Sulfur atom modification on thymine improves the specificity and sensitivity of DNA polymerization

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Supporting information

ORF1ab gene sequence:

5'-

GGGACAACCAAUCACUAAUUGUGUUAAGAUGUUGUGUACACACACUGGUACUGGUCAGGCAAUAA
CAGUUACACCGGAAGCCAAUAUGGAUCAAGAAUCCUUUGGUGGUGCAUCGUGUUGUCUGUACUGC
CGUUGCCACAUAGAUCAUCCAAAUCCUAAAGGAUUUUGUGACUUAAAAGGUAAGUAUGUACAAAU
ACCUACAACUUGUGCUAAUGACCCUGUGGGUUUUACACUUAAAAACACAGUCUGUACCGUCUGCG
GUAUGUGGAAAGGUUAUGGCUGUAGUUGUGAUCAACUCCGCGAACCCAUGCUUCAGUCAGCUGAU
GCACAAUCGUUUUUAAACGGGUUUGCGGUGUAAGUGCAGCCCGUCUUACACCGUGCGGCACAGGC
ACUAGUACUGAUGUCGUAUACAGGGCUUUUGACAUCUACAAUGAUAAAGUAGCUGGUUUUGCUAA
AUUCCUAAAAACUAAUUGUUGUCGCUUCCAAGAAAAGGACGAAGAUGACAAUUUAAUUGAUUCUU
ACUUUGUAGUUAAGA-3'

N gene sequence:

5'-

AUGAUGAACCGACGACGACUACUAGCGUGCCUUUGUAAGCACAAGCUGAUGAGUACGAACUUAUG
UACUCAUUCGUUUCGGAAGAGACAGGUACGUUAAUAGUUAAUAGCGUACUUCUUUUUCUUGCUUU
CGUGGUAUUCUUGCUAGUUACACUAGCCAUCCUUACUGCGCUUCGAUUGUGUGCGUACUGCUGCA
AUAUUGUUAACGUGAGUCUUGUAAACCUUCUUUUUACGUUUACUCUCGUGUUAAAAAUCUGAAUU
CUUCUAGAGUUCCUGAUCUUCUGGUCUAAACGAACUAAA-3'

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	GGGGAACTTCTCCTGCTAGAAT	□
	N-R	CAGACATTTTGCTCTCAAGCTG	□
	N-P	TTGCTGCTGCTTGACAGATT	5'-FAM, 3'TAMRA

