Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Universal probe system for low-abundance point mutation detection based on endonuclease IV

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Materials

All the DNA strands and probes were synthesized and purified by Sangon Co, (Shanghai,China). The sequences are demonstrated in Table S1. Endonuclease IV(endo IV), ThermoPol Reaction Buffer (20 mM Tris–HCl, 10 mM KCl, 10 mM (NH_4) 2 SO4,2 mM $MgSO_4$ and 0.1% Triton X-100, pH 8.8) were purchased from New England Biolabs(MA, USA). Taq PCR Master Mix(2X) and DNase/RNase-free deionized water were purchased from BBI,China. DNA purification kit was purchased from Tiangen Biotech Co. (Beijing, China). The clinical samples were provided by Wuhan Union hospital.

Evaluation of the discrimination capability of the system between MT and WT

The template-A1(120nM), trigger-A1(100nM), template-B1(120nM), trigger-B1(100nM), blocker(1200nM), endo IV(0.004U), AP-probe(100nM) and target DNA were added to make up with the reaction system. ThermPol reaction buffer and ddH2O made the total volume to 50ul. Template-A1 and trigger-A1, template-B1 and trigger-B1, target DNA and blocker were incubated at 85°C for 5 minutes, then 55°C for 5 minutes, and 37°C for 1h in advance to form a double-stand structure. And the AP-probe and endo IV were added to the system and fluorescence was detected by microplate reader. (BioTek, American). The parameter of the microplate reader was set as follow: excitation wavelengths was 485 nm, emission wavelengths was 528 nm, gain level was 90 at 37°C and detection time was 30min to 2h.

Detection of low-abundance MT targets

Dilute MT with WT to prepare different concentrations (100 nM, 10 nM, 5 nM, 1 nM, 0.5 nM, 0.1 nM, 0.05 nM and 0) of target DNA. And the method for detecting fluorescence intensity was same as before.

Detection of EGFR L858R in human genomic DNA after PCR

The human genomic DNA was extracted from the cancer tissue using the DNA purification kit(Tiangen, Beijing, China). Then the DNA was sequenced to determine the point mutation. Two-step PCR method was used to amplify the target strand, according to Tang. The first step was a conventional PCR. The primers were demonstrated in table S1. The procedures: pre-denaturation at 95 ℃ for 1 minute, deneturation at 95 ℃ for 15 seconds, annealing at 60℃ for 30 seconds, extensions at 72 ℃ for 30 seconds, for 40 cycles. We diluted the product of the first step for 100 times as the template for the second step. Follow as Tang's method, we added of 25ul of PCR mix, 1000nM for forward primer and 100nM for the reverse primer (the sequence in Table S1), 1ul of template and ddH2O to make the volume to 50ul. After the two-step PCR, the fluorescence intensity was detected as the methods demonstrated before.

Optimization of the concentration of the blocker strand of PTEN R130Q (G>A) mutation point

