Supporting Information

Archaeal replicative primases can perform translesion DNA synthesis

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Supplementary results

Archaeal replicative primases display an "extender" TLS activity relevant to the bypass of UV-induced DNA damages

Efficient bypass of the replication blocking DNA lesions relays on two basic functionalities characteristic for the TLS polymerases. First, TLS polymerase must be able to incorporate nucleotides opposite the damage. Second, the polymerase must be efficient in extending of the primer bearing the 3' terminal base paired with the damage. Although most TLS DNA polymerases are highly specialized in bypassing of particular types of lesions and can perform both of the above TLS functions. Some of TLS polymerases are specialized exclusively in insertion ("inserter" TLS polymerases) or extension step ("extender" TLS polymerases) only (1,2). For example, human DNA polymerase kappa (Pol κ) is unable to bypass cyclobutane pyrimidine dimers (CPDs) and O^6-methyl deoxyguanosines (m6dGs) as it is inefficient in inserting nucleotide opposite these replication blocking lesions (3). However, Pol κ is efficient in extending the primers with 3' termini annealed opposite the CPDs and m6dGs (3). To understand further the TLS capabilities of archaeal replicases and primases in bypass of UV induced DNA lesions, we tested the extender TLS activities of these enzymes.

Eukaryotic replicases are capable of incorporating up to two deoxyadenosines (dAA) opposite the CPD before eventually stalling (4,5). Therefore, we next tested if the archaeal replicase (Afu-Pol B) or primase active subunit (Afu-Pri S) and primase holoenzyme (Afu-Pri S/L) could efficiently extend a primer containing one (dA) or two

(dAA) 3' terminal deoxyadenosines annealed opposite the CPD. Although Afu-Pol B was unable to extend these substrates, Afu-Pri S and Afu-Pri S/L were capable of extending such primers (Figure S2A and Figure S2B). Interestingly, when these substrates were used to assay the TLS activity of Pfu-Pri S/L, we observed accumulation of full-length product in both cases (Figure S1E and S1F). In the case of Afu-Pri S and Afu-Pri S/L, the most efficient extension was observed for primers containing two 3' terminal deoxyadenosines (dAA) annealed opposite to the CPD. Importantly, the observed extension rate was comparable with efficiency of extension of the same primer annealed to non-damaged DNA template. To determine if Afu-Pri S/L could access and extend primers annealed to the CPD after stalling of the replicative polymerase, we pre-incubated the damaged substrate with Afu-Pol B and the processivity factor, proliferating cell nuclear antigen (PCNA), and assayed bypass synthesis performed by Afu-Pri S/L. Despite the presence of stalled replisome components, the replicative primase (Afu-Pri S/L) was still capable of traversing the CPD (Figure S2C). Together, these data indicate the possibility of interplay between the replicative polymerase and primase to facilitate TLS bypass of CPDs during archaeal replication.

Next, we measured the capacity of archaeal replicases (Afu-Pol B and Pol D) and primases (Afu-Pri S/L and Pfu-Pri S/L) to replicate past the second most frequent UV induced DNA modification, 6-4PPs. This DNA damage is potent replication blockage, which cannot be extended by the action of single canonical TLS DNA polymerase (2,6). TLS past the 6-4PP lesion requires the collaborative effort of two TLS DNA polymerases where the first performs insertion opposite the 6-4PP (an "inserter" polymerase) and the second extends the primer bearing 3' terminal base annealed with the dimer (an "extender" polymerase) (1,2). None of the tested archaeal enzymes were able to bypass the 6-4PP (Figure S3D). Interestingly, Afu-Pol D displayed ability to incorporate a single incoming nucleotide opposite 3' base of the 6-4PP (Figure S5A). Single nucleotide incorporations opposite the dimer revealed that Afu-Pol D incorporated preferentially deoxyadenosine (dA), doxyguanosine (dG) and, less efficiently, deoxythymidine (dT).

Next, we tested the “extender” TLS ability using four synthetic primer-template substrates, where the primer bearing all four possible bases at its 3’ end was annealed with 3’ thymine of 6-4PP lesion (Figure S4A-D). Both of tested archaeal DNA polymerases, Afu-Pol B and Pol D were unable to extend any of the 6-4PP containing substrates. In contrast, the archaeal primases (Afu-Pri S/L and Pfu-PriS/L) displayed a distinct “extender” TLS ability when 3’ terminal cytosine or thymine of a primer annealed to the 3’ terminal base of 6-4PP. This “extender” TLS activity was more efficient in the case of a primer with a 3’ terminal cytosine resulting in an N+7 extension product (Figure S4B). The extension of a primer terminating with a 3’ thymine annealed opposite with 6-4PP lesion resulted in somehow less pronounced N+5 product formation, mirroring the TLS activity observed for human PrimPol (7-9).

