Electronic Supplementary Material (ESI) for Chemical Communications

Stepping Towards Highly Flexible Aptamers: Enzymatic Recognition Studies of Unlocked Nucleic Acid Nucleotides

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Additional Gel images:

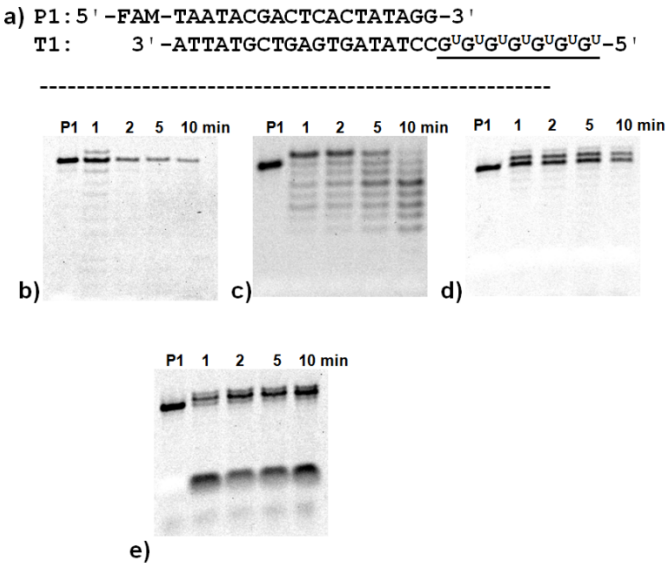

Figure S1. Reading of UNA-G nucleotides by polymerases. a) Primer P1 and template T1 sequences; b) KOD DNA polymerase; c) Phusion DNA polymerase; d) Klenow DNA polymerase; e) Taq DNA polymerase.

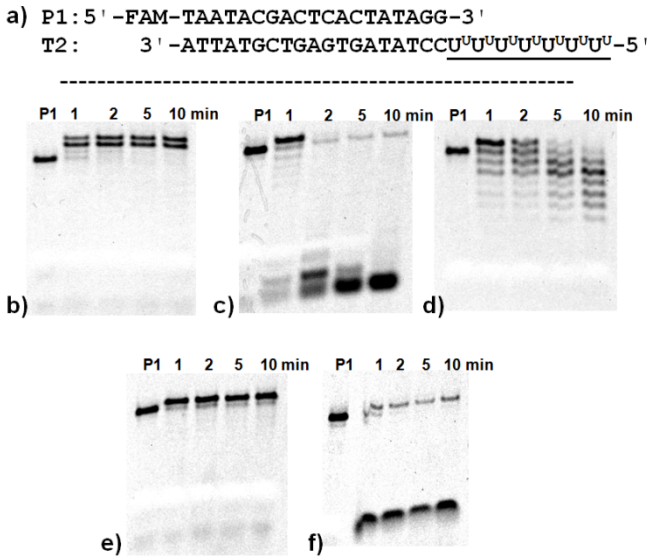

Figure S2. Reading of UNA-U nucleotides by polymerases. a) Primer P1 and template T2 sequences; b)Therminator DNA polymerase; c) KOD DNA polymerase; d) Phusion DNA polymerase; e) Klenow DNA polymerase; f) Taq DNA polymerase.