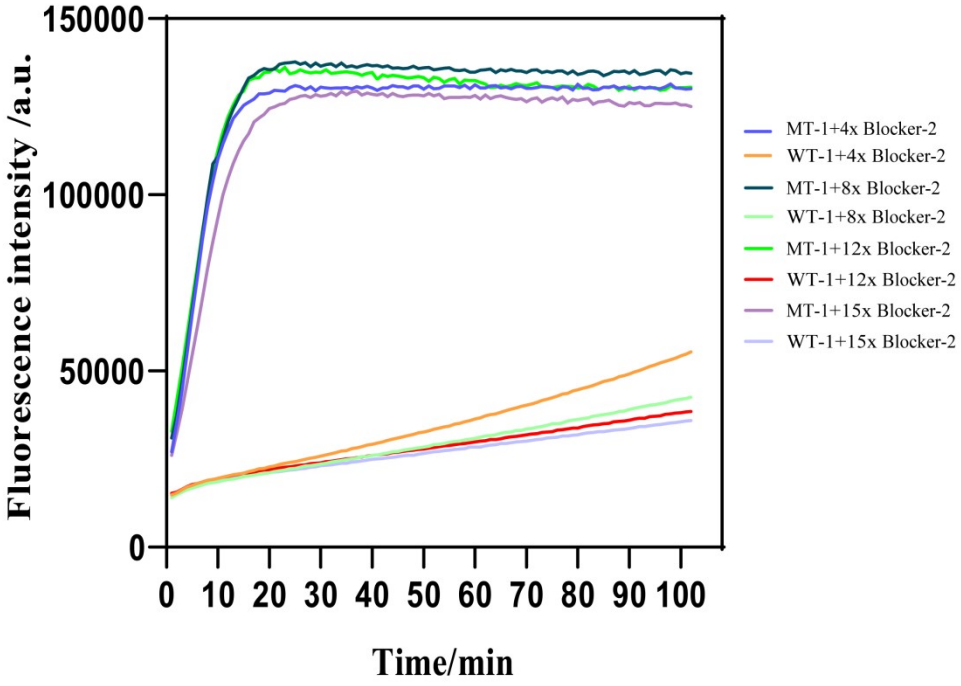

Figure S1 The fluorescence curve for WT-1/MT-1 reaction process of different concentration of Blocker-2 (4x/8x/12x/15x, 1x: 200nM).

Optimization of the concentration of Endo IV of PTEN R130Q (G>A) mutation point

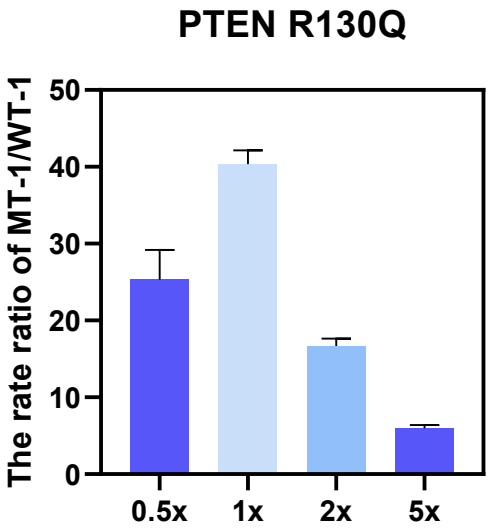

Figure S2 The rate ratio of MT-1/WT-1 under the different concentration of Endo IV. (1x: 0.004U)

Optimization of the concentration of the blocker strand and the design of the template-A strand of EGFR L858R mutation point

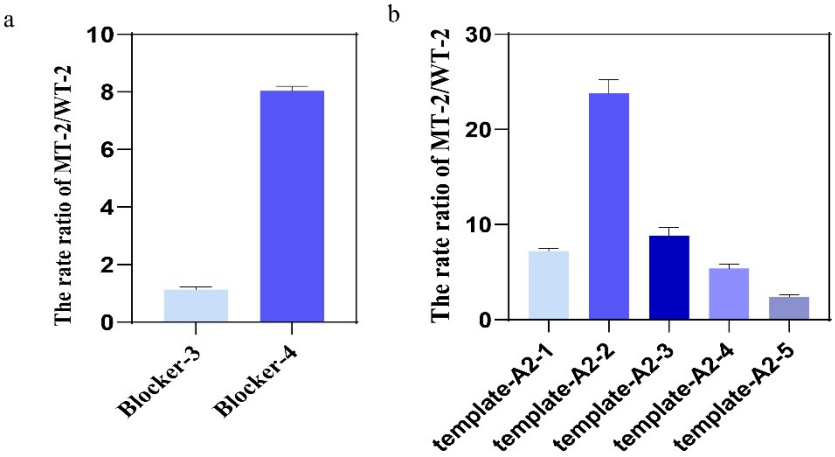

Figure S3 Optimization of the system of EGFR L858R mutation point detection. (a) Choose the better blocker strand for the detection. The length of the toehold of the template-A strand is 12nt. (b) Choose the better design of the template-A strand.

