Supplementary Data

Exonuclease III- amplified 4-way strand migration system for low-abundance deletion mutation

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SI 1 Supplementary Materials

1. 1 Sequences of oligonucleotides used in this work.

Table S1. The core DNA sequences of used in detecting EGFR exon 19 del

Strand Name	Sequence (5'-3')
WT- EGFR-15nt	TTAAA ATTCCCGTCGCTATC AAG GAA TTA AGA GAA GCA ACATCTCCGAAAGCC AACAA
MT- EGFR-15nt	TTAAA ATTCCCGTCGCTATCAAGACATCTCCGAAAGCC AACAA
D-Probe-FAM-EGFR-15nt	AAGACATCTCCGAAAGCC AACAA
D-Probe-BHQ- EGFR-15nt	GGCTTTCGGAGA TGT CTTGATAGCGACGGGAAT
Blocker- EGFR-15nt	TGT TGC TTC TCT TAA TTC CTT
PCR-WT- EGFR-15nt	GACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGCTATCAAGGAATT AAGAGAAGCAACATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTCTG CTTT
PCR-MT-EGFR-15nt	GACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGCTATCAAAACATC TCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTCTGCTTT
PCR-FP- EGFR-15nt	GACTCTGGATCCCAGAAG
PCR-RP- EGFR-15nt	AAAGCAGAAACTCACATCGA
Specific-RP- EGFR-15nt	GGCTTTCGGAGATGTTTTG
Plasmid- EGFR-15nt	pUC57

Table S2. The core DNA sequences of used in detecting PBRM1 c.3923-3930del

Strand Name	Sequence(5'-3')
WT- PBRM1-8nt	TTCACCCAGAAGAAACAGAGCATGAGCCCACAAAAATGTTCTACAAAAAAGAAG TATTT
MT- PBRM1-8nt	TTCACCCAGAAGAAACAGAGCATGAAAATGTTCTACAAAAAAGAAGTATTT
D-Probe-FAM- PBRM1-8nt	GCATGAAAATGTTCTACAA
D-Probe-BHQ- PBRM1-8nt	AGAACATTTTCATGCTCTGTTTCTT
Blocker-1- PBRM1-8nt	AGAACATTTTTGTGGGCTGTAC
Blocker-2- PBRM1-8nt	AGAACATTTTTGTGGGCT
Blocker-3- PBRM1-8nt	AGAACATTTTTGTGGGCTCATGGTAC

PCR-WT- PBRM1-8nt	GCCCCATCTTCATTCACCCAGAAGAAACAGAGCATGAGCCCACAAAAATGTTCTA
	CAAAAAAGAAGTATTTCTGAGTAATCTGGAAGAAACCTGCCCCATGA
PCR-MT- PBRM1-8nt	GGCCCCATCTTCATTCACCCAGAAGAAACAGAGCATGAAAATGTTCTACAAAAA
	AGAAGTATTTCTGAGTAATCTGGAAGAAACCTGCCCC
PCR-FP- PBRM1-8nt	CCCCATCTTCATTCACCCAGA
PCR-RP- PBRM1-8nt	GGGGCAGGTTTCTTCCAGAT

Table S3. The core DNA sequences of used in detecting EGFR c.2127-2129del

Strand Name	Sequence(5'-3')
WT- EGFR-3nt	AGCTCTCTTGAGGATCTTGAAGGAAACTGAATTCAAAAAGATCAAAGTGCTGGG
	CTCCG
MT- EGFR-3nt	AGCTCTCTTGAGGATCTTGAAGGATGAATTCAAAAAGATCAAAGTGCTGGGCTCC
D-Probe-FAM- EGFR-3nt	G
D-Probe-BHQ- EGFR-3nt	AGGATGAATTCAAAAAGATC
	TTTTTGAATTCATCCTTCAAGATCCT
Blocker-1-EGFR-3nt	GAATTCAGTTTCCTTCATCTA
Blocker-2- EGFR-3nt	TGAATTCAGTTTCCTTCAATCTA
Blocker-3 EGFR-3nt	TTGAATTCAGTTTCCTTCAAGTCTA

Table S4. The core DNA sequences of used in detecting different length of deletion mutation of PBRM1

