Electronic Supplementary Material (ESI) for Analyst
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Supplementary Information

Kinetics-based Detection of SNP for EGFR Mutation by Extension Reactions and T7 Exonuclease Mediated Isothermal Amplification

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Reagents

The PCR kit was purchased from Zoman Biotech Co. (Beijing, China). The forward, reverse primers and the probe were synthesized in Sangon Co., China. The Exonuclease I and Cac8I restriction endonuclease were supplied by New England Biolabs (USA). The Klenow Fragment (exo-) was purchased from Enzymatics (USA) and the T7 Gene 6 Exonuclease was purchased from Affymetrix (USA).

Low abundance detection of EGFR mutation with PCR amplicons

In a 200 μL PCR tube, 40 μL ddH_2O, 5 μL 10× Taq Buffer, 1 μL dNTPs mixture (10 mM each), 1 μL Taq DNA polymerase (2.5 U/ μL), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM) and 1 μL mixed genome DNA template (26 ng/ μL) were added and mixed. The sequence of the forward primer was 5'-GCTCAGAGCCTGGCATGAA-3' and the sequence of the reverse primer was 5'-CATCCTCCCCTGCATGTGT-3'. The procedures for PCR amplification were: 95 ºC 15min, 35 cycles of 95 ºC 20s, 60 ºC 30s and 72 ºC 1min, followed by the further extension at 72 ºC for 3min. After the amplification by PCR, we added 1.5 μl Exonuclease I (30 U) and incubated at 37 ºC for 30 min followed by inactivating at 85 ºC for 20 min to remove the excess of the primers. Then we added 5.9 μl 10× CutSmart Buffer and 1.5 μl Cac8I (7.5 U) and incubated at 37 ºC for 1 h, followed by inactivating at 70 ºC for 20 min to cut the amplicons and leave the SNP site at the 3' terminus. Next we added 0.6 μl forward primer (10 μM) and 3 μl dNTPs (10 mM) to perform the one cycle asymmetric PCR and obtain the single stranded target with a SNP at its 3' terminus. We further added 1 μl 3285 mM NaCl, 1 μl 657 mM $MgCl_2$ and 1 μl 200 μM probe to anneal to the target. We took 5 μl from the mixture and added 0.5 μl 10× Blue Buffer as well as 0.28 μl Klenow Fragment (exo-) (14 U) and incubated at 37 ºC for 15 min. After the incubation, we added 1.46 μl T7 exonuclease (3.68 U/ μl T7 exonuclease and 4.63× T7 Gene 6 Exonuclease Buffer) to the mixture and detected the change of fluorescence intensity at 25 ºC.

Principles for calculating the reaction rate

The T7 exonuclease assisted signal amplification was a linear amplification process, i.e.,

$$\text{reaction rate} = \frac{d[Product]}{dt} = \frac{d(n \times Signal)}{dt} = n \times \frac{d(Signal)}{dt} \quad (n \text{ is a constant}) \qquad [1]$$

From equation [1], the reaction rate was proportional to the first derivative of the reaction curve. We first calculated and found out the maximum first derivative from the burst phase of the curve. Then we fit the data points to a linear model with the k value around the maximum first derivative value. The k value from the linear model was denoted as the reaction rate. We then utilized the k value to differentiate the matching target and the target with SNP.

Table S1. DNA sequences used in the experiment. The SNP was in red color and underlined.

Name	Sequence(5' → 3')
Probe sequences for Klenow fragment (exo-) extension	
P-FAM	(FAM)TGCGCGTTATACCGCAGTCCGG AGTGGCTAACG
P-BHQ-1	CGGTATAACGCGCA(BHQ1)
Target sequences for Klenow fragment (exo-) extension	
C14-G	CGTTAGCCACTCCG
C14-A-ter	CGTTAGCCACTCCA
C14-C-ter	CGTTAGCCACTCCC
C14-T-ter	CGTTAGCCACTCCT
C14-A-sec	CGTTAGCCACTCAG
C14-G-sec	CGTTAGCCACTCGG
C14-T-sec	CGTTAGCCACTCTG
Probe sequences for optimization of T7 exonuclease	
P-FAM-BHQ-1	(FAM)TT(BHQ1)CGCGTATTACCGCAG TCCGGAGTGGCTAACG
Target sequences for optimization of T7 exonuclease	
anti-probe	AAAACGTTAGCCACTCCGGACTGCG GTAATACGCGAA
Probe sequences for Klenow fragment (exo-) with T7 exonuclease-assisted target cycling	
P-FAM-BHQ-1	(FAM)TT(BHQ1)CGCGTATTACCGCAG

