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Supporting Information

Enabling lateral flow readout for DNA strand displacement by disassembling chemical labels

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Experimental Section

DNA oligonucleotides. The DNA oligonucleotides used in this study were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Fluorophore (FAM-) and quencher (BHQ1 and Biotin-) modified DNA oligonucleotides were purified by Sangon Biotech Co., Ltd. (Shanghai, China) using high-performance liquid chromatography (HPLC). Sequences and modifications of all oligonucleotides are listed in Table S1.

Buffer conditions. DNA oligonucleotides were re-suspended by dissolving oligonucleotides using 1 × tris-EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, purchased from Sangon) and then stored at −20 °C. Unless indicated otherwise, 1 × TE buffer containing 10 mM $MgCl_2$ and 0.5% (v/v) Tween-20 (Sangon) was used as the reaction buffer. Tween-20 was used to prevent the potential loss of DNA oligonucleotides during dilution and pipetting.

Probe preparation. All probes for toehold-mediated DNA strand displacement (TMDSD) and toehold-exchange were prepared by mixing equal concentrations of quencher and biotin dual-labeled P strand and FAM-labeled S strand in reaction buffer. The mixture at a final concentration of 10 μM was annealed in a Monad thermocycler using an annealing program of heating to 95 °C for 5 min and gradually cooling to room temperature at a constant rate over a period of 1hr. Once prepared, probes were stored in 4 °C until use.

Fluorescence analysis of TMDSD. For a typical TMDSD reaction, a 100 μL reaction mixture containing 20 nM triple-labeled probe, varying concentrations of target sequence, and reaction buffer was added to the well of a 96-well microplate. Fluorescence was measured immediately at 37 °C using a BioTek Cytation 5 cell imaging multimode microplate reader with a data acquisition rate of one read per minute for 1 h. The excitation/emission wavelength was set to be 485/525 nm.

Fabrication of lateral flow strips. The lateral flow strip was assembled by four components: the sample pad, the conjugate pad, the nitrocellulose membrane (Whatman®, purchased from Sigma Aldrich), and the absorbent pad. The sample pad (17 mm × 30 cm) was saturated with a buffer containing 0.25% Triton X-100, 0.05 M Tris-HCl, and 0.15 M NaCl.

Anti-FAM modified gold nanoparticles (1.16 nM) with 810 μL were loaded to the conjugate pad (8 mm × 30 cm). Streptavidin (2 mg/mL) was dispensed on the nitrocellulose membrane at the control line (C-line), and the goat anti-rabbit IgG (4 mg/mL) was dispensed at the test line (T-line) using the XYZ platform dispenser HM3030 (Shanghai Kinbio Tech Co., Ltd.). All the membranes and pads were dried at 37 °C for 2 h. Finally, the sample pad, conjugate pad, nitrocellulose membrane, and absorption pad were assembled on a plastic adhesive backing (60 mm × 30 cm). Each part overlaps 2 mm to ensure the solution is migrating through the strip during the assay. Strips with a 3 mm width were cut by using the cutting instrument ZQ2002.

Lateral flow assay for strand displacement. To enable lateral flow readout for TMDSD or toehold-exchange reactions, varying concentrations of target sequence were mixed with triple-labeled probe at a final concentration of 30 nM. After incubation at 37 °C for 1 hr, 20 μL of reaction mixture was loaded to the sample pad of lateral flow strip followed by 30 μL 4 × SSC washing buffer. The colored bands were developed over a period of 10 min and then recorded by the EPSON V500 scanner. The relative band intensities of each of the lateral flow strip were measured using ImageJ software (National Institutes of Health). The relative band intensity was calculated as the ratio of the mean band intensities between T-line and C-line.

Discrimination of CYP2C19*2 mutation in buccal swab samples. All buccal swabs were collected from healthy volunteers. Genomic DNAs in buccal swab samples were extracted by TIANamp Swab DNA Kit (Tiangen Biotech Co., Ltd. (Beijing, China)), strictly following the manufacturer's protocols. Recombinase polymerase amplification (RPA) reactions were performed using a TwistAmp® Basic Kit (TwistDX Inc. England). Briefly, the 50 μL RPA reaction contained 29.5 μL of rehydration buffer, 2.5 μL of 10 μM forward primer, 2.5 μL of 10 μM reverse primer, one freeze-dried reaction pellet, 5 μL of genomic DNA, 8 μL of RNase-free water and 2.5 μL of 280 mM magnesium acetate (last added). After a short centrifugation and vertexing, the mixture was incubated at 37 °C for 20 min. To convert the double-stranded RPA amplicon into single-stranded sequence, RPA amplicons (15 μL) were mixed with 600 nM DNA equalizer probes in reaction buffer in a 0.2 mL PCR tube, adjusting volume to 90 μL. The mixture was incubated at 95 °C for 5 min and then cooled down to 4 °C for 2 min. For fluorescence readout, the mixture was finally mixed with 20 nM CYP-probe in 100 μL reaction buffer in a 96-well microplate. Fluorescence was measured immediately using a BioTek Cytation 5 cell

