Supplementary material

Fig. S1 Amino acid sequence alignment of the exonuclease domain of AtPolIs versus KF-DNAP I. The conserved exonuclease motifs I, II, and II are underlined. Amino acid involved in the two-metal ion metal ion mechanism for exonucleolysis in KF-DNAP I are indicated by an arrow.

Fig. S2 Amino acid sequence alignment of the polymerase domain of AtPolIs versus –DNAPs I from *E. coli*, *T. aquaticus*, and *G. stearothermophilus*. The subdomain organization is indicated by color arrows as follow: thumb, green; palm, brown; and fingers, purple. The three unique amino acid insertions in AtPolIs are indicated by blue, red and yellow boxes respectively.

Fig. S3 Amino acid sequence alignment of the polymerase domain of representative POPs from plants, protists and algae in comparison to KF-DNAP I. Three insertion are indicated by a black line.

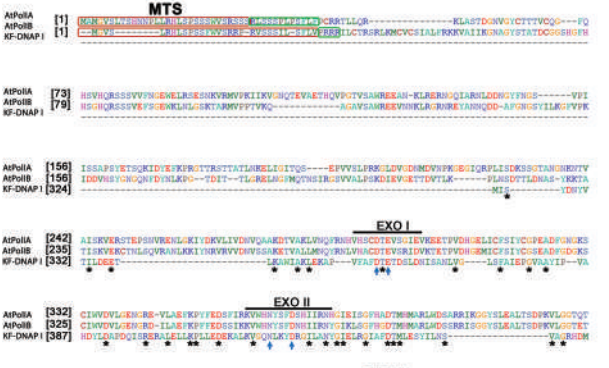
Fig. S4 Deletion of the disorder region in AtPolIs increase protein purification yields with out altering polymerization, exonuclease and TLS activities A) SDS-PAGE gel showing purified AtPolIs result of the removal of the N-terminal disorder region (residues 31 to 257). These constructs resemble the Klenow fragment of *E. coli* DNAP I and are dubbed KF-AtPolIs **B) Comparison between polymerization and exonuclease activities of full-length and KF-AtPols.** Time course reaction from 15 to 60 sec showing the exonuclease (lanes 1 to 12) and the polymerase (lanes 13 to 24) activity of AtPolIs in comparison to KF-AtPolIs. Exonucleolysis started by the addition of 2mM $MgCl_2$, whereas primer extension started by addition of 2mM $MgCl_2$ and 50 μM of each dNTP. **C)** Primer extension reactions by by full-length and KF-AtPolIs on a primer-template contain an AP site (tetrahydrofuran) in the templating position located immediately after the 3'-OH end of the primer. Time points were taken at 5, 10, 20, 40 and 80 sec after the addition of $MgCl_2$ Both full-length and Klenow fragment AtPolIs bypass an AP site (lanes 1 to 20) The migration of the 19-mer primer and full length extension 28-mer product are labeled.

Fig. S6 SDS-PAGE showing the purification of KF AtPolIB deletions. 10%SDS-PAGE gel showing the purification of KF-AtPolIB deletions with molecular weight between 85 to 95 KDa.

Fig. S7 Amino acid sequence alignment of the polymerase domain of AtPolIs in comparison to POLQ. Three insertions in POLQ and AtPolIs are indicated by a red and green line respectively.

Table S1 Oligonucleotides used in this study

Fig S1



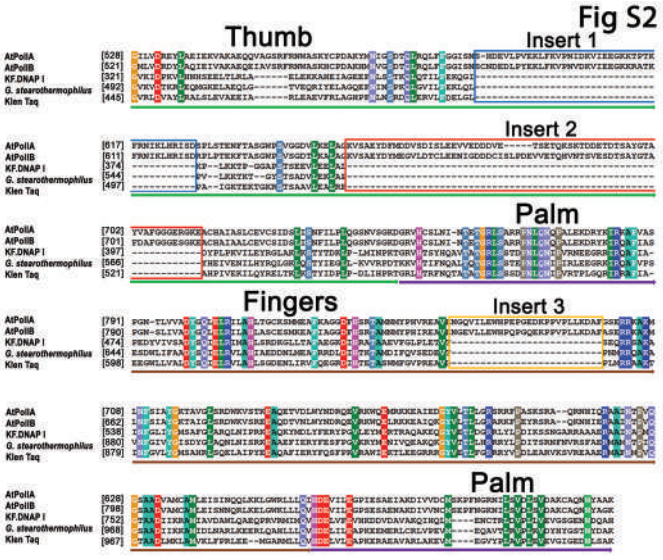


Fig S2
Thumb
Insert 1
Insert 2
Palm
Fingers
Insert 3
Palm
AtPolIA
AtPolIB
KF.DNAP I
G. stearothermophilus
Klen Taq