Strand Name	Sequence(5'-3')
MT- PBRM1-8nt	TTCACCCAGAAGAAACAGAGCATGAAAATGTTCTACAAAAAAGAAGTATTT
D-Probe-FAM- PBRM1-8nt	GCATGAAAATGTTCTACAA
D-Probe-BHQ- PBRM1-8nt	AGAACATTTTCATGCTCTGTTTCTT
WT- PBRM1-8nt	TTCACCCAGAAGAAACAGAGCATGAGCCCACAAAAATGTTCTACAAAAAAGAAG
WT- PBRM1-7nt	TATTT
WT- PBRM1-6nt	CAGAAGAAACAGAGCATGGCCCACAAAAATGTTCTACAAA
WT- PBRM1-5nt	CAGAAGAAACAGAGCATGCCCACAAAAATGTTCTACAAA
WT- PBRM1-4nt	CAGAAGAAACAGAGCATGCCACAAAAATGTTCTACAAA
WT- PBRM1-3nt	CAGAAGAAACAGAGCATGCACAAAAATGTTCTACAAA
WT- PBRM1-2nt	CAGAAGAAACAGAGCATGACAAAAATGTTCTACAAA

WT- PBRM1-1nt	CAGAAGAAACAGAGCATGCAAAAATGTTCTACAAA
	CAGAAGAAACAGAGCATGAAAAATGTTCTACAAA
Blocker- PBRM1-8nt-4	AGAACATTTTTGTGGGCTCATGCTCTGTGTAC
Blocker- PBRM1-7nt	AGAACATTTTTGTGGGCCATGCTCTGTGTAC
Blocker- PBRM1-6nt	AGAACATTTTTGTGGGCATGCTCTGTGTAC
Blocker- PBRM1-5nt	AGAACATTTTTGTGGCATGCTCTGTGTAC
Blocker- PBRM1-4nt	AGAACATTTTTGTGCATGCTCTGTGTAC
Blocker- PBRM1-3nt	AGAACATTTTTGTCATGCTCTGTGTAC
Blocker- PBRM1-2nt	AGAACATTTTTGCATGCTCTGTGTAC
Blocker- PBRM1-1nt-1	AGAACATTTTTCATGCTCTGTGTAC
Blocker- PBRM1-1nt-2	TAGAACATTTTTCATGCTCTGTTGTAC

SI 2 Supplementary Notes

2.1 Optimize the buffer for Exonuclease III

According to the instruction of Exonuclease III (Exo III), 1X Exo III buffer (10mM Bis-tris (pH 7.0),10mM MgCl2,1mM DTT) was different from 1X ThermoPol buffer. ThermoPol buffer was usually used in nucleic acid detection research. So, we compared Exo III buffer with ThermoPol buffer in Exo III-assisted toehold probe system. The mutation abundance of 10%and 0% were detected by the system with two buffer separately. The results were shown in Fig. S1. ThermoPol buffer was better for detecting by Exo III-assisted toehold probe system.

2.2 Optimize the concentration for Exonuclease III

According to the principle of the detection method, we needed to optimize the concentration of Exo III. Exo III was diluted by 60, 80,100, and 120 times, and other experimental condition were fixed as the before. In order to obtain the optimal concentration, 10% group and 1% group were used to determine the effect of amplification.0% group was used to analyze the background signal. The results were shown in Fig. S2 a, b, c. Exo III diluted by 100 times was the optimal concentration.

2.3 Deletion mutation specific primer for sanger sequencing

In order to verify the results of the PCR products detected by Exo III-assisted toehold probe system were better than sanger sequencing. In addition to the conventional primer-mediated sanger sequencing which can detect MT and WT, we also sequenced the PCR products by deletion mutation specific primer which can detect MT and cannot detect WT. The sequencing results deletion mutation specific primers were shown in Fig S3. No matter sanger sequencing with conventional primer or deletion mutation specific primers, the low mutation abundances (1%-0%) cannot be distinguished.

SI 3 Supplementary Figures.