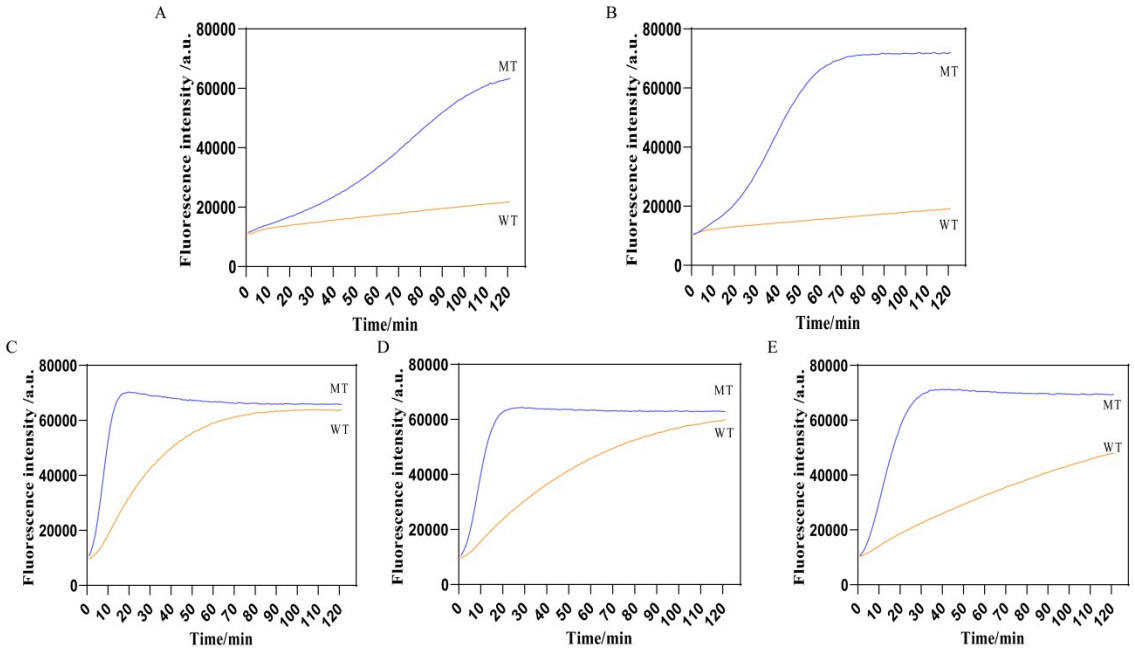

Figure S4 The fluorescence curve of WT-2/MT-2 reaction process with different design of the template-A strand. The template-A strands used from a to e are template-A2-1 to template-A2-5 in turn.

Optimization of the design of the trigger-A strand of PTEN rs1473918395 mutation point

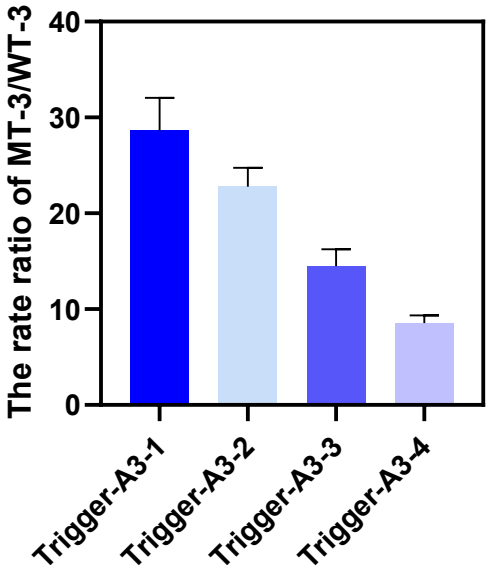

Figure S5 The rate ratio of MT-3/WT-3 under the different design of Trigger-A3

The replicates of the experiments on LOD of the three mutation points

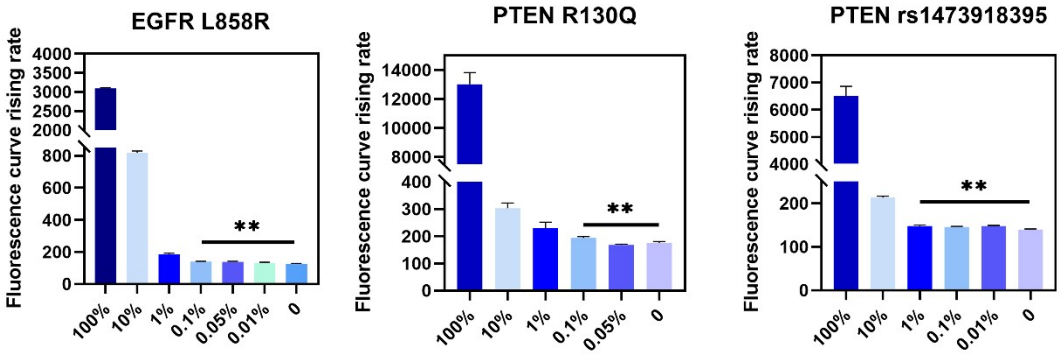

Figure S6 The results of the experiments on LOD of each mutation point. Each group were repeated in three times. The LOD of EGFR L858R/PTEN R130Q/PTEN rs1473918395 is 0.1%/0.1%/1% in turn.