Furthermore, we investigated the “extender” 6-4PP bypass activity of Afu enzymes and Pfu replicative primase by examining single base incorporation opposite the 5’ thymine of the 6-4PP. Initially, we used a primer bearing a 3’ terminal cytosine annealed with the 3’ base of the 6-4PP. Notably, both of the primases performed incorporation of the correct dA and incorrect dT nucleotide opposite the 5’ base of the 6-4PP (Figure S5B). Next, we used a primer with a 3’ terminal thymidine annealed with the 3’ base of the 6-4PP. Afu-Pri S/L primase displayed error-prone incorporation of dC and dG (Figure S5C). Interestingly, this “extender” TLS signature was observed previously for human PrimPol (7-9). In contrast, Pfu-Pri S/L was an inefficient “inserter” when tested on the above 6-4PP containing primer-template showing inefficient incorporation of dG opposite the 5’ base of the dimer (Figure S5C). In order to test further the “extender” TLS activity of the archaeal enzymes, we used the primer bearing two adenine bases at the 3’ end annealed with the 6-4PP dimer and the primer with 3’ end deoxyguanosine annealed with the first undamaged templating base located after the lesion. All the tested archaeal replicative polymerases and primases were unable to extend these 6-4PP containing DNA substrates (Figure S5D and S5E).

