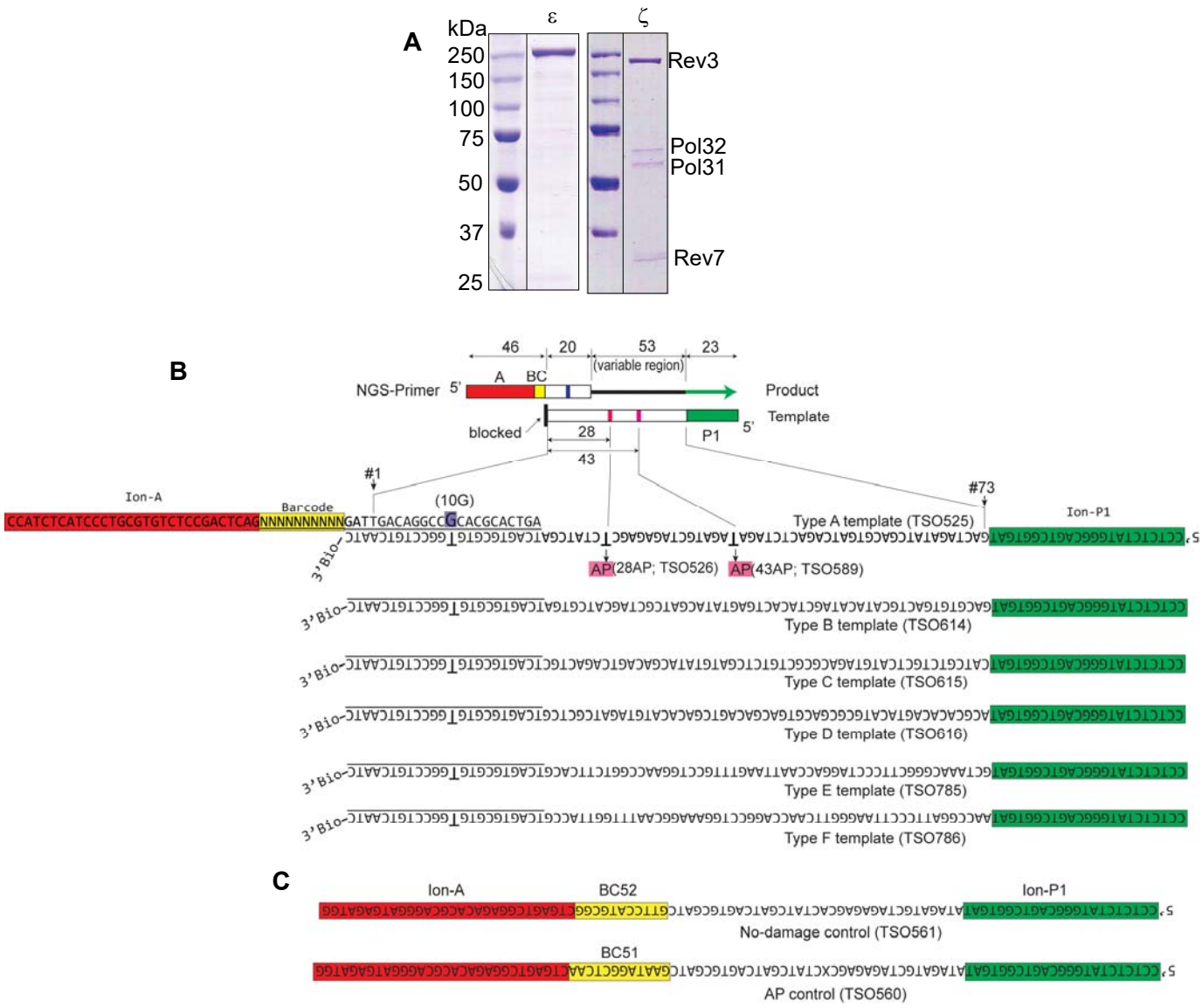

Figure S1. (**A**) Proteins purified for this study. Pol ε (0.84 μg) and four-subunits complex of Pol ζ (0.39 μg of Rev3) were separated by SDS-PAGE and stained by coomassie brilliant blue R250. (**B**) NGS substrates. Type A to Type F templates share the Ion-P1 adaptor (green box) and primer-binding site (underlined), but have different sequences between them (from #21 to #73 in the Figure). The variable regions in type A –D templates were excluding repeated nucleotides, and type E and F contain homodimers (like AA). (**C**) Control oligonucleotides that have two adaptors. TSO560 has an abasic site at the position indicated by "X".