Gel Analysis of Canonical and S-modified DNAs

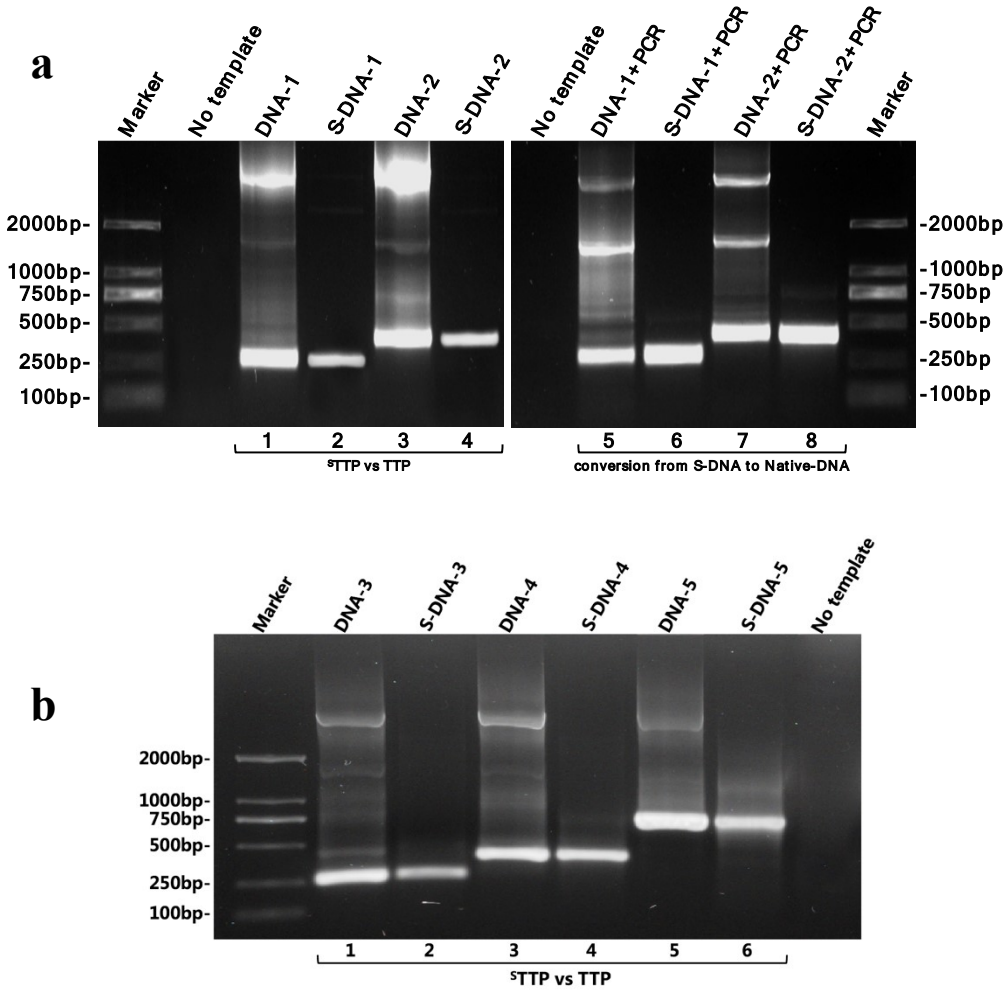

Figure S1. Inhibition of non-specific DNA amplification with ^{S}TTP; **a.** agrose gel analysis of the canonical and S-modified DNAs after PCR amplification. DNA-1 template was prepared by PCR with canonical dNTPs, plasmid pFS 255 and DNA-1 primers (forward primer: 5'-CCTCTTCCGACCATCAAGCAT-3'; reverse primer: 5'-CGTCATCAAAATCACTCGCATCAAC-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 ^{S}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 ^{S}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. **b.** PCR amplification results

using the other three templates.

Sequenced Canonical and S-modified DNAs from PCR

DNA-1 (1-100): 1-
TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCA
GTGTTCC-100

S-DNA-1 (1-100): 1-
TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCA
GTGTTCC-100

DNA-1 (101-200): 101-
TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGG
TTTGGT-200

S-DNA-1 (101-200): 101-
TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGG
TTTGGT-200

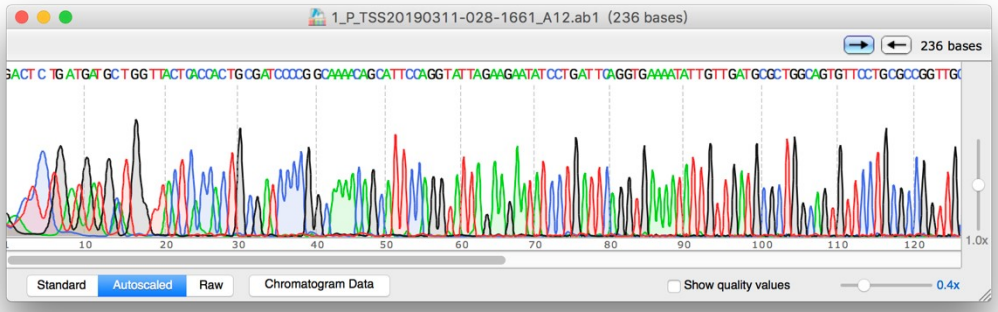

Figure S2. Sanger sequencing result of DNA-1. In DNA-1 experiment, the PCR DNA was prepared with DNA-1 template, DNA-1 primers, TTP, and the other canonical dNTPs. The PCR DNA-1 was sequenced, and the resulted sequence was identical to template DNA-1.

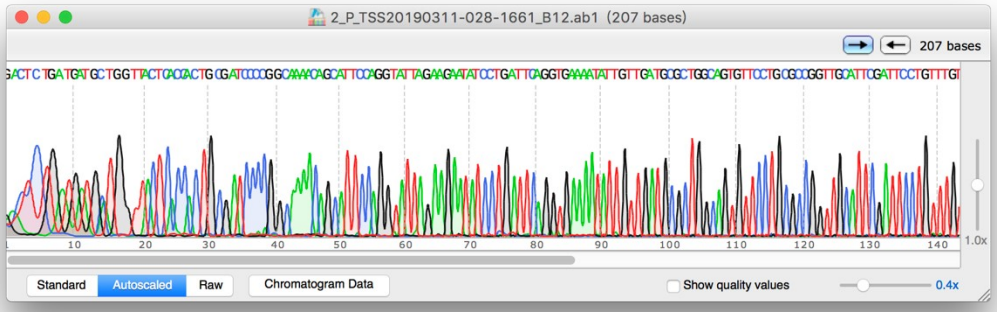

Figure S3. Sanger sequencing result of S-DNA-1. In S-DNA-1 experiment, the PCR DNA was prepared with DNA-1 template, DNA-1 primers, ^{S}TTP, and the other canonical dNTPs. The PCR S-DNA-1 was sequenced, and the resulted sequence was identical to template DNA-1.

