Supplementary Information

Invasive Reaction Assisted Strand-displacement Signal Amplification for Sensitive DNA Detection

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Experimental section

Materials and instruments: 3-(morpholino) propanesulfonic acid (MOPS), Tween-20, tris base, Nonidet P40, and dithiothreitol (DTT) were purchased from Amresco Inc. (OH, USA). Dimethyl sulfoxide (DMSO) was purchased from Sigma (LO, USA). Klenow fragment exo- was obtained from New England Biolabs (MA, USA). ExTaq Hot Start Version was purchased from TaKaRa (Dalian, China). QIAamp DNA FFPE Tissue Kit was obtained from Qiagen (MD, USA). Formalin-fixed, paraffin-embedded (FFPE) specimens of non-small cell lung cancer patients were from Jinling Hospital, Medical School of Nanjing University. The usage of non-small cell lung cancer FFPE specimens was agreed by Ethics Committee of Jinling Hospital. Primers and probes were purchased from TaKaRa (Dalian, China), and listed in Table S1. All solutions were prepared in deionized and sterilized water. Other chemicals were of a commercially extra-pure grade. MJ Opticon 2 continuous fluorescence detector was purchased from MJ research corporation (USA).

Table S1. The sequences of oligonucleotides used in the study.

Name	Sequences（5′-3′）
Target-1	CAG CAG TTT GGC CCG CCC AAA ATC TGT GAT CTT GAC ATG
Up	CAT GTC AAG ATC ACA GAT TTT GGG CC
Dp	CGG GCG TTG GGC CAA ACT GCT G-P
Flap	CGG GCG TTG
MB	FAM-TCT TGG AGT CTG AAA GAG AGC AAC GCC CGG AGA CTC CAA GA-DABCYL
Primer	TCT TGG AG
PCR primer 1	GGAACGTACTGGTGAAAACACCGC
PCR primer 2	TGCATGGTATTCTTTCTCTTCCGCACC

Notes: P represents the oligonucleotide modified with phosphate.

Real sample treatment: Three to four freshly cut sections of FFPE tissue, each with a thickness of about 10 μm, were as the starting material for DNA extraction by using QIAamp DNA FFPE

Tissue Kit. The concentrations of extracted DNA were measured by UV absorption. PCR was carried out by adding 10 ng extracted DNA into PCR mixture containing 1×EX Taq Buffer, 0.25 mM dNTP, 0.4 μM PCR primer 1 and PCR primer 2, 1 U of ExTaq HS. The reaction mixture was incubated in 95 °C for 30 s and then subjected to 30 thermal cycles of 95 °C for 10 s, 60°C for 10 s. The total volume of PCR reaction was 25 μL. The amplicons were diluted 1000-fold with deionized and sterilized water as the template for invasive reaction.

Invasive reaction: The invasive reaction was carried out by adding target DNA (Target 1 in Table S1 or PCR products) into the reaction mixture containing 10 mM MOPS (pH 7.5), 0.05 % Tween-20, 0.05 % Nonidet P40, 0.1 μM upstream probe (Up in Table S1), 1 μM downstream probe (Dp in Table S1) and 7.5 mM $MgCl_2$. After 95 °C for 5 min, 100 ng of *Afu* flap endonuclease was added to the mixture and incubated at 63 °C for 2 h. The total volume of the reaction is 10 μL.

Molecular beacon assisted strand-displacement amplification: After invasive reaction, the products were added into strand-displacement reaction mixture consisting of 100 nM primer (Primer in Table S1), 100 nM molecular beacon (MB in Table S1), 2.5 U of Klenow fragment exo$^-$, 100 mM dNTPs, 6% DMSO, 1 mM DTT and 5 mM $MgCl_2$ in 50 mM Tris-HCl (pH 8.0) and incubated at 37°C. Readout was carried out at MJ Opticon 2 continuous fluorescence detector (MJ research corporation, USA).