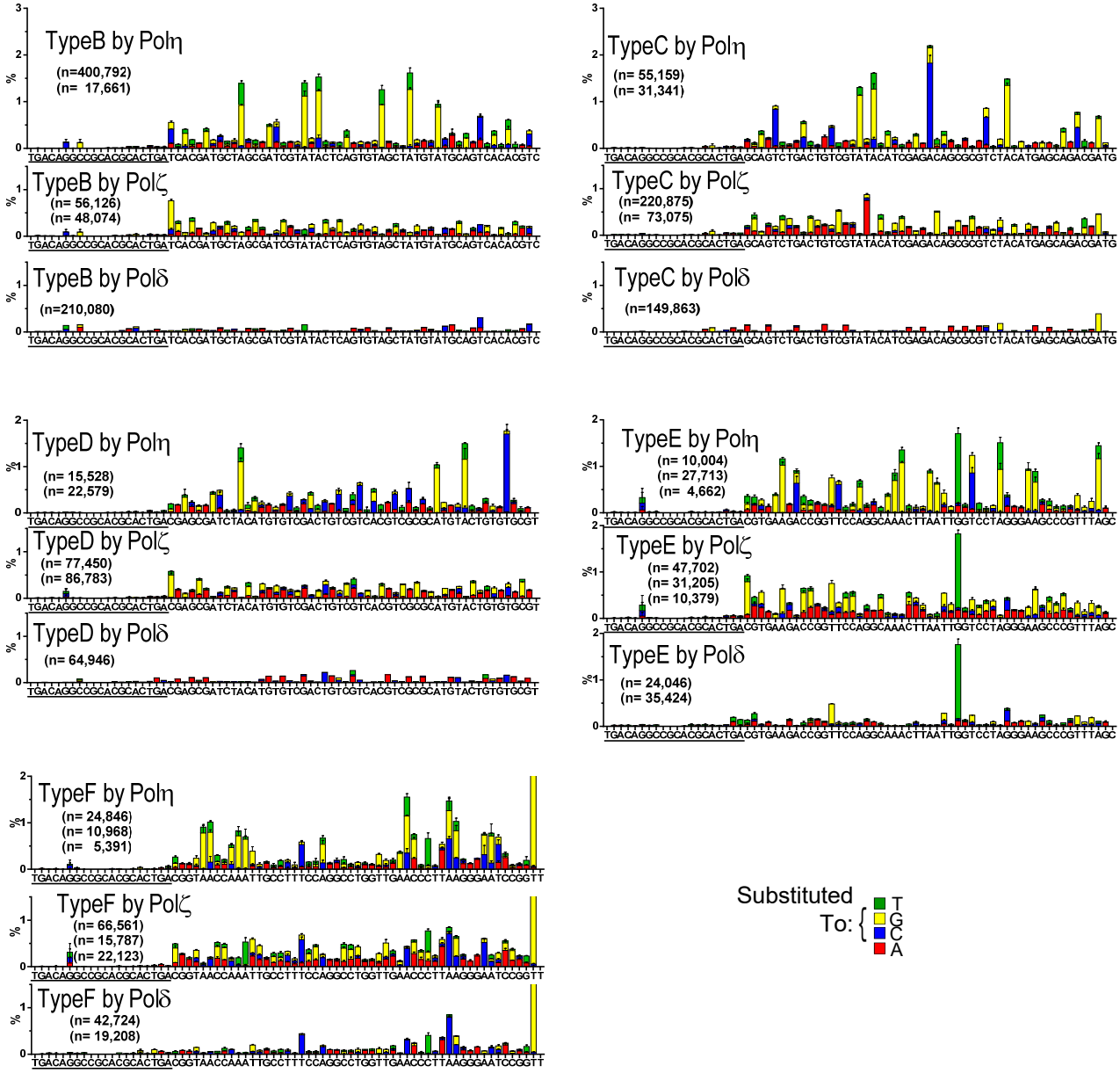

Figure S2. Same experiments as in Figure 2 were repeated with five undamaged templates (Type B to F) by indicated polymerases. Error bars are SD (n=2 to 3).

