Supplementary for:

Simultaneous Identification of Point Mutations via DNA Ligase-mediated Gold Nanoparticle Assembly

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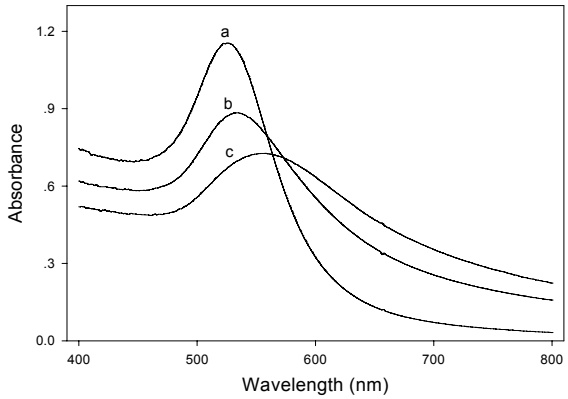

Figure S1 UV-vis spectra of the reaction mixture. Curve a, before hybridization; curve b, after incubating at 55°C for 60 min; curve c, after incubating at 40°C for another 60 min. The reaction mixture was composed of 208 nM of T4, 242 nM of T2 and 75 μL of P1, P2, P3 and P4 each. The hybridization of T2 and T4 with gold nanoparticle-tagged probes was carried out in 0.3 M KCl - 0.1% (v/v) Triton X-100 - 30 mM Tris-HCl solution (pH 7.8).

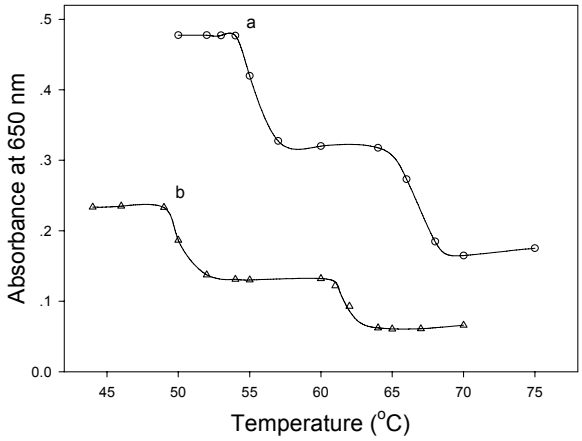

Figure S2 The melting analysis of nanoparticle aggregates in the 0.3 M KCl and 0.1 M KCl solution, respectively. Treatment of samples: for curve a, the reaction mixture containing 75 μL of each gold probe (P1, P2, P3, P4) and 208 nM of T4 and 242 nM of T2 was first incubated at 55°C for 60 min, and then at 40°C for another 60 min; for curve b, 100 μL of the reaction mixture described in curve a was diluted to 300 μL with a 0.1% (v/v) Triton X-100 - 30 mM Tris-HCl dilution buffer (pH 7.8). Absorbance values at 650 nm were recorded at 1°C interval with a holding time of 0.5 min/deg.

