

## Selenium-atom-modified thymidine enhances specificity and sensitivity of DNA polymerization and detection

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### Experimental Section

#### Synthesis :

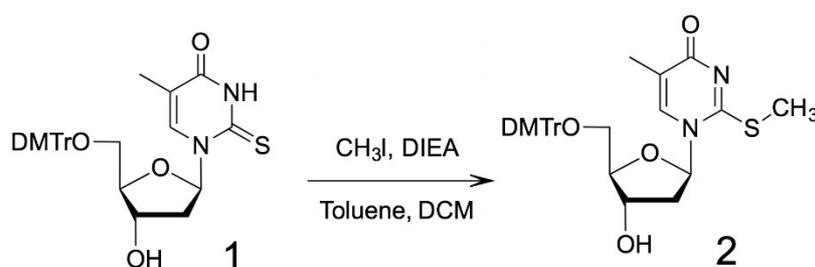
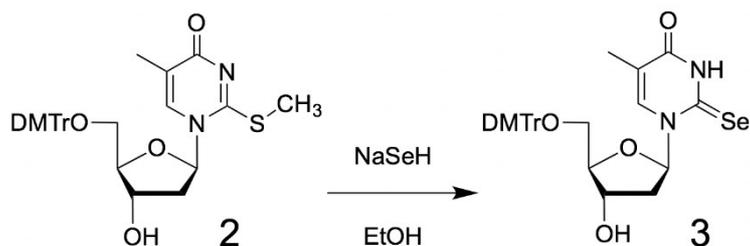


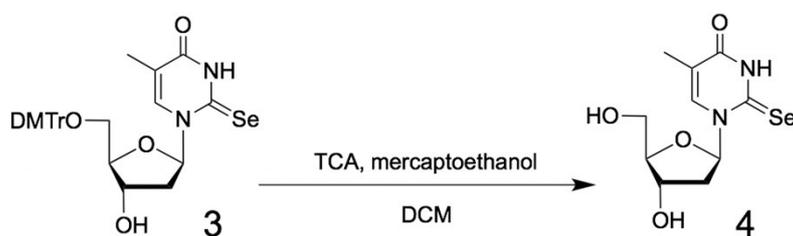
Figure S1. Synthesis of 5'-DMTr-MeST (2)

**Synthesis of 2:** **1** (1 g, 1.78 mmol) was dissolved in dry dichloromethane (DCM, 10 mL), followed by addition of toluene (5 mL). N,N-diisopropylethylamine (DIEA, 0.69 g, 5.34 mmol) and iodomethane (0.76 g, 5.35 mmol) was then added to the reaction mixture at room temperature. The reaction was monitored by thin layer chromatography (TLC) plate (10% methanol in dichloromethane, R<sub>f</sub>= 0.4) and completed in 1 h. Methanol (5 mL) was poured into the mixture and stirred for 5 minutes to quench the reaction. The organic phase was evaporated under reduced pressure, DCM (50 mL) was then added. The organic layer was washed once with ddH<sub>2</sub>O (50 mL) and then three times with Saturated sodium bicarbonate aqueous solution (50 mL). The organic phase was dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by flash column chromatography (5% methanol in dichloromethane) and pure **2** was obtained in 89% yield (0.92 g, 1.59 mmol), which was analyzed by NMR and MS, the results are consistent with our previous work.



**Figure S2.** Synthesis of 5'-DMTr-SeT (**3**)

**Synthesis of 3:** A solution of NaSeH was generated by addition of absolute ethanol (5 mL) to selenium (0.41 g, 5.19 mmol) and sodium borohydride (NaBH<sub>4</sub>, 0.25 g, 6.61 mmol) at 0°C. The reaction was completed in 0.5 h and a clear solution was formed. The ethanolic solution was added to **2** (0.5 g, 0.87 mmol) and the mixture was stirred overnight under argon. The reaction mixture was then concentrated under reduced pressure and ethyl acetate (5 mL) was added to the residue. The organic layer was washed with water three times (3 × 30 mL), and then dried over anhydrous magnesium sulfate. Purification was performed by flash column chromatography (5% methanol in dichloromethane) and the light yellow **3** was obtained in 83% yield (0.44 g, 0.72 mmol), which was analyzed by NMR and MS, the results are consistent with our previous work.



**Figure S3.** Synthesis of 2-Se-T nucleoside (<sup>Se</sup>T, **4**).

**Synthesis of 4:** **3** (0.22 g, 0.38 mmol) was placed in a dried round-bottom flask (10 mL), followed by addition of dichloromethane (5 mL, DCM) containing mercaptoethanol (0.2 g, 2.56 mmol, 7 eq.). Trichloroacetic acid (TCA) solution (1 mL of 10% TCA in DCM, m/v) was cautiously added dropwise to the flask. The reaction mixture was then stirred for 10 minutes, the precipitate was formed, and the reaction was monitored by TLC plate (10% methanol in dichloromethane, R<sub>f</sub>= 0.3). After the reaction completion, the precipitate was filtered and washed with a small amount of DCM, offering pure **4** in 95% yield (0.10 g, 0.34 mmol), which was analyzed by NMR and MS (Figure S4-S6);

<sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>) δ: 13.00 (s, 1H), 8.08 (s, 1H), 6.94 (t, J = 6.5 Hz, 1H), 5.28 (d, J = 4.1 Hz, 1H), 5.20 (t, J = 4.9 Hz, 1H), 4.27 (dq, J = 7.4, 3.8 Hz, 1H), 3.87 (q, J = 3.3 Hz, 1H), 3.66

(qdd,  $J = 12.1, 5.0, 3.3$  Hz, 2H), 2.30 (ddd,  $J = 13.4, 6.2, 3.7$  Hz, 1H), 2.05 (dt,  $J = 13.3, 6.5$  Hz, 1H), 1.80 (d,  $J = 1.2$  Hz, 3H);

$^{13}\text{C}$ -NMR (101 MHz, DMSO- $d_6$ )  $\delta$ : 173.62, 160.48, 137.21, 117.45, 92.75, 88.68, 70.25, 61.18, 13.21;

Molecular formula:  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_4\text{Se}$ ; measured  $[\text{M}+\text{H}]^+$ : 307.0190 (calc. 307.0197), error: 2.3 ppm; measured  $[\text{M}+\text{Na}]^+$ : 329.0012 (calc. 329.0017), error: 1.5 ppm.

