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Supplementary information

High-efficiency and high-fidelity ssDNA circularisation via the pairing of five 3′-terminal bases to assist LR-LAMP for the genotyping of single-nucleotide polymorphisms

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Table S1. The nucleic acids sequences used in this study.

Name		Sequence
sDNA1		5'-CGCTCTGAACAAAG-3'
sDNA2		5'-CGCTCTGAACAAAGT-3'
sDNA3 / sDNA3-A		5'-CGCTCTGAACAAAGTC-3'
sDNA3-T		5'-CGCTCTGAACATAGTC-3'
sDNA3-C		5'-CGCTCTGAACACAGTC-3'
sDNA3-G		5'-CGCTCTGAACAGAGTC-3'
sDNA4		5'-CGCTCTGAACAAAGTCA-3'
sDNA5		5'-CGCTCTGAACAAAGTCAT-3'
sDNA6		5'-CGCTCTGAACAAAGTCATA-3'
sDNA7		5'-CGCTCTGAACAAAGTCATAA-3'
sDNA8		5'-CGCTCTGAACAAAGTCATAAT-3'
sDNA9		5'-CGCTCTGAACAAAGTCATAATC-3'
sDNA10 / sDNA10-A		5'-CGCTCTGAACAAAGTCATAATCT-3'
sDNA10-T		5'-CGCTCTGAACATAGTCATAATCT-3'
sDNA10-C		5'-CGCTCTGAACACAGTCATAATCT-3'
sDNA10-G		5'-CGCTCTGAACAGAGTCATAATCT-3'
l-DNA62-A		5'P-TGTTCAGAGCGACCCAAACCCACCCAACCTCCTCCTTCCTCCTCCGTCGAAGATTATGACTA-3'
l-DNA62 / l-DNA62-T		5'P-TGTTCAGAGCGACCCAAACCCACCCAACCTCCTCCTTCCTCCTCCGTCGAAGATTATGACTT-3'
l-DNA62-C		5'P-TGTTCAGAGCGACCCAAACCCACCCAACCTCCTCCTTCCTCCTCCGTCGAAGATTATGACTC-3'
l-DNA62-G		5'P-TGTTCAGAGCGACCCAAACCCACCCAACCTCCTCCTTCCTCCTCCGTCGAAGATTATGACTG-3'
5'-sDNA-A		5'-GAACAAAGTCATAATCTT-3'
5'-sDNA-T		5'-GAACTAAGTCATAATCTT-3'
5'-sDNA-C		5'-GAACCAAGTCATAATCTT-3'
5'-sDNA-G		5'-GAACGAAGTCATAATCTT-3'
l-DNA38		5'P-GTTGTGCCAACTCCCTTGTGAATCGTGCGGAAACGCTC-3'
sDNA38-A		5'-TTGGCACAACAAGCG-3'
sDNA38-T		5'-TTGGCACAACTAGCG-3'
sDNA38-C		5'-TTGGCACAACCAGCG-3'
sDNA38-G		5'-TTGGCACAACGAGCG-3'
MT-DNA		5'-TCGAAGATTATGACTTATAGTGAAAGTATT-3
WT-DNA		5'-TCGAAGATTATGACTTTTAGTGAAAGTATT-3
l_M-DNA		5'P-AAGTCATAATCTTCCCTATCCTACCCAACCACCGAAACTCACCTCACCTCACAGGAAACTAT-3'
l_W-DNA		5'P-AAGTCATAATCTTCCCTATCCTACCCAACCACCGAAACTCACCTCACCTCACAGGAAACTAA-3'
LAMP	FIP	5'-AAGTCATAATCTTCTTTTTGGTGGTTGGGTAGGATAGG-3'
primers	BIP	5'-TAGTTTCCTGTGTTTTTCCGAAACTCACCTCACCT-3'
PCR	SNP-F	5'-CTGGCGCAAATTCTTCCTGA-3'
primers	SNP-R	5'-CGTCCACAGAGCGAGTTTTC-3'

Table S2. The comparison of the LR-LAMP method with other LAMP-based assays for SNPs detection