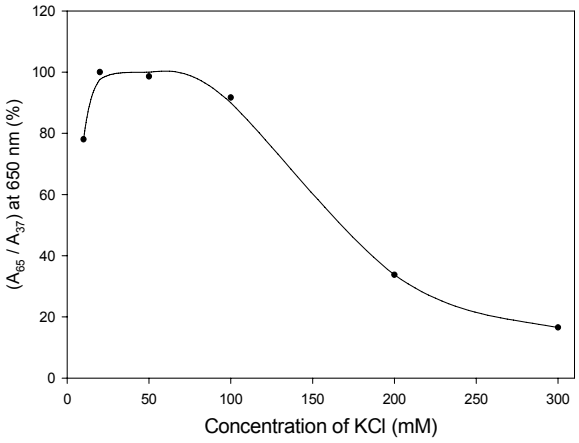

Figure S3 The effect of K^+ concentration on the ligation efficiency. Treatment of samples: the reaction mixture containing P3 (5'-SH-$(CH_2)_6$-cgttactgccctgtggggca-3'), P4 and T3 was incubated at 55 °C for 60 min. Then the solution was diluted to 600 μL with the concentration of 0.032 U/ μL *E. coli* DNA ligase, 4 mM Mg^{2+}, 0.05% BSA, 0.1 mM NAD^+ and different K+ concentrations. After ligation at 37 °C for 30 min, the reaction mixture was heated to denature the formed DNA-linked aggregates at 65 °C. The absorbance at 650 nm was recorded before (A_{37}) and after (A_{65}) denaturing. To quantitatively measure the efficiency of the ligase in such optimization experiments, one could use the ratio (in percent) of the absorbance of the ligase-treated nanoparticle aggregate solution after heat denaturing at 65 °C to initial absorbance of the solution at 650 nm. That is, the ligase efficiency is defined as $\frac{A_{65}}{A_{37}} \times 100\%$, where A_{37} is the absorbance measured at 37 °C for the nanoparticle aggregate solution of target T3 with probes P3 and P4 subjected to the ligase reaction, and A_{65} is the absorbance of the solution on heat denaturing at 65 °C.This ligase efficiency measure is based on the fact that, when the ligase reaction is performed for all perfectly matched duplex, the oligonecleotide-labeled gold nanoparticles are covalently linked together and would not melt apart and show no absorbance decrease on denaturing treatment.

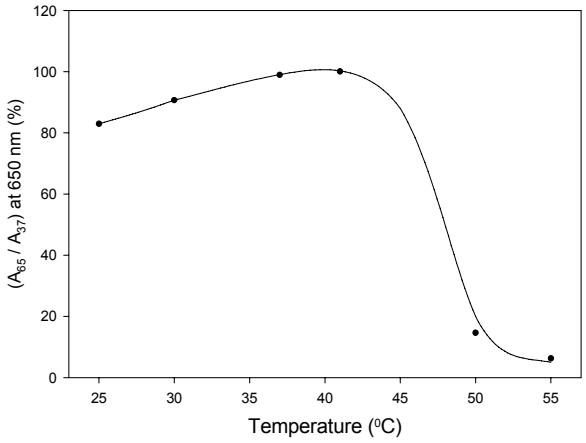

Figure S4 The effect of temperature on the ligation efficiency. Treatment of samples: the reaction mixtures containing P3 (5'-SH-$(CH_2)_6$-cgttactgccctgtggggca-3'), P4 and T3 were incubated at 55 °C for 60 min. Then these solutions were diluted to 600 μL with the concentration of 0.032 U/ μL *E. coli* DNA ligase, 4 mM Mg^{2+}, 0.05% BSA, 0.1 mM NAD^+ and 50 mM K^+. After ligation at different temperature for 30 min, the experiments were carried out as described in Figure S2.

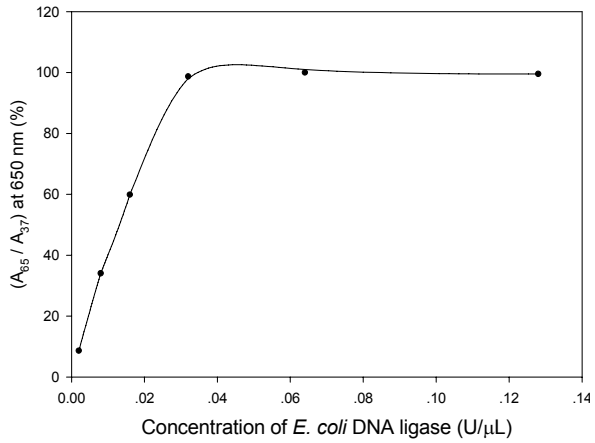

Figure S5 The effect of concentration of *E. coli* DNA ligase on the ligation efficiency. Treatment of samples: the reaction mixtures containing P3 (5'-SH-$(CH_2)_6$-cgttactgccctgtggggca-3'), P4 and T3 were incubated at 55 °C for 60 min. Then these solutions were diluted to 600 μL with the concentration of 4 mM Mg^{2+}, 0.05% BSA, 0.1 mM NAD^+, 50 mM K^+ and different *E. coli* DNA ligase concentration. After ligation at 37 °C for 30 min, the experiments were carried out as described in Figure S2.