TCCGGAGTGGCTAACG	
Target sequences for Klenow fragment (exo-) with T7 exonuclease-assisted target cycling	
T-match	AAAACGTTAGCCACTCCG
T-mismatch-A	AAAACGTTAGCCACTCCA
T-mismatch-C	AAAACGTTAGCCACTCCC
T-mismatch-T	AAAACGTTAGCCACTCCT

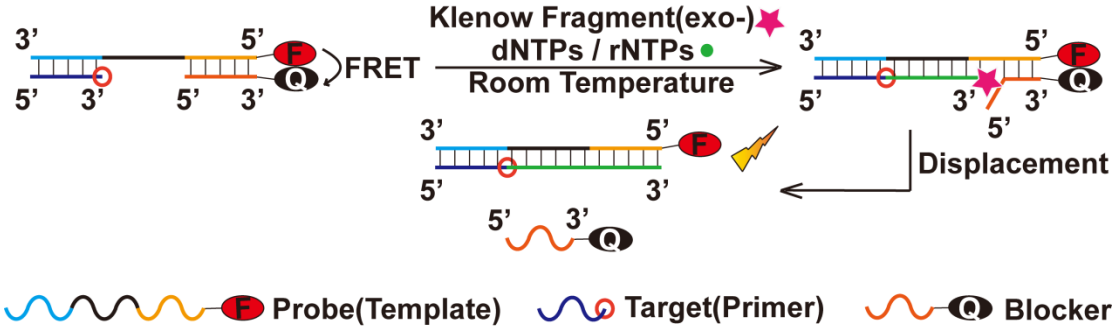

Figure S1. The mechanism of the reaction with only Klenow fragment (exo-) to measure the capability of discriminating SNP.

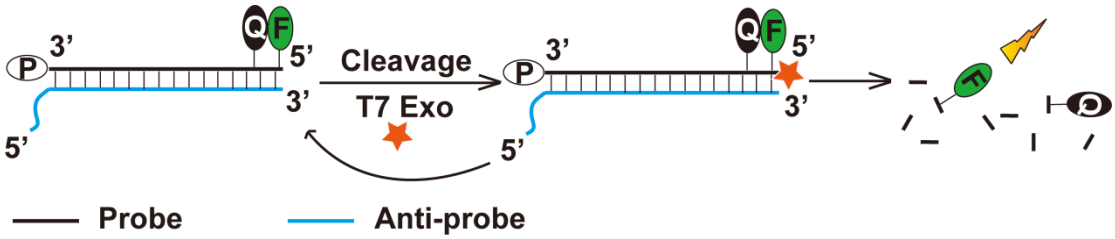

Figure S2. Optimization of the reaction conditions in T7 exonuclease-assisted target cycling process.

Table S2. Rate constants and discrimination factors of different types of single-base mismatching targets in the SNP detection with only Klenow fragment (exo-).

	Type of mismatching base pair	$k_{obs}\times10^{-3}$ (s^{-1})	Discrimination Factor [a]
Mismatch at the target terminus	C:T	11±2	101
	C:A	7.3±0.5	152
	C:C	1.5±0.2	536
Mismatch next to the target terminus	G:T	3.4±0.3	326
	G:A	6.4±0.5	173
	G:G	4±1	278

[a] $k_{obs,\ match}$ = 1.11±0.09 s^{-1}, Discrimination Factor = $\frac{\boldsymbol{k_{obs,match}}}{\boldsymbol{k_{obs,mismatch}}}$