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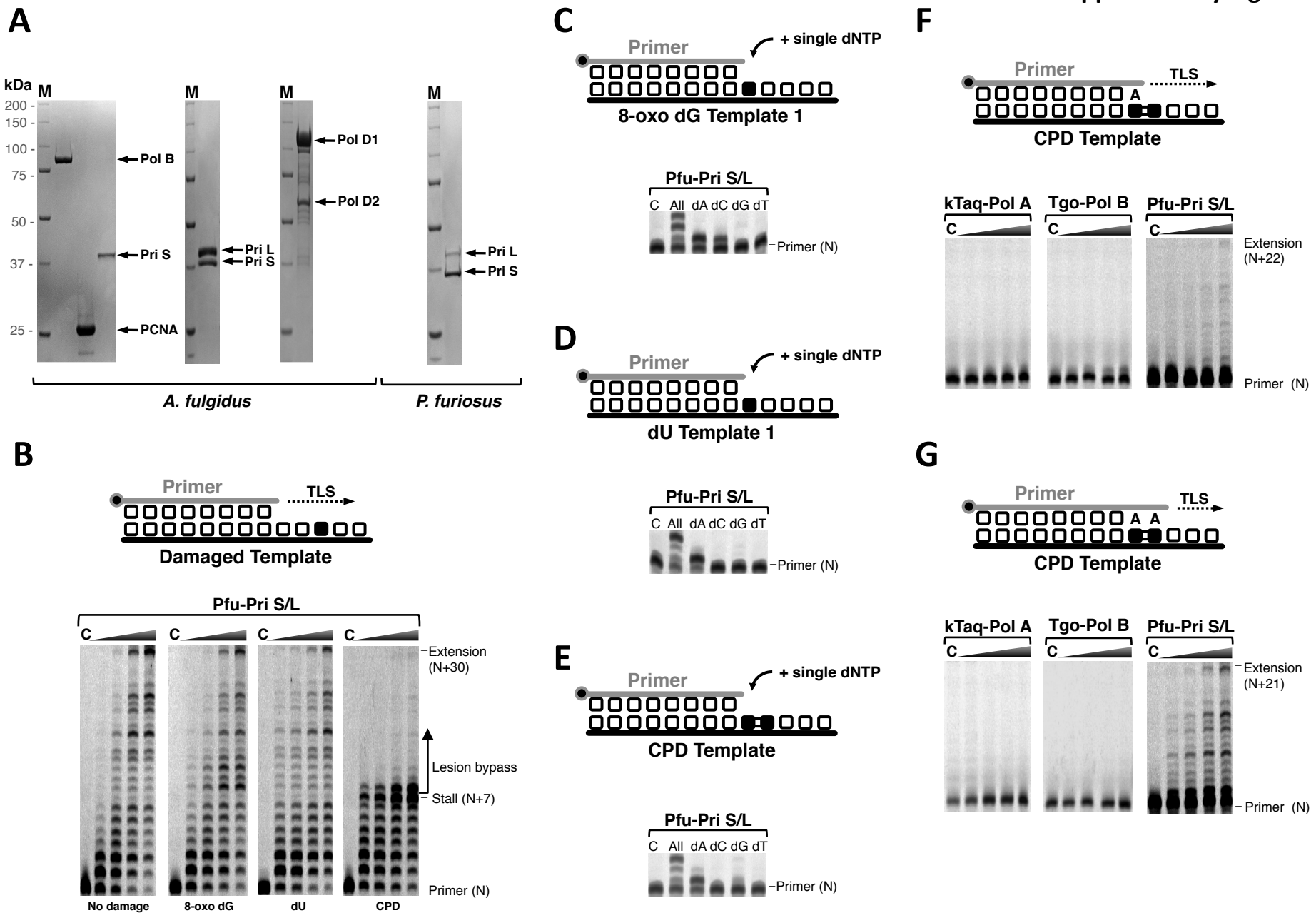
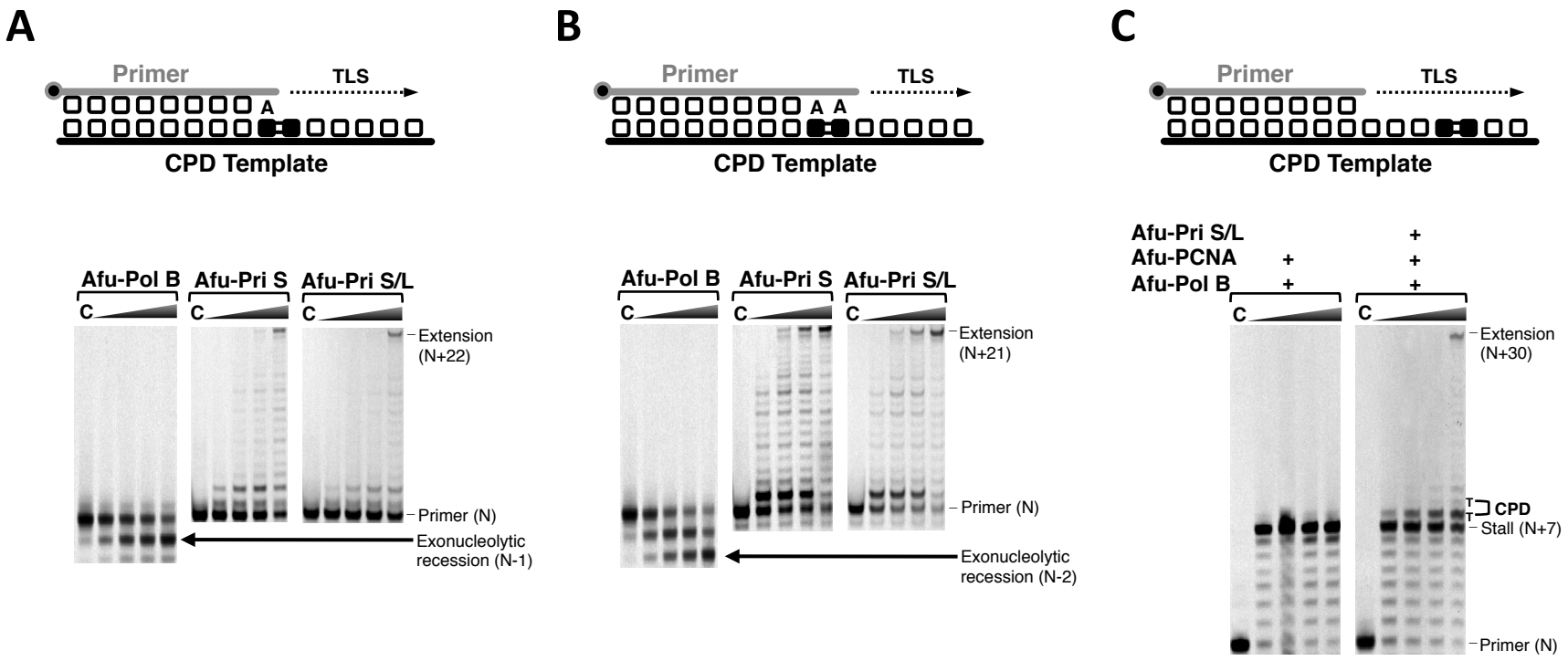
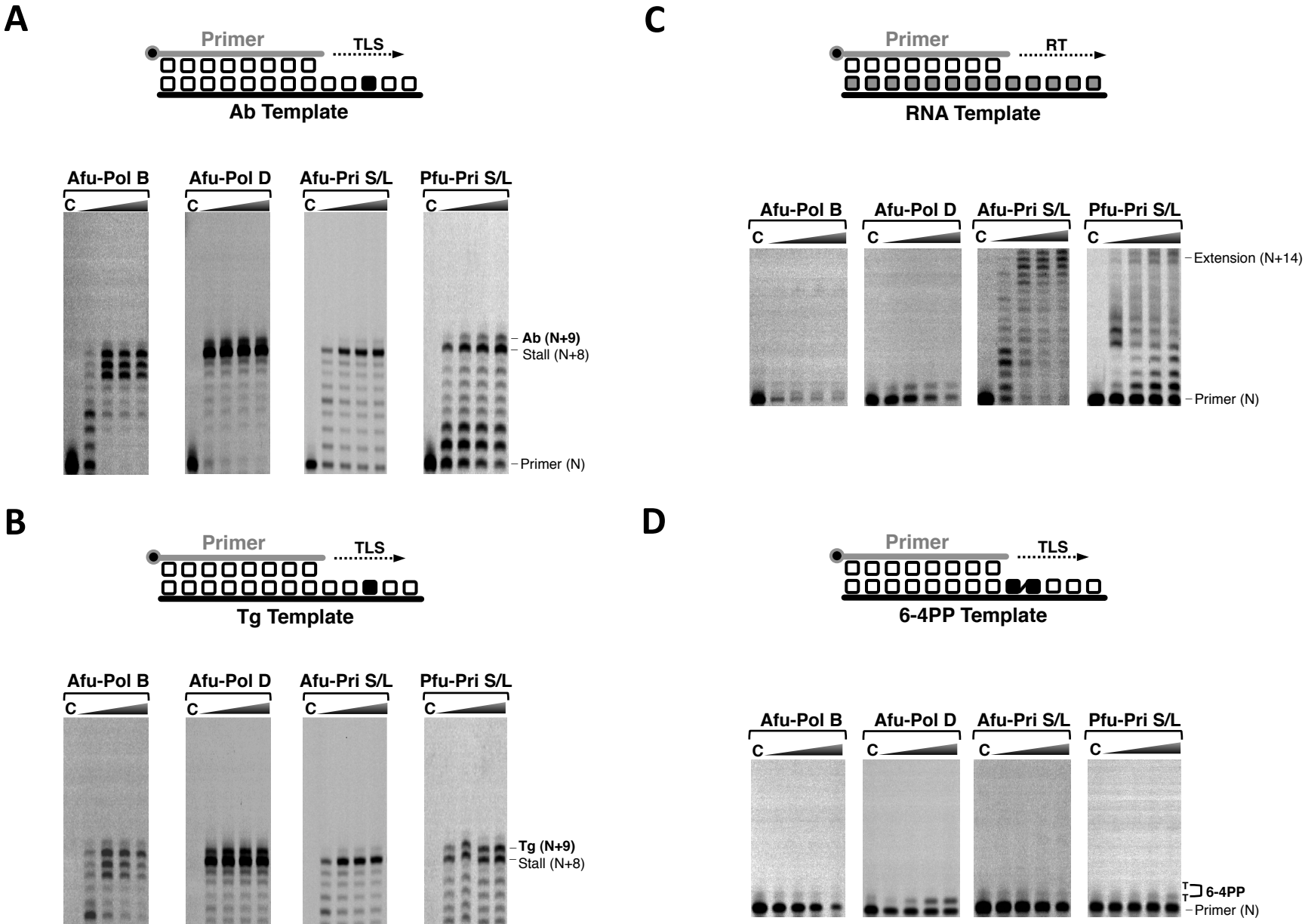
Supplementary Figure S1