Fig. S3

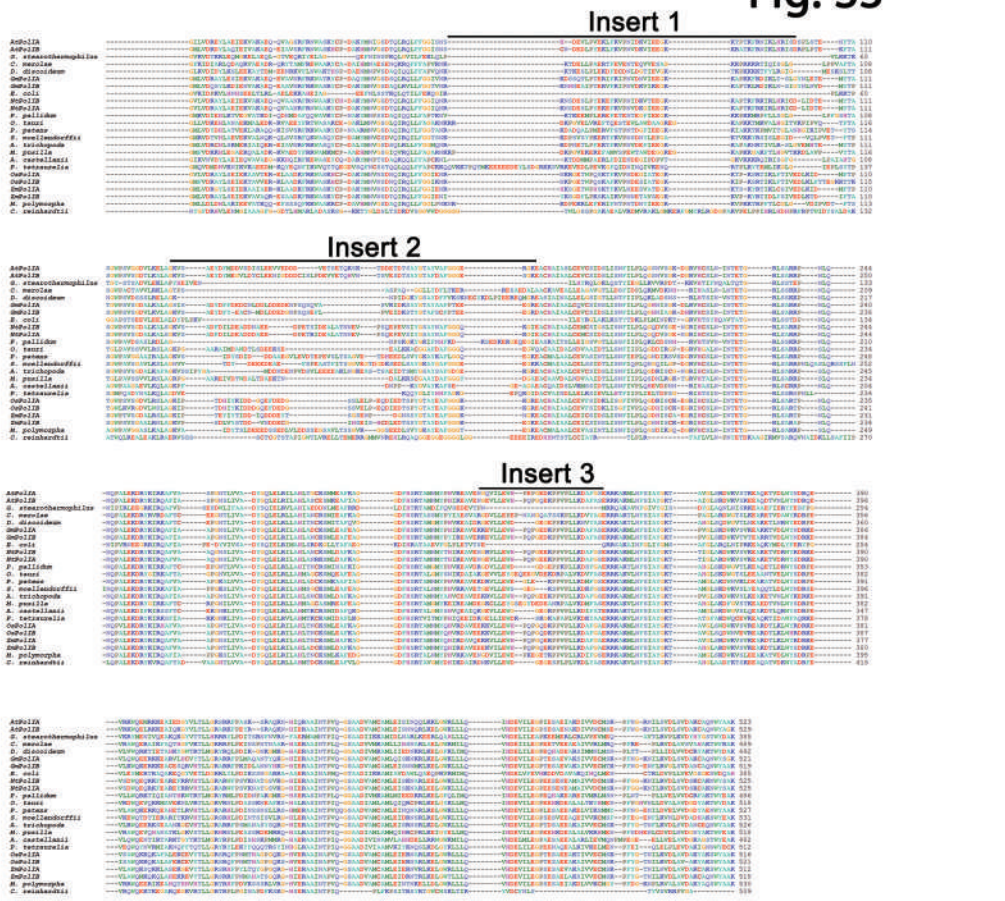
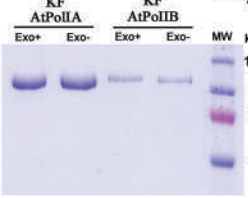
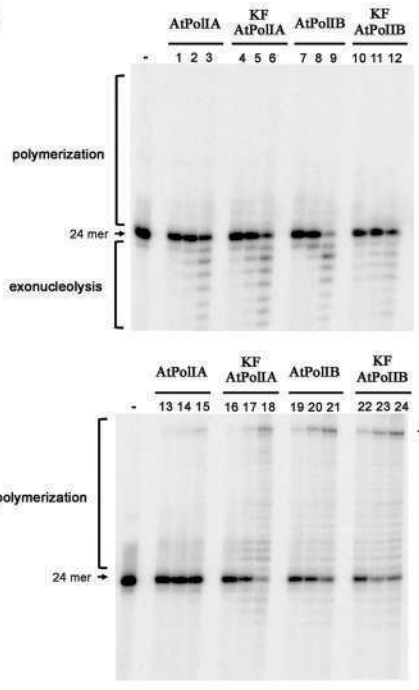