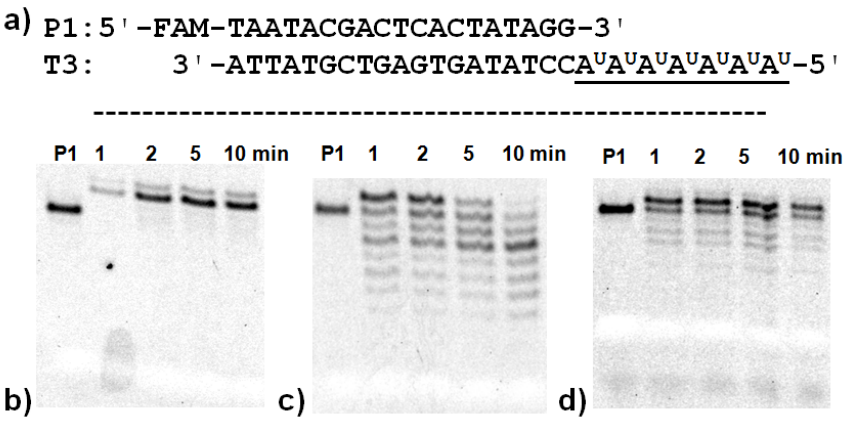

Figure S3. Reading of UNA-A nucleotides by polymerases. a) Primer P1 and template T3 sequences; b)Therminator DNA polymerase; c) Phusion DNA polymerase; d) Klenow DNA polymerase.

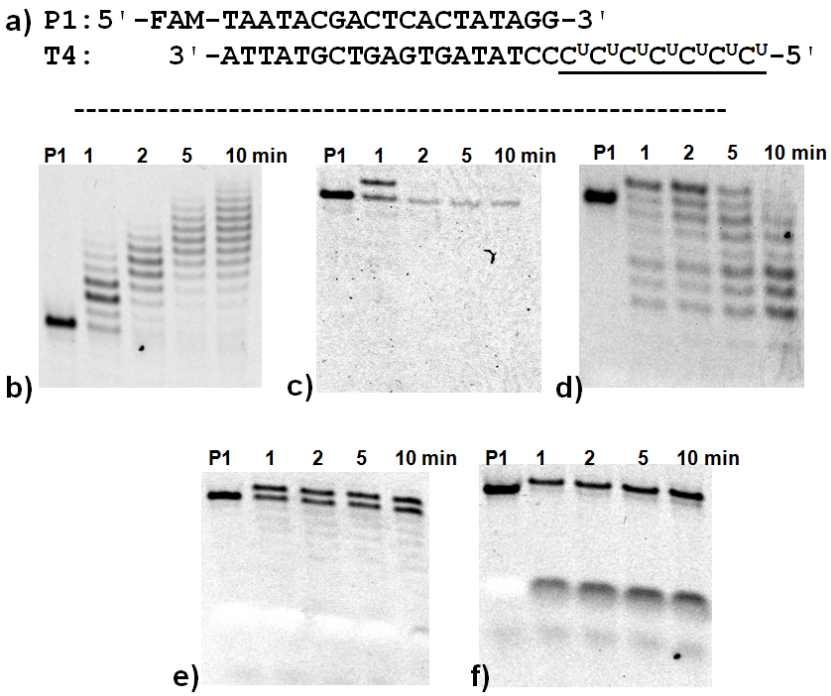

Figure S4. Reading of UNA-C nucleotides by polymerases. a) Primer P1 and template T4 sequences; b)Therminator DNA polymerase; c) KOD DNA polymerase; d) Phusion DNA polymerase; e) Klenow DNA polymerase; f) Taq DNA polymerase.

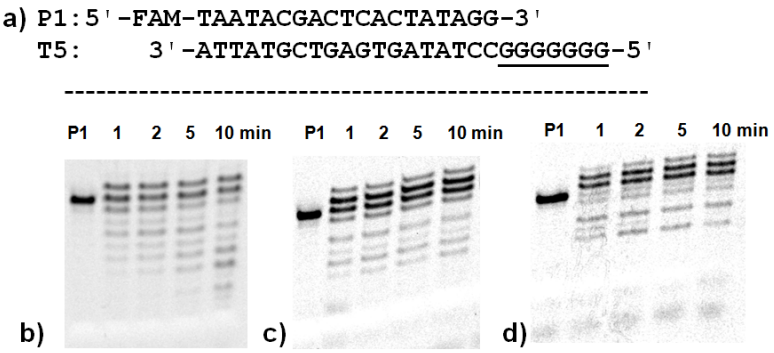

Figure S5. Incorporation of UNA nucleotides by polymerases. a) Primer P1 and template T5 sequences; b) KOD DNA polymerase; c) Phusion DNA polymerase; d) Klenow DNA polymerase.