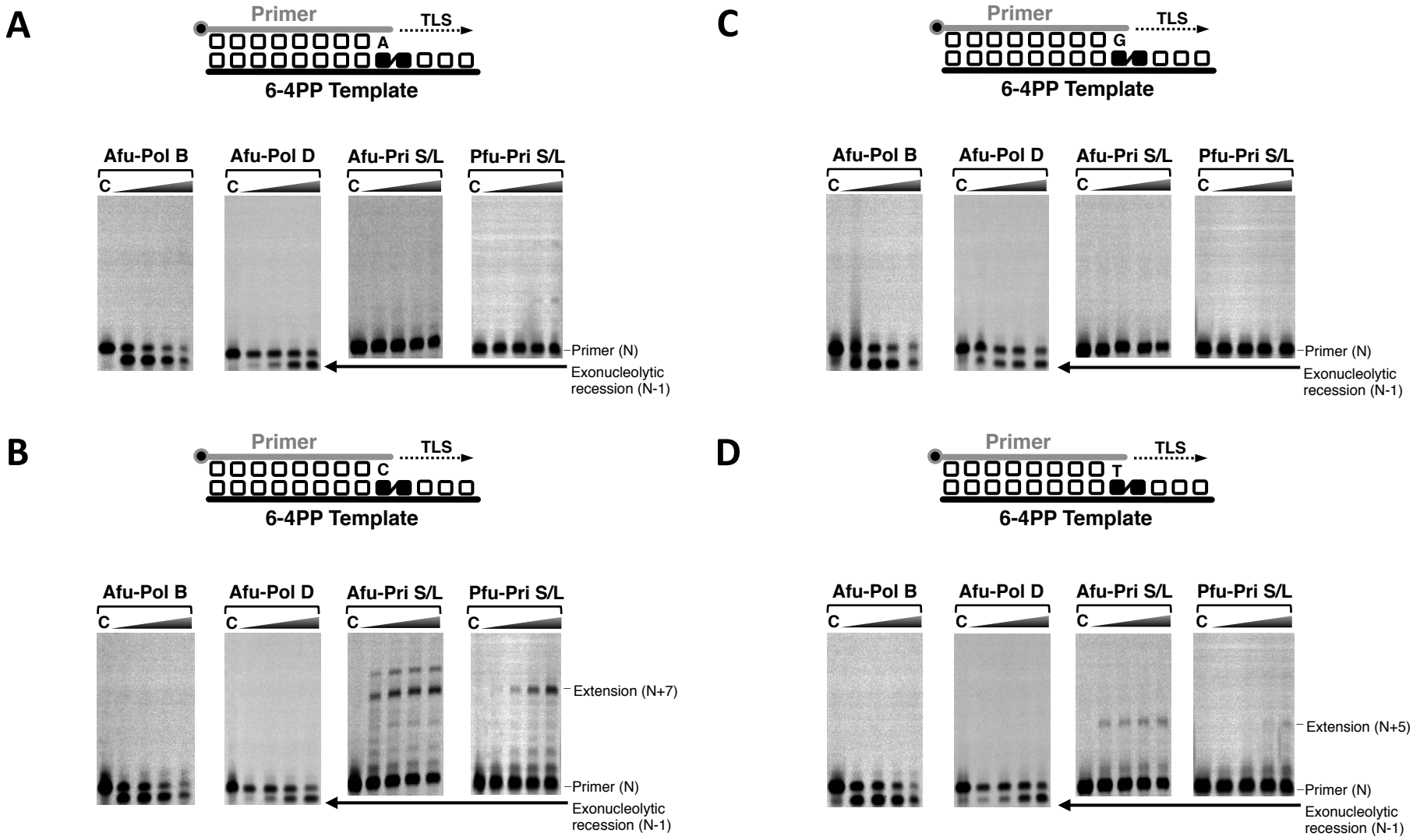
Supplementary Figure S1. (A) Purification of recombinant archaeal proteins. **(B)** TLS past 8-oxo-2'-deoxyguanosine (8-oxo dG), deoxyuracil (dU) and cis-syn thymine-thymine cyclobutane pyrimidine dimer (CPD) performed by *P. furiosus* replicative primase (Pfu-Pri S/L). **(C)** Single nucleotide incorporation opposite the 8-oxo dG lesion performed by Pfu-Pri S/L. **(D)** Single nucleotide incorporation opposite the dU lesion performed by Pfu-Pri S/L. **(E)** Single nucleotide incorporation opposite the CPD lesion performed by Pfu-Pri S/L. **(F)** Polymerization observed for the primer annealed with the first (3') base of the dimer. The reaction was carried out using Pfu-Pri S/L and two reference enzymes: kTaq-Pol A (bacterial repair family A DNA polymerase) and Tgo-Pol B exo$^-$ (exonuclease deficient variant of archaeal family-B DNA polymerase). (**G**) DNA synthesis observed for the primer containing two 3' terminal adenines annealed opposite the CPD dimer. The reaction was carried out as above using Pfu-Pri S/L, kTaq-Pol A and Tgo-Pol B exo$^-$. Description of the gel panels: C- denotes no enzyme control. The triangles above gel panels indicate time course of the polymerisation (30s, 2', 5', 10'). The single nucleotide incorporations were terminated after 5 minutes.