Methods	Sensitivity	Accuracy	Result analysis	Reference
PE-LAMP	1.66 fM	0.1%	Fluorescence	S1
AS-LAMP	166 aM	—	Agarose gel electrophoresis	S2
LAMP-Invader-AuNP	1.66 fM	—	Naked eye	S3
mLAMP	100 aM	0.1%	Fluorescence	S4
PA-LAMP	22 aM	0.01%	Fluorescence	S5
ARMS-SNP LAMP	10 pg	—	Naked eye	S6
LAMP-MC	—	—	Melting curve analysis	S7
SNP-LAMP	10 pg	0.5%	Agarose gel electrophoresis/ LFD strip	S8
LR-LAMP	100 aM	0.01%	Fluorescence / Naked eye	This work

References:

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S3: Y. Lu, X. Ma, J. Wang, N. Sheng, T. Dong, Q. Song, J. Rui, B, Zou and G. Zhou, *Biosens. Bioelectron.*, 2017, **90,** 388-393.

S4: Y. Ren, Y. Li, X. Duan, H. Wang and Z. Li, *ChemistrySelect*, 2019, **4**, 1423-1427.

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S7: C. Drogou, F. Sauvet, M. Erblang, L. Detemmerman, C. Derbois, M. C. Erkel, A. Boland, J. F. Deleuze, D. Gomez-Merino and M. Chennaoui, *Biotechnol. Rep.*, 2020, **26**, e00468.

S8: S. Yongkiettrakul, J. Kampeera, W. Chareanchim, R. Rattanajak, W. Pornthanakasem, W. Kiatpathomchai and D. Kongkasuriyachai, *Parasitol. Int.*, 2017, **66**, 964-971.

Table S3. The isolates of *Phytophthora infestans* used in this study.

Isolate	Origin (xxx)	*RPA190* 1145 SNP genotype
AH16-10	Anhui	T
AH16-17	Anhui	T
AH16-18	Anhui	T
CQ2016	Chongqing	T/A
HN16-1	Henan	T/A
HB1501	Hebei	T
HIY1602	-	A
NL07434	-	T
YN02809	Yuannan	T/A
YN21206	Yuannan	T/A

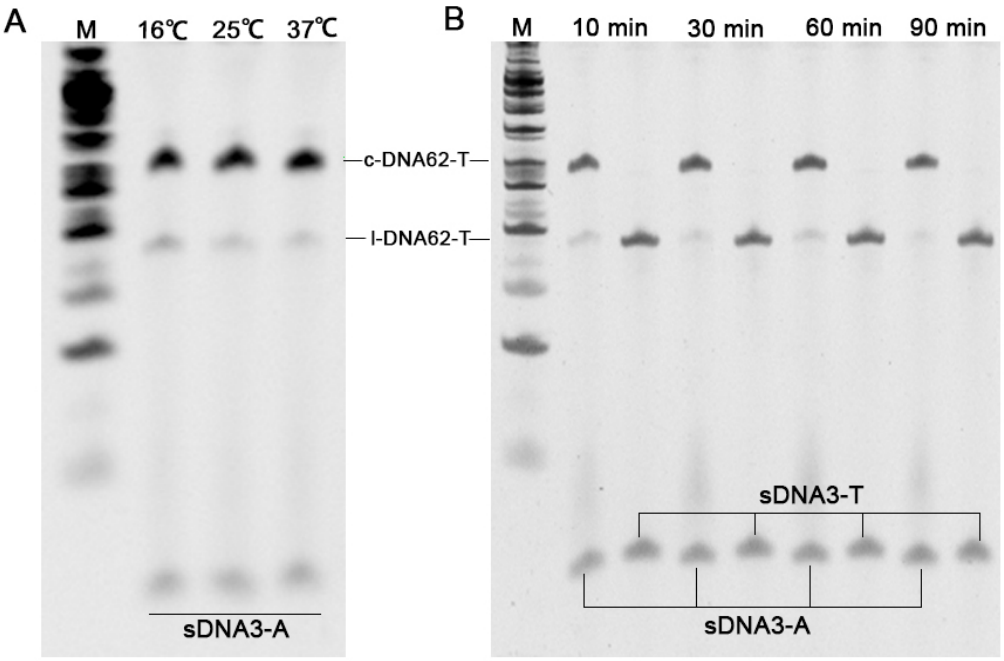

Figure S1. Effects of (A) ligation temperature and (B) ligation time on the efficiency of circularization of l-DNA62-T. Experiments were carried out in 1×T4 DNA ligase with T4 DNA ligase (70 U), and the products were separated on 15% denaturing polyacrylamide gels. l-DNA_{62}:splint ratio = 1:2 (0.25:0.5 μM).