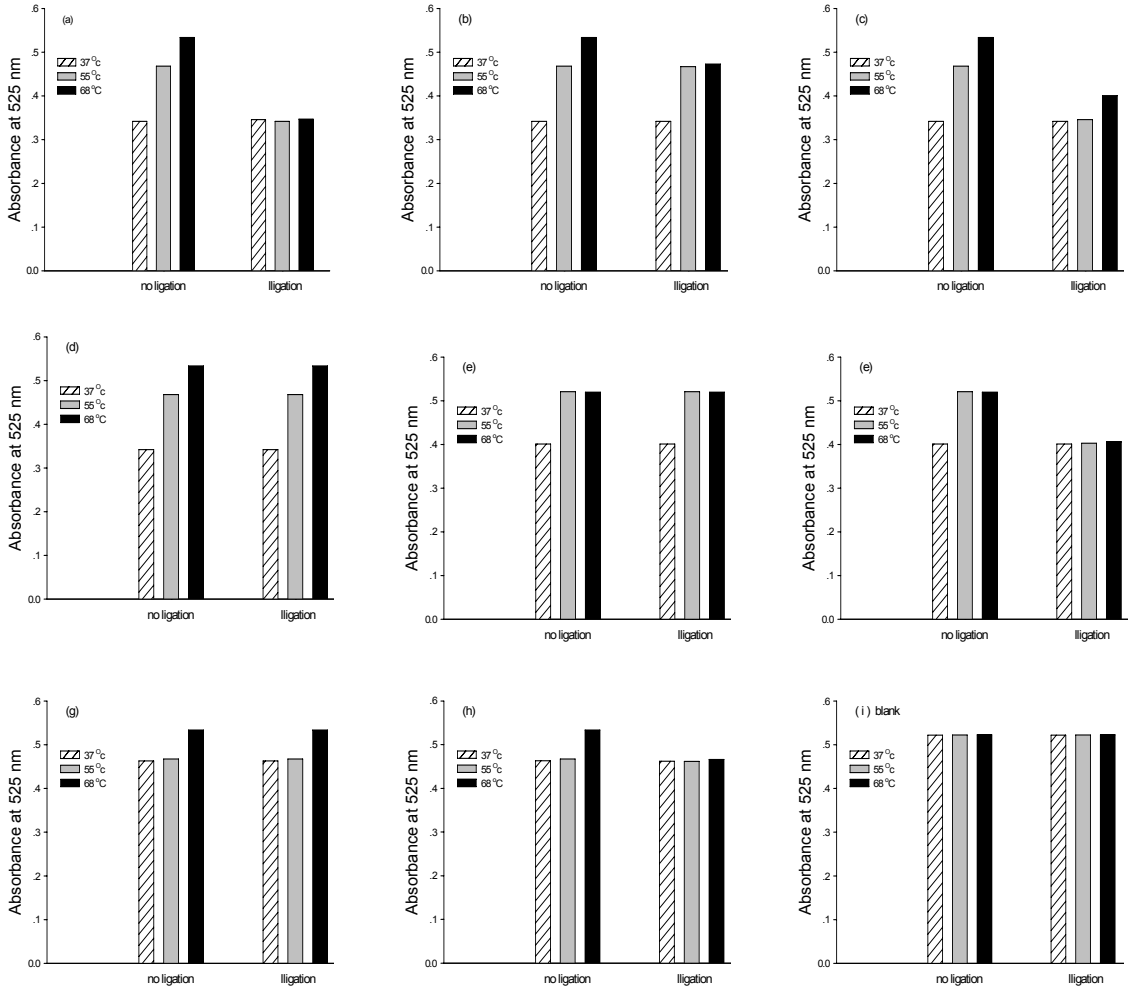

Figure S6 Ligation analysis of perfect-match or mismatch targets using Au nanoparticle probes. Treatment of samples: the reaction mixture contains 25 μL of each gold probe (P1, P2, P3, P4). (a), 121 nM of T2 + 104 nM of T4; (b), 121 nM of T1 + 104 nM of T4; (c), 121 nM of T2 + 104 nM of T3; (d), 121 nM of T1 + 104 nM of T3; (e), 121 nM of T1; (f), 121 nM of T2; (g), 104 nM of T3; (h), 104 nM of T4; (i), blank. The reaction mixtures were first incubated at 55 °C for 60 min, and then at 40 °C for another 60 min. Then these solutions were diluted to 300 μL with the concentration of 0.12 U/ μL E.coli DNA ligase, 4 mM Mg^{2+}, 0.05% BSA, 0.1 mM NAD^{+} and 100 mM K^{+}. After ligation at 37 °C for 30 min, the melting analysis was carried out. For 'no ligation', the reaction mixture contained DNA target, but the ligase was not added; for 'ligation', the reaction mixture contained DNA target, moreover, the ligation process was carried out.