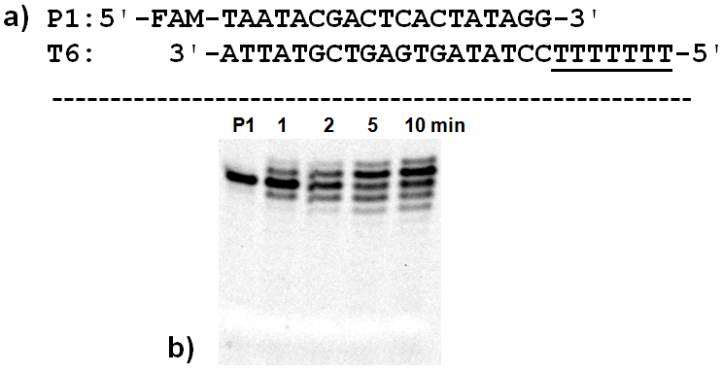

Figure S6. Incorporation of UNA nucleotides by polymerases. a) Primer P1 and template T6 sequences; b) Therminator DNA polymerase.

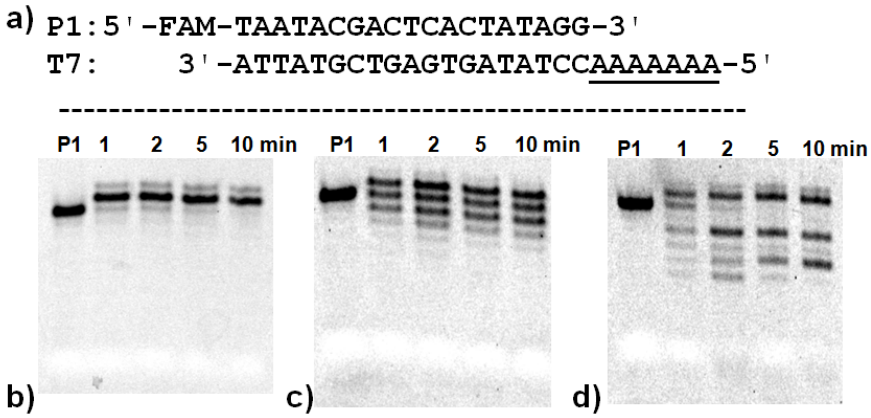

Figure S7. Incorporation of UNA nucleotides by polymerases. a) Primer P1 and template T7 sequences; b) Therminator DNA polymerase; c) Phusion DNA polymerase; d) Klenow DNA polymerase.

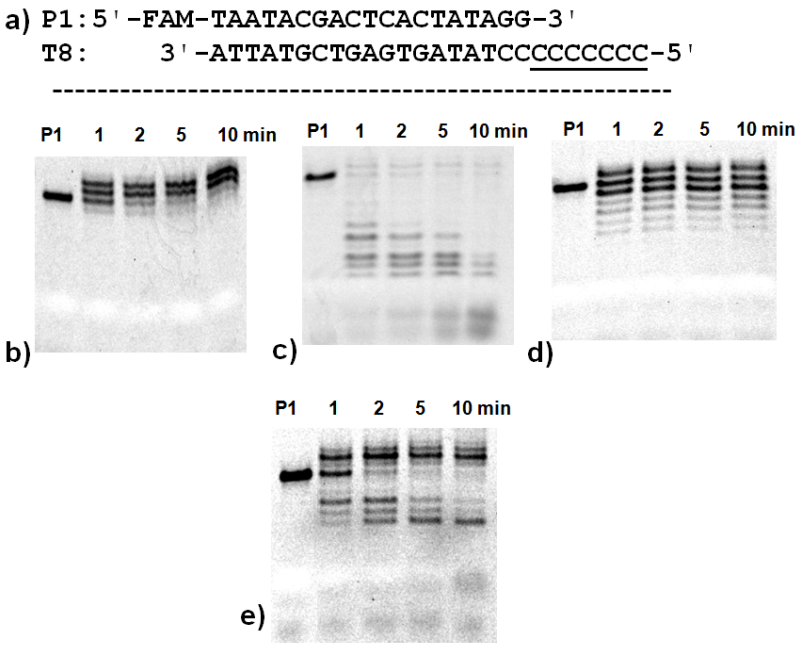

Figure S8. Incorporation of UNA nucleotides by polymerases. a) Primer P1 and template T8 sequences; b) Therminator DNA polymerase; c) KOD DNA polymerase; d) Phusion DNA polymerase; e) Klenow DNA polymerase.