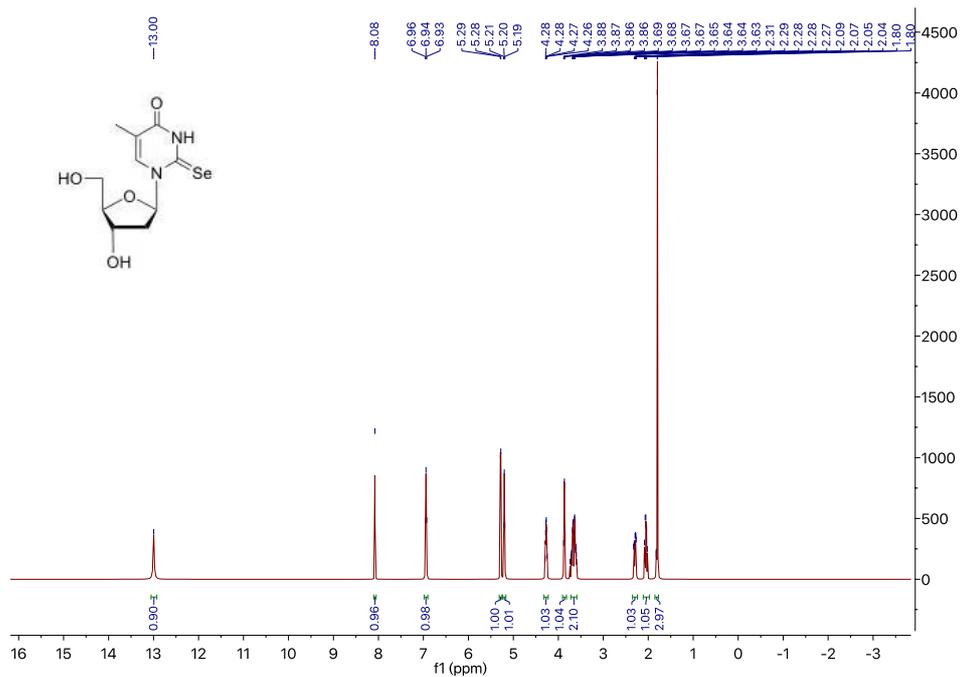


Figure S4.  $^1\text{H}$ -NMR of  $\text{SeT}$ .

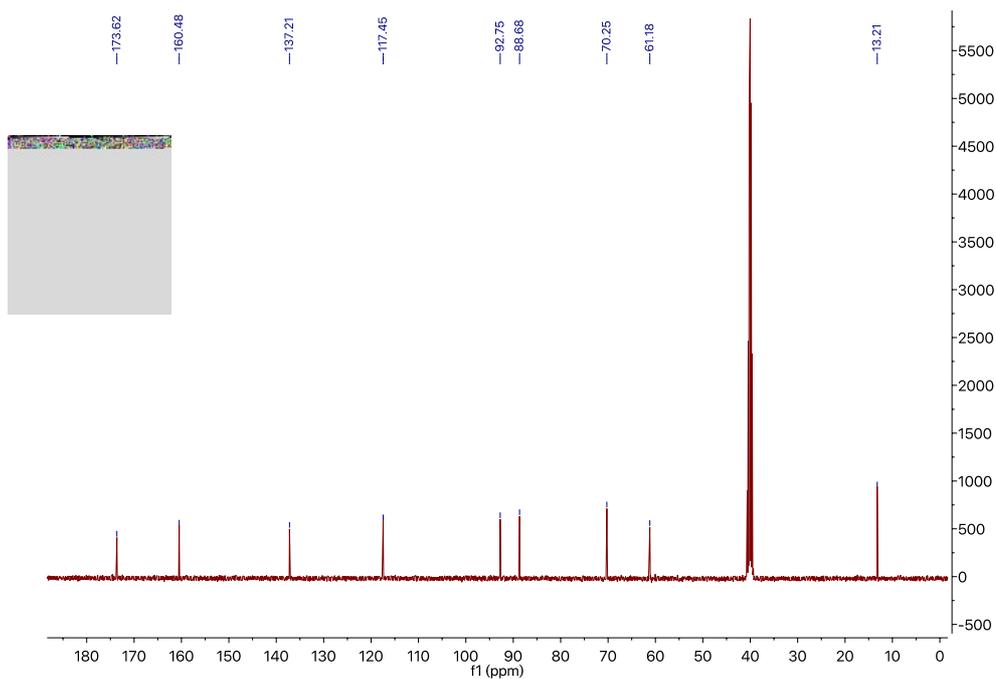


Figure S5.  $^{13}\text{C}$ -NMR of  $\text{SeT}$ .

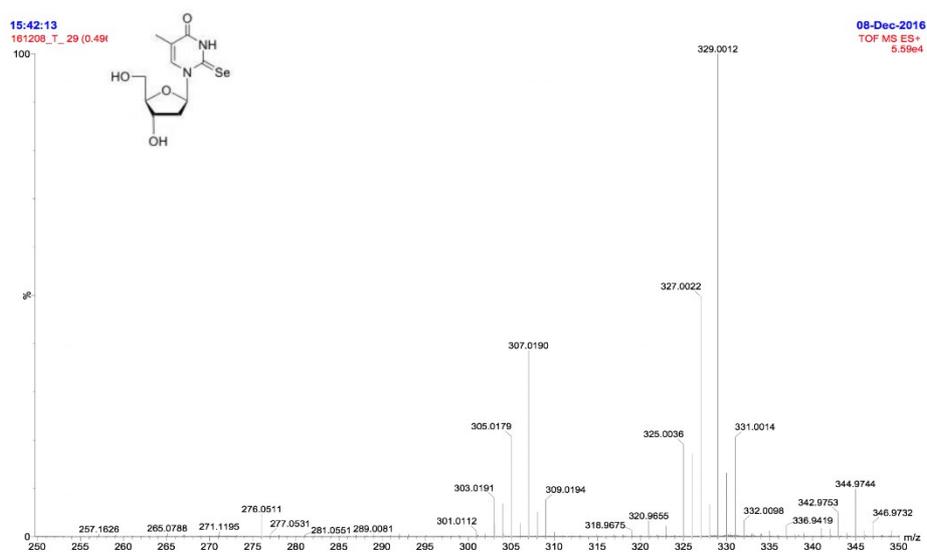
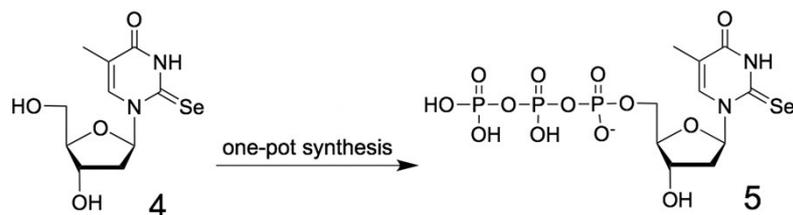


Figure S6. Mass spectrum of  $\text{SeT}$ . Molecular formula:  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_4\text{Se}$ ; measured  $[\text{M}+\text{H}]^+$ : 307.0190 (calc. 307.0197), error: 2.3 ppm; measured  $[\text{M}+\text{Na}]^+$ : 329.0012 (calc. 329.0017), error: 1.5 ppm.



