

# A colorimetric method for point mutation detection using high-fidelity DNA ligase

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## ABSTRACT

The present study reported proof-of-principle for a genotyping assay approach that can detect single nucleotide polymorphisms (SNPs) through the gold nanoparticle assembly and the ligase reaction. By incorporating the high-fidelity DNA ligase (Tth DNA ligase) into the allele-specific ligation-based gold nanoparticle assembly, this assay provided a convenient yet powerful colorimetric detection that enabled a straightforward single-base discrimination without the need of precise temperature control. Additionally, the ligase reaction can be performed at a relatively high temperature, which offers the benefit for mitigating the non-specific assembly of gold nanoparticles induced by interfering DNA strands. The assay could be implemented via three steps: a hybridization reaction that allowed two gold nanoparticle-tagged probes to hybrid with the target DNA strand, a ligase reaction that generates the ligation between perfectly matched probes while no ligation occurred between mismatched ones and a thermal treatment at a relatively high temperature that discriminate the ligation of probes. When the reaction mixture was heated to denature the formed duplex, the purple color of the perfect-match solution would not revert to red, while the mismatch gave a red color as the assembled gold nanoparticles disparted. The present approach has been demonstrated with the identification of a single-base mutation in codon 12 of a K-ras oncogene that is of significant value for colorectal cancers diagnosis, and the wild-type and mutant type were successfully scored. To our knowledge, this was

the first report concerning SNP detection based on the ligase reaction and the gold nanoparticle assembly. Owing to its ease of operation and high specificity, it was expected that the proposed procedure might hold great promise in practical clinical diagnosis of gene-mutant diseases.

## INTRODUCTION

Single nucleotide polymorphisms (SNPs) are the most abundant form of genetic variation and occur once every 100–300 bases (1). Many pathogenic and genetic diseases are associated with changes in the sequence of particular genes (2). Identification of these single-base mutations can realize the medical diagnosis of the diseases. Up to now, many techniques have been developed for SNP detection. Conventional procedures involved the implementation of mass spectrometry or gel electrophoresis for the discrimination of fragments produced by endonuclease cleavage (3,4). These methods are time-consuming and of relatively high cost. Other approaches for SNP detection are primarily built on the following three mechanisms for the allele-specific discrimination. One is to inspect the thermal stability of the hybridized product via precise temperature control. Typical examples include the allele-specific DNA microarray (5,6) and the allele-specific molecular beacon assay (7,8). Another is built on the allele-specific extension or exonuclease cleavage mediated by the DNA polymerase. Methods of this kind are exemplified by the allele-specific TagMan assay (9) and the template-directed dye terminator incorporation assay (10). The third bases the single-base discrimination on the allele-specific ligation reaction mediated by the DNA ligase. Methods of this type comprise ligase detection reaction (11–13) and single-pair fluorescence resonance energy transfer detection (2). Among the existing

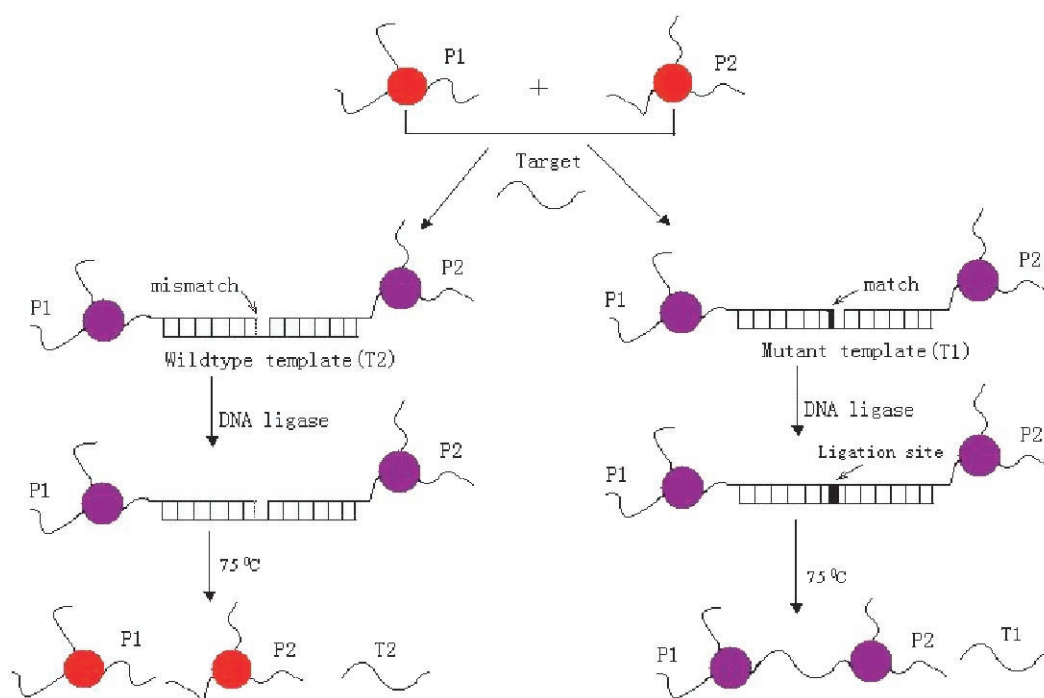
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methods, the DNA enzyme-based approaches have attracted increasing interest due to its cost efficiency, ease of operation and rapidness of implementation. Moreover, the incorporation of DNA enzymes offers additional advantages of high fidelity and improved sensitivity.