DNA-2 (100-100): 1-TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCC-100

S-DNA-2 (100-100): 1-TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCC-100

DNA-2 (101-200): 101-TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

S-DNA-2 (101-200): 101-TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

DNA-2 (201-300): 201-TGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTC-300

S-DNA-2 (201-300): 201-TGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTC-300

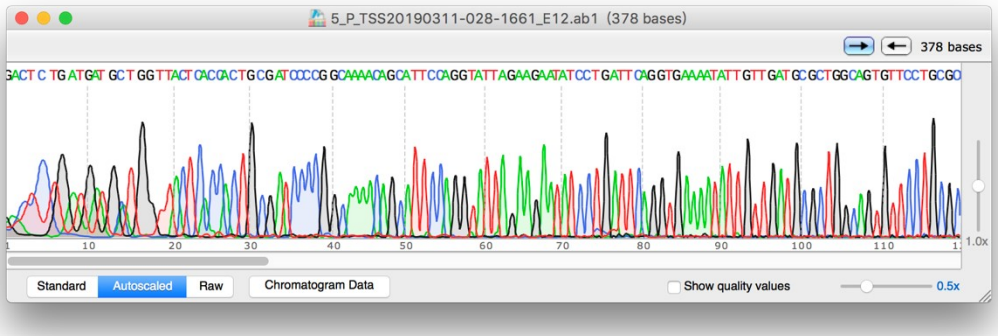

Figure S4. Sanger sequencing result of DNA-2. In DNA-2 experiment, the PCR DNA was prepared with DNA-2 template, DNA-2 primers, TTP, and the other canonical dNTPs. The PCR DNA-2 was sequenced, and the resulted sequence was identical to template DNA-2.

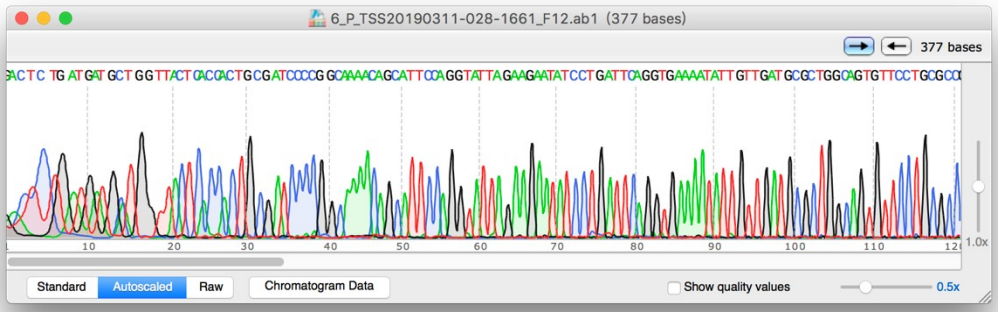

Figure S5. Sanger sequencing result of S-DNA-2. In S-DNA-2 experiment, the PCR DNA was prepared with DNA-2 template, DNA-2 primers, ^{S}TTP, and the other canonical dNTPs. The PCR S-DNA-2 was sequenced, and the resulted sequence was identical to template DNA-2.

DNA-1/PCR (1-100): 1-TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCC-100

S-DNA-1/PCR (1-100): 1-TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCC-100

DNA-1/PCR (101-200): 101-TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

S-DNA-1/PCR (101-200): 101-TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

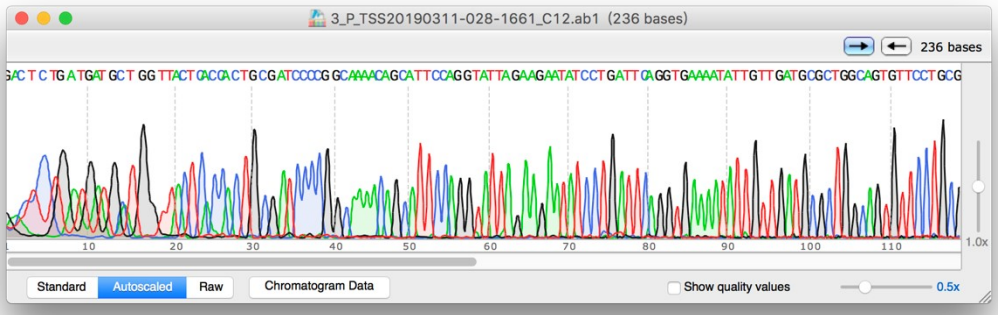

Figure S6. Sanger sequencing result of DNA-1/PCR. In the experiment, the DNA-1/PCR was prepared with PCR DNA-1 (as template, after 50-times dilution) from **Figure S2**, DNA-1 primers, and all canonical dNTPs. The DNA-1/PCR was sequenced, and the resulted sequence was identical to template DNA-1.