The gel electrophoresis of the MT/WT extracted by the clinical samples.

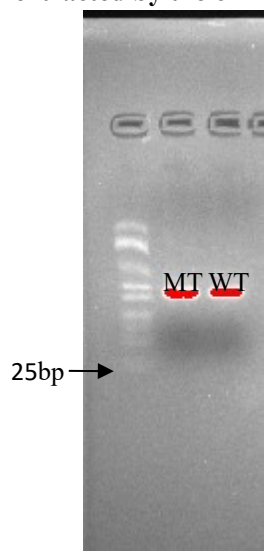

Figure S7 The first lane: EGFR L858R MT; The second lane: EGFR L858R WT

Sanger sequencing of mutations from genomic DNA of lung cancer patient and normal genomic DNA from normal people.

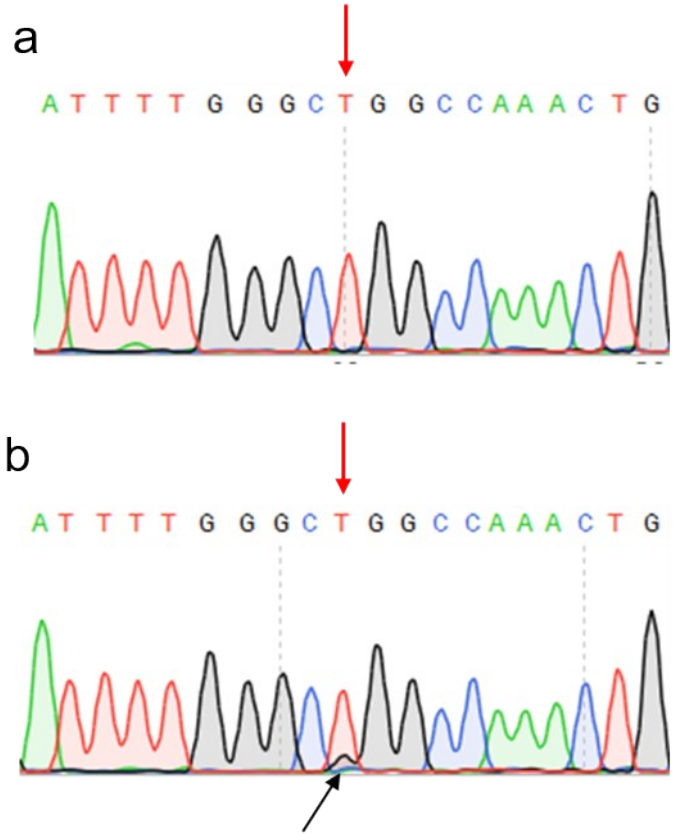

Figure S8 (a) Sanger sequencing result of EGFR L858R mutation of normal genomic DNA from normal people. (b) Sanger sequencing result of EGFR L858R mutation from genomic DNA of endometrial cancer patient.