Recently, Mirkin and co-workers (14,15) reported a novel and elegant method for detecting polynucleotides that utilized the distance-dependent optical properties of aggregated Au nanoparticles functionalized with oligonucleotides. They also discussed the melting properties of DNA-linked gold nanoparticle assemblies containing mismatch, deletion or insertion, and explored the possibility of discrimination of gene mutation (16). This system was exploited by Murphy *et al.* (17) for the selective colorimetric discrimination of a cystic fibrosis-related genetic mutation. Rothberg *et al.* (18) showed that after single-stranded DNA adsorbed on negatively charged gold nanoparticles, the particles could be stabilized against salt-induced aggregation. The phenomenon formed the basis for a colorimetric assay to identify specific sequences and SNPs (18). Also based on the optical properties of aggregated Au nanoparticles, Storhoff and co-workers (19) developed a scatter-based 'spot-and-read' method for ultrasensitive detection of DNA target of zeptomole quantities. Later, they coupled this scatter-based method with a further silver amplification step for the discrimination of SNP (20,21). However, all these newly emerging methods were based on the melting temperature difference of the duplex between the allele-specific probe and the DNA target, which involved stringency temperature control, thereby requiring skilled personnel and relatively long analysis time. Therefore, it might be inconvenient for these techniques in real-world clinical applications.

Furthermore, they might be difficult for the discrimination of single-base imperfections that exhibited insignificant melting temperature difference.

In this paper, we described a novel colorimetric detection system based on a high-fidelity Tth DNA ligase (13,22,23), as the basis of a rapid, highly specific and practical approach capable of detecting point mutation in clinical diagnosis. The detection scheme is illustrated in Scheme 1. This assay uses an allele-specific discriminating probe and a common probe, both immobilized on the surface of gold nanoparticles by strong sulfur–Au adsorption. Hybridization of the target strand with the probes results in the formation of an extended polymeric Au nanoparticle–polynucleotide aggregate, triggering a red to purple color change in the solution. A perfect match between the base at the 3' end of the discriminating probe and the target allows the ligase to covalently join the two adjacent probes that flanked the mutation site, while a mismatch does not. When the reaction mixture is heated to denature the duplex formed, the purple color of the perfect-match solution does not revert to red, while the mismatch remains in red color as the assembled gold nanoparticles dispart. Then, the single-point mutation is discriminated via direct visual colorimetry or ultraviolet spectrophotometric measurements. Compared with existing techniques based on melting temperature differentiation, this proposed method offers the advantage for straightforward single-base discrimination without the need of precise temperature control. In addition, the thermally stable ligase enables the hybridization reaction to be performed at a relatively high temperature, ensuring the non-specific assembly of gold nanoparticles induced by interfering DNA strands could be maximally mitigated.



**Scheme 1.** Illustration of the Au nanoparticle assembly and ligase reaction-based assay. The Au nanoparticles were chemically modified with 5'- or 3'-(alkanethiol)-capped oligonucleotides (primer). These primers flank a single-base mutation on the target template. High-fidelity DNA ligase (Tth DNA ligase) covalently joins the two adjacent primers when perfectly matched to the template, resulting in a purple color after annealing at 75°C. Conversely, for the unligated primers, a red color of separated gold nanoparticles was observed. Only two particles are shown aligning on a target strand, in reality, large extended networks of Au nanoparticles are formed.

**Table 1.** Oligonucleotides synthesized in this experiment<sup>a</sup>

Probe 1 (P1)	5'-HS-(CH <sub>2</sub> ) <sub>6</sub> - TAAAA CTT GTG GTA GTT GGA GCT G[A]-3'
Probe 2 (P2)	5'-pT GGC GTA GGC AAG AGT GCC CT-(CH <sub>2</sub> ) <sub>3</sub> -SH-3'
Probe 3 (P3)	3'-(SH)-(CH <sub>2</sub> ) <sub>3</sub> -TCC GAT GGC TCC GAG GTC GAA-(FITC)-5'
Probe 4 (P4)	5'-HS-(CH <sub>2</sub> ) <sub>6</sub> -TAAAA CTT GTG GTA GTT GGA GCT G[T]-3'
Target 1 (T1)	3'-TTTT GAA CAC CAT CAA CCT CGA C[T]A CCG CAT CCG TTC TCA CGG G-5'
Target 2 (T2)	3'-TTTT GAA CAC CAT CAA CCT CGA CCA CCG CAT CCG TTC TCA CGG G-5'

<sup>a</sup>The target and probe used in this paper (except P3) were designed according to ref. (2) with some modifications. The designed DNA sequences were used for detection of point mutation in codon 12 of the K-ras gene associated with colorectal cancer. For this particular mutation, the second position in codon 12, GGT, coding for glycine, mutates to GAT coding for aspartate or GTT coding for valine. The circled base in P1, P4 and T1 indicate the mutant base. p in P2 represents phosphate at 5'. FITC in P3 is fluorescein iso-thiocyanate.

