Electronic Supplementary Material (ESI) for Analyst.

Supplementary Materials for

Recombinase Assisted Loop-mediated Isothermal DNA Amplification

Gangyi Chen, Rong Chen, Sheng Ding, Mei Li, Jiayu Wang, Jiawei Zou, Feng Du, Juan Dong, Xin Cui, Xin Huang, Yun Deng and Zhuo Tang*

Experimental Section

Chemicals and Oligonucleotides. All chemicals were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). All oligonucleotides including modified probe were ordered from Sangon Biotech (Shanghai, China). **Pfu** DNA polymerase was purchased from Vazyme Biotech (Nanjing, China). **Taq** DNA polymerase and pEASY®-T1 Cloning Kit were purchased from TransGen Biotech (Beijing, China). **Bsm** DNA polymerase (Large Fragment), Polynucleotide kinase (**PNK**) were purchased from Thermo Fisher (China). [γ -^{32}P]**ATP** was purchased from Furui Biological Engineering (Beijing, China).

Protein Expression and Purification. The tthrecA gene was obtained by overlap PCR using gene fragment that was synthesized from Sangon Biotech (Shanghai) Co., Ltd. Then the amplified tthrecA gene fragment was cloned into pET28a vector at the site between *Nde*I and *Hind*III restriction sites. The recombinants were screened by PCR using T7 promoter specific primers, TAATACGACTCACTATAGG and GCTAGTTATTGCTCAGCGG. The positive clones were further confirmed by restriction analysis and DNA sequencing. The recombinant plasmid was designated as pET28a-*tth*recA. After that, the plasmid pET28a-*tth*recA was transformed into ER2566 competent cells (NEB, USA). Clones were inoculated into 10ml LB medium and grew overnight, then expand to 1L LB medium and cultivated until OD600 reach 0.6. Proteins were expressed by inducing with 0.5mM IPTG for 24h at 16℃, and then the induced cells were pelleted at 6000 × g for 10 min. The harvested cells were resuspended in starting buffer (20 mM Tris–HCl (pH 8.0), 500mM NaCl) and sonicated in a sonicator (Omni Sonic Ruptor 400, GA, USA) at 30% amplitude for one second on/off for 35 min on ice. The sonicated lysate was spun at 12000 × g for 20 min at 4℃ and the supernatant obtained was used for purification of the expressed ***Tth*RecA** protein. As ***Tth*RecA** is known to be thermostable, so we take advantage of this property to purify it. The supernatants of the sonicated lysate was heated at 75℃ for 30 min, after which the samples were centrifuged at 20000 × g for 20 min. Then the supernatants filtered through 0.45 μm millipore filter and purified using Ni–agarose His-tag protein purification kit according to the instruction. The purified ***Tth*RecA** protein was

analyzed on 12% SDS–PAGE to determine the purity and concentrated to a final concentration of 2μg/μl in the storage buffer (20mM Tris-HCl PH 8.0, 100mM NaCl, 1mM DTT, 0.1mM EDTA) with Amicon Ultra-15-10Kd (Merck & Co. Inc.).

Strand exchange assay. First, oligonucleotides X1 and its complementary sequence X2 were mixed with the final concentration of 6μM in the following condition: 20mM Tris-HCl (7.6), 1mM MgCl2, 1mM DTT and heat to 94℃ for 5min, then let cool to room temperature to form duplex which was designated as ds. Then, 0.15 μM FAM-labelled oligonucleotide with the same sequence of X1 (designated as F-X1) was incubated with ***Tth*RecA** in the following condition: 20mM Tris-HCl (7.6), 1mM $MgCl_2$, 1mM DTT at 60°C for 5min, followed by adding ds to final concentration of 0.15 μM and elevating $MgCl_2$ concentration to 10mM. The reaction mixture was incubated at 60 °C for 10min and treated with equivalent volume of acid equilibrated phenol/chloroform. After centrifuging, the supernatant was precipitated with ethanol at -20°C for 30min and centrifuged for 10 min at 14000 rpm. Final DNA pellet was dried by rotary vacuum desiccator and resuspended in 10μL of sterile water and 10μL 2 x DNA loading buffer (10mM EDTA, 12%(v/v) Glycerol, 0.03%(w/v) Xylene Cyanol FF, 0.03%(w/v) Bromophenol Blue), followed by electrophoresis on 10% native polyacrylamide gel and analyzed by Typhoon FLA 7000 IP (GE Healthcare).

Preparation of plasmid targets. 6 oligonucleotides S1, S1C, S2, S2C, S3, S3C for overlap PCR to obtain **BRAF** gene that could hybridize with each other was synthesized according to the sequence of **BRAF** gene as shown in **Table S1**. Then put 0.1 μM each of these oligonucleotides together into a reaction mixture containing **Taq** DNA polymerase (1U), dNTP (0.2mM), 1 x **Taq** buffer (20mM Tris-HCl pH 8.3, 20mM KCl, 100mM $(NH4)_2SO_4$, 20mM $MgSO_4$). The reaction was incubated in a PCR machine with the program: 94℃ 30s, 50℃ 30s, 72℃ 30s, repeat for 20 times for overlap PCR. Then, 1 μL of the product was used as the template for further PCR reaction and amplification using the S1 and S3C as primers (**Table S1**). The acquired **BRAF** fragment was cloned into pEASY-T1 vector and transformed into *E. coli* Trans 5α competent cell according to the manufacturer's instructions. The **BRAF** insert recombinant was verified by sequencing at Sangon Biotech (Shanghai, China). The

correct plasmids that served as templates were prepared using the AxyPrep Plasmid Miniprep Kit and were designated as **T1-BRAF**.