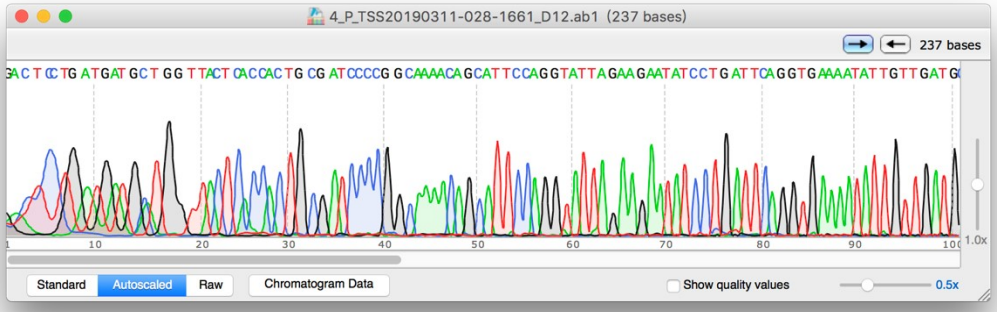

Figure S7. Sanger sequencing result of S-DNA-1/PCR. In the experiment, the S-DNA-1/PCR was prepared with PCR S-DNA-1 (as template, after 50-times dilution) from **Figure S3**, DNA-1 primers, and all canonical dNTPs. The S-DNA-1/PCR was sequenced, and the resulted sequence was identical to template DNA-1.

DNA-2/PCR (1-100): 1-TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCC-100

S-DNA-2/PCR (1-100): 1-TGGTTACTCACCACTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCC-100

DNA-2/PCR (101-200): 101-TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

S-DNA-2/PCR (101-200): 101-TGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGT-200

DNA-2/PCR (201-300): 201-TGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTC-300

S-DNA-2/PCR (201-300): 201-TGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTC-300

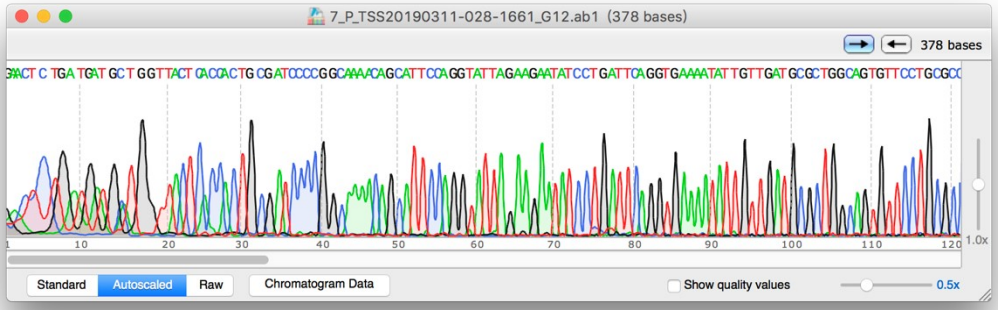

Figure S8. Sanger sequencing result of DNA-2/PCR. In the experiment, the DNA-2/PCR was prepared with PCR DNA-2 (as template, after 50-times dilution) from **Figure S4**, DNA-2 primers, and all canonical dNTPs. The DNA-2/PCR was sequenced, and the resulted sequence was identical to template DNA-2.

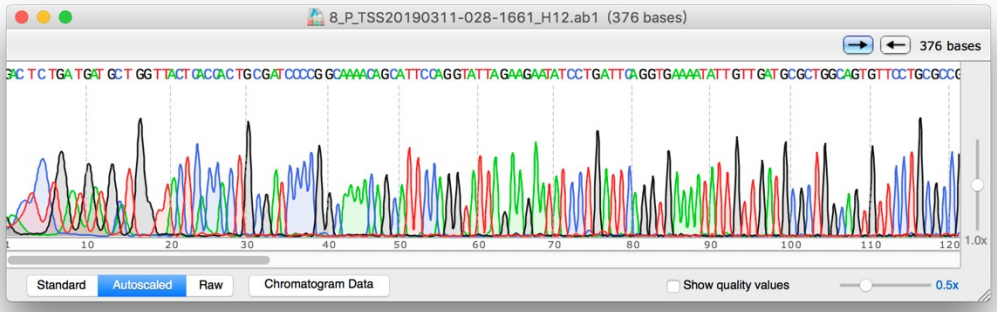

Figure S9. Sanger sequencing result of S-DNA-2/PCR. In the experiment, the S-DNA-2/PCR was prepared with PCR S-DNA-2 (as template, after 50-times dilution) from **Figure S5**, DNA-2 primers, and all canonical dNTPs. The S-DNA-2/PCR was sequenced, and the resulted sequence was identical to template DNA-2.