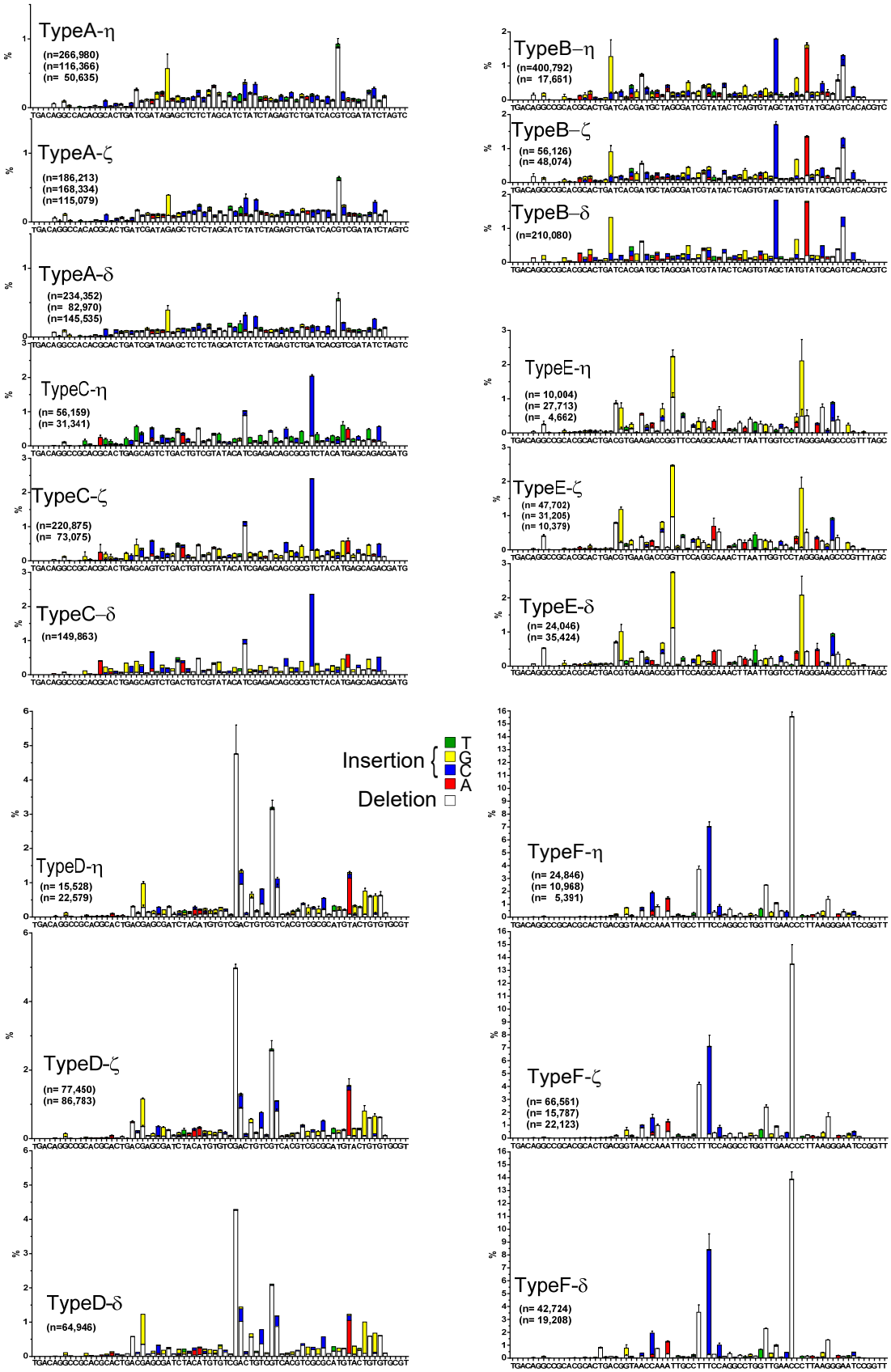

Figure S3. Rates of single nucleotide insertions and deletions on six undamaged templates (type A-F) by Polη, Polζ, and Polδ. For insertions, positions of colored bars indicate the positions after which insertions were observed.

