Supplementary Information

Amplification of G-quadruplex DNAzymes using PCR-like temperature cycles for specific nucleic acid and single nucleotide polymorphism detection

1. Experimental Section

Materials and Reagents. The oligonucleotides (Table 1, Table S1 and Table S2) were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). The concentrations of the oligonucleotides were represented as single-stranded concentration. Single-stranded concentration was determined by measuring the absorbance at 260 nm. Molar extinction coefficient was determined using a nearest neighbour approximation (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer). Hemin, H_2O_2, 2,2′-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS), Triton X-100, tris(hydroxymethyl) aminomethane (Tris), were obtained from Sigma. All chemical reagents were of analytical grade and used without further purification.

Specific nucleic acid and SNP detection. PCR-like cycles were carried out on Life express TC-48/H/(t) Thermal Cycler (Hangzhou Dahe Thermo-Magnetics Co., Ltd., China) with 25 μL of reaction mixtures consisted of 2 × Taq buffer (40 mM Tris-HCl (pH = 8.4), 40 mM KCl, 20 mM $(NH_4)_2SO_4$), 8 mM $MgCl_2$, 0.2 mM dGTP, 2.4 μM of the probe, 0.2 μM of the target, 2.5 U Taq polymerase. the reaction mixtures were heated to 95 °C for 5 min, then were subjected to 20 cycles under following conditions: 95 °C for 30 s, 60 °C for 30 s. When the cycles completed, Triton X-100 was added to a final concentration of 0.002% (w/v) and the reaction mixtures were diluted to 87 μL by water. In order to ensure the formation of G-quadruplex structures by extended probes, the mixtures were heated at 98 ^{o}C for 15 min, cooled slowly to 25 ^{o}C, and then incubated at 25 ^{o}C for 1 h. To this solution was added 50 μM hemin (2 μL) and the reaction mixtures were held for another 1h at 25 ^{o}C. Then, 40 mM ABTS

(7.4 μL) and 60 mM H_2O_2 (3.6 μL) were added. The color of the reaction mixtures was recorded by a digital camera, and the absorption intensity at λ = 419 nm was followed using a TU-1901 UV-Vis Spectrophotometer after the reaction had run for 4 min.

When the competition strategy was used in the SNP detection, the probe mixtures (for example the mixture of Pro-G, iPro-C, iPro-T, and iPro-A) were used. The four probes have the same concentration. Other procedures are identical to those described above.

Circular dichroism (CD) study. The PCR-like cycles were conducted as described as above. When the cycles completed, The reaction mixtures were diluted to 100 μL by water. The mixtures were heated at 98 ^{o}C for 15 min, cooled slowly to 25 ^{o}C, and then incubated at 25 ^{o}C overnight. 30 tubes of such reaction mixtures were combined and the CD spectrum was measured on a Jasco J-715 spectropolarimeter at room temperature. Spectra were recorded between 225 and 320 nm in 1 mm pathlength cuvettes. Spectra were averaged from 3 scans, which were recorded at 100 nm/min with a response time of 1s and a bandwidth of 0.1 nm.

UV-visible spectrum detection of hemin. The PCR-like cycles were conducted as described as above. When the cycles completed, Triton X-100 was added to a final concentration of 0.002% (w/v) and the reaction mixtures were diluted to 98 μL by water. The mixtures were heated at 98 ^{o}C for 15 min, cooled slowly to 25 ^{o}C, and then incubated at 25 ^{o}C for 1 h. To this solution was added 50 μM hemin (2 μL) and the reaction mixtures were held at 25 ^{o}C overnight. The absorption spectrum of hemin was recorded by the UV-visible spectrophotometer in the wavelength range from 350 to 450 nm.