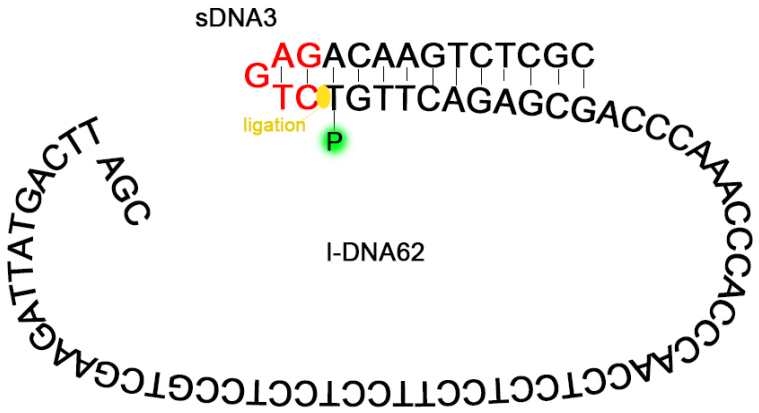

Figure S2. Analysis of the I-DNA62+sDNA3 (78 nt) bands. The 3'-terminal base (GAGTC) of the sDNA3-G strand can form a hairpin structure, and the remaining bases are paired with the I-DNA62 strand as shown, and then the gap is ligated by T4 DNA ligase to form a 78-nt single strand.

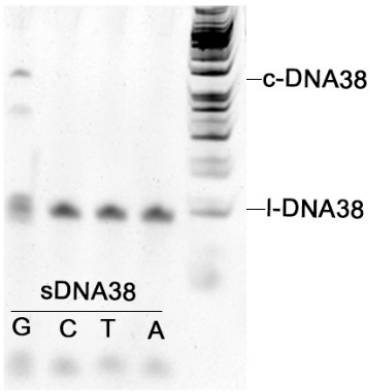

Figure S3. The fidelity of the cyclization with 38-base l-DNA. The experiments were carried out in 1×T4 DNA ligase with T4 DNA ligase (70 U) at 37℃ for 10 min, and the products were separated on 15% denaturing polyacrylamide gels. l-DNA:sDNA ratio = 1:2 (0.25:0.5 μM).

