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Supporting Information

Highly convenient and highly specific-and-sensitive PCR using Se-atom modified dNTPs

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Experimental Section

dNTPs, DNA polymerase and PCR components in PCR

2'-Deoxynucleoside 5'-(alpha-P-seleno)-triphosphates (dNTPαSe), including dATPαSe, dCTPαSe, dGTPαSe and dTTPαSe (TTPαSe), used in this study were synthesized as the one-pot convenient strategy used in previous research[1]. Four canonical dNTPs were purchased from Sangon Biotech. All the primers used in PCR and multiplex PCR were synthesized by Sangon Biotech. The DNA polymerase for PCR and multiplex PCR was Taq DNA polymerase and purchased from TransGen Biotech. The template DNA was were human genomic DNA extracted from Hela cells. Primer sequences were showed in **Table S1** and **Table S2**.

Suppression of nonspecific amplification with dNTPαSe in PCR

Reactions in **Fig. 1** were performed with 0.075 U/μL Taq DNA polymerase, the basic Taq DNA polymerase buffer (20 mM Tris–HCl (pH 8.3), 20 mM KCl, 10mM $(NH_4)_2SO_4$ and 2 mM $MgCl_2$), primers (0.2 μM in **Fig. 1A** and **1B**, 2 μM in **Fig. 1C** and **1D**), templates (20 pg/μL pEGFP-C1 plasmid DNA in **Fig. 1A**, 200 pg/μL HeLa cell genomes DNA in **Fig. 1B,** background DNAs [0.3 ng/μL pEGFP-C1 plasmid DNA for vent (exo-), vent and LA Taq or 0.3 ng/μL HeLa cell genomes DNA for Phusion in **Fig. 1C**], dNTPs (all four canonical dNTPs (each 200 μM); one (or two) of four dNTPαSe and other three (or two) canonical dNTPs (each 200 μM); four canonical dNTPs (each 200 μM) and four dNTPαSe (each 200 μM)). PCR thermal cycles were used as follows: 3 min at 94°C for DNA initial denaturation, followed by 30 cycles for 30 s at 94°C, 30 s at 55°C and 30 s (or 150 s) at 72°C, and a final extension step at 72°C for 5 min. The PCR products were electrophoresis analyzed in 1 or 2% agarose gel with ethidium bromide staining. The discrimination efficiency of by-product suppression using dNTPαSe was measured by the reaction rate ratio between the reactions with dCTP and dCTPαSe. The reaction rates were calculated on quantified gray values of the gel lanes with ImageJ.

Optimization of component concentrations with dNTPαSe

Reactions in **Fig. 2** were performed with the basic Taq DNA polymerase buffer, various concentrations

of Taq DNA polymerase, primers, templates and dNTPs. PCR thermal cycles were used as follows: 3 min at 94°C for DNA initial denaturation, followed by 30 cycles for 30 s at 94°C, 30 s at 55°C and 30 s (or 150 s) at 72°C, and a final extension step at 72°C for 5 min. The PCR products were electrophoresis analyzed in 1 or 2% agarose gel with ethidium bromide staining.

Optimization of thermal conditions with dNTPαSe

Reactions in **Fig. 3** were performed with 0.075 U/μL Taq DNA polymerase, the basic Taq DNA polymerase buffer, 0.2 μM primers, templates 200 pg/μL (HeLa cell genomes DNA), 200 μM dNTPs. PCR thermal cycles were used as follows: 3 min at 94°C for DNA initial denaturation, followed by 30 cycles for 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, and a final extension step at 72°C for 5 min. The PCR products were electrophoresis analyzed in 2% agarose gel with ethidium bromide staining.

Increasing successful rate of primer design and PCR optimization with dNTPαSe

Reactions in **Fig. 4** were performed with 0.075 U/μL Taq DNA polymerase, the basic Taq DNA polymerase buffer, 0.2 μM primers, templates (200 pg/μL of HeLa cell genomes DNA), 200 μM dNTPs. PCR thermal cycles were used as follows: 3 min at 94°C for DNA initial denaturation, followed by 30 cycles for 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and a final extension step at 72°C for 5 min. The PCR products were electrophoresis analyzed in 2% agarose gel with ethidium bromide staining.