## MATERIALS AND METHODS

### Oligonucleotides and reagents

All oligonucleotides (Table 1) were purchased from Takara Biotechnology Co., Ltd (Dalian, China) and were used as received. Thermal ligase (Tth DNA ligase) was from ABgene (UK).  $\beta$ -Nicotinamide adenine dinucleotide (oxidized, NAD<sup>+</sup>) was from ICN (Germany). Tris was from Roche. Trisodium citrate, Triton X-100, NaCl, KCl and MgCl<sub>2</sub> were purchased from Amresco (Solon, OH). Deionized water was obtained through a Nanopure Infinity ultrapure water system (Barnstead/thermolyne Corp, Dubuque, IA) and had an electric resistance >18.3 M $\Omega$ . Gold nanoparticles with ~13 nm diameter were prepared following literature procedures (16). The size of gold nanoparticles was verified by TEM (Hitachi TEM 800, Japan).

### Preparation of 3'- or 5'-(alkanethiol)oligonucleotide-modified Au nanoparticles

Gold nanoparticle probes were prepared following literature procedures with minor modifications (24). Briefly, 2.5 OD of (alkanethiol)oligonucleotide (P1, 4 nmol/OD; P2, 4.76 nmol/OD) was added to 5 ml of 13 nm Au nanoparticle solution. After ~16 h, the colloid solution was brought to 10 mM in phosphate (NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>) by adding 0.1 M pH 7 concentrated buffer. In the subsequent salt aging process, colloids were first brought to 0.05 M in NaCl by dropwise addition of 2 M NaCl solution and allowed to stand for 8 h, salted to 0.1 M and allowed to age for another 8 h, then salted to 0.2 M for standing 8 h, and finally salted to 0.3 M in NaCl. To remove excess thiol-DNA, the solution was centrifuged for 25 min at 15 000 r.p.m. 16106  $\times g$ . Following removal of the supernatant, the red oily precipitate was then washed with a solution 0.3 M in KCl, and 20 mM in Tris-HCl (pH 8.3). After being washed two times, the colloid was redispersed in 5 ml of 0.3 M KCl-0.1% (v/v) Triton X-100-20 mM Tris-HCl (pH 8.3) solution, and stored in a refrigerator (4°C).

### Point mutation detection

In a typical experiment, appropriate oligonucleotide target was added to 100  $\mu$ l of gold probe solutions containing 50  $\mu$ l of each gold probe. After mixing, the solutions were heated to 65°C for 60 min and then were diluted to 300  $\mu$ l with a ligation buffer containing 0.225 U/ $\mu$ l Tth DNA ligase, 1.5 mM NAD<sup>+</sup>, 15 mM MgCl<sub>2</sub>, 0.1% (v/v) Triton X-100 and 20 mM Tris-HCl (pH 8.3). The mixtures were heated to 65°C for 30 min and then were cooled rapidly to 4°C and 3  $\mu$ l of 1.05 M Trisodium

citrate was added. Finally, after 5 min of denaturing at 75°C, the solutions were rapidly immersed in ice water and then colorimetric or UV-vis (Mutispec-1501 Shimadzu Hyper UV equipped with a EYEL4 Water Bath, Japan) analysis was performed.

### Genomic DNA extraction from cell lines

Genomic DNA was extracted from cell lines HT29 (wild type) and SW480 (GTT mutation). The cell lines were cultured at 37°C in RPMI 1640 (GIBCO BRL) supplemented with 10% fetal calf serum (FCS) and 100 U/ml penicillin/streptomycin. Harvested cells (about 10<sup>7</sup>) were suspended in DNA extraction buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, pH 8.0) with 0.5% SDS and 200  $\mu$ g/ml proteinase K and incubated at 37°C for 24 h. The samples were centrifuged. Three volumes of EtOH were then added to precipitate DNA from the supernatant. The precipitated DNA was washed with 70% EtOH and resuspended in TE buffer (10 mM Tris-HCl, pH 7.2, 2 mM EDTA, pH 8.0).