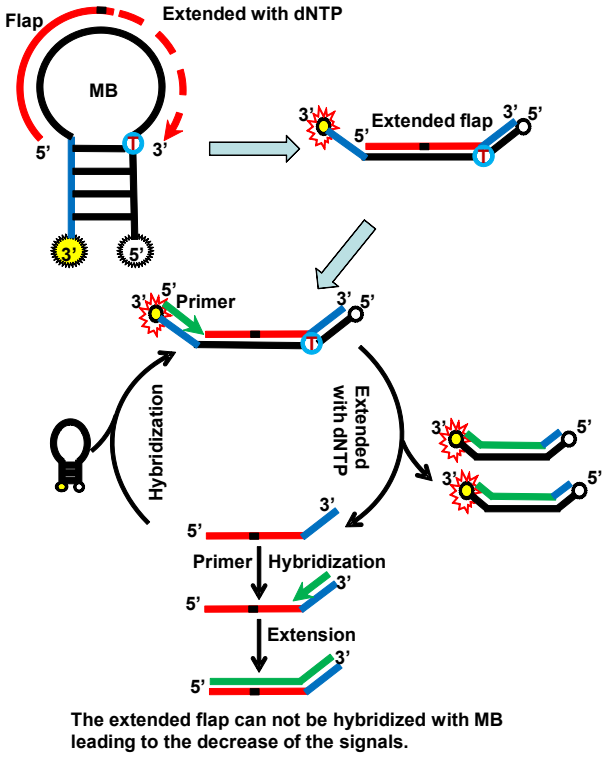

Figure S1. The illustration of strand-displacement in IRASA using four kinds of dNTPs for extension. The amplified flaps are firstly extended to the 5'-end of the beacon so that the 3'-end of extended flaps contain a fragment, which is complementary to the primer. After primer extension, the displaced strand can be extended by the primer to form double strand products, which cannot be captured by another beacon, leading to a decreased displacement-rate.

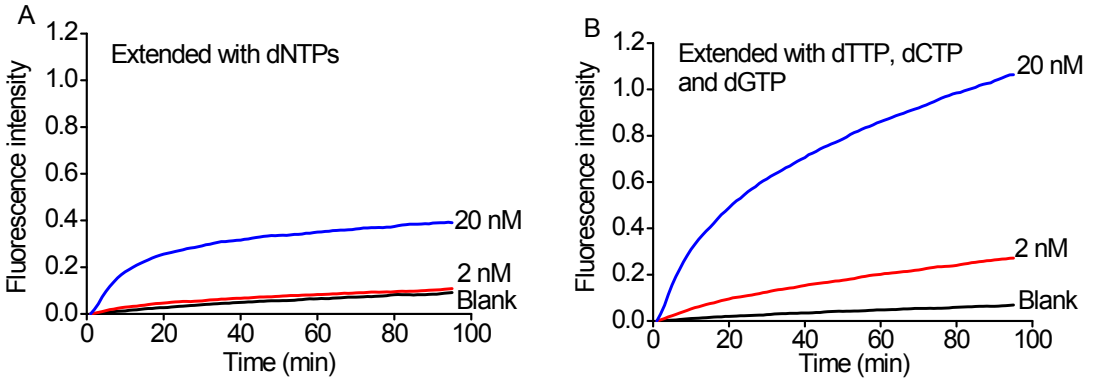

Figure S2. The time-course of IRASA for detecting 2 nM and 20 nM flaps by using dNTPs (dATP, dTTP, dCTP and dGTP) (A) and dTTP, dCTP and dGTP (B) for extension. Blank is the reaction without flaps.

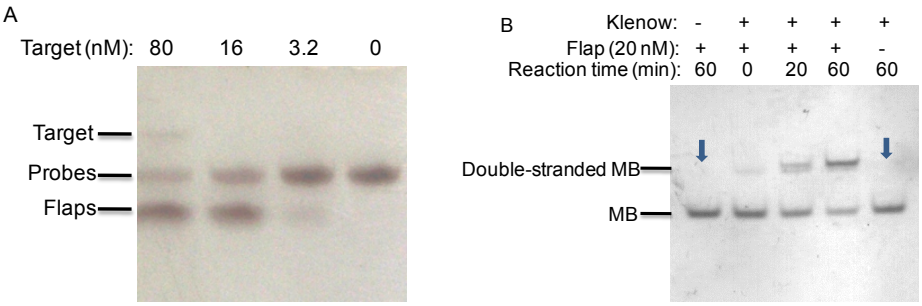

Figure S3. Denaturing PAGE image for analyzing invasive reaction products at different target-concentrations (A) and non-denaturing PAGE image for analyzing the products from the strand-displacement amplification with different components and at different reaction time (B). The band of flap was observed in the invasive cleavage products at 80, 16 and 3.2 nM targets, but not observed without target, indicating a target-specific cleavage. The amount of double-stranded MB produced by extension reaction increased along with the reaction time, but no double-stranded MB was observed without Klenow or flaps, indicating a flap-specific strand-displacement reaction.