imaging multimode microplate reader with a data acquisition rate of one read per minute for 1 h. The excitation/emission wavelengths were set to be 485/525 nm. For lateral flow readout, the reaction mixture was loaded to the sample pad followed by washing using 30 μL of 4 × SSC buffer. Data visualization and analysis were performed using the above-mentioned protocol.

Native PAGE Analysis of RPA amplicons. RPA amplicons were analyzed using 6 % PAGE gel. 5 μL of RPA product was mixed with 1 μL loading buffer before loading on gel. After electrophoresing in 1× TAE buffer at 120 V for 35 min, the gel was stained and visualized using GenoSens 2200.

Table S1. Sequences of oligonucleotides used in study.

	Name	Sequences (5' → 3')	Modification
Model target for TMDSD	Target	TTCCTGTTGTAGATTCTTATTATTCATTG	
	P	TTCCTGTTGTAGATTCTTATTA	5'-BHQ1, 3'-Biotin
	S	CAATGAATAATAAGAATCTACAACAGGAA	3'-FAM
Toehold-exchange for EGFR L858R mutation	Correct target (L858R)	AGTTTGGCC**C**GCCCAAAATCTGTGATCTTGAC	
	Spurious target (wild-type)	AGTTTGGCC**A**GCCCAAAATCTGTGATCTTGAC	
	P	TACATGTTTGGCCCGCCCAA	5'-BHQ1, 3'-Biotin
	S	ACAGATTTTGGGCGGGCCAAACATGTA	3'-FAM
	Spurious target (mut-1)	AGTTTGGCCCGCCCAAAATCTG**A**GATCTTGAC	
	Spurious target (mut-5)	AGTTTGGCCCGCCCAAAA**A**CTGTGATCTTGAC	
	Spurious target (mut-10)	AGTTTGGCCCGCC**A**AAAATCTGTGATCTTGAC	
	Spurious target (mut-14)	AGTTTGGCC**A**GCCCAAAATCTGTGATCTTGAC	
	Spurious target (mut-18)	AGTTT**A**GCCCGCCCAAAATCTGTGATCTTGAC	
	Spurious target (mut-22)	A**A**TTTGGCCCGCCCAAAATCTGTGATCTTGAC	
RPA primers for analyzing CYP2C19*2	Forward primer	TTCTCTTAGATATGCAATAATTTTCCCACT	
	Reverse primer	TTTCTCCAAAATATCACTTTCCATAAAAGCAAG	
DNA equalizer probes and toe-hold-exchange probe for CYP2C19*2	DEP1	TATTTCCCAGGAACCCATAA	
	DEP2	CAAATTACTTAAAAAC	
	DEP3	ATCAATGATAGTGGGAAAATTATTGCATATCTAAGAGAA	
	DEP4	TTTCTCCAAAATATCACTTTCCATAAAAGCAAG	
	CYP2C19*2-S	TAATTTGTTATGGGTTCCTGGGAATGTAG	3'-FAM
	CYP2C19*2-P	CTACATTCCCAGGAACCCATAA	5'-BHQ1, 3'-Biotin

Table S2. Comparison of LFA-based nucleic acid analysis platforms harnessing sandwiched hybridization complexes or strand displacement.

Methods	Type of LFAs	Sensitivity	Specificity to SNV	Comment	Reference
LFNAB	sandwich	60 pM miRNA	Not demonstrated	Sandwiched hybridization complex is needed to generate LFA signal	1
SD-LAMP	sandwich	110 E.coli cells / μL with LAMP amplification	Not demonstrated	A special design is required to convert strand displacement to sandwiched binding complex	2
CASLFA	sandwich	150 copies of genomic DNA with PCR amplification	Not demonstrated	Cas9 and sgRNA are used to generate a sandwiched binding complex	3
HCR-LFA	sandwich	2 nM DNA with HCR amplification	Not demonstrated	Hybridization chain reaction is used to generate a sandwiched binding complex	4
CHA-LFA	sandwich	1 pM miRNA with CHA amplification	Yes	Catalytic hairpin assembly is used to generate a sandwiched binding complex	5
SHERLOCK	DCL	2 aM RNA with RPA amplification	Yes	Cas13 and crRNA are used to mediate trans-cleavage of chemically labeled substrates	6
TMDSD-based DCL	DCL	6 nM DNA	Yes	Direct separation of chemical labels using TMDSD	This work