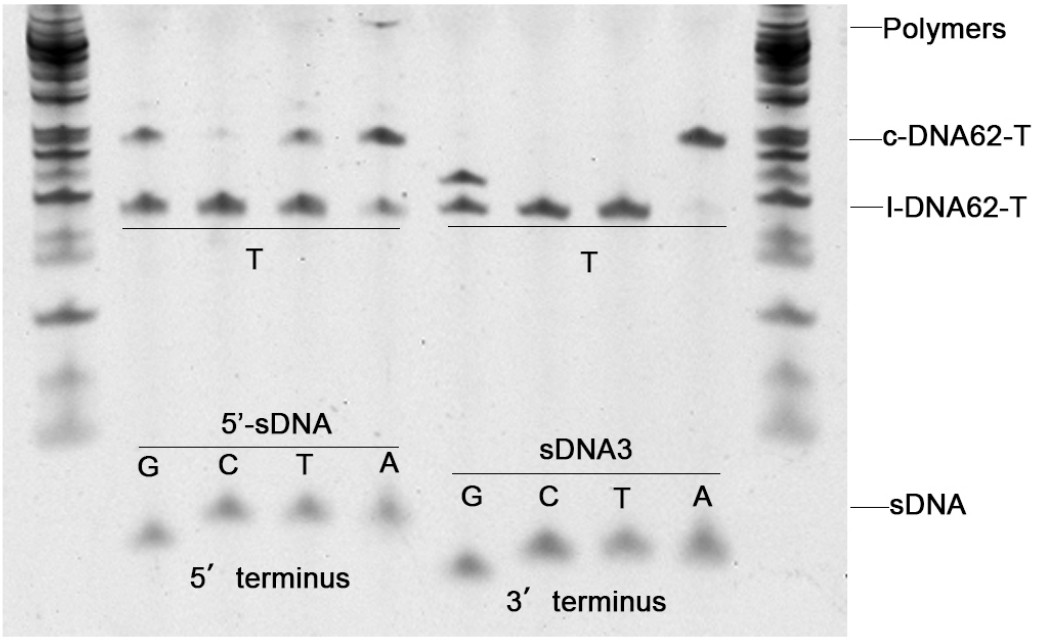

Figure S4. Comparison of the fidelity of the two cyclization structures. “5′ terminus” i.e., the 3′ terminus of l-DNA62 had a long-paired base, whereas the 5′ terminus exhibited pairing using only five bases; “3′ terminus” i.e., the 5′ terminus of l-DNA62 had a long-paired base, whereas the 3′ terminus exhibited pairing using only five bases. The experiments were carried out in 1×T4 DNA ligase with T4 DNA ligase (70 U) at 37℃ for 10 min, and the products were separated on 15% denaturing polyacrylamide gels. l-DNA:sDNA ratio = 1:2 (0.25:0.5 μM).