Table S1 The sequences used in the experiment

Name	Sequences
MT-1	TCACTGTAAAGCTGGAAAGGGACAAACTGGTGTA
WT-1	TCACTGTAAAGCTGGAAAGGGACGAACTGGTGTA
Template-A1	TCCGCATTCCAGTTTGTCCCTTTCCAGCTTTACAGTGA
Blocker-1	CAGTTCGTCCCT
Blocker-2	ACCAGTTCGTCCCTT
Trigger-A1	CAAACTGGAATGCGGA
Template-B1	CAGCCACTTCCGCATTCCAGTTTGTC
Trigger-B1	AATGCGGAAGTGGCTG
MT-2	TGTCAAGATCACAGATTTTGGGCGGGCCAAACTGC
WT-2	TGTCAAGATCACAGATTTTGGGCTGGCCAAACTGC
Template-A2-1	TCCGCATTTTGGCCCGCCCAAAATCTGTGATCTTGACA
Template-A2-2	TCCGCATTTTGGCCCGCCCAAAATCTGTGATCTTGA
Template-A2-3	TCCGCATTTTGGCCCGCCCAAAATCTGTGATCT
Template-A2-4	TCCGCATTTTGGCCCGCCCAAAATCTGTGATC
Template-A2-5	TCCGCATTTTGGCCCGCCCAAAATCTGTGAT
Trigger-A2	GCGGGCCAAAATGCGGA
Template-B2	CAGCCACTTCCGCATTCCAGTTTGTC
Blocker-3	TTTGGCCAGCCCAAA
Blocker-4	TTGGCCAGCCCAA
MT-3	GAGAACTGTCATGTACCCAATACTCCTTACTT
WT-3	GAGAACTGTCATGTACCCACTACTCCTTA CTT
Template-A3	TCCGAATTGGAGTATTGGGTACATGACAGTTCTC
Trigger-A3-1	CAATACTCCAATTCGGA
Trigger-A3-2	CCAATACTCCAATTCGGA
Trigger-A3-3	CCCAATACTCCAATTCGGA
Trigger-A3-4	ACCCAATACTCCAATTCGGA
Template-B3	CAGCCACTTCCGCATTGGAGTATTG
Blocker-5	TAAGGAGTAGT
AP-probe	FAM CAGCC-CTTCCG BHQ
L858R FP	ACTTGGAGGACCGTCGC
L858R RP	GCTGGCTGACCTAAAGCC
Amplicon of EGFR L858R	ACTTGGAGGACCGTCGCTTGGTGCACCGCGACCTGGCAG CCAGGAACGTACTGGTGAAAACACCGCAGCATGTCAAG ATCACAGATTTTGGGCTGGCCAAACTGCTGGGTGCGGAA GAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAG GTGGCTTTAGGTCAGCCAGC
No senses sequence	TGAGATAACCTTCCCTGTATAGACGTCAACTTAC

The ΔG calculation of the reactions in theory

WT/Blocker + Template-A/Trigger-A → WT/Template-A + Trigger-A (1)

MT/Blocker + Template-A/Trigger-A → MT/Template-A + Trigger-A (2)

Trigger-A + Template-B/Trigger-B → Trigger-A/Template-B + Trigger-B (3)

Table S2 The ΔG value of the reaction in theory

Target	ΔG of reaction (1) (kcal/mol)	ΔG of reaction (2) (kcal/mol)	ΔG of reaction (3) (kcal/mol)
PTEN R130Q MT	-1.93	/	-1.58
PTEN R130Q WT	/	13.69	-1.58
EGFR L858R MT	-1.30	/	-4.01
EGFR L858R WT	/	1.57	-4.01
PTEN rs1473918395 MT	-1.33	/	-1.01
PTEN rs1473918395 WT	/	1.20	-1.01

Table S3. Comparison with other normal methods for ALP detection.

Strategy	Time	Cost	The limit of detection	Reference
Sanger sequencing	Several days	Expensive (The equipment is expensive. The operators need to be trained)	5-10%	[1,11]
NGS (Next generation sequencing)	Several days	Expensive (The equipment is expensive. The operators need to be trained)	1%	[2,11]
COLD-PCR	Several hours	Expensive (The temperature control ability and sample processing ability of the instrument have higher requirements)	0.1%	[3]
BM-PCR	2-3h	Low	0.5%-5%	[4]
A branch-migration based fluorescent probe	10-20min	Medium (The probe is not universal.)	0.3%-1%	[5]
BM-PCR with endonuclease IV-assisted target recycling probe/blocker system	50min	Medium (BM-PCR, λ exo and Endo IV are needed, which make the cost high)	0.1%	[6]
Universal probe-	1-2h	Low	5%	[7]

based melting curve analysis under an interlocked DNA cascade system				
Universal star-probe detection system	30-50min	Medium (The second structure of the probe system will affect the result. High cost of system reset and optimization)	5%	[8]
G-quadruplex colorimetric universal probe detection system	60min	Medium (The probe and Hemin are needed)	0.5%	[9]
Quantitative Real-Time PCR Assay Based on TaqMan-MGB Probes	2-3h	Medium (The probe is not novel and the method have to combine with RT-PCR)	0.1%-4%	[10]
The universal probe system we designed	20-50min	Low (The probe is universal, the concentration of Endo IV is low and do not need the expensive detection platform)	0.1%	This work

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