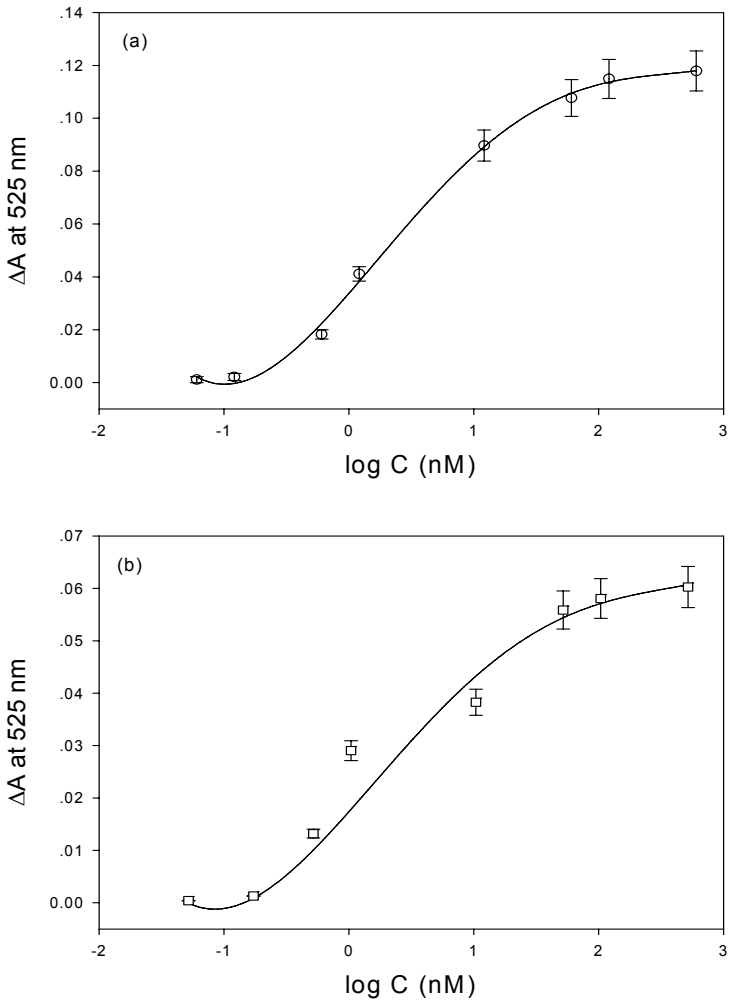

Figure S7 Quantitative analysis of the targets gene concentration using UV-vis spectrophotometry. For figure 3s (a) (or figure 3s (b)), the reaction mixture containing a certain concentration of T2 (or T4) and 25 μL of each gold probe (P1, P2, P3, P4) was first incubated at 40°C (or 55 ^{o}C) for 60 min, and then the solution was diluted to 300 μL with the concentration of 0.12 U/ μL *E. coli* DNA ligase, 4 mM Mg^{2+}, 0.05% BSA, 0.1 mM NAD^{+} and 0.1 M K^{+}. After ligation at 37°C for 30 min, the reaction mixture was heated to denature the formed DNA-linked aggregates at 55°C (or 68^{o}C). The absorbance at 525 nm was recorded before (37^{o}C) and after (55^{o}C or 68^{o}C) denaturing. The standard deviations obtained by five repeated measurements were shown as the error bars.

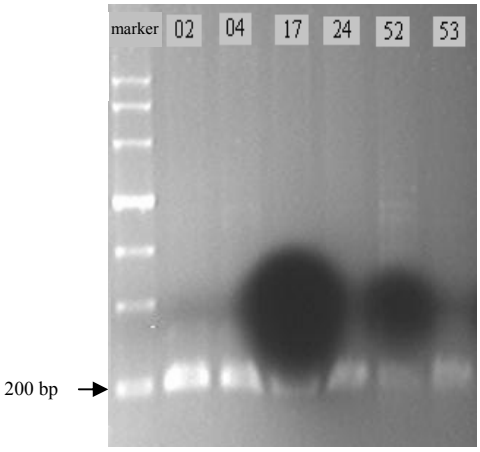

Figure S8 The sol-gel image of PCR products. 1.2% TBE Agarose Gel. Sample 02, 2 years old; sample 04, 4 years old; sample 17, 17 years old; sample 24, 24 years old; sample 52, 52 years old; sample 53, 53 years old.

The GenBank reference sequence (accession number: AY128650) :

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