Fig. S4

A


KF AtPolIIA
KF AtPolIIB
Exo+
Exo-
Exo+
Exo-
MW
kDa
130
95
72
55

B


AtPolIIA
KF AtPolIIA
AtPolIIB
KF AtPolIIB
- 1 2 3 4 5 6 7 8 9 10 11 12
polymerization
24 mer
exonucleolysis
AtPolIIA
KF AtPolIIA
AtPolIIB
KF AtPolIIB
- 13 14 15 16 17 18 19 20 21 22 23 24
45 mer
polymerization
24 mer

C

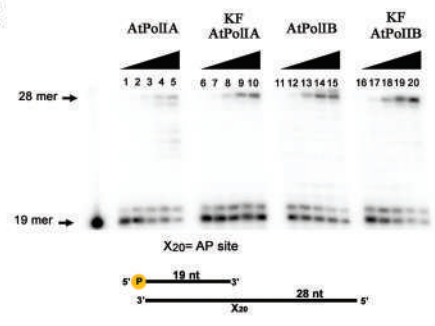
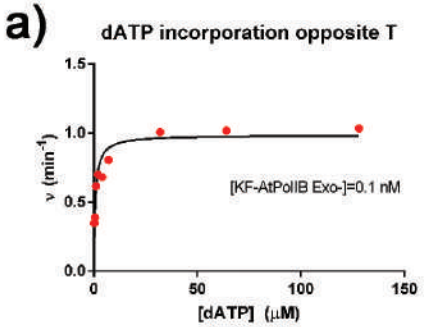
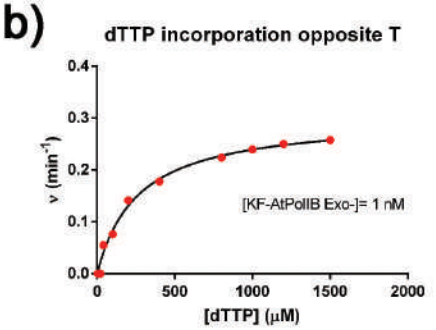
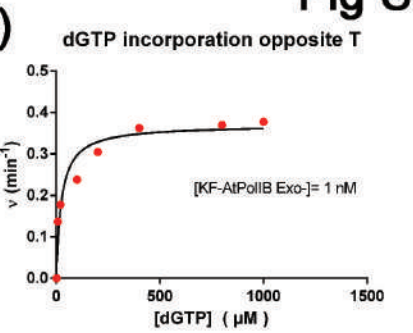
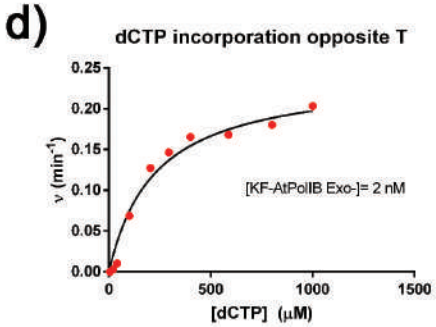

AtPolIIA
KF AtPolIIA
AtPolIIB
KF AtPolIIB
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
28 mer
19 mer
X20= AP site
5'
P
19 nt
3'
3'
28 nt
5'
X20

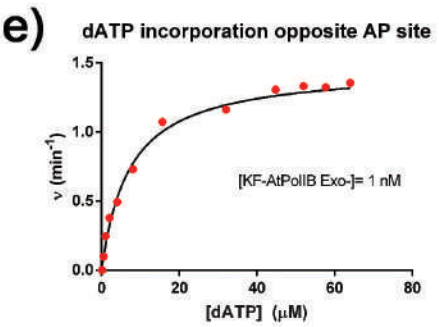
Fig S5

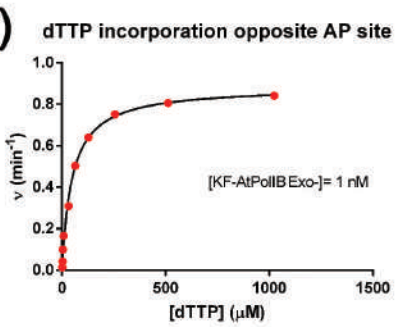

a)
dATP incorporation opposite T
v (min-1)
[KF-AtPolIB Exo-]=0.1 nM
[dATP] (μM)

