Electronic Supplementary Material (ESI) for Chemical Communications


# Synthesis of Selenomethylene-Locked Nucleic Acid (SeLNA)-Modified Oligonucleotides by Polymerases

**Megan Wheeler,[a] Antoine Chardon,[a] Astrid Goubet,[a] Kunihiko Morihiro,[b] Sze Yee Tsan,[a] Stacey L. Edwards,[a] Tetsuya Kodama,[b,c] Satoshi Obika[b] and Rakesh N. Veedu*[a]**

## Additional Gel images:

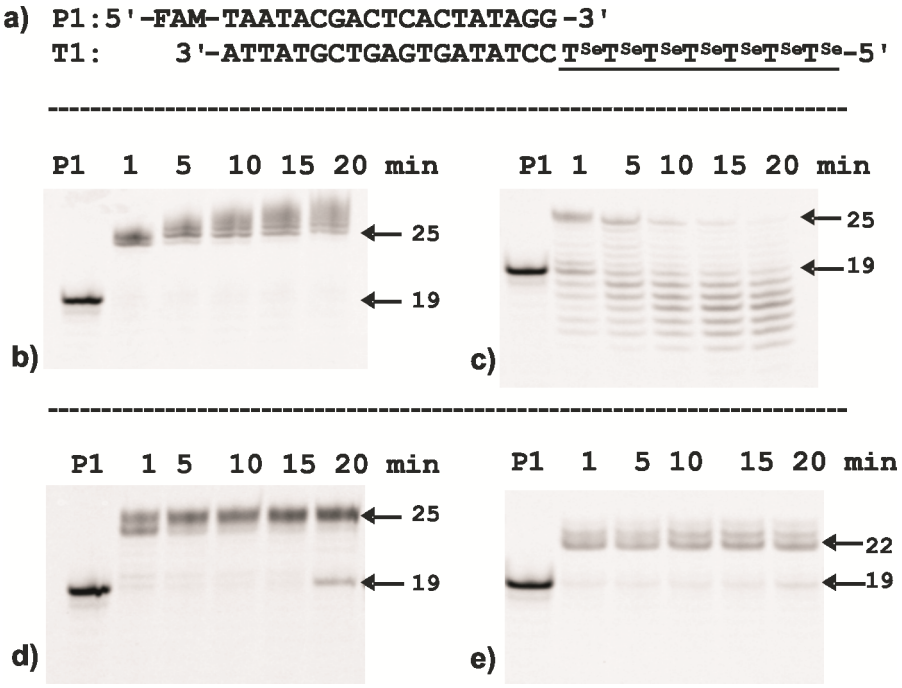



**Figure S1**. Reading of SeLNA-T nucleotides by polymerases. a) Primer P1 and template T1 sequence; b) Therminator DNA polymerase; c) Phusion DNA polymerase; d) Klenow DNA polymerase; e) *Taq* DNA polymerase. SeLNA-T nucleotides are underlined and denoted by 'Se' as superscript

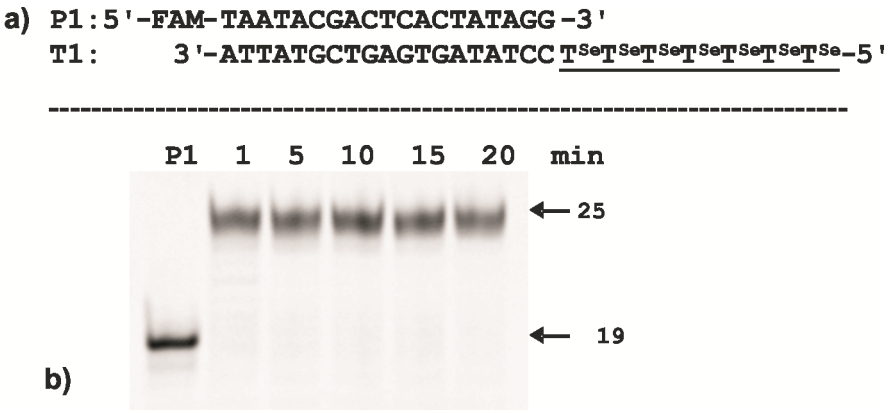



**Figure S2**. SeLNA-T nucleotide reading by KOD XL DNA polymerase without supplementing $MnCl_2$. a) Primer P1 and template sequence T1 (for reading); b) Incorporation of dA opposite to SeLNA-T of the template strand.