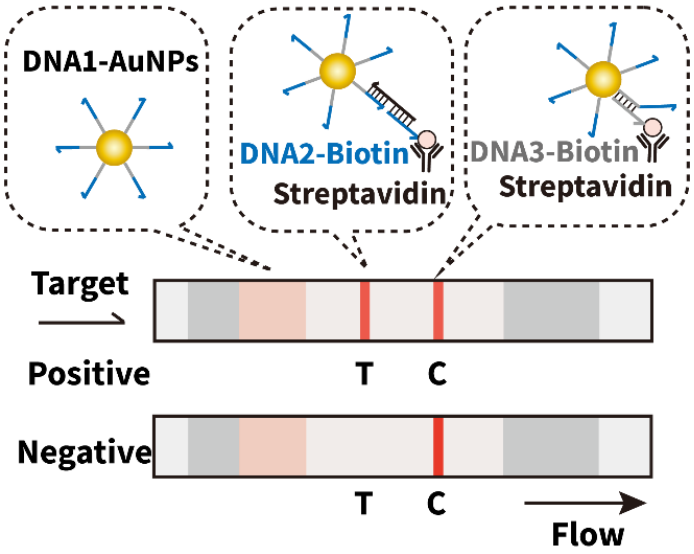

Figure S1. Schematic illustration of the classic sandwiched binding-based lateral flow strip for nucleic acid testing.

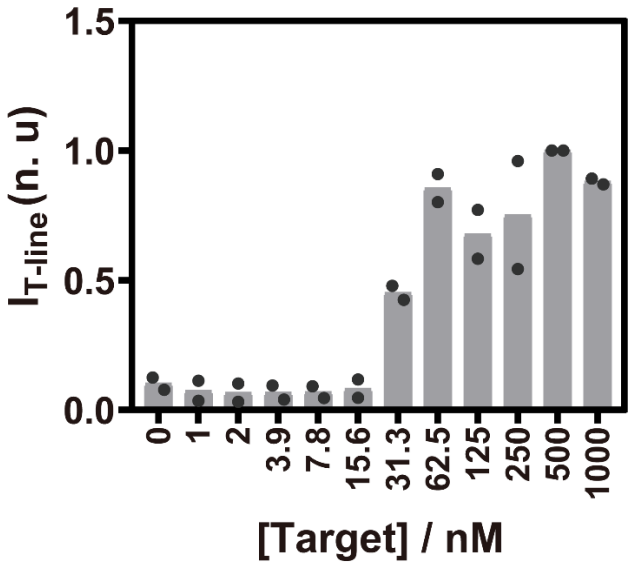

Figure S2. Intensities of T-line plotted as a function of target concentrations. Each bar represents the mean of duplicate measurements.

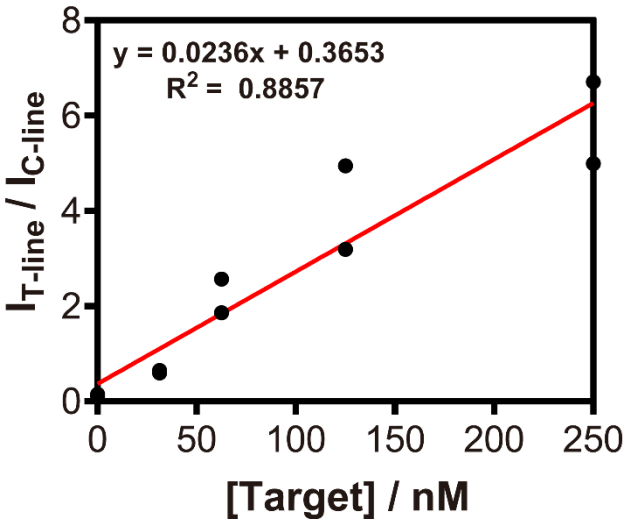

Figure S3. Calibration curve of the TMDSD reaction using LFA readout.