T to A transversion in plasmid **T1-BRAF** (The corresponding V600E mutant in **BRAF** gene) was constructed by overlap PCR amplification starting with the wild-type plasmids and site-specific mutagenic primers (MF and MR, **Table S1**) in the following condition: 20 mM Tris-SO_4 (PH 9.2), 40 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 2% Glycerol and 1U Pfu. The amplification program was as follows: 95ºC for 2 min, then 30 cycles for 95ºC 20s, 55ºC 20s and 72ºC 4 min, then 72ºC for 5min. 5 μl of the products were transformed into Tran 5α and determined by sequencing. The correct mutant plasmid was designated as **T1-BRAF-M**.

The plasmid used to verify the specificity of **ProofMan** probe was obtained by following method. Two primers (NF and NR in **Table S1**) were designed to target the adjacent sequence of the site where the **ProofMan** probe bind. First, NF and NR were labelled with phosphate group at 5' end in the reaction mixture containing 1 μM NP or NR, 50 mM Tris-HCl (pH 7.8), 40 mM NaCl, 10 mM $MgCl_2$, 1 mg/mL BSA, 1mM ATP and 10 units of Polynucleotide kinase (PNK) and incubated at 37 °C for 1 h. The labelled probe was purified by 10% denaturing polyacrylamide gel and quantified using spectrophotometer. Then PCR reaction was performed in the following condition: 20 mM Tris-SO_4 (PH 9.2), 40 mM KCl, 10 mM $(NH4)_2SO_4$, 2 mM $MgSO_4$, 2% Glycerol, 0.2 μM NP and NR each, 0.1 nM **T1-BRAF** plasmid and 1 U **Pfu**. The amplification program was as follows: 95ºC for 2 min, then 30 cycles for 95ºC 20s, 55ºC 20s and 72ºC 4 min, then 72ºC for 5min. The amplification products were purified using EasyPure PCR Purification Kit (TransGen Biotech, Beijing, China) and quantified using spectrophotometer. Then the purified products were ligated in the condition: 50 mM Tris-HCl（pH 7.5）, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 0.5 μM purified PCR products and 400 U T4 DNA ligase. The ligation products were transformed into Trans 5α competent cell. The correct ligation recombinants were verified by sequencing and designated as **T1-BRAF-N**.

Establishment of RALA method. Enzymatic components for the **RALA** assays contain ***Tth*RecA** and **Bsm**. After verifying the strand exchange function of ***Tth*RecA**,

the feasibility of **RALA** method was tested at 60℃. The reaction mixture was as follows: 1.6 μM each of primer (F1C-F2 and B1C-B2), 0.8 mM dNTPs, 5% PEG-20M, 20 mM Tris-HCl (pH 8.8), 60 mM KCl, 10 mM $(NH_4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 0.2 x SYBR Green I, 0.16 U/μl of **Bsm** DNA polymerase, 0.006 μg/μl ***Tth*RecA** (if included), 0.4 mM ATP (if included) and 10^8 copies of recombinant plasmids **T1-BRAF** as template. For sequence-specific detection of **RALA**, the reaction mixture was as follows: 6 ng/μl ***Tth*RecA**, 0.4 mM ATP , 1.6 μM each of each of primer (**F1C-F2** and **B1C-B2**), 0.2 μM **ProofMan** probe **P2**, 0.8 mM dNTPs, 5% PEG-20M, 20 mM Tris-HCl (pH 8.8), 60 mM KCl, 10 mM $(NH_4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 0.16 U/μl of **Bsm** DNA polymerase, 0.01 U/μl of **Pfu** DNA polymerase and varied amount of plasmids as indicated. The optimization experiments were illustrated in Fig. S7 and S8.

Optimize the number of 3' end mismatch of probe. The probe **P1** was isotope labelled with phosphorus-32 in the reaction mixture containing 1 μM **P1**, 50 mM Tris-HCl (pH 7.8), 40 mM NaCl, 10 mM $MgCl_2$, 1 mg/mL BSA, 10 μCi [γ -^{32}P]ATP and 0.5 U/μl of Polynucleotide kinase (PNK) at 37 °C for 1 h. The labelled probe was purified by 10% denatured polyacrylamide gel. Then, the probe extended on synthesized single-stranded template with varied mismatches(T0-T4) in the presence or absence of **Pfu** as indicated. The assay was carried out in a total 20 μl reaction mixture containing 20 mM Tris–HCl (pH 8.8), 60 mM KCl, 5 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 200 μM dNTPs, 0.16 U/μl **Bsm** and 0.01 U/μl **Pfu** as indicated, 0.5 μM probe **P1** and 0.5 μM template (T0-T4) respectively, and final mixture solution was kept in the PCR thermocycle instrument (C1000 Thermal Cycler, Bio-Rad, USA) to perform the reaction at 60°C for 20 min. After the reaction, 10 μl aliquot of each reaction was analyzed by denatured polyacrylamide gel electrophoresis. Then the gel was analyzed on Typhoon FLA 7000 IP (GE Healthcare) using Phosphorimaging and Fluorescence mode.

Visual inspection of target DNA using RALA. For visual inspection, 10^8 copies of plasmids were used as template and amplified in the following condition: 2.0 μM each of turn-back primer (F1C-F2 and B1C-B2, Table S1), 0.5 μM **ProofMan** probe, 6 ng/μl

***Tth*RecA**, 0.6 mM ATP, 0.8 μM each boost primers (LBa and LBb, Table S1), 0.8 mM dNTPs, 5% PEG-20M, 20 mM Tris-HCl (pH 8.8), 60 mM KCl, 10 mM $(NH_4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 0.24 U/μl **Bsm**, 0.02 U/μl **Pfu**, 120 μM Hydroxynaphthol blue, the reaction was performed at 59℃ for 15 minutes before 20 X SYBR Green I was added. Then the reaction tube were observed by naked eyes directly under UV irradiation or under natural light.