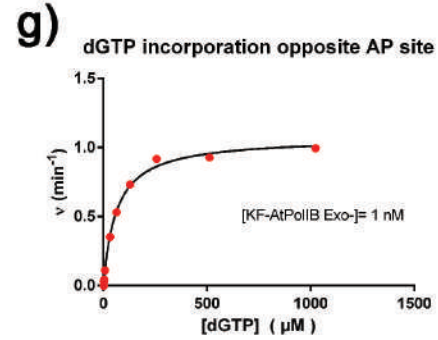

b)
dTTP incorporation opposite T
v (min-1)
[KF-AtPolIB Exo-]= 1 nM
[dTTP] (μM)

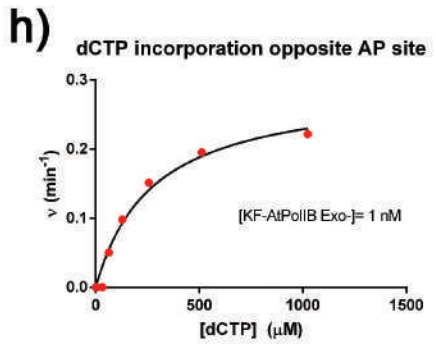

c)
dGTP incorporation opposite T
v (min-1)
[KF-AtPolIB Exo-]= 1 nM
[dGTP] (μM)


d)
dCTP incorporation opposite T
v (min-1)
[KF-AtPolIB Exo-]= 2 nM
[dCTP] (μM)


e)
dATP incorporation opposite AP site
v (min-1)
[KF-AtPolIB Exo-]= 1 nM
[dATP] (μM)


f)
dTTP incorporation opposite AP site
v (min-1)
[KF-AtPolIB Exo-]= 1 nM
[dTTP] (μM)


g)
dGTP incorporation opposite AP site
v (min-1)
[KF-AtPolIB Exo-]= 1 nM
[dGTP] (μM)


h)
dCTP incorporation opposite AP site
v (min-1)
[KF-AtPolIB Exo-]= 1 nM
[dCTP] (μM)

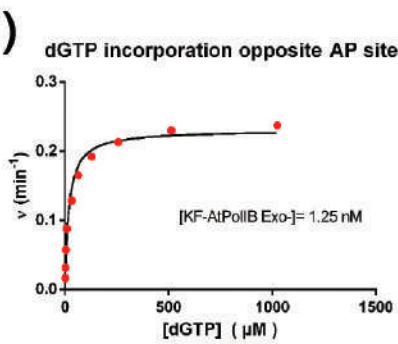

i)
dGTP incorporation opposite AP site-AC
v (min-1)
[KF-AtPolIB Exo-]= 1.25 nM
[dGTP] (μM)