A
Primer
TLS
A
CPD Template
Afu-Pol B
Afu-Pri S
Afu-Pri S/L
C
Extension (N+22)
Primer (N)
Exonucleolytic recession (N-1)
B
Primer
TLS
A A
CPD Template
Afu-Pol B
Afu-Pri S
Afu-Pri S/L
C
Extension (N+21)
Primer (N)
Exonucleolytic recession (N-2)
C
Primer
TLS
CPD Template
Afu-Pri S/L
Afu-PCNA
Afu-Pol B
+
C
Extension (N+30)
T
T
CPD
Stall (N+7)
Primer (N)

Supplementary Figure S2. (A) Extension of the primer annealed with the first (3') base of the CPD performed *A. fulgidus* primase (Pri S/L) and replicase (Pol B). (**B**) Extension of the primer annealed with two bases of the CPD performed *A. fulgidus* primase (Pri S/L) and replicase (Pol B). **(C)** Polymerisation performed by *A. fulgidus* Pol B/PCNA complex is strongly inhibited by the UV lesion (left panel) but DNA synthesis is partially restored when primase (Pri S/L) is added to the reaction (right panel). Description of the gel panels: C- denotes no enzyme control. The triangles above gel panels indicate time course of the polymerisation (30s, 1', 5', 10').