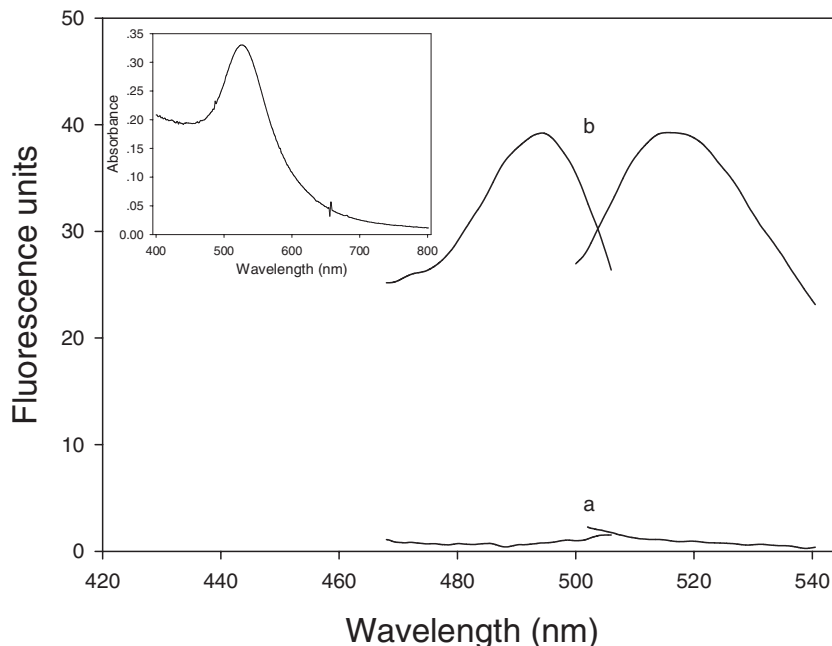
### PCR amplification of genomic DNA

PCR amplification was performed in 50  $\mu$ l of 10 mM Tris-HCl buffer (pH 8.3) with 10 mM KCl, 4.0 mM MgCl<sub>2</sub>, 250  $\mu$ M dNTPs, 1  $\mu$ M forward and reverse primers (50 pmol for each primer), as well as ~40 ng of genomic DNA extracted from the cell lines. The following primers were used: Ex.1.3 forward: 5'-AACCTTATGTGTGACATGTTCTAATATAGTCAC-3'; Ex.1.4 reverse: 5'-AAAATGGTCAGAGAAACCTTTATCTGTATC-3' (2). Amplification was achieved by thermal cycling for 40 cycles at 95°C for 30 s, 60°C for 30 s, 72°C for 1 min and a final extension at 72°C for 10 min. PCR products were purified by Agar Gel DNA Purification Kit (GIBCO BRL).

## RESULTS AND DISCUSSION

### Preparation and properties of Au nanoparticles modified with alkanethiol-capped oligonucleotides

Gold nanoparticles (~13 nm) were chemically modified with 5'-alkylthiol-capped 25-base or 3'-alkylthiol-capped 21-base oligonucleotides. These oligonucleotide-modified nanoparticles exhibited similar optical properties to those reported in the literature (15,16). After modification, only a slight shift in the surface plasmon band from 520 to 525 nm was observed. This shift might not be due to surface modification, rather than the effect of centrifugation on the particle size distribution or



**Figure 1.** The UV-vis spectra of P3-modified Au nanoparticles in aqueous solution (inset:  $\lambda_{\text{max}} = 525 \text{ nm}$ . According to literature (27), the  $\epsilon$  is  $\sim 6 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$  per particle. The Au nanoparticle concentration is about 0.55 nM. The surface area of 13 nm-Au nanoparticle is then estimated to be  $\sim 5.3 \times 10^{-12} \text{ cm}^2$ ) and their fluorescence spectra before (curve a) and after (curve b) the Au nanoparticles were dissolved using a solution of KCN +  $\text{K}_3\text{Fe}(\text{CN})_6$  (28). The detection of fluorescence for curve b, 20  $\mu\text{l}$  of 0.2 M KCN–2 mM  $\text{K}_3\text{Fe}(\text{CN})_6$  was added into 100  $\mu\text{l}$  of P3-modified Au nanoparticle solution. The concentration of FITC-modified P3 was determined as 34.6 nM using fluorescence standards.

other factors (16). The surface coverage of probe DNA could be estimated using probe 3 (Table 1). The P3-modified nanoparticles were obtained as described in Materials and Methods. The UV-vis spectra of P3-modified Au nanoparticles in aqueous solution and its fluorescence before and after the Au nanoparticles dissolved by use of a solution of KCN and  $\text{K}_3\text{Fe}(\text{CN})_6$  are shown in Figure 1. One observes that there is no significant fluorescence for the P3-modified Au nanoparticles, while the fluorescence is substantially enhanced as the Au nanoparticles are dissolved. This is due to the fact that the fluorescence of fluorescein iso-thiocyanate (FITC) is not only strongly quenched by the Au nanoparticles and the dye molecules themselves (self-quenching), but also abated by the inner filtration effect of the Au nanoparticle solution. Based on the calculated surface area of Au nanoparticles and the determined concentration of FITC-modified P3, the number of oligonucleotides attached to an individual nanoparticle was estimated from the experimental data as  $\sim 60$ , i.e. the surface coverage of probe DNA is  $\sim 1.25 \times 10^{13} \text{ strand/cm}^2$ .