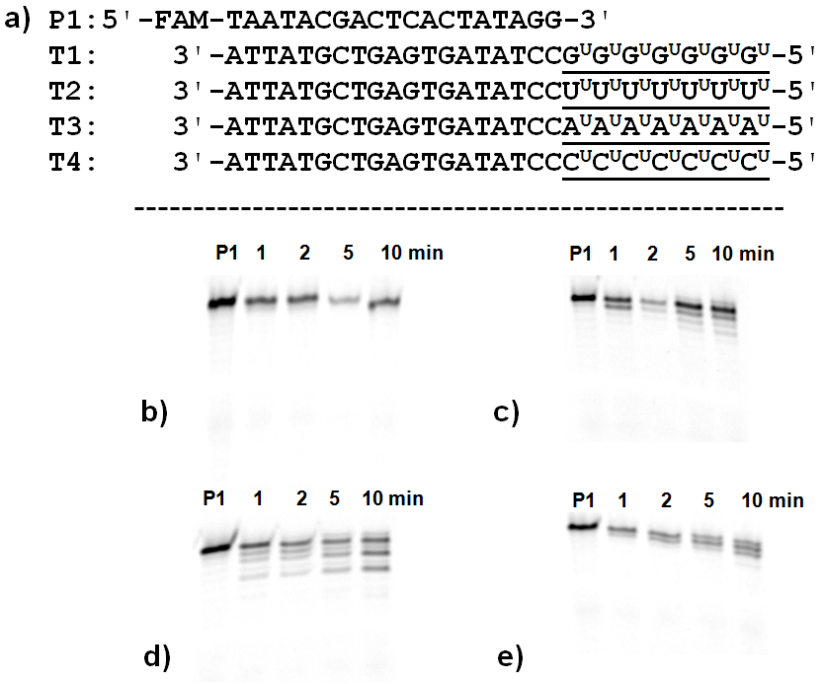

Figure S9. Fidelity of reading UNA-nucleotides by Therminator DNA polymerase. a) Primer P1 and template sequences; b) with template T1 and dGTP instead of dCTP; c) with template T2 and dTTP instead of dATP; d) with template T3 and dATP instead of dTTP; e) with template T4 and dCTP instead of dGTP.

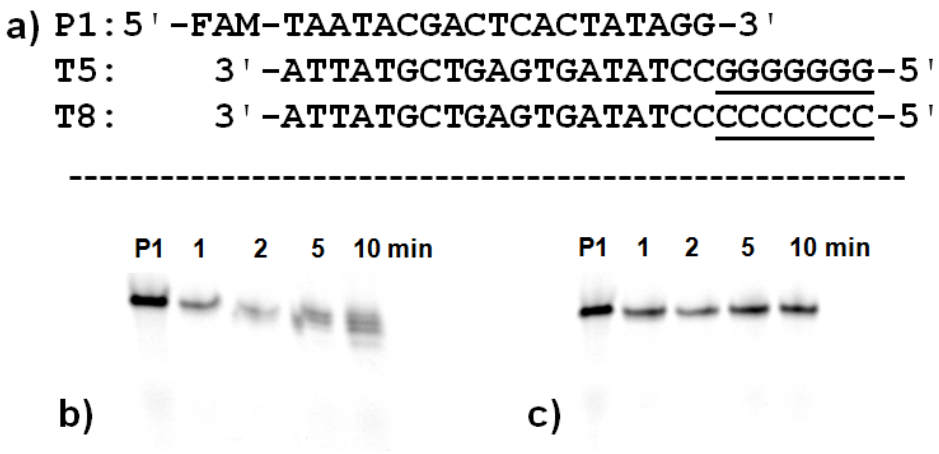

Figure S10. Fidelity of UNA-nucleotide incorporations by Therminator DNA polymerase. a) Primer P1 and template sequences; b) with template T5 and UNA-GTP instead of UNA-CTP; c) with template T8 and UNA-CTP instead of UNA-GTP.