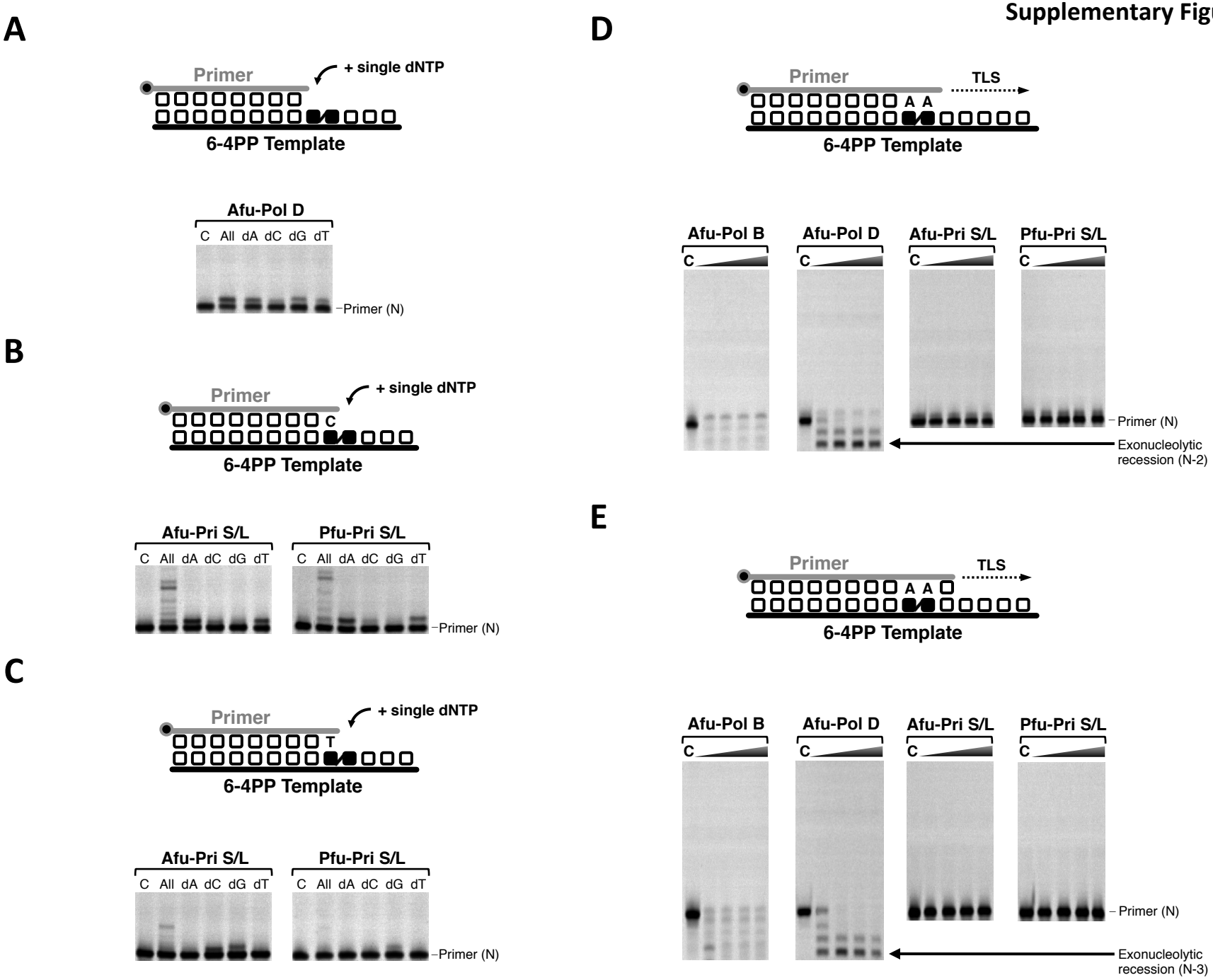

A
Primer
TLS
Ab Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
Ab (N+9)
Stall (N+8)
Primer (N)
B
Primer
TLS
Tg Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
Tg (N+9)
Stall (N+8)
Primer (N)
C
Primer
RT
RNA Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
Extension (N+14)
Primer (N)
D
Primer
TLS
6-4PP Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
T
T
6-4PP
Primer (N)

Supplementary Figure S3 (A) TLS past an abasic site (Ab), performed by *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). **(B)** TLS past thymine glycol (Tg), performed by *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). **(C)** Primer extension on RNA template (measuring reverse transcriptase activity of the tested enzymes), performed by *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). **(D)** TLS past 6-4 photo product lesion (6-4PP), performed by *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). Description of the gel panels: C- denotes no enzyme control. The triangles above gel panels indicate time course of the polymerisation (30s, 2', 5', 10').