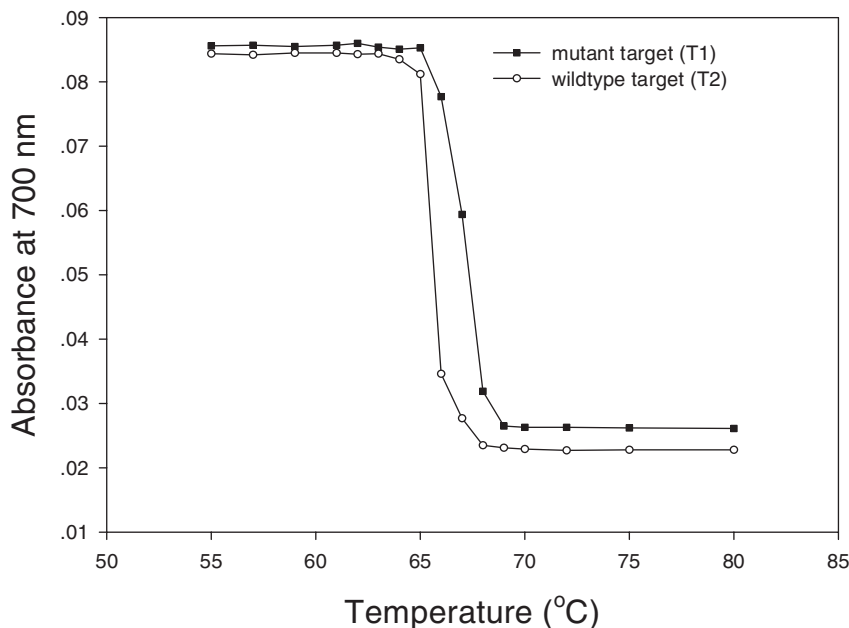
#### Hybridization of wild-type or mutant template with Au nanoparticle probes

The salt concentration is one of important factors affecting the hybridization of the template with Au nanoparticle probes. When the hybridization was performed in a solution containing 0.1 M KCl, 0.1% (v/v) Triton X-100 and 20 mM Tris–HCl (pH 8.3) at 65°C, even after 24 h, the color or the UV-vis spectra of the mutant template and the wild-type template did not noticeably change (the UV-vis spectra at 25°C were similar to that at 65°C, data not shown). It was shown that the hybridization of template with Au nanoparticle probes could

not be carried out under these conditions. As the KCl concentration was increased to 0.3 M, only after 60 min, the solutions color changed to purple. This color change might be attributed to the formation of large DNA-linked 3D aggregates of Au nanoparticles, which led to a red shift in the surface plasmon resonance. Detailed explanation about this phenomenon can be found in the literature previously reported (16,24). Therefore, throughout the experiments the hybridization was performed under 65°C in 0.3 M KCl Tris–HCl buffer.

#### Melting analysis

The thermal ligase was shown to have the optimal nick-closing activity at a temperature about 65°C (13). Therefore, it is essential to ensure the duplex formed between the nanoparticle-tagged probes and the template does not dissociate at such a temperature. Otherwise, the ligation reaction would not be taking place. So, the melting analysis of the duplex was investigated. This was performed using a Mutispec-1501 Shimadzu Hyper UV equipped with a EYEL4 Water Bath. It is observed that the spectra of Au nanoparticle aggregates show a substantial alteration at  $\sim 68$  and 66°C, respectively, for the perfectly matched and single-base mismatched duplex. A detailed melting analysis at 700 nm (16) gave more straightforward evidence. As shown in Figure 2, the melting curve clearly reveals that the melting temperatures ( $T_m$ ) for the perfect match and the single-base mismatch are  $\sim 68$  and 66°C, respectively, indicating that the duplex formed between the nanoparticle-tagged probes and the template does not dissociate at 65°C. Therefore, the ligation temperature was set to 65°C in the present colorimetric detection procedure.



**Figure 2.** Comparison of the normalized thermal dissociation curves for Au nanoparticle probes (P1 and P2) with a fully complementary mutant template (T1) and a wild-type target containing a single-base mismatch (T2). Treatment of samples: 2  $\mu\text{l}$  of 13.65 pmol/ $\mu\text{l}$  T1 or 11.35 pmol/ $\mu\text{l}$  T2 was added to 100  $\mu\text{l}$  of gold probe solutions containing 50  $\mu\text{l}$  of each gold probe. After mixing, the solutions were heated to 65°C for 60 min and then were diluted to 300  $\mu\text{l}$  with a 0.1% (v/v) Triton X-100–20 mM Tris–HCl dilution buffer (pH 8.3). Subsequently, the melting analysis was carried out. Absorbance values at 700 nm were recorded at 1°C interval with a holding time of 0.5 min/deg from 25 to 80°C. Here, only the region from 55 to 80°C is shown.