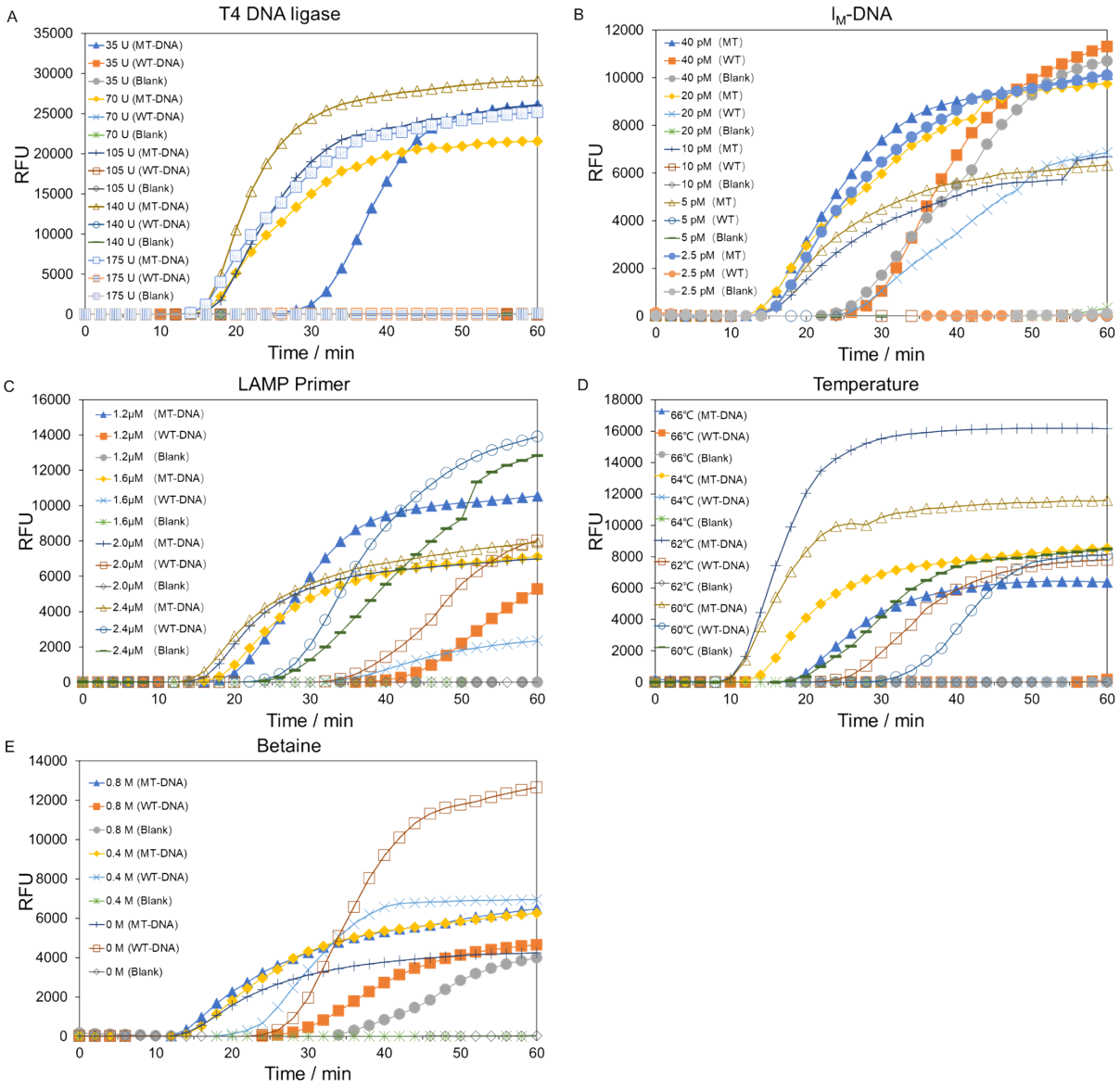

Figure S5. Optimization of LR-LAMP reaction conditions for SNP detection. The effects of the concentrations of I_M-DNA (2.5, 5, 10 and 20 pM) and T4 DNA ligase (70, 105, 140 and 175 U) on the ligation, as well as those of the concentrations of LAMP primers (1.2, 1.6, 2, 2.4 μM each) and betaine (0, 0.4, and 0.8 M) and reaction temperature (60℃, 62℃, 64℃ and 66℃) on LAMP amplification. The MT-DNA and WT-DNA target concentrations were 1 pM.

