATGGATGC	5′-ATCTGCCCACATACCTCTAGTGACTCC
R81C	5′-TTCTACTGGCACAAGTTTGTGAGTCCCGTGGG	5′-CCCACGGGACTCACAAACTTGTGCCAGTAGAA
E82D	5′-TCTACTGGCACAAGTTCGTGATTCCCGTGGGAA	5′-TTCCCACGGGAATCACGAACTTGTGCCAGTAGA

Supplementary Table 2 – Base substitution error spectrum of truncated pol η mutants as calculated from forward gap filling assay. Values given are the calculated frequency of errors per 10,000 bases copied. Rates relative to wild type are given in parentheses. Values in red indicate rates greater than 5 fold above wild type rate. Values in green are rate less than 5 fold the wild type rate. For changes that were not observed, rates were calculated as if a single instance was found and the values presented as less than or equal to (≤) values.

Base	Mutation From → To	Mispair Template·dNTP	Error Rate (x 10^{-4}) WT	M14V	Q38A	Q38V	Y52E	R61A	S62A	S62G	R81C	E82D
A	A → C	A·dGTP	**72**	65 (0.9X)	16 (0.2X)	53 (0.7X)	6 (0.1X)	22 (0.3X)	22 (0.3X)	35 (0.5X)	54 (0.8X)	26 (0.4X)
	A → G	A·dCTP	**60**	27 (0.5X)	40 (0.7X)	21 (0.4X)	6 (0.1X)	11 (0.2X)	38 (0.6X)	35 (0.6X)	38 (0.6X)	16 (0.3X)
	A → T	A·dATP	**150**	65 (0.4X)	88 (0.6X)	126 (0.8X)	6 (0.04X)	22 (0.1X)	22 (0.1X)	52 (0.3X)	87 (0.6X)	58 (0.4X)
	A → N	A·dNTP	**283**	157 (0.6X)	144 (0.5X)	200 (0.7X)	18 (0.1X)	56 (0.2X)	49 (0.2X)	122 (0.4X)	179 (0.6X)	100 (0.4X)
C	C → A	C·dTTP	**5**	18 (3.6X)	52 (10.4X)	13 (2.6X)	≤ 5 (1.0X)	≤ 5 (1.0X)	18 (3.6X)	19 (3.8X)	≤ 4 (0.8X)	9 (1.8X)
	C → G	C·dCTP	**10**	≤ 4 (0.4X)	7 (0.7X)	9 (0.9X)	20 (2.0X)	≤ 5 (0.5X)	≤ 4 (0.4X)	5 (0.5X)	9 (0.9X)	≤ 4 (0.4X)
	C → T	C·dATP	**63**	44 (0.7X)	78 (1.2X)	26 (0.4X)	20 (0.3X)	18 (0.3X)	57 (0.9X)	75 (1.2X)	26 (0.4X)	17 (0.3X)
	C → N	A·dNTP	**78**	62 (0.8X)	130 (1.7X)	47 (0.6X)	39 (0.5X)	18 (0.2X)	75 (1.0X)	99 (1.3X)	40 (0.5X)	26 (0.3X)
G	G → A	G·dTTP	**75**	28 (0.4X)	100 (1.3X)	77 (1.0X)	≤ 6 (0.1X)	6 (0.1X)	11 (0.1X)	54 (0.7X)	28 (0.4X)	22 (0.3X)
	G → C	G·dGTP	**13**	17 (1.3X)	25 (1.9X)	≤ 5 (0.4X)	6 (0.5X)	≤ 6 (0.5X)	23 (1.8X)	18 (1.4X)	17 (1.3X)	11 (0.8X)
	G → T	G·dATP	**13**	17 (1.3X)	42 (3.2X)	11 (0.8X)	6 (0.5X)	6 (0.5X)	6 (0.5X)	24 (1.8X)	11 (0.8X)	16 (1.2X)
	G → N	A·dNTP	**100**	62 (0.6X)	167 (1.7X)	88 (0.9X)	13 (0.1X)	12 (0.1X)	40 (0.4X)	97 (1.0X)	57 (0.6X)	49 (0.5X)
T	T → A	T·dTTP	**20**	22 (1.1X)	163 (8.2X)	38 (1.9X)	10 (0.5X)	23 (1.2X)	40 (2.0X)	24 (1.2X)	18 (0.9X)	21 (1.1X)
	T → C	T·dGTP	**385**	335 (0.9X)	345 (0.9X)	457 (1.2X)	166 (0.4X)	164 (0.4X)	397 (1.0X)	297 (0.8X)	375 (1.0X)	235 (0.6X)
	T → G	T·dCTP	**24**	26 (1.1X)	33 (1.4X)	≤ 4 (0.2X)	24 (1.0X)	5 (0.2X)	9 (0.4X)	8 (0.3X)	31 (1.3X)	38 (1.6X)
	T → N	A·dNTP	**429**	383 (0.9X)	540 (1.3X)	495 (1.2X)	200 (0.5X)	191 (0.4X)	445 (1.0X)	334 (0.8X)	423 (1.0X)	295 (0.7X)
			227	**173 (0.8X)**	**255 (1.1X)**	**215 (0.9X)**	**73 (0.3X)**	**74 (0.3X)**	**172 (0.8X)**	**169 (0.7X)**	**182 (0.8X)**	**122 (0.5X)**