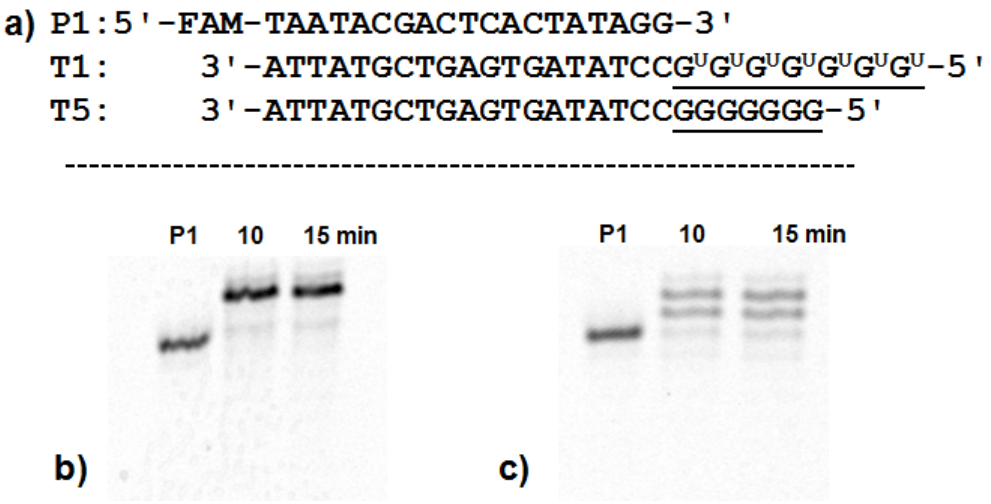

Figure S11. UNA-nucleotide reading and incorporations by Therminator DNA polymerase without supplementing $MnCl_2$. a) Primer P1 and template sequences T1 (for reading) and T5 (for incorporation); b) Incorporation of dC opposite to UNA-G; c) Incorporation of UNA-C opposite to dG of the template strand.

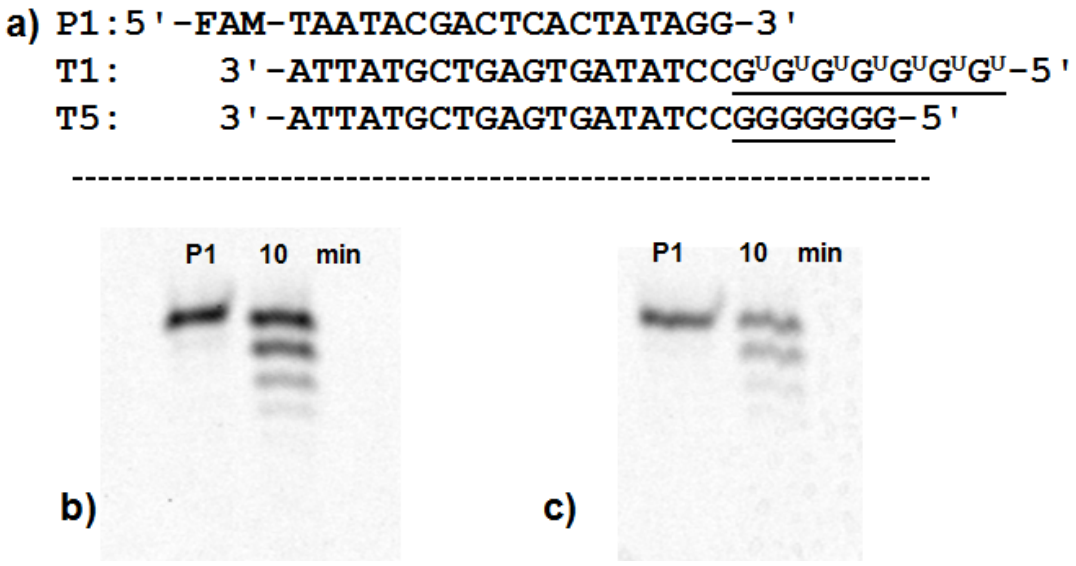

Figure S12. UNA nucleotide reading and incorporations by Therminator DNA polymerase without adding any nucleoside triphosphates. a) Primer P1 and template sequences; b) With template T1; c) With template T5.