### Probe design

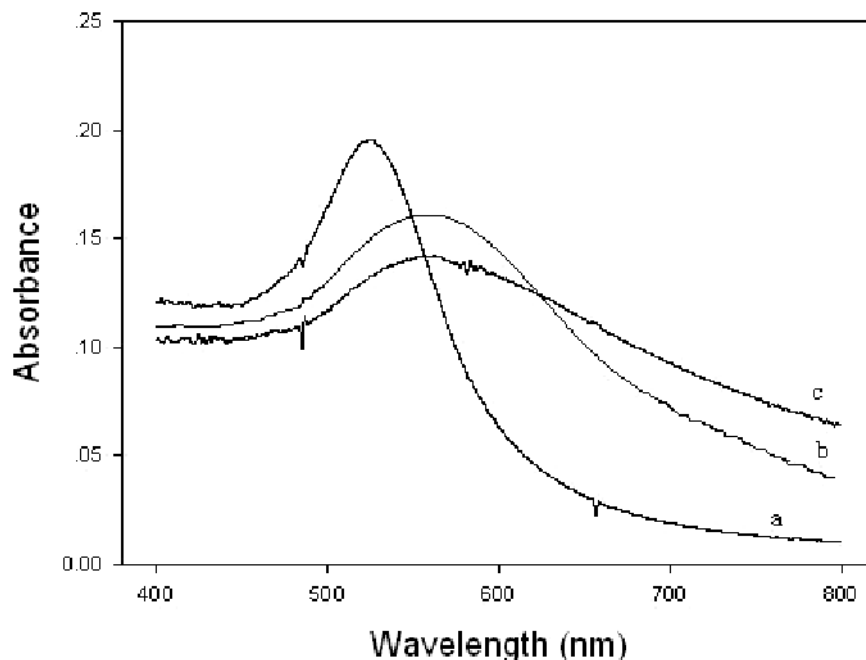
The assay conditions require the probes to be ligated at 65°C and disassociate from the target over 75°C. Therefore, appropriate design of the probes is needed in order to ensure them to function optimally in the assay conditions for various analytical targets. A common criterion for the designed probes is that the formed duplex should have a melting temperature 5–10°C higher than the ligation reaction temperature, i.e. 65°C. This criterion guarantees the probe-target hybrid does not melt apart at 65°C such that the ligation reaction could be performed, while the unligated probes disassociate from the target over 75°C such that the point mutation could be discriminated. The melting temperature of the probe-target hybrid depends upon the GC content and the length of the probes. When the target composition is G/C rich, appropriate short probes could be selected such that the duplex does not disassociate at 65°C while melts apart at 75°C. With the appropriately designed probes, the hybridization/ligation for G/C-rich targets can be performed consistently under the aforementioned conditions. In common practice, the probe sequence consists of 15–30 nt. The melting temperature of the formed duplex can be estimated using the ‘percent-GC’ rule or ‘nearest neighbor’ rules, while are available in most probe or primer design software packages.

### Ligation detection of wild-type or mutant target using Au nanoparticle probes

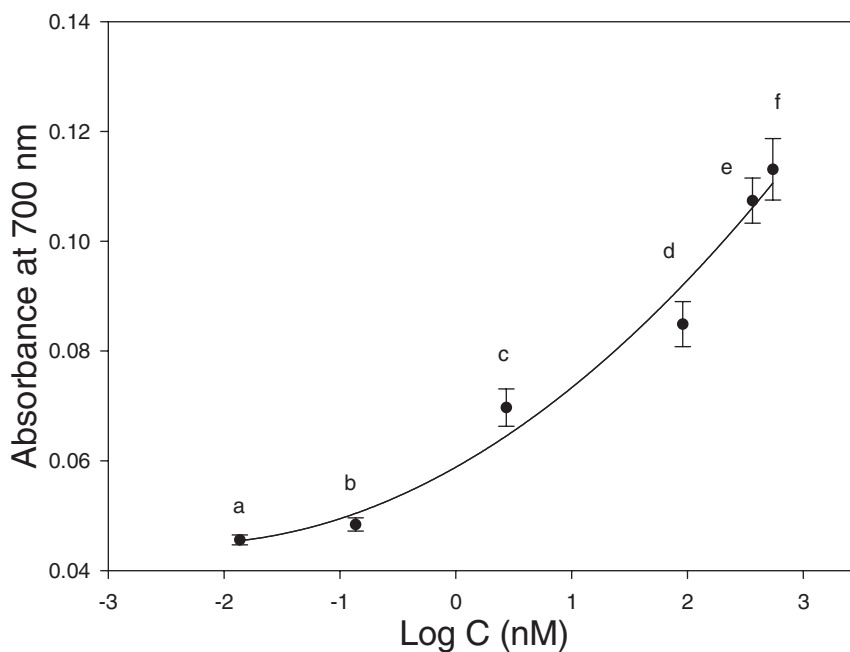
Reactions catalyzed by DNA ligases comprise three steps: adenylation of the ligase in the presence of ATP or NAD<sup>+</sup>, transferring the adenylyl moiety to the 5'-phosphate of the nicked DNA substrate (deadenylation) and sealing the nick through the formation of a phosphodiester bond. As