Table S1. Synthetic DNA oligos. Type-A to F templates have same sequences except for the internal 53-nt region (variable region; Figure 1A and Figure S1B). Ion Torrent primers have same sequences except for the barcode (BC).

Name	Sequence (5'-3')	Use	Source
TSO525	CCTCTCTATGGGCAGTCGGTGATGACTAGATATCGACGT GATCAGACTCTAGATAGATGCTAGAGAGCTCTATCGATC AGTGCGTGTGGCCTGTCAATC/3Bio/	Template (Type-A) 3'-Biotin Undamaged	IDT
TSO526	CCTCTCTATGGGCAGTCGGTGATGACTAGATATCGACGT GATCAGACTCTAGATAGATGCTAGAGAGCXCTATCGATC AGTGCGTGTGGCCTGTCAATC/3Bio/	Template (Type-A) 3'-Biotin 28AP (indicated as "X")	IDT
TSO589	CCTCTCTATGGGCAGTCGGTGATGACTAGATATCGACGT GATCAGACTCTAGAXAGATGCTAGAGAGCTCTATCGATC AGTGCGTGTGGCCTGTCAATC/3Bio/	Template (Type-A) 3'-Biotin 43AP (indicated as "X")	IDT
TSO614	CCTCTCTATGGGCAGTCGGTGATGACGTGTGACTGCATA CATAGCTACACTGAGTATACGATCGCTAGCATCGTGATC AGTGCGTGTGGCCTGTCAATC/3Bio/	Template (Type-B) 3'-Biotin Undamaged	IDT
TSO615	CCTCTCTATGGGCAGTCGGTGATCATCGTCTGCTCATGT AGACGCGCTGTCTCGATGTATACGACAGTCAGACTGCTC AGTGCGTGTGGCCTGTCAATC/3Bio/	Template (Type-C) 3'-Biotin Undamaged	IDT
TSO616	CCTCTCTATGGGCAGTCGGTGATACGCACACAGTACATG CGCGACGTGACGACAGTCGACACATGTAGATCGCTCGTC AGTGCGTGTGGCCTGTCAATC/3Bio/	Template (Type-D) 3'-Biotin Undamaged	IDT
TSO785	CCTCTCTATGGGCAGTCGGTGATGCTAAACGGGCTTCCC TAGGACCAATTAAGTTTGCCTGGAACCGGTCTTCACGTC AGTGCGTGTGGCCTGTCAATC[BtnTg]	Template (Type-E) 3'-Biotin Undamaged	Sigma
TSO786	CCTCTCTATGGGCAGTCGGTGATAACCGGATTCCCTTAA GGGTTCAACCAGGCCTGGAAAGGCAATTTGGTTACCGTC AGTGCGTGTGGCCTGTCAATC[BtnTg]	Template (Type-F) 3'-Biotin Undamaged	Sigma
TSO590	GGTAAGGTAACGATTGACAGGCCACACGCACTGA	Primer for Gel assay	Fisher
TSO686	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGAA CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC2)	Fisher
TSO687	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGATT CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC3)	Fisher
TSO688	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGAT CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC4)	Fisher
TSO689	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGAA CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC5)	Fisher
TSO690	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTT CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC6)	Fisher
TSO691	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATT CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC7)	Fisher
TSO692	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCGATAA CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC8)	Fisher
TSO693	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAA CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC9)	Fisher
TSO694	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAA CGATTGACAGGCCGCACGCACTGA	Primer For IonTorrent (BC10)	Fisher