Fig S6

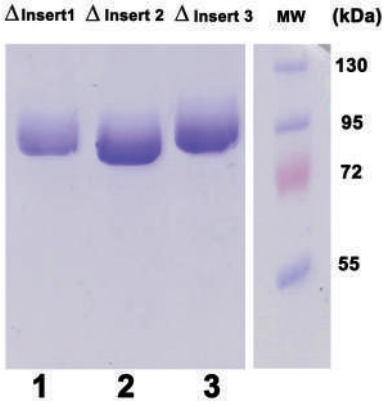


Fig S7

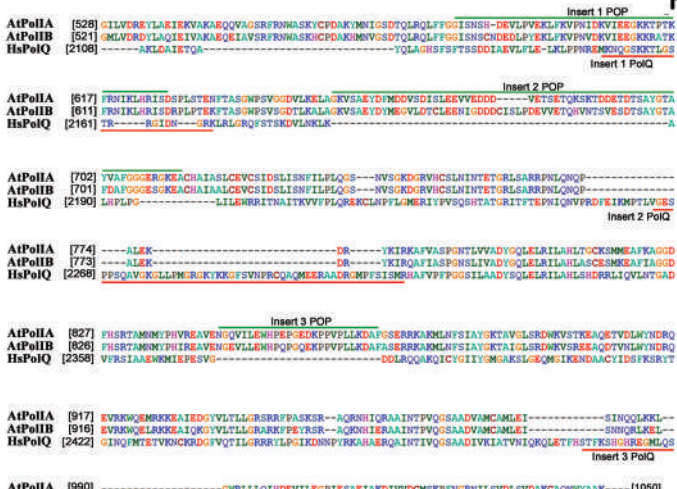

Insert 1 POP
AtPolIA [528] GILVDREYLAEIEKVAKAEQQVAGSRFRNWASKVCPDAKVMNIGSDTQLRQLFFGGISNSH-DEVLPVEKLFKVPNIDKVIEEGKKTPTK
AtPolIB [521] GMLVDRDYLAQIEIVAKAEQEIAVSRFRNWASKHCPDAKHMNVGSDTQLRQLFFGGISNSCNDEDLPYEKLFKVPNVDKVIEEGKKRATK
HsPolQ [2108] ------AKLDAIETQA----------YQLAGHSFSFTSSDDIAEVLFLE-LKLPPNREMQNQGSKKTLQS
Insert 1 PolQ
Insert 2 POP
AtPolIA [617] FRNIKLHRISDSPLSTENFTASGWPSVGGDVLKELAGKVSAEYDFMDDVSDISLEEVVEDDD-----VETSETQKSKTDDETDTSAYGTA
AtPolIB [611] FRNIKLHRISDRPLPTEKFTASGWPSVSGDTLKALAGKVSAEYDYMEGVLDTCLEENIGDDDCISLPDEVVETQRVNTSVESDTSAYGTA
HsPolQ [2161] TR-----RGIDN---GRKLRLGRQFSTSKDVLWKLK----------A
AtPolIA [702] YVAFGGGERGKEACHAIASLCEVCSIDSLISNFILPLQGS---NVSGKDGRVWCSLNINTETGRLSARRPNLQWQP----------
AtPolIB [701] FDAFGGGESGKEACHAIAALCEVCSIDSLISNFILPLQGS---NVSGKDGRVWCSLNINTETGRLSARRPNLQWQP----------
HsPolQ [2190] LHPLPG----------LILEWRRITNAITKVVFPLQRSKCLNPFLGMERIYPVSQSHTATGRITFTEPNIQNVPRDFEIKMPTLVGES
Insert 2 PolQ
AtPolIA [774] ----ALEK----------DR-----YKIRKAFVASPGNTLVVADYGQLELRILAHLTGCKSMMEAFKAGGD
AtPolIB [773] ----ALEK----------DR-----YKIRQAFIASPGNSLIVADYGQLELRILAHLASCESMKEAFIAGGD
HsPolQ [2268] PPSQAVCKGLLPMGRGKYKKGFSVNPRCQAQMEERADRGMPFSISMRHAFVPFPGGSILAADYSQLELRILAHLSHDRRLIQVLNTGAD
Insert 3 POP
AtPolIA [827] FHSRTAMNMYPHVREAVENGQVILEWHPEPGEDKPPVPLLKDAFGSERRKAKMLNFSIAYGKTAVGLSRDWKVSTKEAQSTVDLWYNDRQ
AtPolIB [826] FHSRTAMNMYPHIREAVENGEVLLEWHPQPQQEKPPVPLLKDAFASERRKAKMLNFSIAYGKTAIGLSRDWKVSRKEAQQTVNLWYNDRQ
HsPolQ [2358] VFRSIAAEWKMIEPESVG----------DDLRQQARQICIYGIYGMGAKSLGEQMSIKENDAACYIDSFKSRYT
AtPolIA [917] EVRKWQKMRKKKEAIEDGYVLTLLGRSRRFPASKSR--AQRNHIQRAAINTPVQGSAADVAMCAMLEI----------SINQQLKKL--
AtPolIB [916] EVRKWQKLRKKKEAIQKGYVLTLLGRARKFPEYRSR--AQKNHIERAAINTPVQGSAADVAMCAMLEI----------SNNQRLKEL--
HsPolQ [2422] GINQFMTSTVKNCKRDGFWQTILGRRRYLPGIKDNNPYRKAHAERQAINTIVQGSAADIVKIATVNIQKQLETFHSTFKSHGHRSCMLQS
Insert 3 PolQ
AtPolIA [990] ----------GWRLLLQIHDEVILEGPIESAEIAKDIVVDCMSKPFNGRNILSVDLSVDAKCAQNWYAAK-----[1050]
AtPolIB [989] ----------GWKLLLQVHDEVILEGPSESAENAKDIVVNCMSEPFNGRNILSVDLSVDAKCAQNWYAGK-----[1049]
HsPolQ [2512] DQTGLSRKRKLQGMFCPIRGGFFILQLHDELLYEVAEEDVVQVAQIVKNEMES----AVKLSVKLKVKVKIGASWGELKDFDV[2590]