Supplemental figures

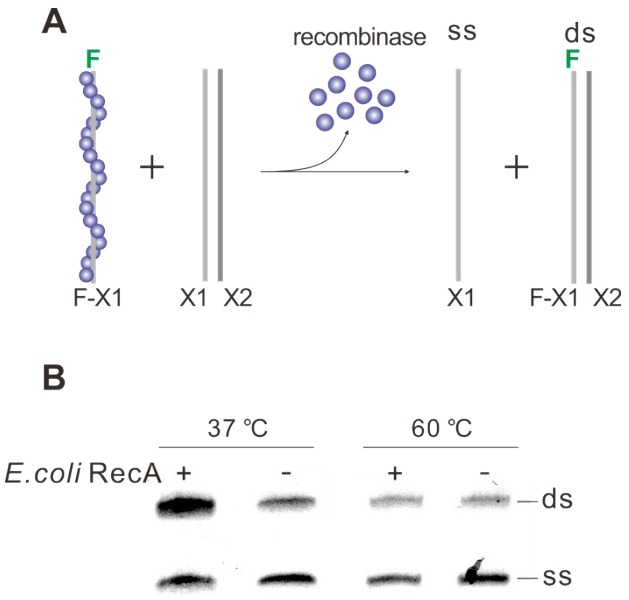


Figure S1. Strand exchange assay of *E.coli* RecA for verifying its activity. (A) the principle of strand exchange assay promoted by recombinase. First, recombinase can form nucleoprotein with the fluorophore-labelled single-stranded DNA (F-X1), then the nucleoprotein will displace one strand of the homologous double-stranded DNA and form a fluorophore-labelled double-stranded DNA. (B) the strand exchange assay results of *E.coli* **RecA**. The experiments were performed at 37℃ and 60℃ in the presence and absence of *E.coli* RecA respectively.

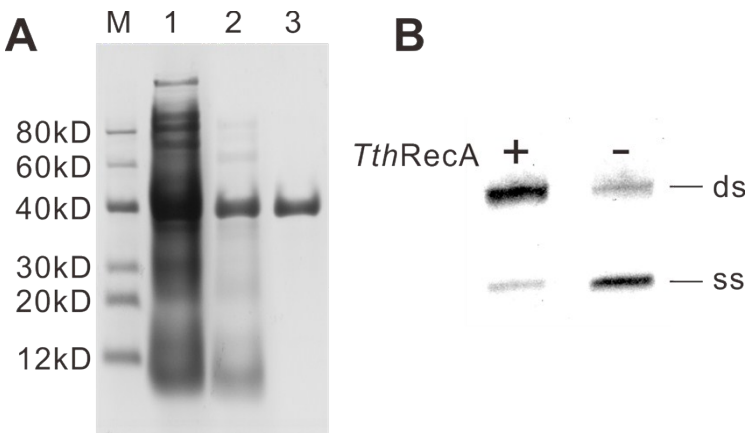


Figure S2. Analysis of the purified *Tth*RecA for its purity and strand exchange activity. (A) SDS-PAGE analysis for extraction fraction of ***Tth*RecA**. M, protein marker with corresponding molecular weight (kD) annotated on the left side; Lane1, the supernatant of sonicated lysate of the bacteria that have been induced to express ***Tth*RecA**; Lane 2, the supernatant of the heat-treated sonicated lysate supernatant. Lane 3, the eluent after Ni–agarose purification of the heat-treated sonicated lysate supernatant. (B) the result of strand exchange assay performed in the presence of the purified ***Tth*RecA** in A. the symbol "+" and "-" represent corresponding reactions were performed in the presence of ***Tth*RecA** and in the absence of ***Tth*RecA** respectively. "ss" and "ds" represent fluorescently labelled single-stranded DNA and double-stranded DNA as shown in **Figure S1A**.

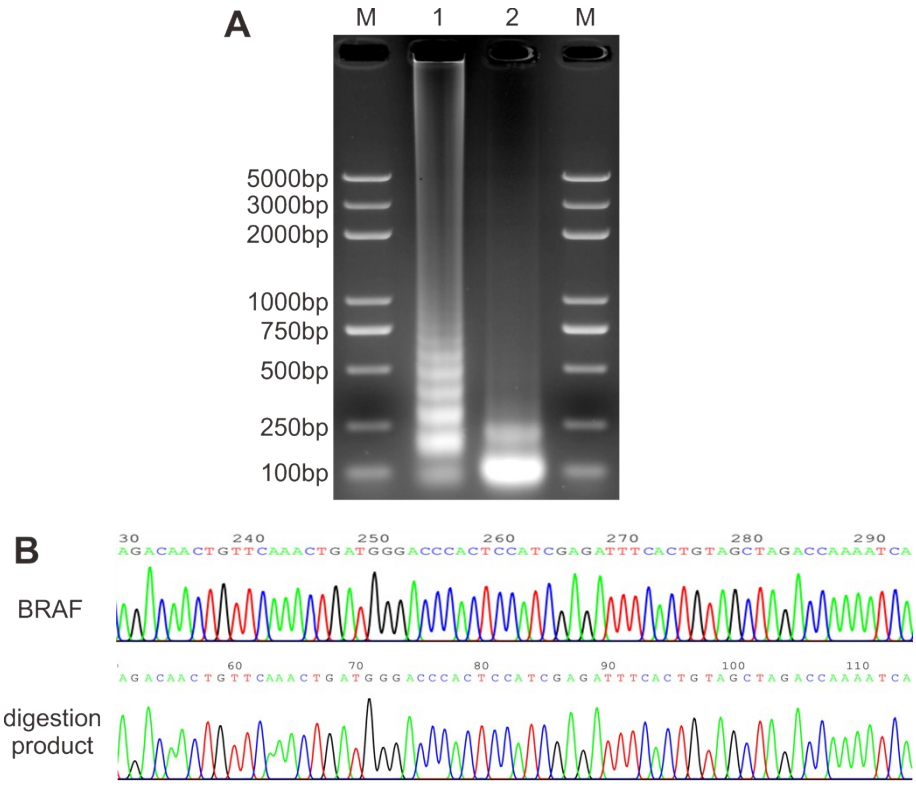


Figure S3. Verification of the products of RALA reaction. (A) agarose gel electrophoresis analysis for the RALA products and its digestion products by *Bam*HI endonuclease. M, DNA marker; lane 1, RALA products as the same in **Fig. 1**; lane 2, the digestion products of reaction in lane 1 by *Bam*HI endonuclease. (B) comparison of the sequence of target and the digestion products. The digestion products in A was purified and cloned into T1 vector and sequenced.