**Figure S7.** One-pot synthesis of <sup>Se</sup>TTP (**5**).

**Synthesis of 5:** To a dried round-bottom flask (10 mL), placed a dried magnetic stir bar and <sup>Se</sup>T (**4**, 52.3 mg, 0.17 mmol). Tributylammonium pyrophosphate (170.4 mg, 0.37 mmol, 2 eqv.) was added into another dried round-bottom flask (25 mL) with a magnetic stir bar. Sealed each flask with a cream rubber septum and wrapped with parafilm. Placed each flask under oil pump to dry overnight at room temperature. 2-Chloro-4H-1,3,2-benzodioxaphosphorin-4-one (40 mg, 0.2 mmol, 1.2 eqv.) was added in another dried round-bottom flask (5 mL) with a magnetic stir bar and dried with a vacuum pump for 15 minutes. After argon washing for three times, anhydrous DMF (0.3 mL) and anhydrous tributylamine (0.6 mL) were injected by a syringe to dissolve tributylammonium pyrophosphate under argon (making Reagent **1**). 2-Chloro-4H-1,3,2-benzodioxaphosphorin-4-one was dissolved in anhydrous DMF (0.6 mL), then it was transferred to Reagent **1** by another syringe and stirred for 30 minutes under argon (making Reagent **2**). Dried **4** was dissolved in anhydrous DMF (0.3 mL) and then transferred to Reagent **2** by a syringe, and the mixture was stirred for 1 h under argon, followed by adding iodine (30 mg, 0.12 mmol) dissolved in a solution (5 mL) of DMF and water (95:5). A stable bright yellow solution was formed. Then, the reaction was stirred for another 0.5 h, before adding triethylamine (54 mg, 0.53 mmol, 3 eqv.) and water (5 mL). The reaction mixture was stirred for 1.5 h and was transferred into a 50-mL tube for ethanol precipitation (e.g., for 1 mL aqueous solution, 0.1 mL of 3M NaCl will be added, followed by adding 3 mL ethanol). Placed the precipitation tubes at -80°C or -20°C for 1 h and centrifuged it at approximately 3000 rpm for 30 min. Removed the supernatant and airdried the white precipitate (for 30 min). Redissolved the precipitate and repeated the precipitation again. Finally, the crude product was purified by HPLC, and **5** (<sup>Se</sup>TTP) was obtained in 12% yield (11.1 mg, 0.02 mmol), which was analyzed by NMR and MS (Figure S8-S11);

<sup>1</sup>H-NMR (400 MHz, deuterium oxide)  $\delta$ : 7.99–7.94 (m, 1H), 7.01 (t,  $J = 6.5$  Hz, 1H), 4.63 (dt,  $J = 6.8, 3.6$  Hz, 1H), 4.21 (d,  $J = 11.6$  Hz, 3H), 3.14 (q,  $J = 7.3$  Hz, 20H), 3.00 (d,  $J = 10.4$  Hz, 1H), 2.35–2.24 (m, 1H), 1.92 (s, 3H), 1.38 (t,  $J = 7.3$  Hz, 1H), 1.22 (t,  $J = 7.3$  Hz, 28H);

<sup>13</sup>C-NMR (101 MHz, deuterium oxide)  $\delta$ : 138.05, 119.41, 92.95, 86.39, 86.30, 70.23, 65.08, 46.66, 42.18, 39.11, 12.24, 8.25;

<sup>31</sup>P-NMR (162 MHz, deuterium oxide)  $\delta$ : -10.10, -11.74, -23.15;

Molecular formula: C<sub>10</sub>H<sub>17</sub>N<sub>2</sub>O<sub>13</sub>P<sub>3</sub>Se; measured [M-H<sup>+</sup>]: 544.9031 (calc. 544.9036), error: 0.92 ppm.



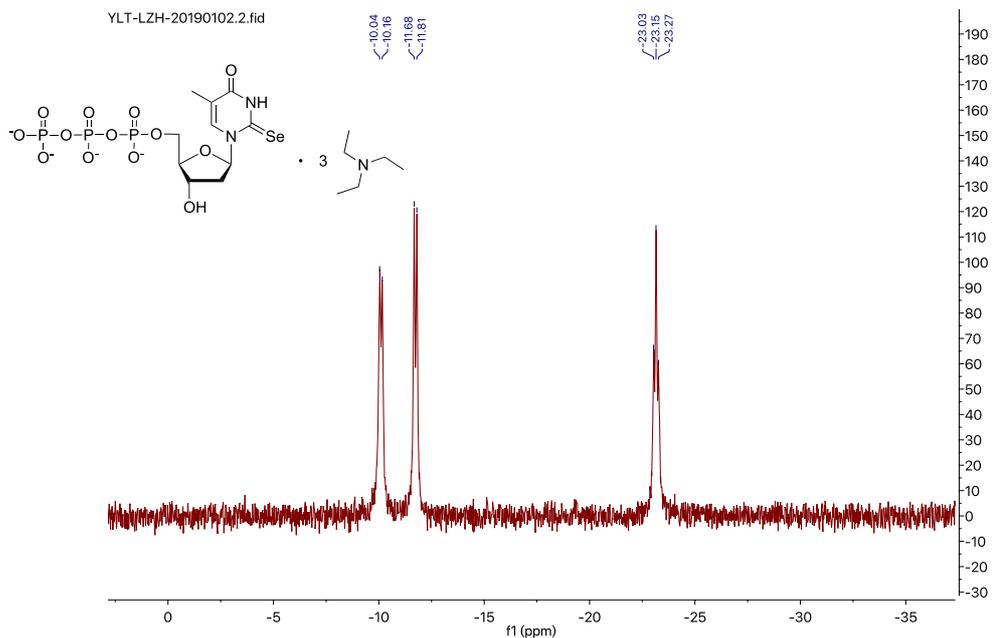


Figure S10.  $^{31}\text{P}$ -NMR of  $\text{Se}^+\text{TTP}$ .

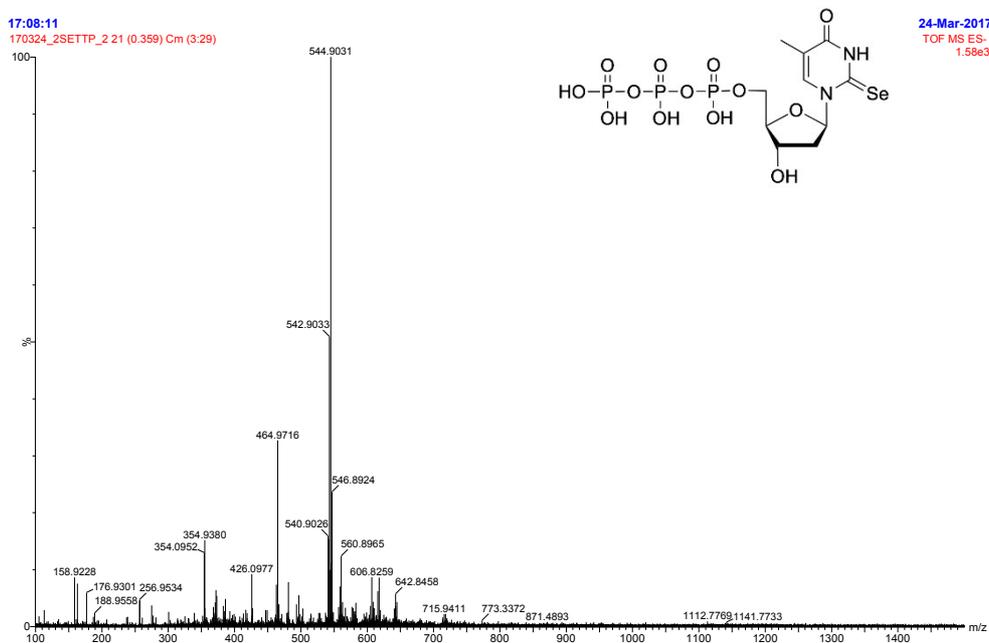


Figure S11: Mass spectrum of  $\text{Se}^+\text{TTP}$ . Molecular formula:  $\text{C}_{10}\text{H}_{17}\text{N}_2\text{O}_{13}\text{P}_3\text{Se}$ ; measured  $[\text{M}-\text{H}^+]$ : 544.9031 (calc. 544.9036), error: 0.92 ppm.

