Supporting Information

PCR Free Multiple Ligase Reactions and Probe Cleavages for SNP Detection of KRAS Mutation with Attomole Sensitivity

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Synthesis of gold nano particles

Au nanoparticles (AuNP) (~ 13 nm) were synthesized by citrate reduction¹ and phosphine capping.² Briefly, a 500 ml aqueous solution of 1 mM HAuCl₄ (Sigma Aldrich)was prepared and brought to reflux under vigorous stirring to which 50 mL of 38.8 mM trisodium citrate was added. Heat removal after 15 minutes was followed by allowing the solution to cool to room temperature. BSP (bis (*p*-sulfonatophenyl) phenylphosphine dihydrate, dipotassium salt. Sterm Chemicals, 150 mg) was added over a period of 5 minutes followed by overnight stirring. Small amounts of a 2 M NaCl solution were added to precipitate the particles. After centrifuging, the solids were washed with 250 mM NaCl and brought up in 0.3 mM BSP in H₂O (~100 nM AuNP). Salt precipitation and NaCl solution wash was repeated, and then the solids were redispersed at 400 nM AuNP (using extinction coefficient at 522 nm of 2.43 x 10⁸ M⁻¹ cm⁻¹) in 10 mM sodium phosphate buffer (pH 7.0). The size and monodispersity of the gold particles were confirmed by performing Transmission Electron Microscopy with JEM 1230 (JEOL).

Modification of gold nano particles with SDH

Thiols of SDH (Refer to Table S1 for the detail of the sequence) were activated by treatment with 10 mM TCEP (Sigma Aldrich) in 10 mM sodium phosphate buffer (pH 7.0) for 30 minutes at room temperature. SDH (4 μ M, 200-fold molar excess) were mixed with 20 nM of AuNPs in 1 mL of buffer for 16 hours at room temperature. To increase the density of SDH coverage on the gold surface, the NaCl concentration was gradually increased up to 0.1 M. After an additional 48 hours, the excess free SDH was removed by centrifugation at 14,000 rpm for 25 minutes in the same buffer, repeated four times. The SDH-modified AuNPs were redispersed in 200 μ L of the phosphate buffer containing 0.1 M NaCl at room temperature

Name	Sequence
P1	5'-CG <u>TACGCCAT</u> -3'
P2	5'OP- <u>CAGCTCCA</u> ACA -3'
SDH	5'(SH)-TGTTGgagcugauggCGTACGTTTTTTTTccaucagc-
(for AuNP immobilization)	(FITC)-3'
Mutant target	5'-TGGAGCTGATGGCGTA-3'
Wild target	5'-TGGAGCTGGTGGCGTA-3'

Table S1, Sequences of oligonucleotides used in the assay

Sequences of P1 and P2 which are complementary to the target sequence are underlined. Lower letters of SDH indicates ribonucleic acid. 35G>A point mutation in the mutant target is the bold fond within the sequences.

Thermal analysis of oligonucleotides

Melting experiments were conducted on a Carry 500 spectrophotometer with a T_m Analysis accessory, and 1 cm path length quartz cuvettes .Temperature was increased by 0.5 °C increments from 25 °C to 90 °C with each step lasting 10 min. Absorbance values at 260 nm were recorded every 0.5 °C. Accuracy of temperature measurements inside of the cuvettes was validated using an external probe with a thermistor thermometer. Before thermal analysis, all the samples were heated to 95 °C lasting for 10 min and then cooled to room temperature slowly.

Gel electrophoresis of oligonucleotides

Electrophoresis of the used oligonucleotides (Table S1) and ligated products was performed by loading the samples into TBE polyacrylamide gel (4~20%) and running the gel at 180 V for 50 min. After electrophoresis, the gel was stained in 1× TBE buffer (pH 8.0) containing 1× SYBR Green I Nucleic Acid Gel Stain (Thermo Fisher Scientific, USA) for 40 min. UV excited fluorescence images of the stained gel were taken using a Davinchi-Gel Imaging System (Davinchi-K, South Korea)

Isothermal Ligase Reaction

For isothermal cyclic ligation of primers, P1 (Table S1) and P2 (Table S1), *E.coli* DNA ligase (0.12 unit /µl in final volume) was added into a solution containing P1 (100 nM), P2 (100 nM), and SDH modified AuNPs (2nM) in 100 µl reaction buffer containing 30 mM Tris-HCl (pH 8.0), 4 mM MgCl₂, 10 mM, (NH₄)₂SO₄, 100 µM NAD and 0.005% BSA in the



Fig. 5 Detection of the point mutation 35 G>A in the KRAS gene. (A) Fluorescence spectra and (B) S/N ratios of the fluorescence amplified products that were isothermally ligated with the SDH in the presence of the mutant target and the wild type target.

presence of various amount of target. After incubation of the mixture for 1 hr at 25 °C, the reaction was stopped by keeping the solution for 10 min at 65 °C

Fluorescence Amplification through Modified Cycling Probe Assay

Fluorescence amplification with the isothermally ligated products were accomplished by performing the modified cycling probe assay (MCP assay). The MCP assay was carried out by addition RNase H, KCl and MgCl₂ into the ligated product . Final concentration of RNase H, KCl and MgCl₂ is 0.043 unit/µl, 75 mM, and 8 mM MgCl₂ respectively in 200 µl of the reaction volume. After keeping the mixture at 37 °C for 1 hr. Fluorescence of each sample was measured using a FluoroMax (Horiba).

References

1. C. K Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, Anal. Chem., 1995, 67, 735.

2. G. Schmid and A. Lehnert, Angew. Chem. 2006, 118, 3381-3384, 1989, 28, 780.