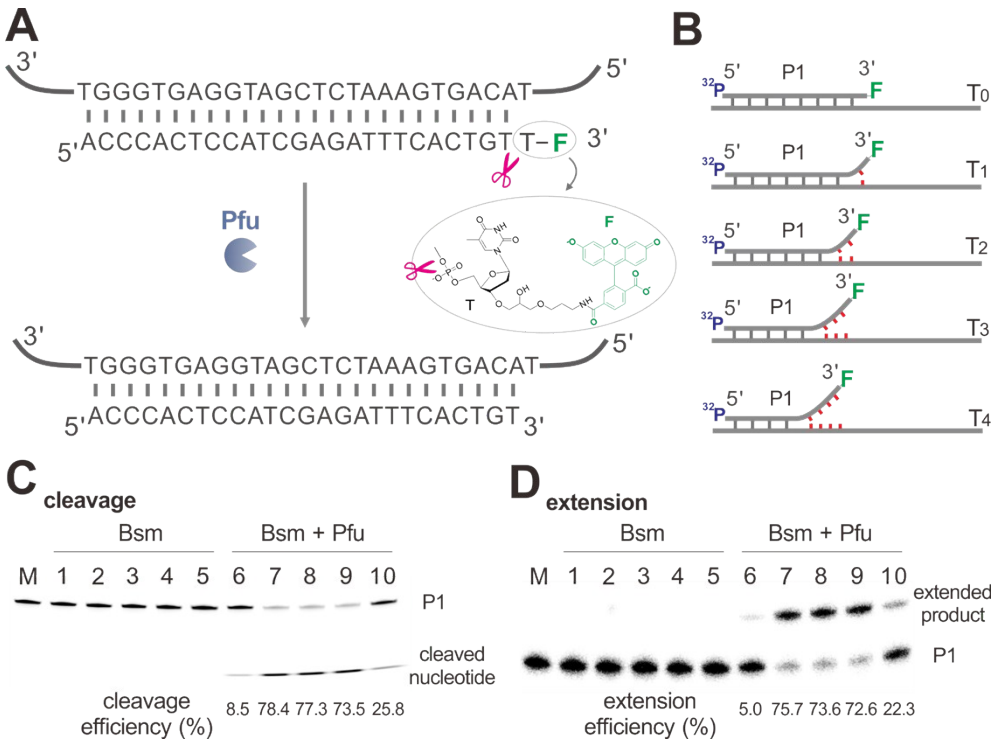


Figure S4. Optimization for the 3' end mismatch number of the probe. (A) The principle of probe cleaving by **Pfu** DNA polymerase with 3'-5' exonuclease activity. The last mismatched nucleotides at the 3' end would be removed by the **Pfu**. (B) Five single-stranded DNAs were synthesized as templates bearing 0 to 4 mismatches with the probe P1 (T0-4 respectively). (C) Cleavage of probe P1 on different templates. Lane 1-5 were reactions performed in the presence of **Bsm** and T0-4 as template respectively. Lane 6-10 were reactions performed in the presence of both **Bsm** and **Pfu** and T0-4 as template respectively. M represent the intact probe P1. The results were obtained by fluorescence mode analysis on Typhoon FLA 7000 IP. (D) Extension of P1 on different templates after being cleaved. The results were obtained by Phosphorimaging mode analysis of the same reactions in (C) on Typhoon FLA 7000 IP.

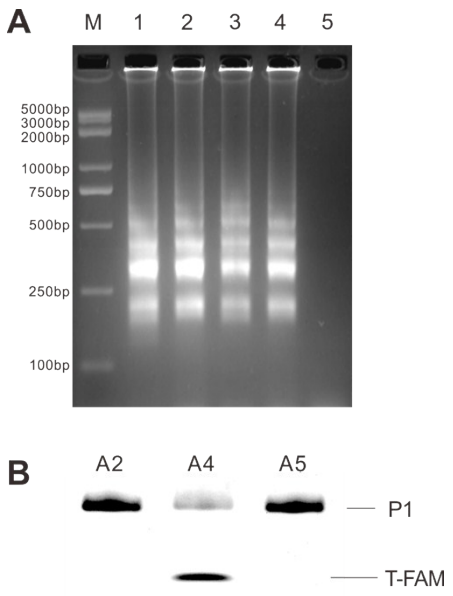


Figure S5. Investigation of the influence on the RALA system by adding probe P1 and Pfu. (A) the agarose gel electrophoresis analysis for the amplification products of the reactions with following conditions: lane 1, **RALA** reaction; lane 2, **RALA** reaction plus probe **P1**; lane 3, **RALA** reaction plus **Pfu** DNA polymerase; lane 4, **RALA** reaction plus both probe **P1** and **Pfu** DNA polymerase; lane 5, reaction same as lane 4 while without target DNA; M, DNA marker with molecular weight annotated on the left side. (B) denatured PAGE analysis for the amplification products of the reaction 2, 4 and 5 in A, followed by detection of fluorescence using Typhoon FLA 7000 IP, the upper band and lower band correspond with the intact probe **P1** and the cleaved nucleotide by **Pfu**.