**Polymerase strand extension by Klenow.** For a 30  $\mu\text{L}$  reaction mixture, in Figure 2b, containing

5'-FAM-primer (0.25  $\mu$ M), template (0.5  $\mu$ M), TTP (0.25 mM) and Klenow (0.1 units/ $\mu$ L); in Figure 2c, containing 5'-FAM-primer (0.25  $\mu$ M), template (0.5  $\mu$ M), <sup>Se</sup>TTP (0.25 mM) and Klenow (0.1 units/ $\mu$ L); in Figure 2e, containing 5'-FAM-primer (0.25  $\mu$ M), template (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM), dCTP (0.25 mM), TTP (0.25 mM) and Klenow (0.1 units/ $\mu$ L); in Figure 2f, containing 5'-FAM-primer (0.25  $\mu$ M), template (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM), dCTP (0.25 mM), <sup>Se</sup>TTP (0.25 mM) and Klenow (0.1 units/ $\mu$ L). Each of the reaction mixture was incubated at 37°C and sampled at specific times respectively. The samples were quenched by loading buffer containing urea (8 M).

**Polymerase strand extension by Taq.** In Figure 3b, we designed 3 experimental groups using template-1, template-2 and template-3, respectively. Each group was divided into 3 equal parts. For a 10  $\mu$ L reaction mixture, in lane 2, containing 5'-FAM-primer (0.25  $\mu$ M), template-1 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 3, containing 5'-FAM-primer (0.25  $\mu$ M), template-1 (0.5  $\mu$ M), canonical dNTPs (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 4, containing 5'-FAM-primer (0.25  $\mu$ M), template-2 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM), TTP (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 5, containing 5'-FAM-primer (0.25  $\mu$ M), template-2 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 6, containing 5'-FAM-primer (0.25  $\mu$ M), template-2 (0.5  $\mu$ M), canonical dNTPs (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 7, containing 5'-FAM-primer (0.25  $\mu$ M), template-1 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM), TTP (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 8, containing 5'-FAM-primer (0.25  $\mu$ M), template-3 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 9, containing 5'-FAM-primer (0.25  $\mu$ M), template-3 (0.5  $\mu$ M), canonical dNTPs (0.25 mM) and Taq (0.1 units/ $\mu$ L); In lane 10, containing 5'-FAM-primer (0.25  $\mu$ M), template-3 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM), TTP (0.25 mM) and Taq (0.1 units/ $\mu$ L). In Figure 3c, TTP was replaced by <sup>Se</sup>TTP to perform the polymerizations. Each of the reaction mixture was incubated at 37°C for 20 minutes. In Figure 3e, containing 5'-FAM-primer (0.25  $\mu$ M), template-1 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM), TTP (0.25 mM) and Taq (0.1 units/ $\mu$ L). In Figure 3f, containing 5'-FAM-primer (0.25  $\mu$ M), template-1 (0.5  $\mu$ M), dATP (0.25 mM), dGTP (0.25 mM), <sup>Se</sup>TTP (0.25 mM) and Taq (0.1 units/ $\mu$ L). Each of the reaction mixture was incubated at 37°C and sampled at specific times respectively. The samples were quenched by loading buffer containing urea (8 M).s

**Polymerase chain reaction (PCR) by Taq.** Plasmid pFS 255 was purchased from <http://www.addgene.org>.

DNA-1 primers (forward primer: 5'-CCTCTTCCGACCATCAAGCAT-3'; reverse primer: 5'-CGTCATCAAAATCACTCGCATCAAC-3');

DNA-2 primers (forward primer: 5'-CCTCTTCCGACCATCAAGCAT-3'; reverse primer: 5'-ACAACCTATTAATTTCCCCTCGTC -3').

For a 50  $\mu$ L reaction mixture in lane 1, containing DNA-1 primers (0.2  $\mu$ M each), template (plasmid pFS255, 50 ng), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM), TTP (0.2 mM) and Taq (0.1 units/ $\mu$ L); In lane 2, containing DNA-1 primers (0.2  $\mu$ M each), template (plasmid pFS255, 50 ng), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM), <sup>Se</sup>TTP (0.2 mM), and Taq (0.1 units/ $\mu$ L); In lane 3, containing DNA-2 primers (0.2  $\mu$ M each), template (plasmid pFS255, 50 ng), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM), TTP (0.2 mM) and Taq (0.1 units/ $\mu$ L); In lane 4, containing DNA-

2 primers (0.2  $\mu$ M each), template (plasmid pFS255, 50 ng), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM),  $S^{32}$ TTP (0.2 mM), and Taq (0.1 units/ $\mu$ L); In lane 5, containing DNA-1 primers (0.2  $\mu$ M each), template (PCR product from Lane 1 and after 50-times dilution), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM), TTP (0.2 mM) and Taq (0.1 units/ $\mu$ L); In lane 6, containing DNA-1 primers (0.2  $\mu$ M each), template (PCR product from Lane 2 and after 50-times dilution), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM), TTP (0.2 mM), and Taq (0.1 units/ $\mu$ L); In lane 7, containing DNA-2 primers (0.2  $\mu$ M each), template (PCR product from Lane 3 and after 50-times dilution), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM), TTP (0.2 mM) and Taq (0.1 units/ $\mu$ L); In lane 8, containing DNA-2 primers (0.2  $\mu$ M each), template (PCR product from Lane 4 and after 50-times dilution), dATP (0.2 mM), dGTP (0.2 mM), dCTP (0.2 mM), TTP (0.2 mM), and Taq (0.1 units/ $\mu$ L). 30 cycle-PCR were performed in lane 1-4 and 10 cycle-PCR were performed in lane 5-8. The samples were performed on 1% agarose gel at 110 V for 35 minutes.

**Denatured PAGE gel analysis.** The samples were performed on 20% denatured PAGE gel at 220 V for 60 minutes. We used Photoshop to calculate the gray value of each band. Relative yield = (the gray value of product) / [(the gray value of product)+(the gray value of primer)]. All the data was import to Prism and Michaelis-Menten equation was used to carry out the curve fitting.

**Denatured PAGE gel analysis.** The samples were performed on 20% denatured PAGE gel at 220 V for 60 minutes. We used Photoshop to calculate the gray value of each band. Relative yield = (the gray value of product) / [(the gray value of product)+(the gray value of primer)]. All the data was import to Prism and Michaelis-Menten equation was used to carry out the curve fitting.

**COVID-19 RNA template.** The template was transcribed with T7 High Yield RNA Synthesis Kit (bought from NEB). The corresponding DNA template was cloned from other two plasmids, containing ORF1ab and N genes sequence of COVID-19 virus. The transcribed RNA template was purified by Monarch RNA Cleanup Kit (bought from NEB).