Suppression of nonspecific amplification in multiplex PCR with dNTPαSe

The multiplex PCRs (**Fig. 5**) were performed with the optimized reaction buffer and conditions including some additives. The thermocycle conditions were: 94°C for 3 min, 30 cycles at 94°C for 30 sec, 60°C for 60 sec, followed by final extension at 72°C for 10 min. And we used slow-down strategy to enhance the efficiency and specificity of multiplex reactions with a slow annealing rate from 94 to 60°C (-0.5°C/sec) [2-4]. The multiplex PCR products were electrophoresis analyzed on 8% polyacrylamide gels with ethidium bromide staining.

Steady-state kinetic study on suppression of mismatched extension with dNTPαSe

The reactions of matched- and mismatched-primer/template extensions using dCTP or dCTPαSe (**Fig. 6** and **Figure S1**) were performed with DNA primer (0.7 μM, final concentration), DNA template (1 μM), Taq (0.075 U/μL, Transgen Co.), dNTPs/ dNTPαSe (200 μM, each of four dNTPs) and 1×basic Taq DNA polymerase buffer for 0-240 min at 72°C. Then the reactions were analyzed by urea-denaturing PAGE (12%) and imaged by FAM-primer fluoresce. Then fluoresce intensity of these bands on PAGE were acquired with ImageJ and analyzed with GraphPad Prism 8.

Study on sensitivity of PCR with dNTPαSe

The quantitative PCR reactions in **Fig. S2** were performed on Roche LightCycler96 with PCR premix (Buffer A, DNA polymerase and dNTPs of KAPA2G Fast HotStart PCR Kit from Sigma-Aldrich in **Fig. S2A** and **S2C**; TB Green™ Premix Ex Taq II from Takara in **Fig. S2B** and **S2D**), SYBR Green I (**Fig. S2A** and **S2C**), DNA primer (0.2 μM, final concentration), DNA template (cDNA reverse transcribed from human total RNA extracted from PC-3 cells), dNTPs (200 μM dCTP/dCTPαSe), and other three canonical dNTPs (each for 200 μM in **Fig. S2A** and **S2C;** 200 μM supplementary dCTP/dCTPαSe in **S2B** and **S2D)**. The thermocycle conditions were: 95°C for 30s, 40 cycles at 95°C for 5 sec, 56°C for 20

sec, 72°C for 20 sec, followed by the melting analysis.

The quantitative PCR reactions in **Fig. 7** were performed on Roche LightCycler96 with PCR premix (TB Green™ Premix Ex Taq II from Takara), DNA primer (0.2 μM, final concentration), DNA template, background DNA (0.6 ng/μL, human genome DNA from HeLa cells) and supplementary dNTPs (dNTP/dNTPαSe, each for 200 μM). The thermocycle conditions were: 95°C for 30s, 40 cycles at 95°C for 5 sec, 56°C for 20 sec, 72°C for 20 sec, followed by the melting analysis. Then, the products were analyzed by agarose gel (2%) with Gel-red staining.

Supplementary figure and tables

Table S1. PCR primer sequences used in specificity, optimization and sensitivity studies with dNTPαSe.