Page Analysis of Canonical and S-modified DNAs

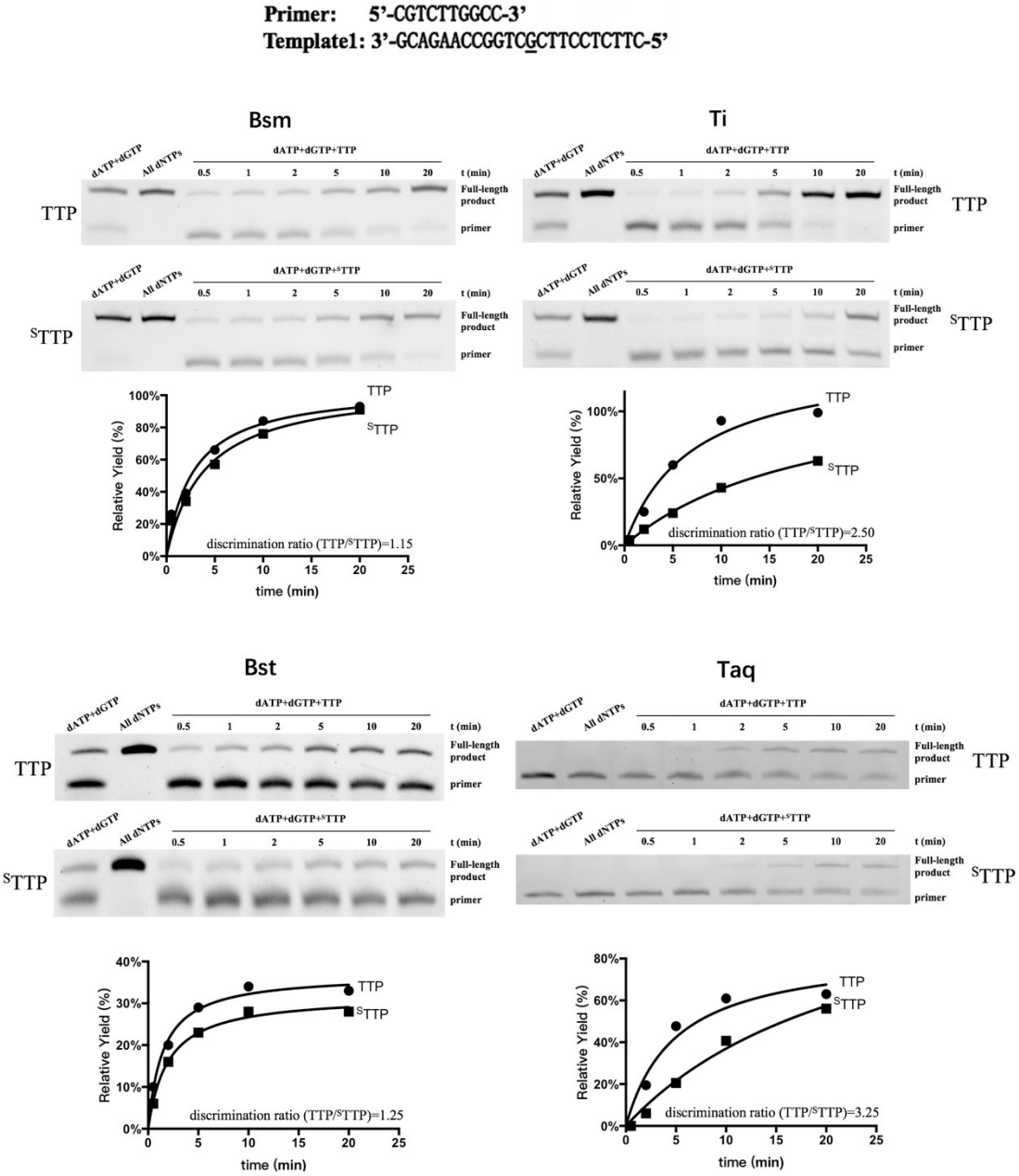

Figure S10. Improving DNA polymerization specificity by ^{S}TTP. The products of DNA extension reactions were analyzed by denaturing PAGE, offering the bands of single-stranded DNAs. Four DNA polymerases (Bsm, Ti, Bst and Taq) were used in these experiments.