A
Primer
TLS
A
6-4PP Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
Primer (N)
Exonucleolytic recession (N-1)
B
Primer
TLS
C
6-4PP Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
Extension (N+7)
Primer (N)
Exonucleolytic recession (N-1)
C
Primer
TLS
G
6-4PP Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
Primer (N)
Exonucleolytic recession (N-1)
D
Primer
TLS
T
6-4PP Template
Afu-Pol B
Afu-Pol D
Afu-Pri S/L
Pfu-Pri S/L
C
Extension (N+5)
Primer (N)
Exonucleolytic recession (N-1)

Supplementary Figure S4. (A) Polymerization observed for the primer bearing 3' terminal adenine annealed with the first (3') base of the dimer. The reaction was carried out using *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). **(B)** Polymerization observed for the primer bearing 3' terminal deoxycytosine annealed with the first (3') base of the dimer. The reaction was carried out using *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). **(C)** Polymerization observed for the primer bearing 3' terminal deoxyguanosine annealed with the first (3') base of the dimer. The reaction was carried out using *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). **(D)** Polymerization observed for the primer bearing 3' terminal thymine annealed with the first (3') base of the dimer. The reaction was carried out using *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). Description of the gel panels: C- denotes no enzyme control. The triangles above gel panels indicate time course of the polymerisation (30s, 2', 5', 10').

Supplementary Figure S5



Supplementary Figure S5. (A) Single nucleotide incorporation opposite the first (3') base of the 6-4PP lesion. The reaction was carried out using family-D replicative polymerase from *A. fulgidus* (Afu-Pol D). **(B)** Single nucleotide incorporation opposite the second (5') base of the 6-4PP lesion. The 3' terminal cytosine of the primer is mismatched with the first (3') base of 6-4PP dimer. The reaction was carried out using archaeal replicative primases from *A. fulgidus* (Afu-Pri S/L) and *P. furiosus* (Pfu-Pri S/L). **(C)** Single nucleotide incorporation opposite the second (5') base of the 6-4PP lesion. The 3' terminal deoxythymidine of the primer is mismatched with the first (3') base of 6-4PP dimer. The reaction was carried out using archaeal replicative primases from *A. fulgidus* (Afu-Pri S/L) and *P. furiosus* (Pfu-Pri S/L). **(D)** Polymerization observed for the primer bearing two adenosines at the 3' end which were annealed with the 6-4PP lesion. The reaction was carried out using *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). **(E)** Polymerization observed for the primer bearing deoxyguanosine at the 3' end which was annealed with the first undamaged base past the 6-4PP lesion. The reaction was carried out using *A. fulgidus* replicative polymerases and primase (Afu-Pol B, Afu-Pol D and Afu-Pri S/L) and *P. furiosus* replicative primase (Pfu-Pri S/L). Description of the gel panels: C- denotes no enzyme control. The triangles above gel panels indicate time course of the polymerisation (30s, 2', 5', 10'). The single nucleotide incorporations were terminated after 5 minutes.

Supplementary Table S1. PCR primers

Amplicon	Forward Primer (5'→3')	Reverse Primer (5'→3')
Afu-Pri S	GTTTCTTCATATGGCAGCAGGTTGTGATTATCAACTTCG	GTTTCTTCTCGAGTTAGGAATCGTAGCTTGCATCCCCTCTGCAAATC
Afu-Pri L	GTTTCTTGAATTCGATGAAATACCTACCCCTTTACCCAATTTTG	GTTTCTTGCGGCCGCTCAACTTTTCGATTTACATTTTTTATAATAAATTAAG
Afu-Pol B	GTTTCTTCATATGGAAAGAGTTGAGGGCTGGCTCATCG	GTTTCTTGCGGCCGCTTAGCTGAAGAATGAATCCAGGCTCATCTG
Afu-Pol D1	GTTTCTTGAATTCGATGGATGCAACTCTTGACAGGTTCTTC	GTTTCTTGCGGCCGCTCAAACGAAATCGGATATTGACACTTG
Afu-Pol D2	GTTTCTTCATATGGTAATTAAAAATATCGATGCCGCAACAG	GTTTCTTCATATGGTAATTAAAAATATCGATGCCGCAACAG
Pfu-Pri S	GTTTCTTCATATGCTGATGAGGGAAGTGACAAAGG	GTTTCTTCTCGAGTTATTCATATTCCAAGGACTCTCTCCACAGTTTATAAG
Pfu-Pri L	GTTTCTTGGATCCGTTAACTTCCATTCTCCCACCTCCATTAAAG	GTTTCTTGCGGCCGCTTACTGTAGAATTCGCTCCTTTCCTCCTTTG
Template 3 preparation	**Phos-**TGTCGTCTGTTCGGTCGTTCCGCGCGTTTCGGTGATGACGGTG	**Biot-**CAGCAACGCGGCCTTTTTACGGTTC