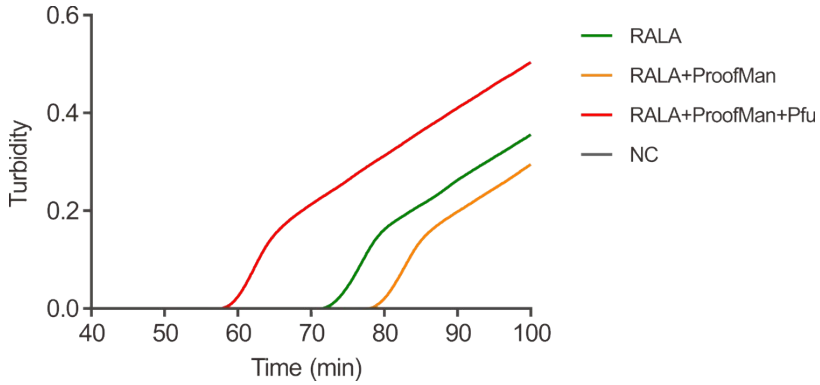


Figure S6. Influence of ProofMan probe and Pfu on RALA reaction. The reactions were performed based on standard **RALA** in the presence of **ProofMan** (orange) or both **ProofMan** and **Pfu** (red) as indicated in the figure and monitored using turbidity meter. Standard **RALA** reaction serve as a control (green).

As shown in the **Figure S5**, when **ProofMan** was added, the amplification efficiency slightly decreased. Maybe, displacement of **ProofMan** from loop influenced the extension speed of **Bsm**. However, when **Pfu** was included, the amplification efficiency greatly increased, which evidently confirmed that **ProofMan** could serve as a boost primer to accelerating **RALA** reaction when cleaved by **Pfu**.

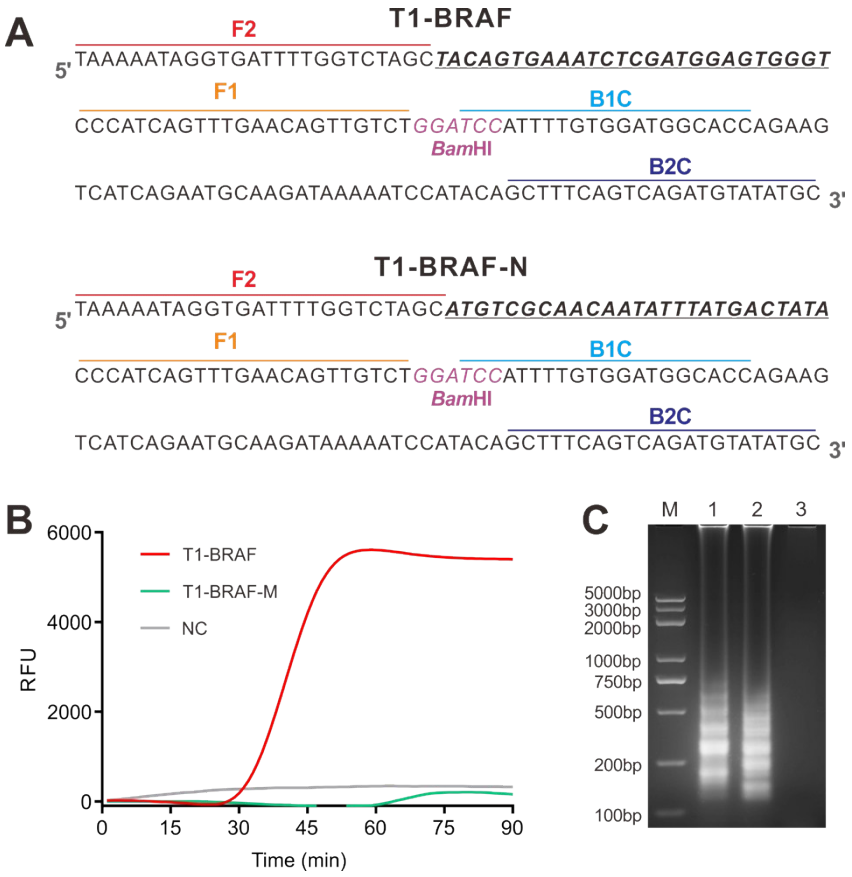


Figure S7. Verification of the specificity of ProofMan probe in RALA reaction. (A) Sequence information alignment of the plasmids **T1-BRAF** and **T1-BRAF-N**. The bold italic sequence in **T1-BRAF** is complementary with **ProofMan**, while this sequence was replaced by an unrelated sequence in **T1-BRAF-N**. (B) The results of real-time monitoring of fluorescence produced in the **RALA + ProofMan** reactions using **T-BRAF** (red) or **T-BRAF-N** (green) as template respectively. Target DNA was not added in another reaction for negative control (gray). (C) agarose gel electrophoresis analysis for the products of the reactions in B; M, DNA marker with molecule weight annotated on the left side; lane 1, **T1-BRAF** as template; lane 2, **T1-BRAF-N** as template; lane 3, negative control.

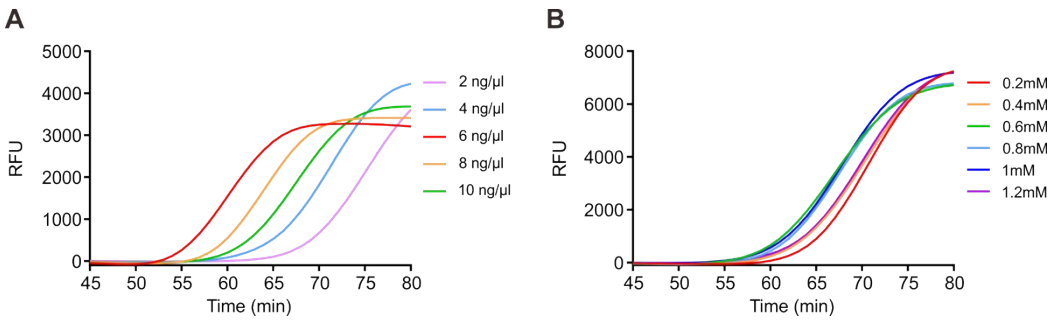


Figure S8. Optimization of the amount of *Tth*RecA and ATP for RALA reaction. (A) optimization of the concentration of ***Tth*RecA** used in **RALA** reaction. The reaction condition was as follows: 20 mM Tris-HCl (pH 8.8), 1 M betaine, 60 mM KCl, 10 mM $(NH4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 1.6 μM turn-back primers each, 0.16 U/μl **Bsm** DNA polymerase, 0.2 x SYBR green I (invitrogen), 0.4 mM ATP and varied concentration of ***Tth*RecA** as illustrated, 10^8 copies plasmids served as template. (B) optimization of the concentration of ATP (the cofactor of ***Tth*RecA**) used in **RALA** reaction. The reaction condition was as follows: 20 mM Tris-HCl (pH 8.8), 1 M betaine, 60 mM KCl, 10 mM $(NH4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 1.6 μM turn-back primers each, 0.16 U/μl **Bsm** DNA polymerase, 0.2 x SYBR green I (invitrogen), 6 ng/μl ***Tth*RecA** and varied concentration of ATP as illustrated 10^8 copies plasmids served as template.