ORF1ab gene sequence:

5'-

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GGGACAACCAAUCACUAAUUGUGUUAAGAUGUUGUGUACACACACUGGUACUGGUCAG
GCAAUACAGUUACACCGGAAGCCAAUAUGGAUCAAGAAUCCUUUGGUGGUGCAUCGU
GUUGUCUGUACUGCCGUUGCCACAUAGAUAUCCAAUCCUAAAGGAUUUUGUGACUU
AAAAGGUAAGUAUGUACAAAUACCUACAACUUGUGCUAAUGACCCUGUGGGUUUUACA
CUUAAAAACACAGUCUGUACCGUCUGCGGUAUGUGGAAAGGUUAUGGCUGUAGUUGU
GAUCAACUCCGCGAACCCAUGCUUCAGUCAGCUGAUGCACAAUCGUUUUUAAACGGGU
UUGCGGUGUAAGUGCAGCCCGUCUACACCGUGCGGCACAGGCACUAGUACUGAUGU
CGUAUACAGGGCUUUUGACAUCUACAAUGAUAAAGUAGCUGGUUUUGCUAAAUUCCUA
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ACUUUGUAGUUAAGA-3'
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N gene sequence:

5'-

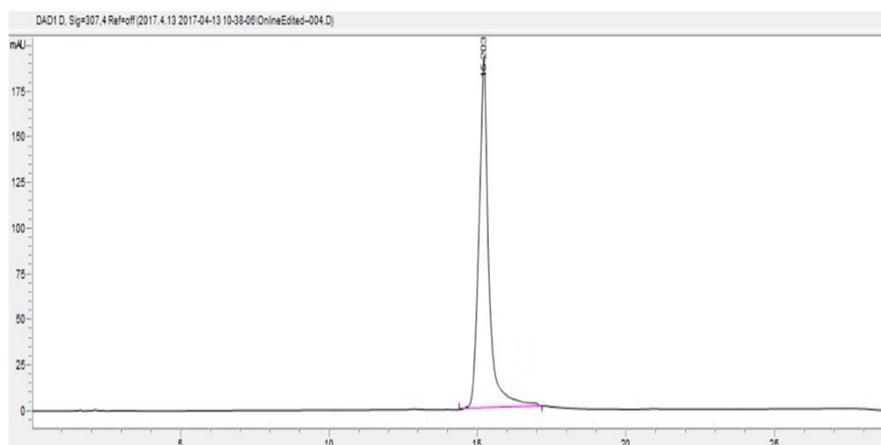
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CUUUUUCUUGCUUUCGUGGUUUCUUGCUAGUUACACUAGCCAUCUACUGCGCUU  
CGAUUGUGUGCGUACUGCGCAAUUUGUUAACGUGAGUCUUGUAAACCUUCUUUUU  
ACGUUUACUCUCGUGUUAAAAUCUGAAUUCUUCUAGAGUUCUGAUCUUCUGGUCUA  
AACGAACUAAA-3'

**RT-qPCR detection for COVID-19 RNA.** The reactions were performed with a commercial COVID-19 kit (containing buffer A, buffer B and enzyme mixture) which targeting ORF1ab and N genes on COVID-19 . For a 25  $\mu$ L reaction mixture in Figure 6a, containing 12  $\mu$ L buffer A, 4  $\mu$ L buffer B, 4  $\mu$ L enzyme mixture, 2  $\mu$ L supplementary (ddH<sub>2</sub>O, TTP or <sup>Se</sup>TTP), 1  $\mu$ L human total RNA and 2  $\mu$ L ddH<sub>2</sub>O. For a 25  $\mu$ L reaction mixture in Figure 6b, containing 12  $\mu$ L buffer A, 4  $\mu$ L buffer B, 4  $\mu$ L enzyme mixture, 2  $\mu$ L supplementary (ddH<sub>2</sub>O, TTP or <sup>Se</sup>TTP), 1  $\mu$ L human total RNA, 1  $\mu$ L COVID-19 RNA and 1  $\mu$ L ddH<sub>2</sub>O. For a 25  $\mu$ L reaction mixture in Figure 6c, containing 12  $\mu$ L buffer A, 4  $\mu$ L buffer B, 4  $\mu$ L enzyme mixture, 2  $\mu$ L supplementary (ddH<sub>2</sub>O, TTP or <sup>Se</sup>TTP), 1  $\mu$ L human total RNA, 1  $\mu$ L clinical viral RNA and 1  $\mu$ L ddH<sub>2</sub>O. RT-qPCR was carried out with a thermal cycler (TianLong, Gentier 96E) with a temperature profile of 50°C for 10 min and 95°C for 5 min, followed by 45 cycles of amplification (95°C for 10 s and 55°C for 40 s).

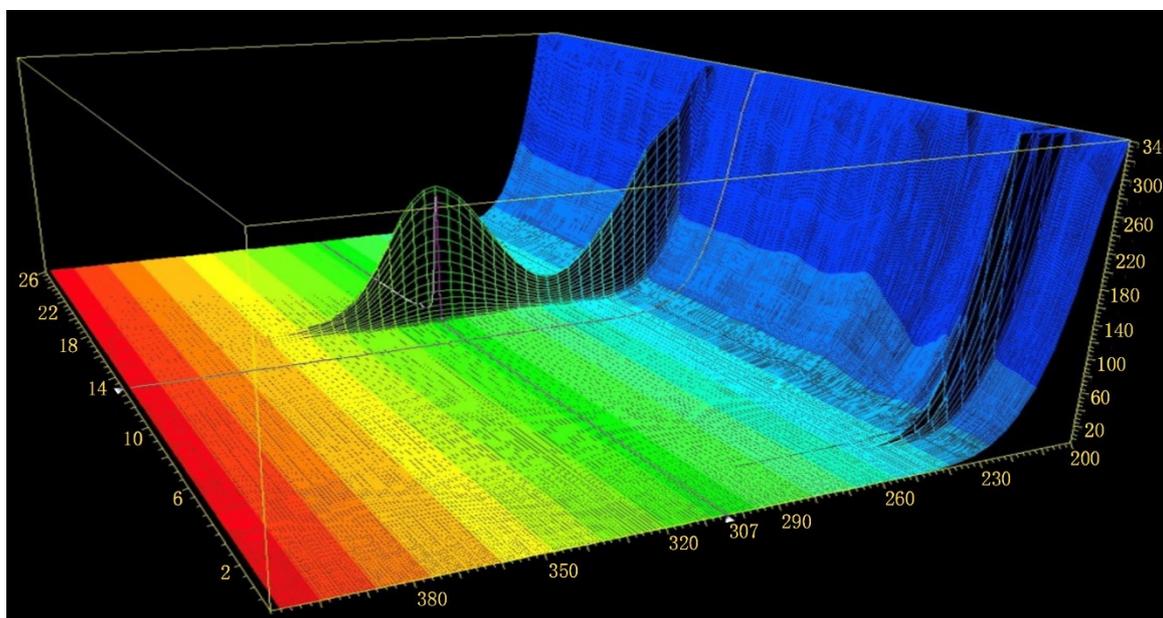
**The melting curves analysis.** The reactions were performed with TaKaRa One Step TB Green® PrimeScript™ RT-PCR kit (Code No. RR066A). For a 20  $\mu$ L reaction mixture, containing 10  $\mu$ L buffer, 0.8  $\mu$ L enzyme mixture, 0.8  $\mu$ L primer, 2  $\mu$ L COVID-19 RNA, 2  $\mu$ L supplementary (ddH<sub>2</sub>O, TTP or <sup>Se</sup>TTP), 1  $\mu$ L human total RNA and 3.4  $\mu$ L ddH<sub>2</sub>O. RT-qPCR was carried out with a thermal cycler (TianLong, Gentier 96E) with a temperature profile of 42°C for 5 min and 95°C for 10 s, followed by 45 cycles of amplification (95°C for 5 s and 60°C for 20 s). The melting process was carried out with a temperature profile from 95°C to 65°C (20°C/s) for 15 s, followed by rising to 95°C at the rate of 0.1°C/s.