Supplementary Table S2. Synthetic primer-template substrates

Figure	Primer (5'→3')	Template (5'→3')
F1C	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCCTTGAAGACCGAACGACCGAACA GACGACA
F1D	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCCTTGAAGACCGAACGACCGAACA GACGACA
F2A	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCC(G)TGAAGACCG AACGACCGAACAGACGACA
F2B	TGTCGTCTGTTCGGTCGTTCGGTCT TCA	CGCGCAGGGCGCACAACAGCC(G)TGAAGACCG AACGACCGAACAGACGACA
F2C	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCC(U)TGAAGACCGAACGACCGAA CAGACGACA
F2D	TGTCGTCTGTTCGGTCGTTCGGTCT TCA	CGCGCAGGGCGCACAACAGCC(U)TGAAGACCGAACGACCGAA CAGACGACA
F2E	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
F2F	TGTCGTCTGTTCGGTCGTTCGGTCT TC	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
F3A	TGTCGTCTGTTCGGTCGTTCGGTCT TCA	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
F3B	TGTCGTCTGTTCGGTCGTTCGGTCT TCAA	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
F3C	TGTCGTCTGTTCGGTCGTTCGGTCT TC	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
F4A	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCC(U)TGAAGACCGAACGACCGAA CAGACGACA
F4B	TGTCGTCTGTTCGGTCGTTC	ACCGCGAACTTGAATTCTAGTTCAGTCTAAATGCTCTCAAGCAC TGAGCAATTCACAACATATGGCTTTCGATTACCGAACGACCGAA CAGACGACA
F4C	TGTCGTCTGTTCGGTCGTTC	ACCGCGAAC(G)TGAATTCTAG(G)TCAGTC(G)AAATGCTC(G)CAA GCAC(G)GAGCAA(G)TCACAACATATGGCT(G)TCGATTACCGAAC GACCGAACAGACGACA
F4D	TGTCGTCTGTTCGGTCGTTC	ACCGCGAAC(U)TGAATTCTAG(U)TCAGTC(U)AAATGCTC(U)CAA GCAC(U)GAGCAA(U)TCACAACATATGGCT(U)TCGATTACCGAAC GACCGAACAGACGACA
S1B	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCCTTGAAGACCGAACGACCGAACA GACGACA CGCGCAGGGCGCACAACAGCC(G)TGAAGACCG AACGACCGAACAGACGACA CGCGCAGGGCGCACAACAGCC(dU)TGAAGACCGAACGACCGAA CAGACGACA CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
S1C	TGTCGTCTGTTCGGTCGTTCGGTCT TCA	CGCGCAGGGCGCACAACAGCC(G)TGAAGACCGAACGACCGAA CAGACGACA
S1D	TGTCGTCTGTTCGGTCGTTCGGTCT TCA	CGCGCAGGGCGCACAACAGCC(U)TGAAGACCGAACGACCGAA CAGACGACA
S1E	TGTCGTCTGTTCGGTCGTTCGGTCT TCA	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
S1F	TGTCGTCTGTTCGGTCGTTCGGTCT TCA	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
S1G	TGTCGTCTGTTCGGTCGTTCGGTCT TCAA	CGCGCAGGGCGCACAACAGCC(T=T)GAAGACCGAACGACCGAA CAGACGACA
S2A	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCC(Ab)TGAAGACCGAACGACCGAA CAGACGACA
S2B	TGTCGTCTGTTCGGTCGTTC	CGCGCAGGGCGCACAACAGCC(Tg)TGAAGACCGAACGACCGAA CAGACGACA

S2C	CACTGACTGTATGATG	CUCGTCAGCAUCUUCAUCAUACAGUCAGUG
S2D	CACTGACTGTATGATG	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S3A	CACTGACTGTATGATGA	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S3B	CACTGACTGTATGATGC	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S3C	CACTGACTGTATGATGG	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S3D	CACTGACTGTATGATGT	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S4A	CACTGACTGTATGATG	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S4B	CACTGACTGTATGATGC	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S4C	CACTGACTGTATGATGT	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S4D	CACTGACTGTATGATGAA	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG
S4E	CACTGACTGTATGATGAAG	CTCGTCAGCATC(T/T)CATCATACAGTCAGTG

Supplementary Table S3. Enzymatically prepared primer-template substrates

Figure	Primer (5'→3')	Template (5'→3')
F4E	TGTCGTCTGTTCGGTCGTTC	CCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCT TTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGG ATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAG CGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTG GCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGG AAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCA CTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGT ATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAA CAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGAT CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCG TCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAA CTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAA TAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGC AGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTAC GCATCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTA CAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCC GCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCG GCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCA TGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGGAACG ACCGAACAGACGACA
F4F	TGTCGTCTGTTCGGTCGTTC	CCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCT TTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGG ATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAG CGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTG GCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGG AAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCA CTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGT ATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAA CAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGAT CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCG TCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAA CTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAA TAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGC AGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTAC GCATCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTA CAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCC GCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCG GCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCA TGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGGAACG ACCGAACAGACGACA