As shown in **Figure S7**, when 6 ng/μl ***Tth*RecA** and 0.6 mM ATP was used in **RALA** reaction, the time to reach the fluorescence threshold was the shortest. Finally, 6 ng/μl ***Tth*RecA** and 0.6mM ATP were chosen for the following experiments.

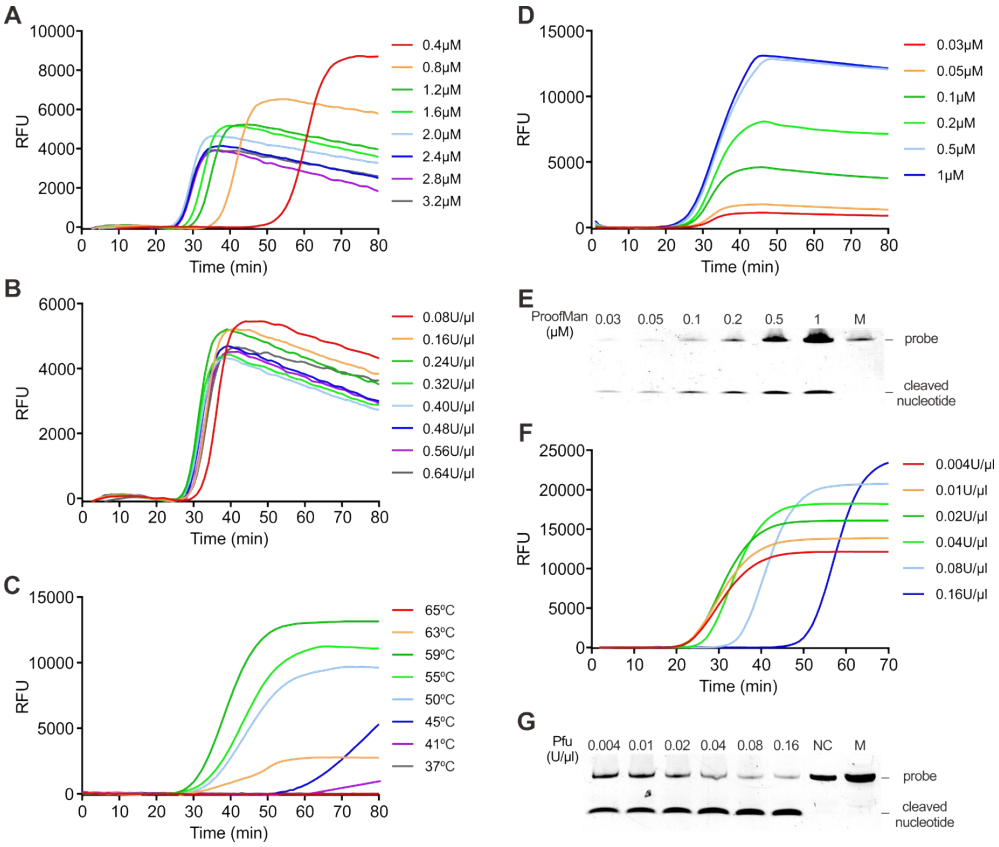
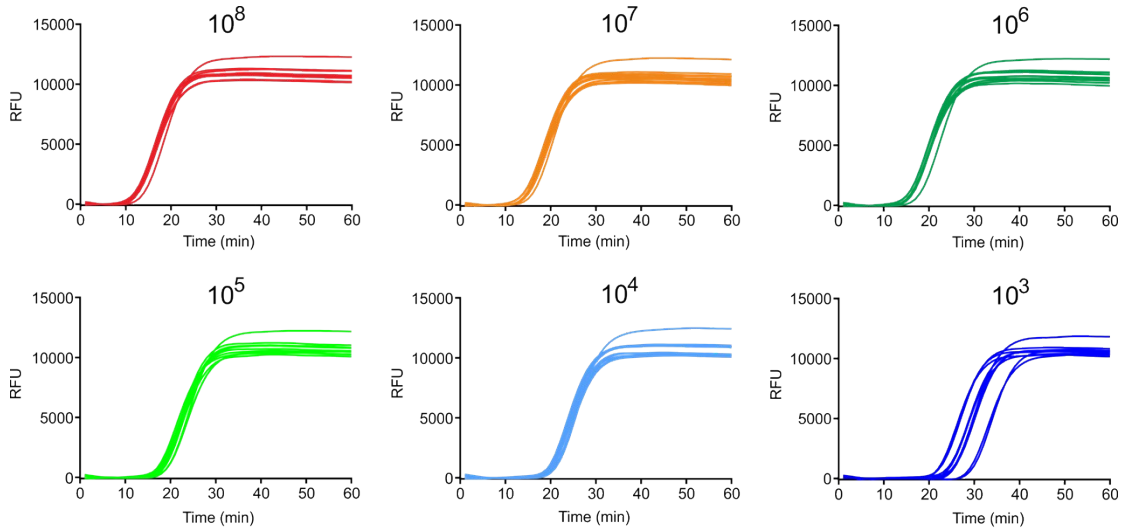