Target gene	Primer	Sequence (5' to 3')	Label
ORF1ab	ORF1ab-F	CCCTGTGGGTTTTACACTTAA	□
	ORF1ab-R	ACGATTGTGCATCAGCTGA	□
	ORF1ab-P	CCGTCTGCGGTATGTGGAAAGGTTATGG	5'-FAM, 3'BHQ1
N	N-F	GGGGAACCTCTCCTGCTAGAAT	□
	N-R	CAGACATTTTGCTCTCAAGCTG	□
	N-P	TTGCTGCTGCTTGACAGATT	5'-FAM, 3'TAMRA

## HPLC Analysis of $\text{Se}^{\text{TTP}}$

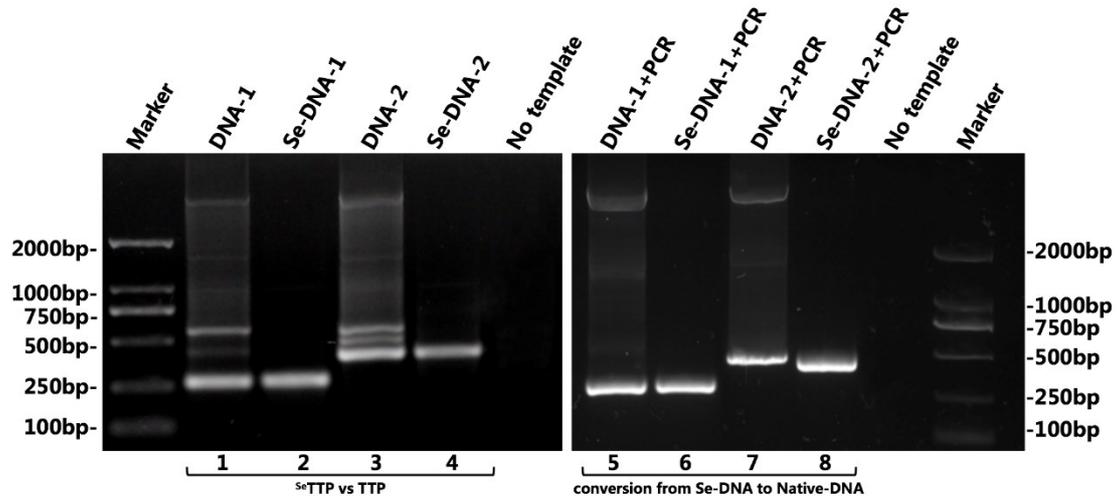


**Figure S12.** HPLC profile of  $\text{Se}^{\text{TTP}}$  (at 307 nm). Agilent 1260 HPLC and Welch Ultimate LP-C18 column were used in this experiment. Buffer A:  $(\text{Et})_3\text{NH}^+\text{CH}_3\text{COO}^-$ , 20 mM, pH 7.0; Buffer B: 50% acetonitrile containing  $(\text{Et})_3\text{NH}^+\text{CH}_3\text{COO}^-$ , 20 mM, pH 7.0. The analysis run from 2% to 15% buffer B in 30 minutes under the flow rate of 1 mL/min. The retention time of  $\text{Se}^{\text{TTP}}$  may around 15 minutes.



**Figure S13.** HPLC profile of  $\text{Se}^{\text{TTP}}$  under 3D full-UV-spectrum analysis. X axis: detection wavelength; Y axis: retention time; Z axis: UV signal intensity. Reverse-phase HPLC analysis was carried out in a C18 analytical column with  $(\text{Et})_3\text{NH}^+\text{CH}_3\text{COO}^-$  buffer (20 mM, pH 7.0; as solvent A) and 50% acetonitrile containing  $(\text{Et})_3\text{NH}^+\text{CH}_3\text{COO}^-$  buffer (20 mM, pH 7.0; as solvent B). The analysis run from 2% to 15% B in 30 minutes, at a gradient of 1 mL/min. The retention time of  $\text{Se}^{\text{TTP}}$  may around 15 minutes.

## Gel Analysis of Canonical and Se-modified DNAs



**Figure S14.** Inhibition of non-specific DNA amplification with  $^{Se}TTP$ ; agarose gel analysis of the canonical and Se-modified DNAs after PCR amplification. DNA-1 template was prepared by PCR with canonical dNTPs, plasmid pFS 255 and DNA-1 primers (forward primer: 5'-CCTCTTCCGACCATCAAGCAT-3'; reverse primer: 5'-CGTCATCAAAAATCACTCGCATCAAC-3'); DNA-2 template was prepared by PCR with canonical dNTPs, plasmid pFS 255 and DNA-2 primers (forward primer: 5'-CCTCTTCCGACCATCAAGCAT-3'; reverse primer: 5'-ACAACCTATTAATTTCCCCTCGTC -3'); In Lane 1, 30-cycle PCR was performed with DNA-1 template and canonical TTP with the other dNTPs; In Lane 2, 30-cycle PCR was performed with DNA-1 template and  $^{Se}TTP$  with the other dNTPs; In Lane 3, 30-cycle PCR was performed with DNA-2 template and canonical TTP with the other dNTPs; In Lane 4, 30-cycle PCR was performed with DNA-2 template and  $^{Se}TTP$  with the other dNTPs; In Lane 5, 10-cycle PCR was performed with canonical dNTPs and the PCR product (from Lane 1 and after 50-times dilution) as template; In Lane 6, 10-cycle PCR was performed with canonical dNTPs and the PCR product (from Lane 2 and after 50-times dilution) as template; In Lane 7, 10-cycle PCR was performed with canonical dNTPs and the PCR product (from Lane 3 and after 50-times dilution) as template; In Lane 8, 10-cycle PCR was performed with canonical dNTPs and the PCR product (from Lane 4 and after 50-times dilution) as template.

## Sequenced Canonical and Se-modified DNAs from PCR

DNA-1: 1-TGGTTACTCACCCTGCGATCCCCGGCAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTC-100

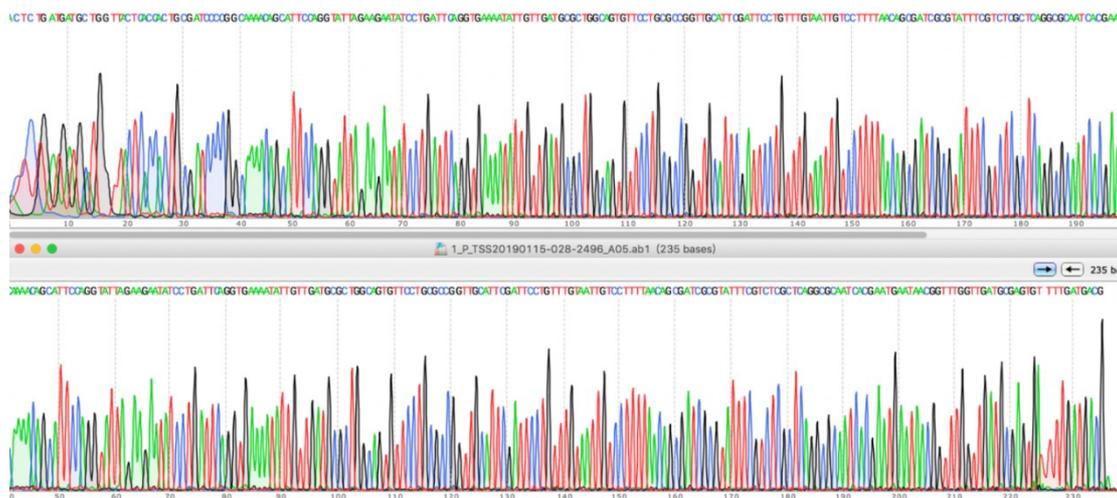
Se-DNA-1: 1-TGGTTACTCACCCTGCGATCCCCGGCAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTC-100