forward primer sequence (5'→3') and its GC content (%)	reverse primer sequence (5'→3') and its GC content (%)	specific product size (bp)	target gene	used in reaction of Figure
TCAAGATCCGCCACAACATC (50.0)	CTGGGTGCTCAGGTAGTG (61.1)	119	eGFP	**1A**
ACCCGCCCCTTGTCAAC (64.7)	CTGGACACCTCAATCAGCTG (55.9)	2410	apoB	**1B, 2A, 3A**
AAGATGCGACCCGATTCAAG (50.0)	CTTGCAGTTGATCCTGGTGG (55.0)	116	apoB	**2B**
TGCAGGCTGAACTGGTGG (61.1)	CCTCCACTGAGCAGCTTG (61.1)	211	apoB	**3B**
CTGAAAAAGTTAGTGAAAGAAGCTC (36.0)	CACACTTACCTCGATGAGG (52.6)	246	apoB	**5B**
TCCTCTCCAGATAAAAAACTCACCAT (38.5)	GATAAACCCACTCAGCATTGTTCTGC (46.2)	262	apoB	**5B**
GCAATCAACCACAGTCATGAACC (47.8)	CCATCCTTCTGAGTTCAGAGACC (52.2)	273	apoB	**5B**
GATCTGGAGAAACAACATATGACCAC (42.3)	GACAAGACAGGCCATATGTGCC (54.5	300	apoB	**5B**
ATTGGCCAAGATGGAATATCTACCAT (40.0)	GGTCAAATTTCATTTCAGCATATGAC (33.3)	320	apoB	**5B**
GTCAAGAGGCGAACACACAAC (52.0)	TTGGACGGACAGGATGTATGC (52.0)	162	c-myc	**S2**
GGGCAAAGGATCCCCATGTA (60)	AAGTCCATAGCACCAAAGCCA (48)	123	HPV-16	**7**

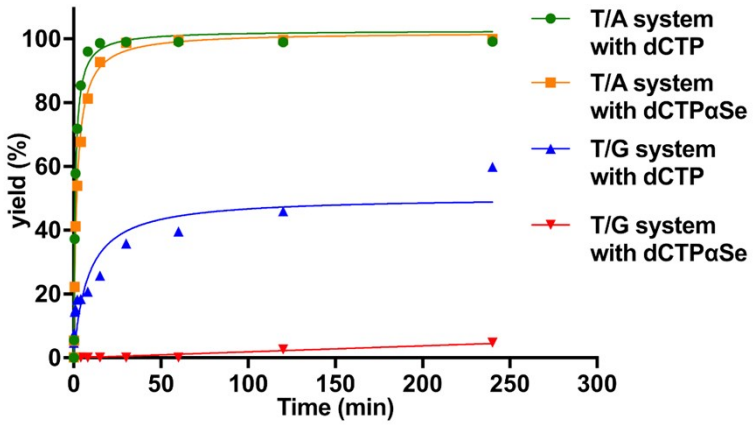
Table S2. Sequences of randomly selected 20 pairs of primers in **Fig. 4** and **Fig. 5**.

Primer pair			specific product size (bp)	nonspecific product	
Number	forward primer sequence (5'→3') and its GC content (%)	reverse primer sequence (5'→3') and its GC content (%)		PCR	Se-PCR
1	TTTTGGGAACACACAAAATCGAAG (37.5)	CTGAAGTCACGGTGTGCAAATG (50.0)	76	-	-
2	GTTGAGAAGCTGATTAAAGATTTGAAA (29.6)	AAGCTCCTCTCCCAAGATGC (55.0)	81	+	-
3	AATAAGTATGGGATGGTAGCACAA (37.5)	CTTCACCAAAGAAGCGGC (55.6)	85	+	-
4	GCCCCGTTTACCATGACC (61.1)	TTGCTATACAGCTGCCCAGTAT (45.5)	89	+	-
5	CTATCATAAGACAAACCCTACAGGG (44.0)	TTCATCCCCAGTGCAGTCATCTTG (50.0)	94	+	-
6	CAGAAAATGATATACAAATTGCATTAGATG (26.7)	ATACTGATCAAATTGTATCATATATGTCTGC (29.0)	98	-	-
7	ACTGAGGCCTACAGGAGAGA (55.0)	CTTCTGCTTGAGTTACAAACTTC (39.1)	110	-	-
8	AAGATGCGACCCGATTCAAG (50.0)	CTTGCAGTTGATCCTGGTGG (55.0)	116	+	-
9	ACCCGCCCCTTGTCAAC (64.7)	TTGTAGGAGAAAGGCAGGAAGAG (47.8)	125	+	-
10	GTTGAGCTGGAGGTTCCC (61.1)	CAAACTCCTCAGAGTTCTTGGTT (43.5)	130	+	-
11	CAACACATTACATTTGGTCTCTACCAC (40.7)	GCGTTGGAGTAAGCGCC (64.7)	135	+	-
12	GCAATGAGCTCATGGCTTC (52.6)	CTTTTTAAGAAGAGGTTTTCTGGG (37.5)	146	+	-
13	GTATGAGCTCAAGCTGGC (55.6)	CAGAAACAACACTTGCTTGG (45.0)	154	+	+