TSO695	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTCGAAT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC11)	Fisher
TSO696	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGTGGTT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC12)	Fisher
TSO697	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAACGGA CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC13)	Fisher
TSO698	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGTGT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC14)	Fisher
TSO699	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGAGGT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC15)	Fisher
TSO700	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGATGA CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC16)	Fisher
TSO701	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTATTCGT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC17)	Fisher
TSO702	**CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGCAATTG CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC18)	Fisher
TSO703	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAGTCGGA CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC19)	Fisher
TSO704	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGATCCAT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC20)	Fisher
TSO705	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCAATTA CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC21)	Fisher
TSO706	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGAGACG CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC22)	Fisher
TSO707	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCCACGAA CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC23)	Fisher
TSO708	**CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCTCATT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC24)	Fisher
TSO709	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTGAGATA CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC25)	Fisher
TSO710	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTACAACCT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC26)	Fisher
TSO711	**CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCATCCG CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC27)	Fisher
TSO712	**CCATCTCATCCCTGCGTGTCTCCGACTCAGATCCGGAAT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC28)	Fisher
TSO713	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGACCACT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC29)	Fisher
TSO714	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAGGTTAT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC30)	Fisher
TSO715	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAAGCTG CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC31)	Fisher
TSO716	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTACACA CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC32)	Fisher
TSO717	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCATTG AACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC33)	Fisher
TSO718	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCATCGT TCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC34)	Fisher
TSO719	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGCCATT GTCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC35)	Fisher

TS0720	**CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGAATCG TCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC36)	Fisher
TS0721	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTGAGAAT GTCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC37)	Fisher
TS0722	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGGACG GACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC38)	Fisher
TS0723	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTAACAATCG GCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC39)	Fisher
TS0724	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACATAA TCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC40)	Fisher
TS0725	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCACTTC GCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC41)	Fisher
TS0726	**CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCACGAAT CGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC42)	Fisher
TS0727	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTGACACC GCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC43)	Fisher
TS0728	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCC AGCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC44)	Fisher
TS0729	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGCTTC CTCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC45)	Fisher
TS0730	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCAGTCCGA ACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC46)	Fisher
TS0731	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGCAAC CACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC47)	Fisher
TS0732	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTAAGAG ACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC48)	Fisher
TS0733	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCTAACAT AACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC49)	Fisher
TS0734	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGACAATG GCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC50)	Fisher
TS0584	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCT TCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC60)	Fisher
TS0585	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGA TCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC61)	Fisher
TS0586	**CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTT CACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC62)	Fisher
TS0587	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGT TCGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC63)	Fisher
TS0588	**CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCC GACGATTGACAGGCCGCACGCACTGA**	Primer For IonTorrent (BC64)	Fisher
TS0560	**CCTCTCTATGGGCAGTCGGTGATATAGATGCTAGAGAGC XCTATCGATCAGTGCGATCGAATAGGCTCAA CTGAGTCGGAGACACGCAGGGATGAGATGG**	Control Oligo (with AP; indicated as "X") For IonTorrent (BC51)	IDT
TS0561	**CCTCTCTATGGGCAGTCGGTGATATAGATGCTAGAGAGC ACTATCGATCAGTGCGATCGTTCCATGCGGCTGA GTCGGAGACACGCAGGGATGAGATGG**	Control Oligo (w/o damage) For IonTorrent (BC52)	IDT

Table S2. Summary of NGS analysis for abasic and undamaged type A templates.

	No-damage (Type A)			28AP (Type A)			43AP (Type A)		
Polymerase	Barcode#	Qualified Read (N)	N with 10G (n)	Barcode#	Qualified Read (N)	N with 10G (n)	Barcode#	Qualified Read (N)	N with 10G (n)
Polη	4	267,526	266,980	22	47,644	47,542	40	19,002	18,934
Polη	10	116,744	116,366	28	48,137	48,046	46	5,964	5,946
Polη	16	50,819	50,635	34	61,912	61,777	61	2,516	2,504
Polζ	5	186,716	186,213	23	216,448	216,014	41	155,208	154,919
Polζ	11	168,710	168,334	29	217,182	216,751	47	114,956	114,727
Polζ	17	115,375	115,079	35	250,922	250,492	62	19,544	19,455
Polη+ζ	6	123,736	123,458	24	78,384	78,248	42	100,393	100,176
Polη+ζ	12	97,916	97,710	30	136,187	135,922	48	235,405	234,770
Polη+ζ	18	123,360	123,126	36	130,582	130,362	63	30,027	29,893
No Pol	7	2,594	2,578	25	2,258	2,245	43	1,224	1,210
No Pol	13	1,318	1,310	31	2,775	2,757	49	2,618	2,600
No Pol	19	2,262	2,242	37	1,823	1,816	64	555	550
Polδ	2	235,223	234,352						
Polδ	8	83,650	82,970						
Polδ	14	145,990	145,535						
Polε	3	235,366	234,284						
Polε	9	167,361	166,884						
Polε	15	115,814	115,497						