DNA-1: 101-TGCGCCGGTTGCATTTCGATTCTGTTTGAATTGTCCTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

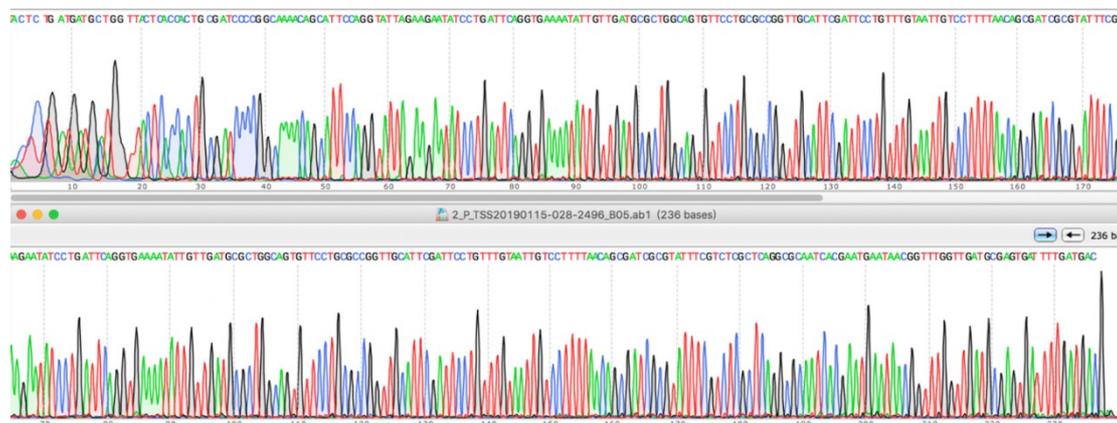
Se-DNA-1: 101-TGCGCCGGTTGCATTTCGATTCTGTTTGAATTGTCCTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

DNA-1: 201-TGATGCGAGTG-211

Se-DNA-1: 201-TGATGCGAGTG-211



**Figure S15.** Sanger sequencing result of DNA-1. In DNA-1 experiment, the PCR DNA was prepared with DNA-1 template, DNA-1 primers, TTP, and the other canonical dNTPs. The PCR DNA-1 was sequenced, and the resulted sequence was identical to template DNA-1.



**Figure S16.** Sanger sequencing result of Se-DNA-1. In Se-DNA-1 experiment, the PCR DNA was prepared with DNA-1 template, DNA-1 primers, <sup>Se</sup>TTP, and the other canonical dNTPs. The PCR Se-DNA-1 was sequenced, and the resulted sequence was identical to template DNA-1.

**DNA-2:** 1-TGGTTACTCACCCTGCGATCCCCGGCAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAAATATTGTTGATGCGCTGGCAGTGTTC-100

**Se-DNA-2:** 1-TGGTTACTCACCCTGCGATCCCCGGCAAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAAATATTGTTGATGCGCTGGCAGTGTTC-100

**DNA-2:** 101-TGCGCCGGTTGCATTTCGATTCTGTTTGTAAATGTCCTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

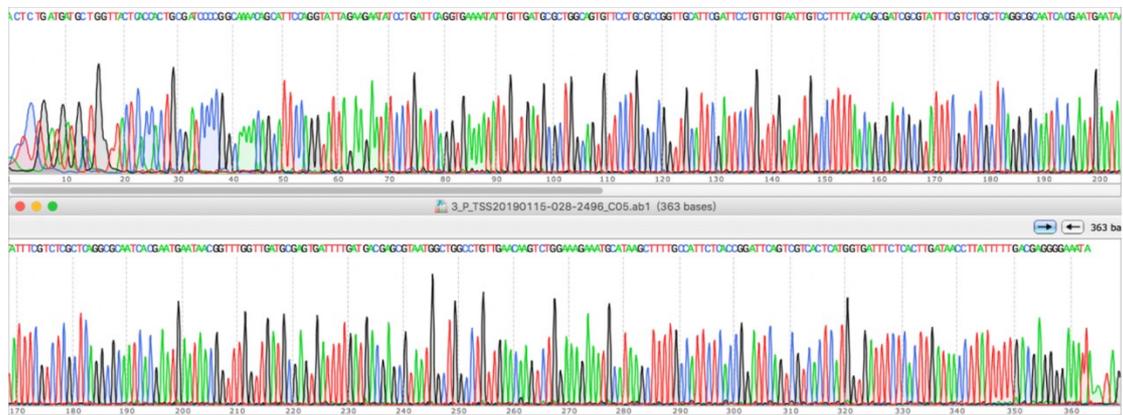
**Se-DNA-2:** 101-TGCGCCGGTTGCATTTCGATTCTGTTTGTAAATGTCCTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

**DNA-2:** 201-TGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGAAAAGAAATGCATAAGCTTTGCCATTCTACCGGATTACAGTCGTC-300

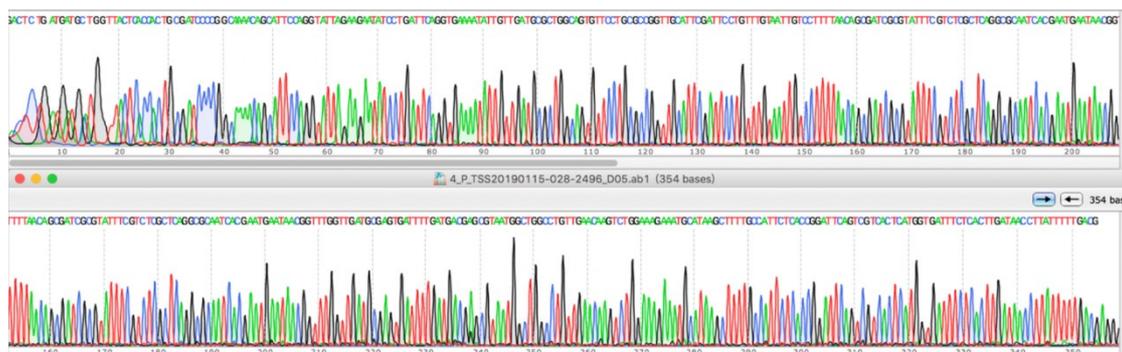
**Se-DNA-2:** 201-TGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGAAAAGAAATGCATAAGCTTTGCCATTCTACCGGATTACAGTCGTC-300

**DNA-2:** 301-ACTCATGGTGATTTCTCACTTGATAACCTATTTTTGACG-340

**Se-DNA-2:** 301-ACTCATGGTGATTTCTCACTTGATAACCTATTTTTGACG-340



**Figure S17.** Sanger sequencing result of DNA-2. In DNA-2 experiment, the PCR DNA was prepared with DNA-2 template, DNA-2 primers, TTP, and the other canonical dNTPs. The PCR DNA-2 was sequenced, and the resulted sequence was identical to template DNA-2.