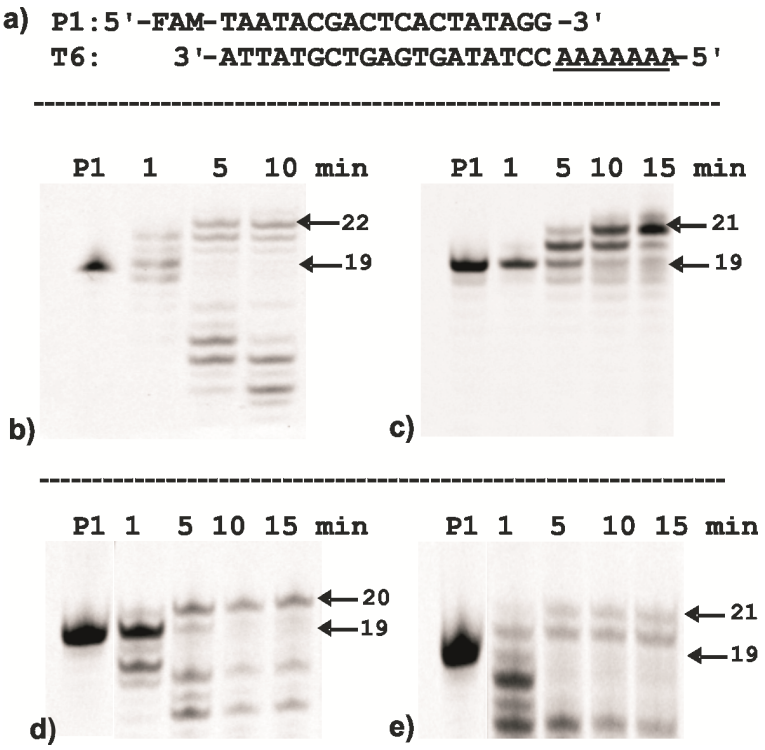



**Figure S3**. Incorporation of SeLNA-T nucleotides by polymerases. a) Primer P1 and template T6 sequence; b) KOD DNA polymerase; c) Therminator DNA polymerase; d) Phusion DNA polymerase; e) Klenow DNA polymerase. Incorporation sites in the template strands are underlined.

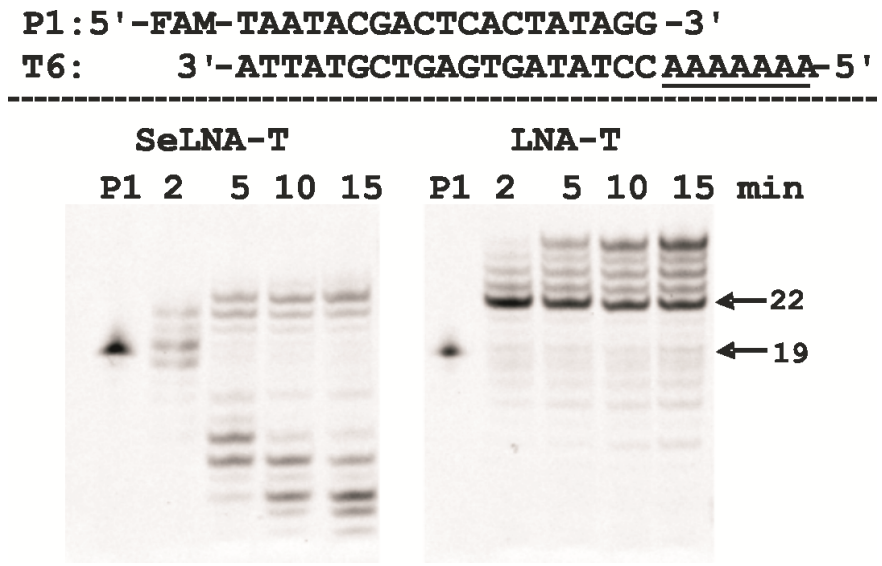



**Figure S4**. Comparision of incorporation efficiencies of SeLNA-T and LNA-T nucleotides by KOD XL DNA polymerase. Primer P1 and template T6 sequence.