shown in Scheme 1, when the template does not perfectly match with Au nanoparticle probes, even at a high concentration of target (e.g. 1  $\mu\text{mol/l}$ ) and a long ligation period (say, 2 h), the nick between the two probes would not be closed by ligase. Hence, the solution color would revert to red after denaturing at a temperature, say 75°C, higher than the melting point. On the contrary, a perfect match between the base at the 3' end of the discriminating probe and the target allows the ligase to covalently join the two adjacent probes flanking the mutation site. Therefore, when heating the reaction mixture to denature the duplex formed at the temperature higher than the melting point, the color of the solution would not revert. The UV-vis spectra of a perfect match sample are shown in Figure 3. After ligating, the absorbance at 700 nm was further substantially increased, indicating that the formed aggregate was getting enlarged. In order to certify the aggregates of the Au nanoparticle probes was resulting from the ligation between Au nanoparticle probes, one can use restriction endonuclease, e.g. Alu I (5'-AG\*CT-3'), to cleavage the formed long DNA strand. After cleavage, the color of the solution would revert to red again.

According to the absorbance at 700 nm after heat denaturing, the target gene containing a single-base mutation, i.e. the perfectly matched target, can be quantified. It is observed from Figure 4 that the absorbance is dynamically correlated to the target concentration in the range from 136 pM to 364 nM. The blank sample (wild-type target) gave an average absorbance of 0.03 at 700 nm after heat denaturing with a relative standard deviation of ~8%. In terms of the 3 $\sigma$  rule, a detection limit about 74 pM was obtained. When the concentration of the target gene is more than 500 pM, an obvious color change can be discriminated by visualization.



**Figure 3.** UV-vis spectra of a perfect match sample. Curve a, before hybridization; curve b, after hybridization; curve c, after ligation. Treatment of samples: for a, 2  $\mu$ l of 13.65 pmol/ $\mu$ l T1 was added to 100  $\mu$ l of gold probe solutions containing 50  $\mu$ l of each gold probe. After mixing, the solutions were diluted to 300  $\mu$ l with a 0.1% (v/v) Triton X-100–20 mM Tris–HCl dilution buffer (pH 8.3). For curves b and c, as described in Materials and Methods.



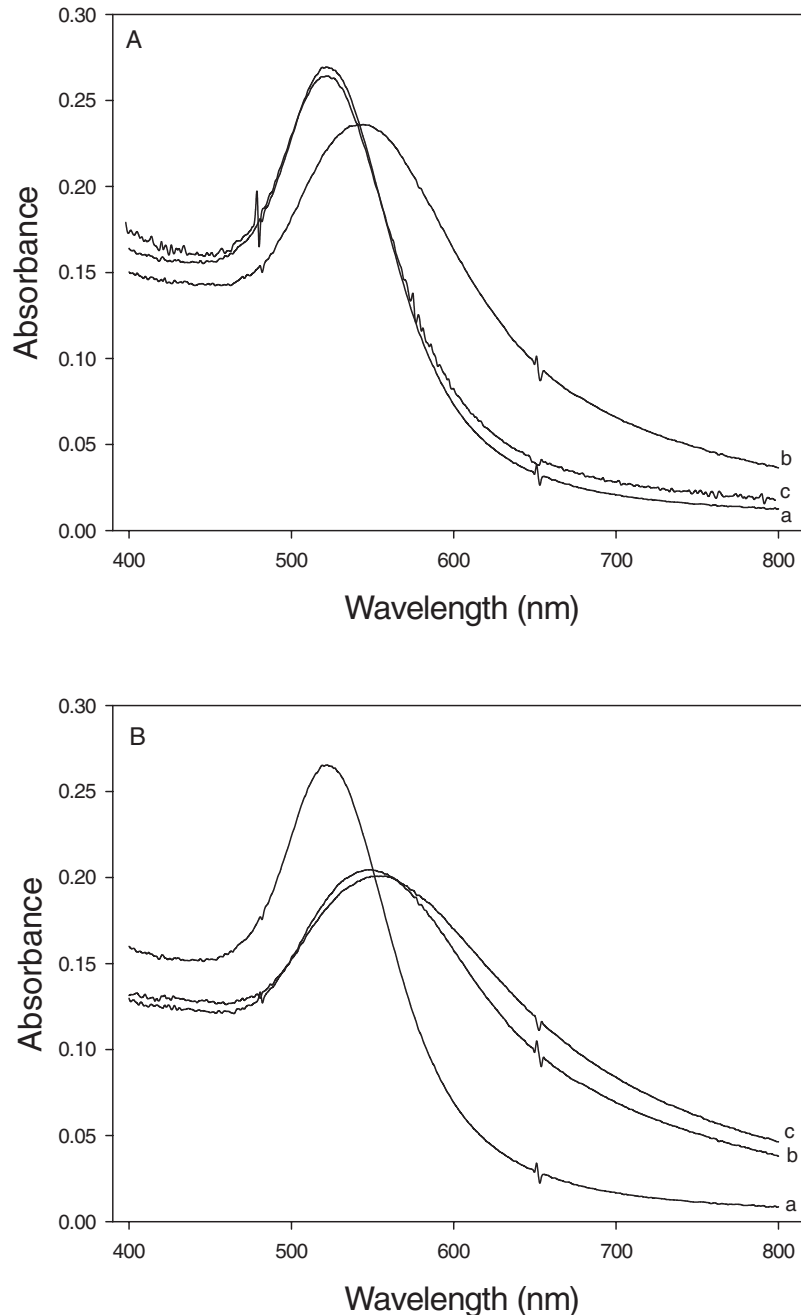
**Figure 4.** Quantitative analysis of the target gene concentration using UV-vis spectrophotometry. The experiment condition was described in Materials and Methods. The standard deviations obtained by five repeated measurements were shown as the error bars. The signal-to-noise ratio for data points a, b, c, d, e and f is 50.7, 40.3, 20.5, 20.7, 26.2 and 20.2, respectively.