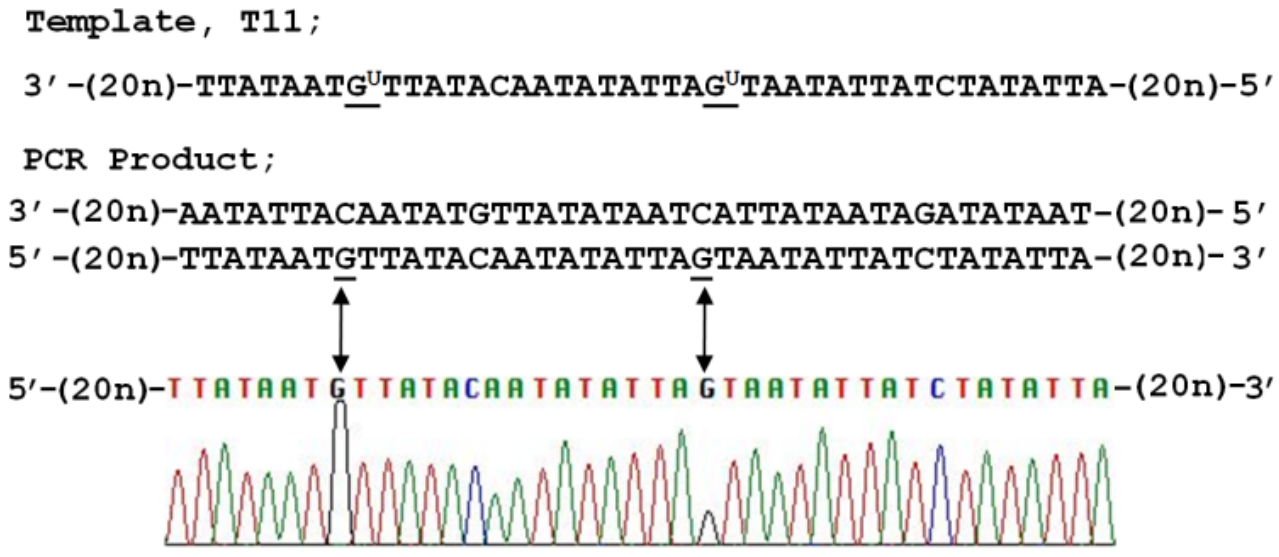

Figure 3. PCR product sequence aligned with the sequencing chromatogram. Positions of DNA-G in the expected positions in the PCR product are underlined, as confirmed by the G nucleotides identified in the sequencing chromatogram.