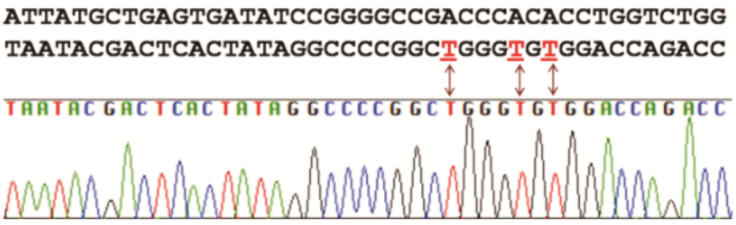


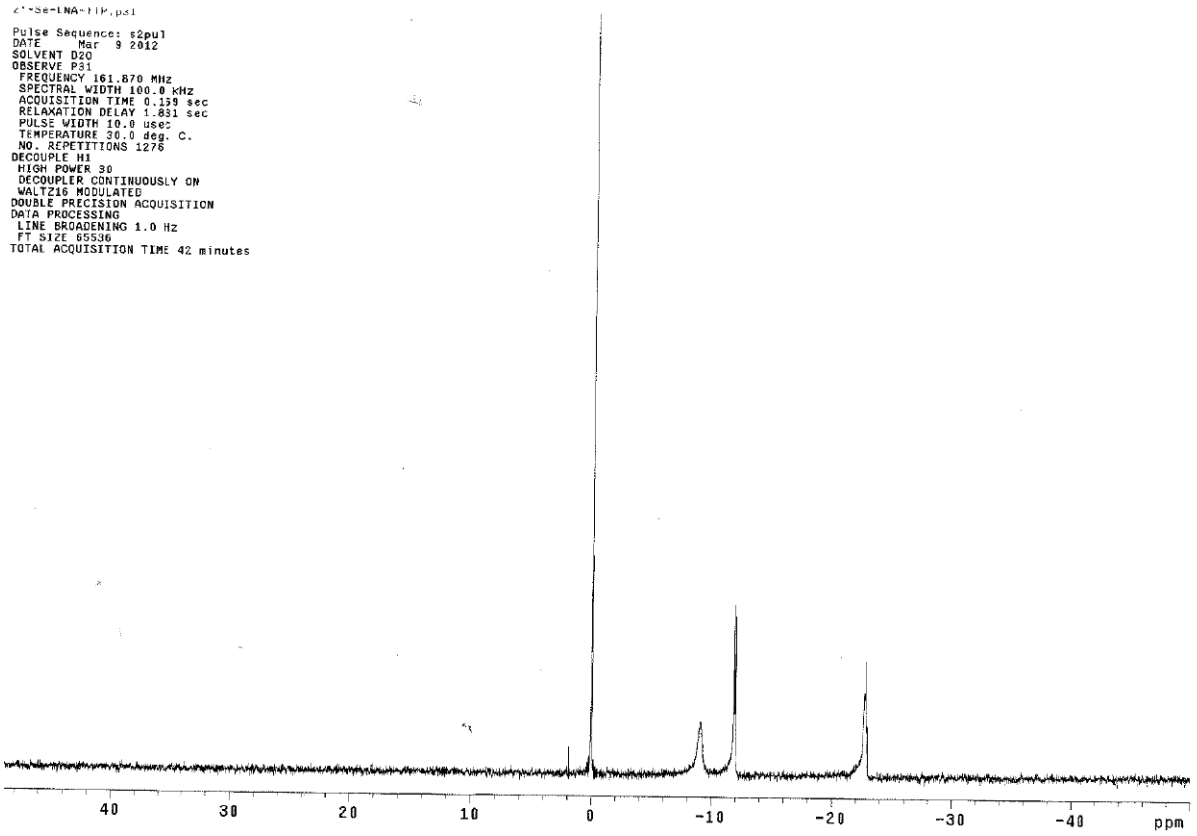

**Figure S5**. Alignment of the sequencing chromatogram with the actual primer extended ssDNA product with SeLNA-T nucleotides. Incorporation sites in the template strands are underlined.