3.1The optimization of Exo III buffer

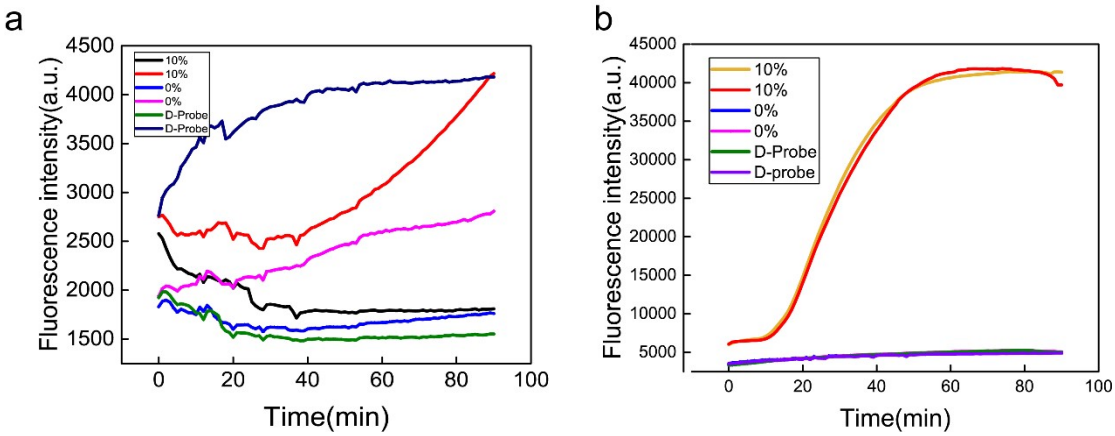

Fig. S1. a) Mutation abundance of 10%，0% and D-Probe were detected by the system with Exo III buffer. b) Mutation abundance of 10%, 0% and D-Probe were detected by the system with ThermoPol buffer 1. Experiments were detected at 37 °C by BioTek, and the gain value was 60. The concentration of Blocker was 1000 nM and the other contents were 300 nM. The dilution ratio of Exo III was 100.

3. 2 The optimization of Blocker.

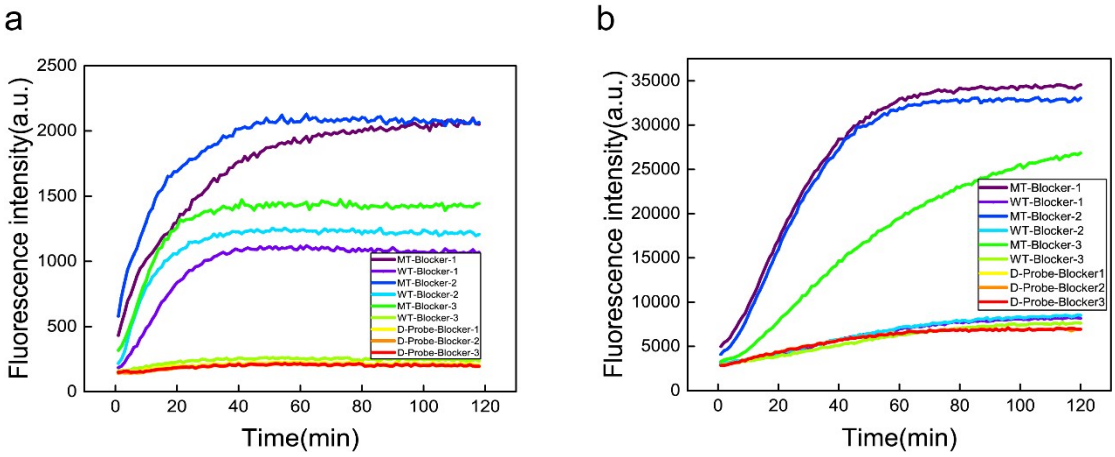

Fig. S2. a) The florescence intensity of PBRM1-8-nt del and the gain value was 120. b) The florescence intensity of EGFR-3-nt del and the gain value was 170. Experiments were detected at 37 °C by Tecan. The concentration of Blocker was 1000 nM and the other contents were 300 nM. The dilution ratio of Exo III was 100.

3.3 The optimization of Exo III concentrtion.

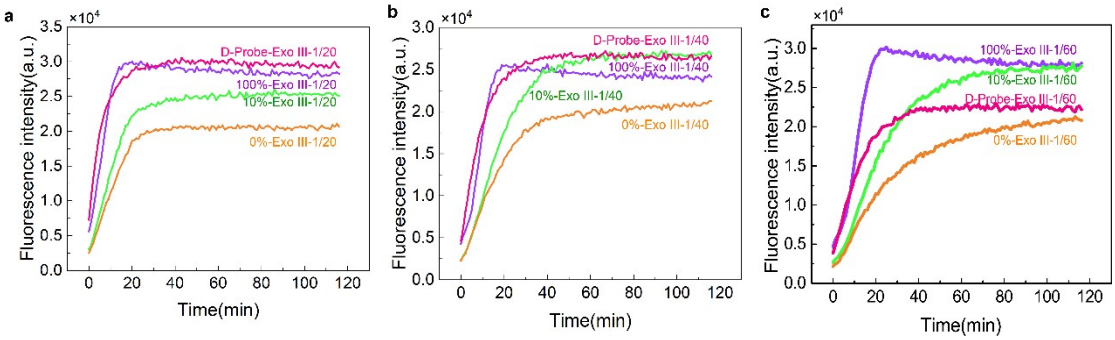

Fig.S3 a, b, c The florescence intensity of PBRM1-8-nt with the dilution ratio of 20,40,60. Experiments were detected at 37 °C by Tecan and the gain value was 170. The concentration of Blocker was 1000 nM and the other contents were 300 nM.