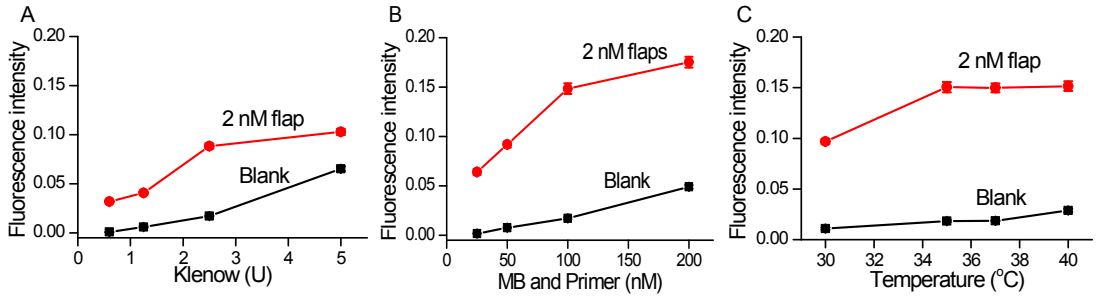

Figure S4. The fluorescence intensities of beacon assisted strand-displacement reaction for 2 nM flaps detection with different units of Klenow (A), serious of MB and primer concentrations (B), and different reaction temperatures (C). The fluorescence intensities were measured at 20-min reaction. Blank is the reaction without flaps.

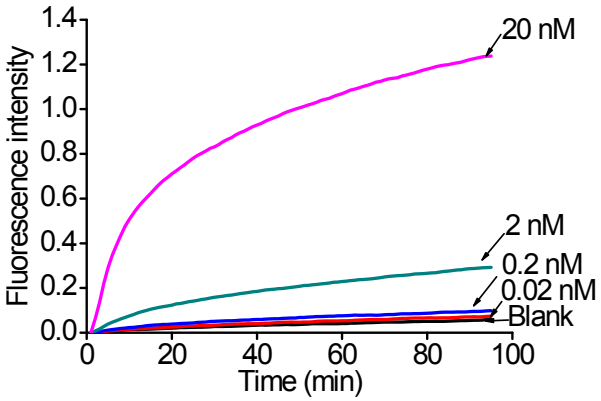

Figure S5. The time-course of beacon assisted strand-displacement reaction for detecting flaps with different concentrations (0.02, 0.2, 2, and 20 nM). Blank is the reaction without flaps.

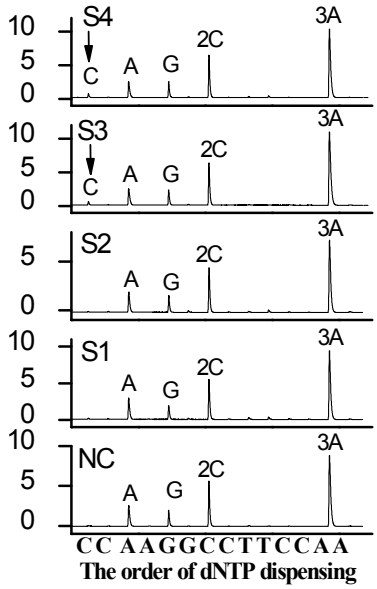

Figure S6. Pyrograms of amplicons from 4 non-small cell lung cancer FFPE specimens and one blood genome DNA of healthy person for EGFR gene c.2573T>G mutation analysis. Pyrosequencing reaction volume was 40 µl, containing 0.1 M tris-acetate (pH 7.7), 2 mM EDTA, 10 mM magnesium acetate, 0.1 mg/ml BSA, 1 mM dithiothreitol, 2 µM adenosine 5'-phosphosulfate, 0.4 mg/ml PVP, 0.4 mM D-luciferin, 2 µM ATP sulfurylase, 5.7×10^8 RLU QuantiLum recombinant luciferase, 18 U/ml Klenow Fragment, and 1.6 U/ml apyrase, The sequencing primer was 5'- GCA CCC AGC AGT TTG GCC-3'. The black arrow marks the mutant base.