## Experimental Section

**Synthesis of SeLNA oligonucleotides**: Templates containing SeLNA-T 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 $^oC$ overnight. The crude oligonucleotides were then purified by RP-HPLC and desalted, prior to use in the enzymatic reactions.

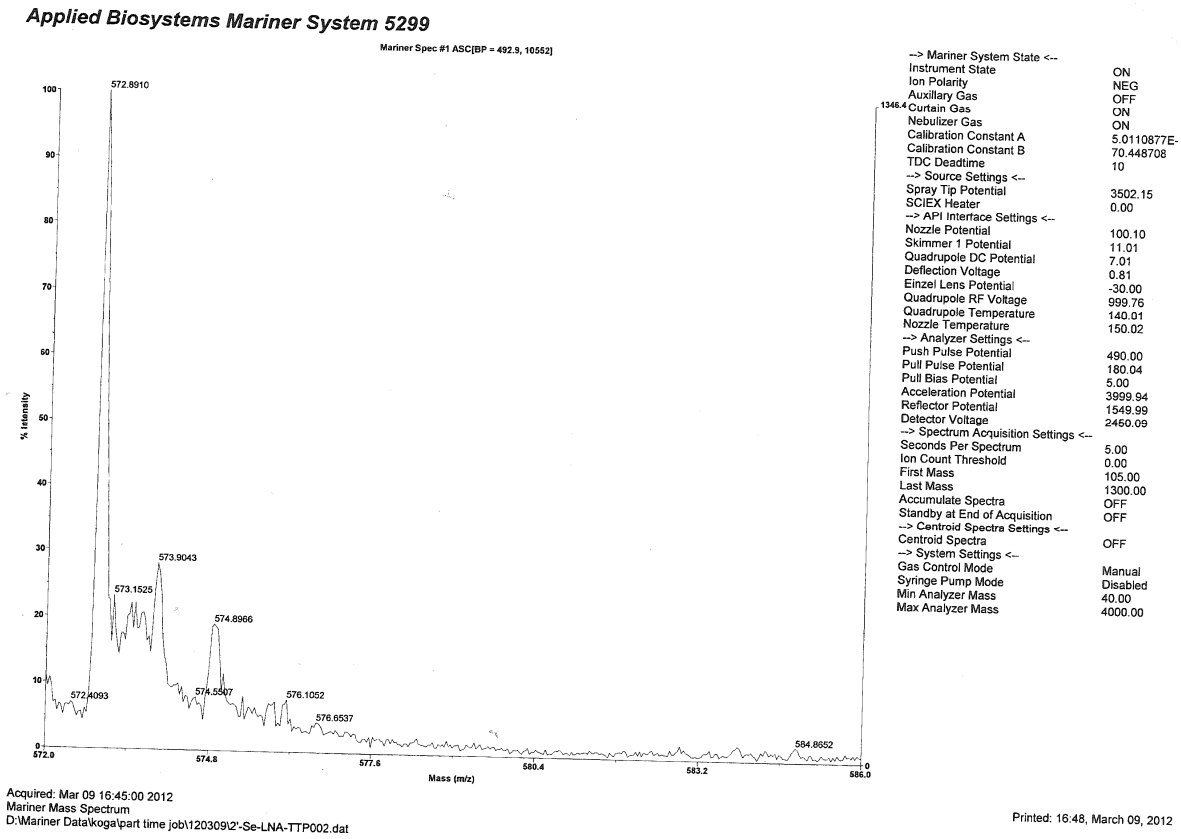
**Synthesis of SeLNA-T nucleoside 5'-triphosphates**: SeLNA triphosphates were synthesized according to a published procedure.[1] Full characterization of SeLNA-TTP is given below.

SeLNA-TTP: $^{31}P$ NMR (1601.8 MHz, in $D_2O$): $\delta$ -9.05 (d, 1P, γ-P), -11.93 (d, 1P, α-P), -22.70 (t, 1P, β-P) ppm.