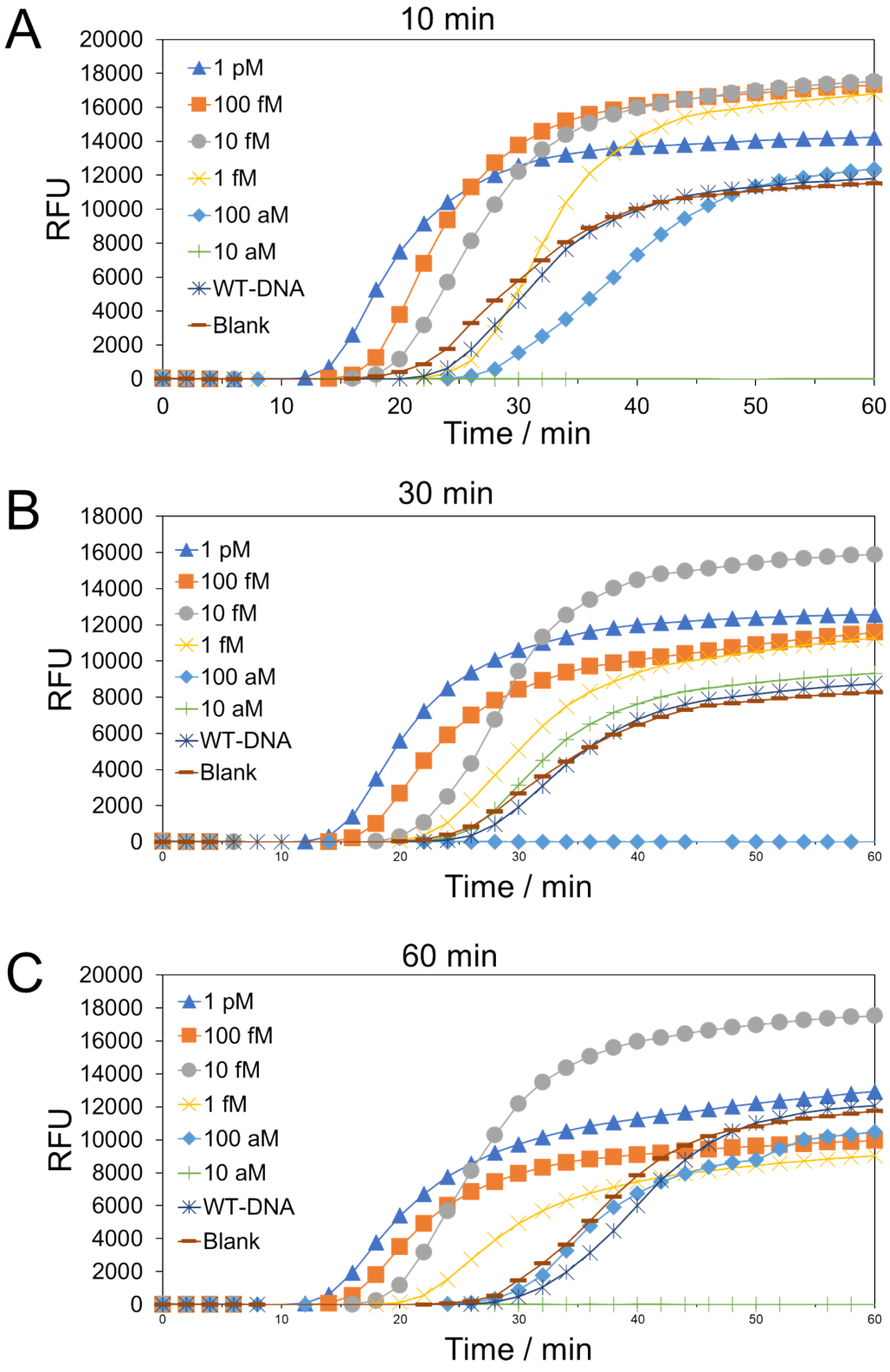

Figure S6. The ligation time was optimized to improve sensitivity. (A) 10 min, (B)30 min, and (C) 60 min. The concentration of MT-DNA was serially diluted from 1pM to 10 aM. WT-DNA target concentrations were 1 pM.

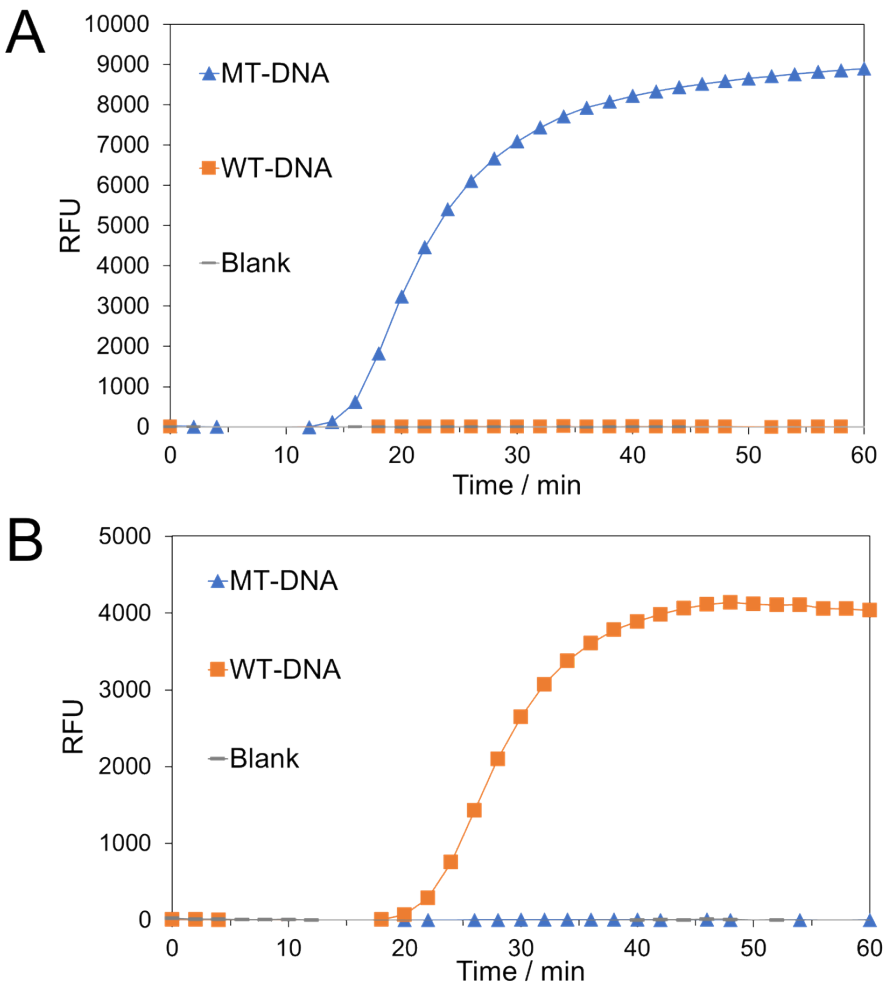

Figure S7. Real-time fluorescence curves obtained from LR-LAMP using the optimum conditions. The MT-DNA and WT-DNA target concentrations were 1 pM. (A) l_M-DNA was used. (B) l_W-DNA was used.