14	AAAGCCTACAGGACACCAAAATA (39.1)	ATCGTTGAAGTTCCTGCTGAATGTC (44.0)	159	-	-
15	TTGGAGAGGTCATCAGGAAGG (52.4)	GTTGGCTACTTCCAGTTTTACTCC (45.8)	167	-	-
16	ATTGGCTTGGAAGGAAAAGG (45.0)	CTGCTCATGTTTATCATCTTTGG (39.1)	177	-	-
17	CATTCAAATATAATCGGCAGAGT (34.8)	CTTAGGTGGCCCATGAGG (61.1)	184	+	-
18	CTGGTGGCAAAACCCTCC (61.1)	CCTCCACTGAGCAGCTTG (61.1)	200	+	-
19	GTACTAAGAAGATGGGCCTCG (52.4)	CTGGACACCTCAATCAGCTG (55.0)	220	+	-
20	ATGAGCAATATTTTATGTGCCCTG (37.5)	TTGAAGATCTCTCGCAGCTG (50.0)	232	+	-
				number of nonspecific reactions	
				14	1

Table S3. The T_m values of DNA and Se-DNA products from PCR reactions.

NO.	Products length (bp)	DNA products	Se(dC)-DNA products	ΔT (T_{m1}-T_{m2}) (°C)
		T_{m1} (°C)	T_{m2} (°C)	
1	76	78.8 ± 0.2	76.7 ± 0.1	2.08 ± 0.1
2	98	82.6 ± 0.1	80.2 ± 0.1	2.4 ± 0.1
3	110	86.6 ± 0.2	81.0 ± 0.1	5.7 ± 0.1
4	159	81.9 ± 0.1	79.4 ± 0.1	2.5 ± 0.1
5	167	82.7 ± 0.1	80.0 ± 0.1	2.7 ± 0.1
6	177	82.4 ± 0.1	79.6 ± 0.1	2.8 ± 0.1
7	232	87.2 ± 0.1	83.5 ± 0.1	3.7 ± 0.1
8	246	81.5 ± 0.1	79.0 ± 0.1	2.4 ± 0.1
9	262	84.2 ± 0.1	81.1 ± 0.1	3.1 ± 0.1
10	273	84.2 ± 0.1	81.1 ± 0.1	3.1 ± 0.1
11	300	81.7 ± 0.1	79.2 ± 0.1	2.4 ± 0.1
12	320	85.1 ± 0.1	82.0 ± 0.1	3.1 ± 0.1