It is noteworthy that the conditions used for the ligase reaction in the present study is somewhat different from the ideal ones (13,22,23). The ideal conditions for the nick-closing activity of the enzyme, as shown in Table 2, comprise a set of reagents that have strong affinity to gold probes. Inclusion

of these reagents would lead to the aggregation of the gold probes. For example, the DNA-coated Au nanoparticles could aggregate immediately in the presence of 1,4-dithiothreitol. Over 65°C, the presence of EDTA would also lead to aggregates of Au-labeled probes. In addition, magnesium ions

**Table 2.** The optimal conditions for the nick-closing activity of the enzyme

Tris-HCl	KCl	MgCl <sub>2</sub>	EDTA	NAD <sup>+</sup>	DTT	Triton X-100	T
20 mM, pH 8.3	0.1 M	10 mM	1 mM	1 mM	10 mM	0.1% (v/v)	65°C



**Figure 5.** UV-vis spectra of the two real samples [(A) HT29; (B) SW480]. Curve a, before hybridization; curve b, after hybridization; curve c, denaturing after ligation. Treatment of samples: 5  $\mu$ l of 90 ng/ $\mu$ l HT29 sample or 80 ng/ $\mu$ l SW480 sample was added to 100  $\mu$ l of gold probe solutions containing 50  $\mu$ l of each gold probe (P2 and P4), so the concentration of HT29 and SW480 samples in the reaction mixture was estimated to be  $\sim$ 54.6 or 48.5 nM, respectively. After mixing, the solutions were heated to 94°C for 5 min and then treated as described in Materials and Methods.

(Mg<sup>2+</sup>) could induce a high background under high temperature (e.g. 75°C). Therefore, in the present study the DTT was abnegated in the reaction buffer, and after ligating, Trisodium citrate was used instead of EDTA to mask Mg<sup>2+</sup> in the reaction

solution so that the signal-to-background ratio could be improved. Furthermore, the utilization of high-fidelity thermal ligase (Tth DNA ligase) allows the ligase reaction to be performed at a relatively high temperature, which offers the

benefit for mitigating the non-specific assembly of gold nanoparticles induced by interfering DNA strands.

### Analysis of genomic DNA

In order to show that the developed method was applicable to real samples, the PCR products of HT29 and SW480 genomic DNA were detected under the aforementioned optimized conditions. The gel electrophoresis image of the two PCR products confirmed the length of these two sequences is 254 bp. Analysis of these two samples using the developed method gave the UV-vis spectra shown in Figure 5. One observed that, after denaturing at a melting temperature, the spectrum for HT29 sample recovers and shows a spectrum characteristic for the separate Au nanoparticles, indicating there is no point mutation in codon 12 in HT29 genomic DNA. On the contrary, the spectrum for SW480 sample exhibits insignificant spectral variations after denaturing at a melting temperature. This implies that the nick between the two adjacent probes was sealed at the mutation site for SW480, that is to say, the codon 12 of K-ras gene is mutant from GGT to GTT. These results were further verified by the sequencing data which showed that the codon 12 for HT29 and SW480 genomic DNA are GGT and GTT, respectively (data not shown), demonstrating that the developed method holds the potential for SNP detection of real genomic DNA samples.

### CONCLUSION

The present study reported a colorimetric method for detection and quantification of target gene containing SNP. This method combines the high-fidelity perfect-match ligation of DNA ligase with the simple yet powerful colorimetric detection of gold nanoparticle assembly. This new, gold nanoparticle assembly and ligase reaction-based detection procedure may prove useful in clinical diagnosis of genetic diseases that contain single nucleotide mutations. The fact that the ligase reaction can be performed in a relative high temperature also offers the possibility of incorporating the developed method into the continuous monitoring of the target in ligase amplification reaction (25,26).

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*Conflict of interest statement.* None declared.

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