Figure S9. Optimization the condition for RALA reaction. standard **RALA+ProofMan** reaction condition was as follows: 20 mM Tris-HCl (pH 8.8), 1 M betaine, 60 mM KCl, 10 mM $(NH4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 1.6 μM turn-back primers each, 0.6 mM ATP, 6 ng/μl ***Tth*RecA**, 0.2 μM probe **P2**, 0.16 U/μl **Bsm** DNA polymerase and 0.01 U/μl **Pfu** DNA polymerase, 10^8 copies of **T-BRAF** plasmids served as the template. (A) standard **RALA+ProofMan** reaction except different concentration of turn-back primers. (B) standard **RALA+ProofMan** reaction except different concentration of **Bsm** DNA polymerase. (C) standard **RALA+ProofMan** reaction performed at different temperature. (D) standard **RALA+ProofMan** reaction except different concentration of probe **P2**. (E) The products of experiment in D was resolved on denatured PAGE for determination of the probe cleaving efficiency at different concentration of **ProofMan** probe. (F) standard **RALA+ProofMan** reaction except different concentration of **Pfu** DNA polymerase. (G) The products of experiment in F was resolved on denatured PAGE for determination of the probe cleaving efficiency at different concentration of **Pfu** DNA polymerase.

From the result of **Figure S8**, the final **RALA+ProofMan** reaction condition was set at: 20 mM Tris-HCl (pH 8.8), 1 M betaine, 60 mM KCl, 10 mM $(NH4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 2.0 μM turn-back primers each, 0.6 mM ATP, 6 ng/μl ***Tth*RecA**, 0.5 μM **ProofMan** probe, 0.24 U/μl **Bsm** DNA polymerase and 0.02 U/μl **Pfu** DNA polymerase.



Target copy	Tt1 (min)	Tt2 (min)	Tt3 (min)	Tt4 (min)	Tt5 (min)	Tt6 (min)	Tt7 (min)	Tt8 (min)	Tt9 (min)	Tt10 (min)	Mean (min)	RSD (%)
10^8	13.98	14.01	14.17	14.49	14.57	14.63	14.67	14.71	14.75	15.93	14.591	3.57
10^7	16.04	16.05	16.13	16.54	16.62	16.63	16.88	16.92	17.14	17.93	16.688	3.28
10^6	17.41	17.45	17.48	17.97	18.03	18.13	18.14	18.2	18.27	19.88	18.096	3.71
10^5	19.34	19.74	20.04	20.33	20.49	20.57	20.71	21.41	21.52	21.54	20.569	3.47
10^4	21.46	21.76	22.1	22.13	22.16	22.37	22.39	22.86	22.93	23.2	22.336	2.27
10^3	24.18	24.37	24.56	26.42	26.61	27.35	27.55	27.63	31.14	31.64	27.145	9.07

Figure S10. Verification for the reproducibility of RALA method. The experiment was performed under the optimized condition as follows: 20 mM Tris-HCl (pH 8.8), 1 M betaine, 60 mM KCl, 10 mM $(NH4)_2SO_4$, 5 mM $MgSO_4$, 0.1% Tween 20, 2.0 μM turn-back primers each, 0.6 mM ATP, 6 ng/μl ***Tth*RecA**, 0.5 μM probe **P2**, 0.24 U/μl **Bsm** DNA polymerase, 0.02 U/μl **Pfu** DNA polymerase and 0.8 μM each of boost primers (LBa and LBb). Different concentration of **T-BRAF** plasmids was used as template and each concentration was repeated for 10 times. Tt values were recorded and the relative standard deviation was calculated for each concentration of target DNA.

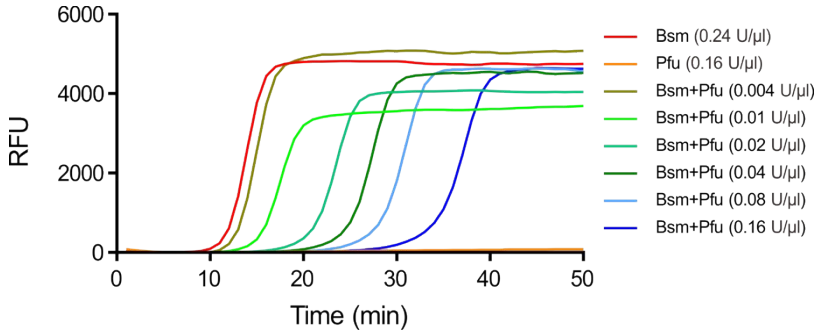


Figure S11. Normal LAMP reaction using different enzymes. All the reactions used same amounts of target DNA (10^8 copies).

From the result of **Figure S11, w**hen **Bsm** was used, normal **LAMP** produced signals in a short time. However, no signals were observed when **Pfu** was used alone. We speculated that this result is due to **Pfu**'s lacking of strand displacement activity that is crucial for **LAMP** reaction. When **Bsm** (0.24 U/μl) and **Pfu** (different concentration) were added into **LAMP** together, all reactions produced signals. However, the time when the signals could be observed was delayed as the increasing of **Pfu** concentration. We attribute this to the competition between **Bsm** and **Pfu** enzyme. When **Pfu** occupy 3' end of primers, extending of primers by **Pfu** would be prevented by downstream double-stranded sequences, and amplification would be affected. When more **Pfu** enzyme are added, more 3' end sites are occupied and lower amplification efficiency are observed.