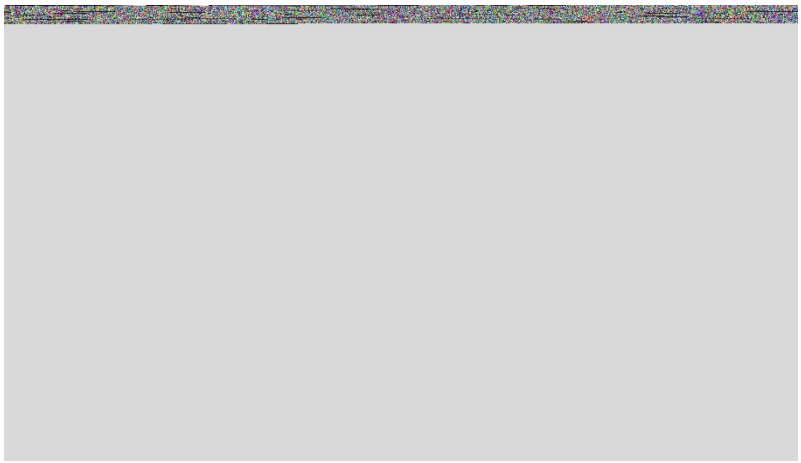
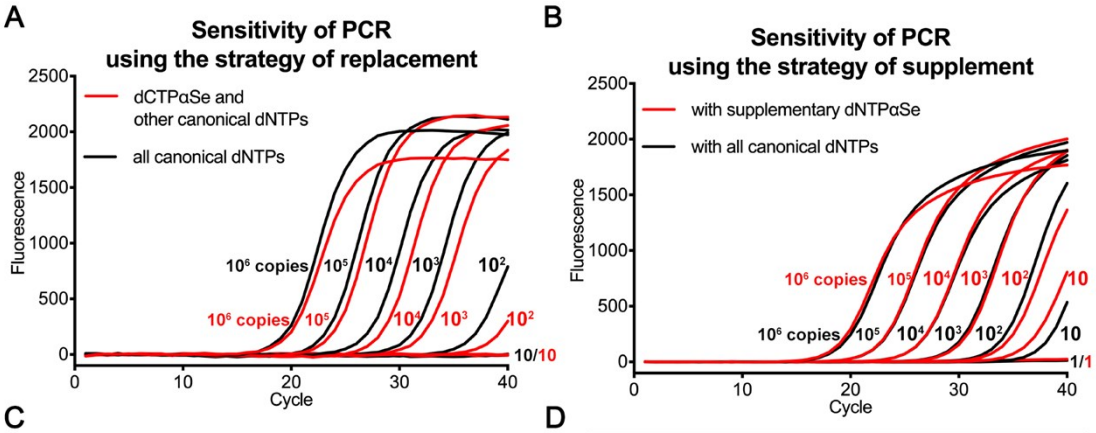


Fig. S1 Quantitative analysis of polymerization inhibition on mismatched primer/template with dCTPαSe.



C

target conc. in cDNA (copy/ reaction)	Ct mean	
	all canonical dNTPs	dCTPαSe and other canonical dNTPs
10^6	19.4±0.2	19.6±0.4
10^5	22.8±0.2	24.0±0.5
10^4	26.9±0.2	28.4±0.5
10^3	30.7±0.4	32.1±0.4
10^2	36.7±0.5	38.7±0.6
10^1	-	-
1	-	-

D

target conc. in cDNA (copy/ reaction	Ct mean	
	all canonical dNTPs	supplementary dNTPαSe
10^6	17.6±0.1	17.5±0.1
10^5	20.9±0.2	20.9±0.2
10^4	24.7±0.1	24.6±0.2
10^3	28.3±0.2	28.6±0.2
10^2	31.6±0.7	32.2±0.4
10^1	35.8±0.6	35.7±1.0
1	-	-

Fig. S2 Sensitivity of PCR with dNTPαSe. (A, B) Fluorescent curves of quantitative PCR with dNTPαSe using the strategy of replacement (A) and supplement (B). (C, D) Cycle of threshold (Ct) values of quantitative PCR with dNTPαSe using the strategy of replacement (C) and supplement (D). The copy number of the target sequence in human cDNA was measured based on the purified and quantified cDNA PCR product, which was used to prepare the curve of Ct values and concentrations.

References:

1. B. Hu, Y. Wang, S. Sun, W. Yan, C. Zhang, D. Luo, H. Deng, L. R. Hu and Z. Huang, *Angew. Chem., Int. Ed.*, 2019, **58**, 7835-7839.
2. R. H. Don, P. T. Cox, B. J. Wainwright, K. Baker and J. S. Mattick, *Nucleic Acids Res.*, 1991, **19**, 4008.
3. D. J. Korbie and J. S. Mattick, *Nat. Protoc.*, 2008, **3**, 1452-1456.
4. U. H. Frey, H. S. Bachmann, J. Peters and W. Siffert, *Nat. Protoc.*, 2008, **3**, 1312-1317.