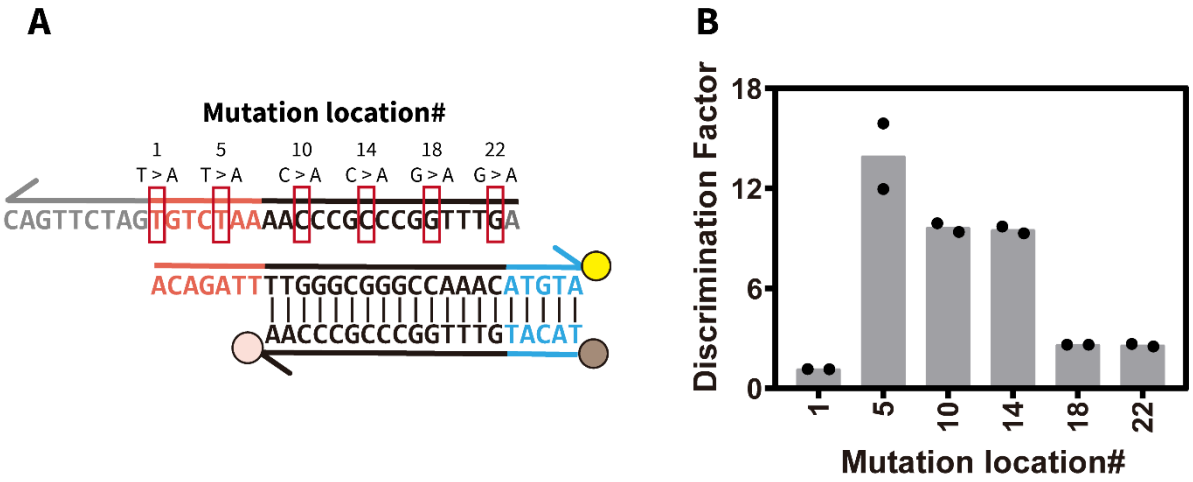

Figure S4. (A) Schematic illustration of location and types of mismatches on the target sequences. (B) Discrimination factors for mismatches at varying locations. The concentration of all correct and spurious targets was fixed at 62.5 nM.

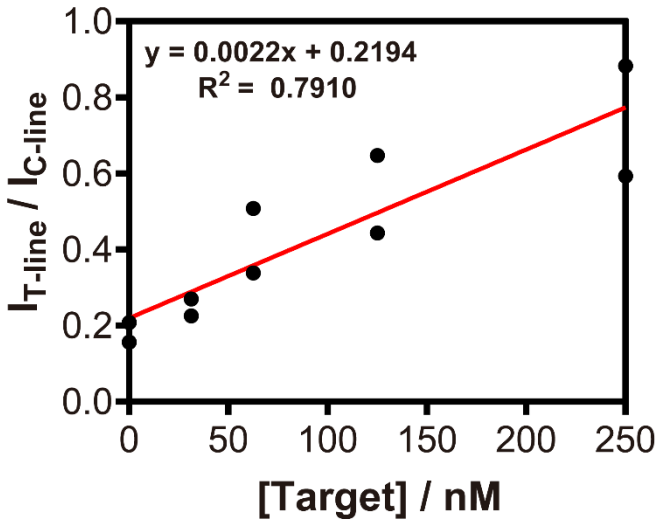

Figure S5. Calibration curve of the toehold-exchange reaction using LFA readout.

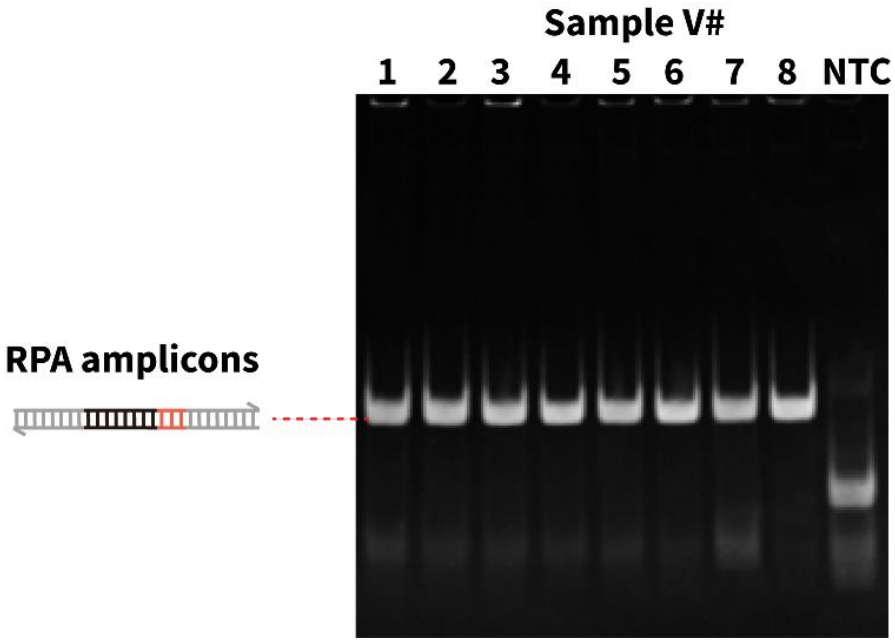

Figure S6. Native PAGE Analysis of RPA amplicons. Lane 1 to 8: buccal swab samples 1 to 8; Lane 9: no template control (NTC).

References

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