**Figure S18.** Sanger sequencing result of Se-DNA-2. In Se-DNA-2 experiment, the PCR DNA was prepared with DNA-2 template, DNA-2 primers, <sup>Se</sup>TTP, and the other canonical dNTPs. The PCR Se-DNA-2 was sequenced, and the resulted sequence was identical to template DNA-2.

DNA-1/PCR: 1-TGGTTACTCACCACCTGCGATCCCCGGCAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTACAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTC-100

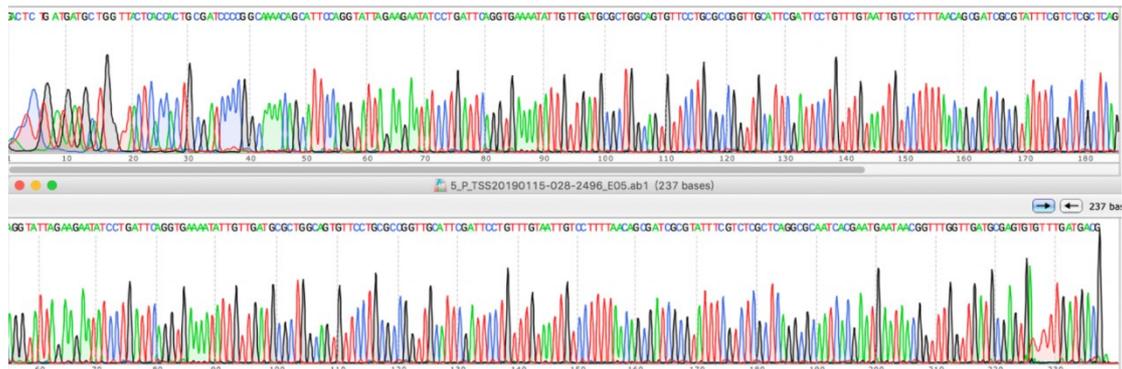
Se-DNA-1/PCR: 1-TGGTTACTCACCACCTGCGATCCCCGGCAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTACAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTC-100

DNA-1/PCR: 101-TGCGCCGGTTGCATTGATTCTGTTTGAATTGTCCTTTAAACAGCGATCGCGTATTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

Se-DNA-1/PCR: 101-TGCGCCGGTTGCATTGATTCTGTTTGAATTGTCCTTTAAACAGCGATCGCGTATTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

DNA-1/PCR: 201-TGATGCGAGTG-211

Se-DNA-1/PCR: 201-TGATGCGAGTG-211



**Figure S19.** Sanger sequencing result of DNA-1/PCR. In the experiment, the DNA-1/PCR was prepared with PCR DNA-1 (as template, after 50-times dilution) from **Figure S15**, DNA-1 primers, and all canonical dNTPs. The DNA-1/PCR was sequenced, and the resulted sequence was identical to template DNA-1.



**Figure S20.** Sanger sequencing result of Se-DNA-1/PCR. In the experiment, the Se-DNA-1/PCR was prepared with PCR Se-DNA-1 (as template, after 50-times dilution) from **Figure S16**, DNA-1 primers, and all canonical dNTPs. The Se-DNA-1/PCR was sequenced, and the resulted sequence was identical to template DNA-1.

DNA-2/PCR: 1-TGGTTACTCACCACCTGCGATCCCCGGCAAACAGCATTCCAGGTATTAGAAGAATATCTTGATTGAGTGGCAGTGGTCC-100

Se-DNA-2/PCR: 1-TGGTTACTCACCACCTGCGATCCCCGGCAAACAGCATTCCAGGTATTAGAAGAATATCTTGATTGAGTGGCAGTGGTCC-100

DNA-2/PCR: 101-TGCGCCGGTTGCATTGATTCTGTTTGAATTGTCCTTTAAACAGCGATCGCGTATTTCTGCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

Se-DNA-2/PCR: 101-TGCGCCGGTTGCATTGATTCTGTTTGAATTGTCCTTTAAACAGCGATCGCGTATTTCTGCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

DNA-2/PCR: 201-TGATGCGAGTGATTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGAAAGAAATGCATAAGCTTTGCCATTCTACCGATTGAGTCGTC-300

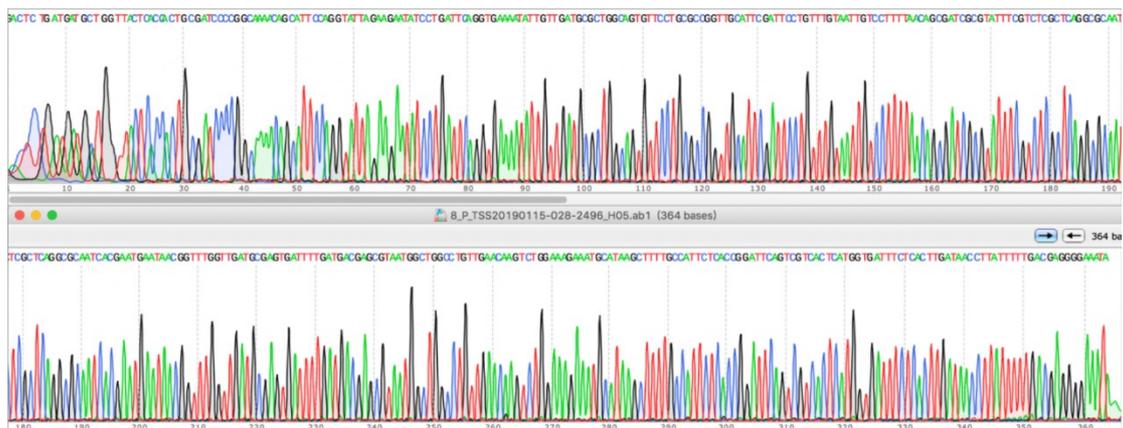
Se-DNA-2/PCR: 201-TGATGCGAGTGATTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGAAAGAAATGCATAAGCTTTGCCATTCTACCGATTGAGTCGTC-300

DNA-2/PCR: 301-ACTCATGGTGATTTCTCACTTGATAACCTTATTTTGGACG-340

Se-DNA-2/PCR: 301-ACTCATGGTGATTTCTCACTTGATAACCTTATTTTGGACG-340



**Figure S21.** Sanger sequencing result of DNA-2/PCR. In the experiment, the DNA-2/PCR was prepared with PCR DNA-2 (as template, after 50-times dilution) from **Figure S17**, DNA-2 primers, and all canonical dNTPs. The DNA-2/PCR was sequenced, and the resulted sequence was identical to template DNA-2.



**Figure S22.** Sanger sequencing result of Se-DNA-2/PCR. In the experiment, the Se-DNA-2/PCR was prepared with PCR Se-DNA-2 (as template, after 50-times dilution) from **Figure S18**, DNA-2 primers, and all canonical dNTPs. The Se-DNA-2/PCR was sequenced, and the resulted sequence was identical to template DNA-2.