3.4 The linear fitting of the rate and mutation abundance

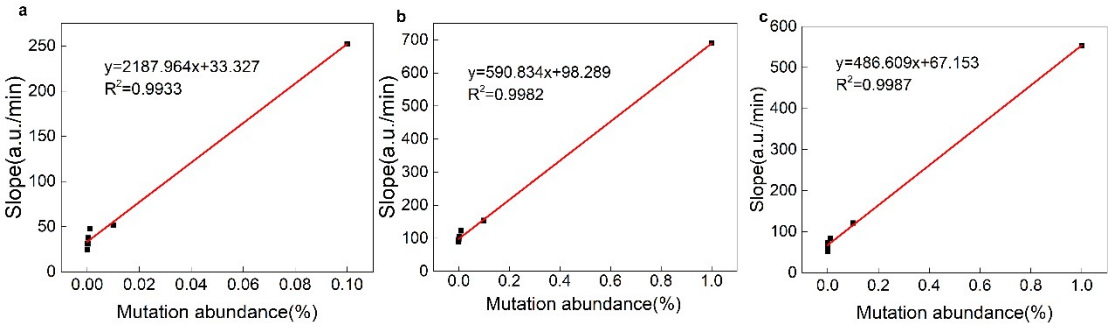

Fig. S4 The linear fitting of EFGR-15-nt del(a), PBRM1-8-nt del(b) and EGFR-3-nt-del(c).

3.5 The free energy of and the conversion of eight types of deletion length

Length of deletion sequence	$\Delta G_{(MT)}$	$\Delta G_{(WT)}$	Combination of WT and Blocker
8	-5.00101	17.2057	100%
7	-5.00354	16.9837	100%
6	-5.17622	16.4317	100%
5	-5.4981	15.6884	100%
4	-6.5819	15.1364	100%
3	-6.7255	14.4164	99%
2	-6.7098	14.2694	99%
1	-7.6753	9.69117	82%

Fig. S5 The free energy difference of MT and WT and binding ratio of Blocker and

WT.

3.6 The sanger sequencing results with ordinary primer

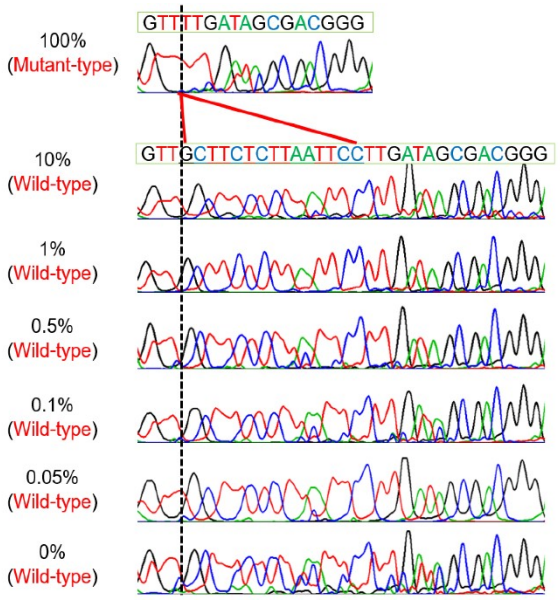

Fig. S6 The result of sanger sequencing with ordinary primer.

3.7 The sanger sequencing results with deletion specific primer

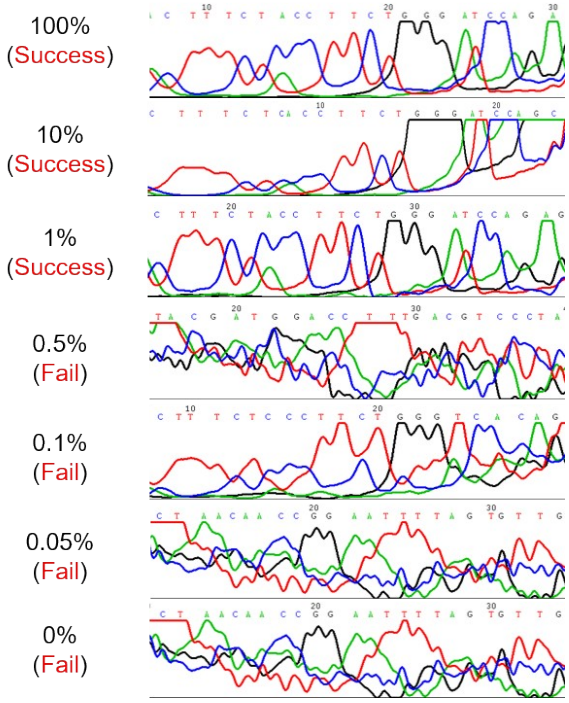

Fig. S7 The result of sanger sequencing with deletion specific primer.