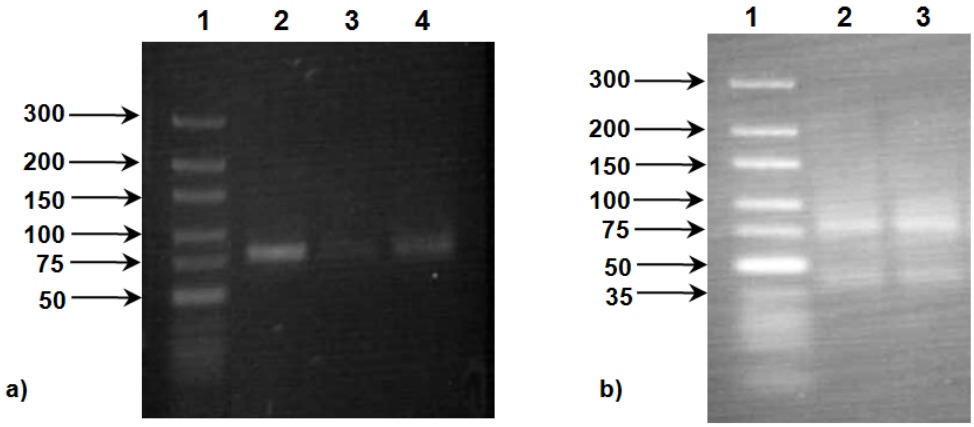

Figure S14. PCR amplification using UNA-modified DNA templates. a) By Phusion DNA polymerase, lane 1: DNA size marker, lane 2: Using template T9, lane 3: Using template T10, lane 4: Using template T11; b) By Therminator DNA polymerase. Lane 1: DNA marker, lane 2: Using template T11 with annealing temperature 45 ^{o}C, lane 3: Using template T11 with annealing temperature 48 ^{o}C.

Experimental Section

Synthesis of UNA oligonucleotides: Templates containing UNA nucleotides were synthesized on an Expedite DNA synthesizer via standard phosphoramidite chemistry in 0.2 µmol scale. The synthesized oligonucleotides were deprotected and cleaved from the solid support by treatment with NH_4OH at 55 ^{o}C overnight. The crude oligonucleotides were then purified by RP-HPLC and desalted, prior to use in the enzymatic reactions. UNA phosphoramidites are commercially available from RiboTask ApS.

Synthesis of UNA nucleoside 5'-triphosphates: UNA triphosphates were synthesized according to a published procedure.[1]

General procedure for primer extension reactions: 5'-FAM labelled primer sequence (purchased from IDT, Coralville, Iowa) was annealed to the templates T1-T11 by mixing primer and template in a molar ratio of 1:2 and heating to 80 ^{O}C for 3 min, followed by slow cooling to room temperature. The extension reaction mixtures were prepared in a total volume of 20 µL containing 1× reaction buffer specific to each polymerase, 2.5 mM $MnCl_2$, 500 µM of dNTP/ 750 µM of UNA-NTP, 3:6 pmol ratio of the annealed primer–template complex and 2.5 U of the DNA polymerase. The reaction mixtures were gently vortexed and incubated at 74 ^{O}C. The reactions were monitored by taking out 2.5 µL of the reaction mixture at 1, 2, 5 and 10 minutes of incubation. The polymerase reactions were quenched by

the addition of an equal volume of loading buffer (8 M urea in 1 × TBE containing 0.05% bromophenol blue). Analysis of the products was performed by 13% 7M urea polyacrylamide gel electrophoresis in the presence of TBE buffer (100 mM Tris, 90 mM Boric acid, 1 mM EDTA) at pH 8.4 followed by fluorescence scanning with an Amersham Typhoon.

General procedure for polymerase chain reaction (PCR): The PCR reaction mixture was prepared in a total volume of 50 µL containing 1× reaction buffer specific to each polymerase, 1 mM $MgCl_2$, 250 µM of dNTPs, 1.5 µM of both forward and reverse primers, 10 nM of UNA-modified DNA template or library and 2.5 U of the DNA polymerase. The reaction mixtures were gently vortexed and then amplified using a thermal cycler (Bio-Rad S-1000). A 25-cycle PCR protocol consisted of three steps, denaturation at 95 ^{o}C for 15 second, annealing at 45-48 ^{o}C for 10 seconds and extension at 72 ^{o}C for 30 seconds. After the polymerase reactions, gel-loading buffer (50% glycerol, 0.05% bromophenol blue, 0.05% xylene cyanol and 10mM EDTA) was added (7.5 µL) and the products were analyzed by 3% agarose gel electrophoresis followed by UV-photography.

Cloning and Sequencing: Purified PCR products (50ng) were cloned into pCR-Blunt (Invitrogen) according to the manufacturer‘s instructions. Plasmid DNA was extracted using QIAprep (Qiagen) and sequenced by the Australian Genome Research Facility (AGRF, Brisbane, Australia).

References:

1. R. N. Veedu, H. V. Burri, P. Kumar, P. K. Sharma, P. J. Hrdlicka, B. Vester, J. Wengel, *Bioorg. Med. Chem. Lett.,* 2010, **20**, 6565.