	Control oligo without damage, no polymerase		
	Barcode#	Qualified Read (N)	N with 10G (n)
No Pol	52	69,892	NA

	Control oligo with AP, no polymerase		
	Barcode#	Qualified Read (N)	N with 10G (n)
No Pol	51	1,541	NA

Table S3. Summary of NGS analysis with type B to F templates.

	No-damage (Type B)			No-damage (Type C)			No-damage (Type D)			No-damage (Type E)			No-damage (Type F)		
	Barcode#	Qualified Read (N)	N with 10G (n)	Barcode#	Qualified Read (N)	N with 10G (n)	Barcode#	Qualified Read (N)	N with 10G (n)	Barcode#	Qualified Read (N)	N with 10G (n)	Barcode#	Qualified Read (N)	N with 10G (n)
Polδ	48	216,737	210,080	49	153,759	149,863	50	66,491	64,946	60	24,145	24,046	61	43,070	42,724
Polδ										63	35,605	35,424	64	19,399	19,208
Polη	19	411,091	400,792	21	56,446	55,159	26	15,978	15,528	28	10,056	10,004	30	25,064	24,846
Polη	32	17,989	17,661	34	32,710	31,341	36	23,196	22,579	38	27,832	27,713	41	11,124	10,968
Polη										43	4,643	4,622	45	5,445	5,391
Polζ	20	56,948	56,126	25	225,590	220,875	27	79,807	77,450	29	47,933	47,702	31	67,407	66,561
Polζ	33	49,059	48,074	35	74,496	73,075	37	89,395	86,783	39	31,353	31,205	42	16,456	15,787
Polζ										44	10,430	10,379	47	22,311	22,123
No Pol										18	452	57			

	Control oligo without damage, no polymerase		
Polymerase	Barcode#	Qualified Read (N)	N with 10G (n)
No Pol	52	10,493	NA

	Control oligo with AP, no polymerase		
Polymerase	Barcode#	Qualified Read (N)	N with 10G (n)
No Pol	51	0	NA

Table S4. Base-substitution rates on undamaged templates.

Data obatined with type A to F templates were averaged after subtraction of background rates obtained in Pol-delta mediated reactions.

Polη				Polζ			
From	**To**	**Avr (%)**	**STD**	**From**	**To**	**Avr (%)**	**STD**
A	C	0.072001	0.231915	A	C	0.018665	0.063343
A	G	0.584463	0.339584	A	G	0.043357	0.085708
A	T	0.152433	0.152056	A	T	0.019069	0.057238
C	A	0.01706	0.017012	C	A	0.072231	0.046962
C	G	0.033472	0.044221	C	G	0.212069	0.080492
C	T	0.050209	0.032468	C	T	0.035905	0.025726
G	A	0.042319	0.026869	G	A	0.058707	0.034413
G	C	0.014348	0.038731	G	C	0.011038	0.015567
G	T	0.004897	0.015081	G	T	0.01668	0.012629
T	A	0.049124	0.029224	T	A	0.090879	0.091182
T	C	0.141028	0.310944	T	C	0.02506	0.021895
T	G	0.062356	0.050967	T	G	0.079522	0.056363
Total substitutions		1.22371		Total substitutions		0.683184	
Mispaired template base (% in total substitutions)				Mispaired template base (% in total substitutions)			
A-template base		20.63461		A-template base		28.6103	
C-template base		5.030889		C-template base		12.65033	
G-template base		8.232498		G-template base		46.86967	
T-template base		66.102		T-template base		11.8697	
Total Misinserted base (% in total substitutions)				Total Misinserted base (% in total substitutions)			
A-Misinsert		8.866698		A-misinsert		32.46823	
C-Misinsert		18.5809		C-misinsert		8.015838	
G-Misinsert		55.59252		G-Misinsert		49.02763	
T-Misinsert		16.95988		T-Misinsert		10.4883	