## Mass Spec Analysis

ESI-MS (m/z): Calced for $C_{11}H_{16}N_2O_{14}P_3Se^-$ [M-H], 572.90; Found, 572.8910



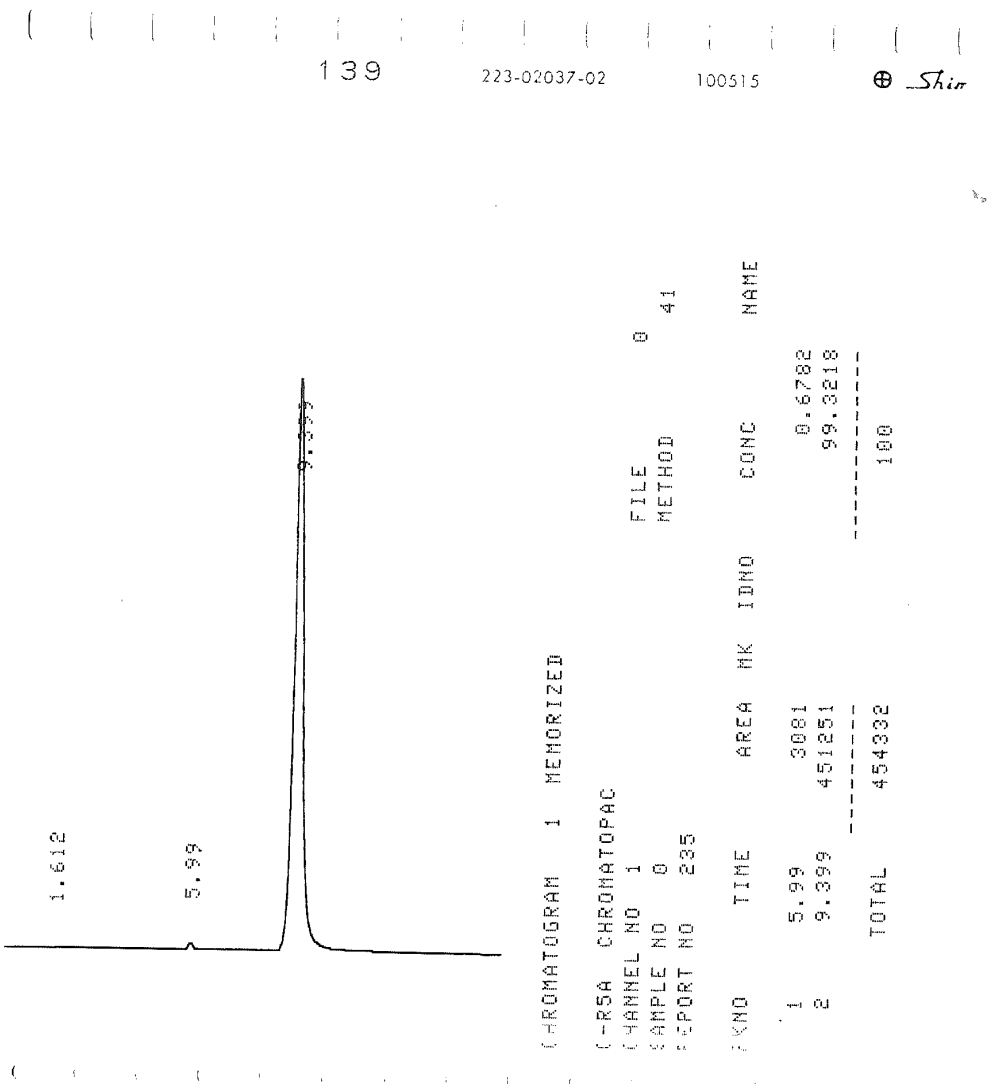

## HPLC

Column: COSMOSIL 5C-18MSII, 150X4.6 mm

Buffer: 32 % Methanol in 20 Mm potassium dihydrogenphosphate, 5 mM terta-n-butylammonium hydrogensulfate (pH 5.0)

Flow: 1.0 mL/Min

Time, 9.399 min; Peak area, 451251; Purity: 99 %




**General procedure for primer extension reactions**: 5'-FAM labelled primer sequence (purchased from IDT, Coralville, Iowa) was annealed to the templates 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 SeLNA-TTP, 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 the specific extension temperature recommended for the polymerases. The reactions were monitored by taking out 2.5 µL of the reaction mixture at different time 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 SeLNA-modified DNA template or the extended DNA 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 55 $^{o}$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, Australia) 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.