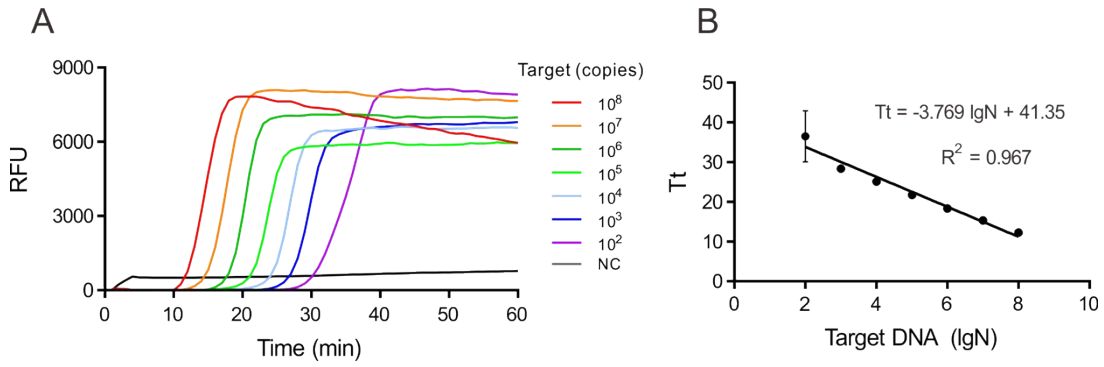


Figure S12. Real-time Normal LAMP reaction. (A) Real-time fluorescence curve of **LAMP** reaction with various amounts of DNA targets. NC: reaction without any DNA target. (B) Threshold time Tt was plotted against logarithm of target DNA amount (N). The solid line indicates linear least squares fitting between 10^2 and 10^8 copies of targets. Error bar represents the variation between three repeated experiments.

Table S1: Sequence information

Description	Labels	Sequences (5' - 3')
Forward turn-back primer	F1C-F2	AGACAACTGTTCAAACTGATGGG-TAAAAATAGGTGATTTTGGTCTAGC
Reverse turn-back primer	B1C-B2	TCCATTTTGTGGATGGCACC-GCATATACATCTGACTGAAAGC
Probe for mismatch optimization experiment	P1	ACCCACTCCATCGAGATTTCACTGTT(**FAM**)
ProofMan probe for sequence-specifici quatitive detection	P2	(**BHQ1**)ACCCACTCCATCGAGATTTCACTGTT(**FAM**)
ProofMan probe for SNP detection	P3	(**BHQ1**)ACCCACTCCATCGAGATTTCT(**FAM**)
Boost primers on B-loop	LBa	GCAAGATAAAAATCCATACA
	LBb	AGAAGTCATCAGAA
BRAF gene fragment	T-BRAF	……TTACACGCCAAGTCAATCATCCACAGAGACC TCAAGAGTAATAATATATTTCTTCATGAAGACCT CACAGTAAAAATAGGTGATTTTGGTCTAGCTACA GTGAAATCTCGATGGAGTGGGTCCCATCAGTTTG AACAGTTGTCTGGATCCATTTTGTGGATGGCACC AGAAGTCATCAGAATGCAAGATAAAAATCCATA CAGCTTTCAGTCAGATGTATATGCATTTGGAATT GTTCTGTATGAATTGATGACTGGACAGTTACCTT ATTCAAACATCAACAACAGGGACCAG……
BRAF mutation surrogate	T-BRAF-M	……TTACACGCCAAGTCAATCATCCACAGAGACC TCAAGAGTAATAATATATTTCTTCATGAAGACCT CACAGTAAAAATAGGTGATTTTGGTCTAGCTACA G**A**GAAATCTCGATGGAGTGGGTCCCATCAGTTTG AACAGTTGTCTGGATCCATTTTGTGGATGGCACC AGAAGTCATCAGAATGCAAGATAAAAATCCATA CAGCTTTCAGTCAGATGTATATGCATTTGGAATT GTTCTGTATGAATTGATGACTGGACAGTTACCTT ATTCAAACATCAACAACAGGGACCAG……
Primers used for obtain mutant T-BRAF-M	MF	TTGGTCTAGCTACAG**A**GAAATCTCGATGGAG
	MR	CTCCATCGAGATTTC**T**CTGTAGCTAGACCAA
Synthetic ssDNA perfectly	T_0	TAGGTGATTTTGGTCTAGCTACAGAGAAATCTCG ATGGAGTGGGT

matched with P1		
Synthetic ssDNA with one mismatch of P1 (red base)	T_1	TAGGTGATTTTGGTCTAGCTACAG**T**GAAATCTCG ATGGAGTGGGT
Synthetic ssDNA with two mismatches of P1 (red base)	T_2	TAGGTGATTTTGGTCTAGCTACAG**TC**AAATCTCG ATGGAGTGGGT
Synthetic ssDNA with three mismatches of P1 (red base)	T_3	TAGGTGATTTTGGTCTAGCTACAG**TCT**AATCTCG ATGGAGTGGGT
Synthetic ssDNA with four mismatches of P1 (red base)	T_4	TAGGTGATTTTGGTCTAGCTACAG**TCTT**ATCTCG ATGGAGTGGGT
Segment for synthesis of BRAF by overlap PCR	S1	TTACACGCCAAGTCAATCATCCACAGAGACCTCA AGAGTAATAATATATTTCTTCATGA
	S1C	ATTTCACTGTAGCTAGACCAAAATCACCTATTTT TACTGTGAGGTCTTCATGAAGAAAT
	S2	CTACAGTGAAATCTCGATGGAGTGGGTCCCATCA GTTTGAACAGTTGTCTGGATCCATT
	S2C	TATGGATTTTTATCTTGCATTCTGATGACTTCTGG TGCCATCCACAAAATGGATCCAGA
	S3	TAAAAATCCATACAGCTTTCAGTCAGATGTATAT GCATTTGGAATTGTTCTGTATGAAT
	S3C	CTGGTCCCTGTTGTTGATGTTTGAATAAGGTAAC TGTCCAGTCATCAATTCATACAGAA
Strand exchange assay	X1	TACGTTAACAAAAAGTCAGATATGGACCTTGCTG CTAAAGGTCTAGGAGCTAAA
	X2	TTTAGCTCCTAGACCTTTAGCAGCAAGGTCCATA TCTGACTTTTTGTTAACGTA
Primers for construction of non-specific plasmid	NF	TTTATGACTATACCCATCAGTTTGAACAGTTG
	NR	TATTGTTGCGACATGCTAGACCAAAATCA