Table S1 Oligonucleotides used in this study

A Primers used for subcloning

A1- N terminal	5'-GGGAAGGGCATATGAGGCTTAGCTCTAGTCCATTACC-3'
A1- C terminal	5'-GGAGGAGGGATCCTTATTTAGCTGCATACCAATT-3'
B1- N terminal	5'-GGGAAGGGCATATGCCACGCCGTCGTATCCTGTGTAC-3'
B1- C terminal	5'-GGGAAGGG GGATCCTTATTTGCCCGCATACC-3'

B Primers used for Site Directed mutagenesis

mutA3-F	5'-GGGAAGGGCATATGCTCGGGAAAATCTATGATAAA-3'
mutA3-R	5'-GGAGGAGGGATCCTTATTTAGCTGCATACCAATT-3'
mutB3-F	5'-GGGAAGGGCATATGCTGAAGAAAATCTACAATCGCGTTC GTGTTGTGGATAATG-3'
mutB3-R	5'-GGGAAGGG GGATCCTTATTTGCCCGCATACC-3'
AExo-F	5'-GGCAGTGAGCGGCATCGAAGTT-3'
AExo-R	5'-GTAGCGCAAGAATGCACATGATT-3'
BExo-F	5'-GGCAGTGTCACGCATCGATGTTAAAAC-3'
BExo-R	5'-GTTGCGCAAGCATGAACCAAGTTAC-3'

C Primers used in DNA polymerization assays

Assay	Polymerase and exonuclease
oligo 24 labeled	5' CGC AGC CCA CCT GCC CAC CTA ACT 3'
oligo 45	5' CCT TGG CAC TAG CGC AGG GCC AGT TAG GTG GGC AGG TGG GCT GCG 3'
	Strand-displacement
oligo 24 labeled	5' CGC AGC CCA CCT GCC CAC CTA ACT 3'
oligo 65	5' CCT TGG CAC TAG CGC ACG ATG CCG CTA AGA ACC TCA GGG CCA GTT AGG TGG GCA GGT GGG CTG CG 3'
oligo 15 blocking	5' CGC AGC CCA CCT GCC 3'
	Abasic site extension
Oligo 21 labeled	5'CGA AGTACT AGT AAC GAC CCT 3'
Positive control	5' ATA TAG GGT ACC GAC TGA AGC ACT GGA CGC CG**T** AAG TCA GGG TCG TTA CTA GTA CTT CG 3'

Abasic fidelity	5' ATA TAG GGT ACC GAC TGA AGC ACT GGA CGC CG [**abasic site]** AAG TCA GGG TCG TTA CTA GTA CTT CG 3'
	Nucleotide insertion opposite canonical an abasic site and Steady-state kinetics
Oligo 24 labeled	5' CGC AGC CCA CCT GCC CAC CTA ACT 3'
FideA	5' CCT TGG CAC TAG CGC AGG GC**A** AGT TAG GTG GGC AGG TGG GCT GCG 3'
FideT	5' CCT TGG CAC TAG CGC AGG GC**T** AGT TAG GTG GGC AGG TGG GCT GCG 3'
FideG	5' CCT TGG CAC TAG CGC AGG GC**G** AGT TAG GTG GGC AGG TGG GCT GCG3'
FideC	5' CCT TGG CAC TAG CGC AGG GG**C** AGT TAG GTG GGC AGG TGG GCT GCG 3'
Oligo 19 labeled	5' TGT TAG CAG ACA AGC CGA T 3'
Abasic 28	5' AAG AGT AC [**abasic site**] ATC GGC TTG TCT GCT AAC A 3'
Long_8A labeled	5' CGC AGC GGA CCT GCC CAC CTA ACT GAT ATA 3'
Abasic45	5' CCT TGG CAC TAG CGC [abasic site]AT ATC AGT TAG GTG GGC AGG TCC GCT GCG 3'
	Non-damaged and damaged template extension by deletions
Long8 labeled	5' CGC AGC GGA CCT GCC CAC CTA ACT GAT AT 3'
C45	5' CCT TGG CAC TAG CGC TAT ATC AGT TAG GTG GGC AGG TCC GCT GCG 3'
Long_8A labeled	5' CGC AGC GGA CCT GCC CAC CTA ACT GAT ATA 3'
Abasic45	5' CCT TGG CAC TAG CGC [abasic site]AT ATC AGT TAG GTG GGC AGG TCC GCT GCG 3'