2. Specific nucleic acid and SNP detection using a probe to which a GGG repeat needs to be added

2.1 Sequences of the oligonucleotides used in this part

Table S1. Sequences of the oligonucleotides used in this part

DNAs		Sequence (from 5′ to 3′)
Probes	Pro-G	TGGGTAGGGCGGGAATCTTACCGGAAGT**G**TTGATAAGATA
	Pro-C	TGGGTAGGGCGGGAATCTTACCGGAAGT**C**TTGATAAGATA
	Pro-T	TGGGTAGGGCGGGAATCTTACCGGAAGT**T**TTGATAAGATA
	Pro-A	TGGGTAGGGCGGGAATCTTACCGGAAGT**A**TTGATAAGATA
	Pro′-G	TGGGTAGGGCGGGAATCTTAGAAGTGTT**G**ATAAgATA
	Pro′-C	TGGGTAGGGCGGGAATCTTAGAAGTGTT**C**ATAAgATA
	Pro′-T	TGGGTAGGGCGGGAATCTTAGAAGTGTT**T**ATAAgATA
	Pro′-A	TGGGTAGGGCGGGAATCTTAGAAGTGTT**A**ATAAgATA
Inhibition Probes	iPro-G	AATCTTACCGGAAGT**G**TTGATAAGATA
	iPro-C	AATCTTACCGGAAGT**C**TTGATAAGATA
	iPro-T	AATCTTACCGGAAGT**T**TTGATAAGATA
	iPro-A	AATCTTACCGGAAGT**A**TTGATAAGATA
	iPro′-G	AATCTTAGAAGTGTT**G**ATAAgATA
	iPro′-C	AATCTTAGAAGTGTT**C**ATAAgATA
	iPro′-T	AATCTTAGAAGTGTT**T**ATAAgATA
	iPro′-A	AATCTTAGAAGTGTT**A**ATAAgATA
Targets	Tar-C	AAATGCCCTATCTTATCAA**C**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar-G	AAATGCCCTATCTTATCAA**G**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar-T	AAATGCCCTATCTTATCAA**T**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar-A	AAATGCCCTATCTTATCAA**A**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar′-C	AAATGCCCTATCTTAT**C**AACACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar′-G	AAATGCCCTATCTTAT**G**AACACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar′-T	AAATGCCCTATCTTAT**T**AACACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar′-A	AAATGCCCTATCTTAT**A**AACACTTCCGGAAACTACTGTTGTTAGACGACG
Mimic product	mProd-G	TGGGTAGGGCGGGAATCTTACCGGAAGTGTTGATAAGATAGGG

2.2 The effect of PCR components on the ABTS-H_2O_2 system

A synthetic oligonucleotide (mProd-G: TGGGTAGGGCGGGAATCTTACCGGA AGTGTTGATAAGATAGGG) was used to mimic the extended product of the probe Pro-G, and the effect of PCR components on the mProd-G-mediated ABTS-H_2O_2 reactions was investigated. The results (Fig. S1) showed that the components of PCR (for example dGTP and Taq DNA polymerase) nearly had no effect on the ABTS-H_2O_2 system.

Experimental detail: 25μL reaction mixtures containing 2 × Taq buffer (40 mM Tris-HCl (pH = 8.4), 40 mM KCl, 20 mM $(NH_4)_2SO_4$), 8 mM $MgCl_2$, 2.4 μM mProd-G, 0.2 mM dGTP, 2.5 U Taq polymerase was prepared. The reaction mixtures were heated to 95 °C for 5 min, then were subjected to 20 cycles under following conditions: 95 °C for 30 s, 60 °C for 30 s. When the cycles completed, Triton X-100 was added to a final concentration of 0.002% (w/v) and the reaction mixtures were diluted to 87 μL by water. The mixtures were heated at 98 ^oC for 15 min, cooled slowly to 25 ^oC, and then incubated at 25 ^oC for 1 h. To this solution was added 50 μM hemin (2 μL) and the reaction mixtures were held for another 1h at 25 ^oC. Then, 40 mM ABTS (7.4 μL) and 60 mM H_2O_2 (3.6 μL) were added. The absorption intensity at λ = 419 nm was followed using a TU-1901 UV-Vis Spectrophotometer after the reaction had run for 4 min.

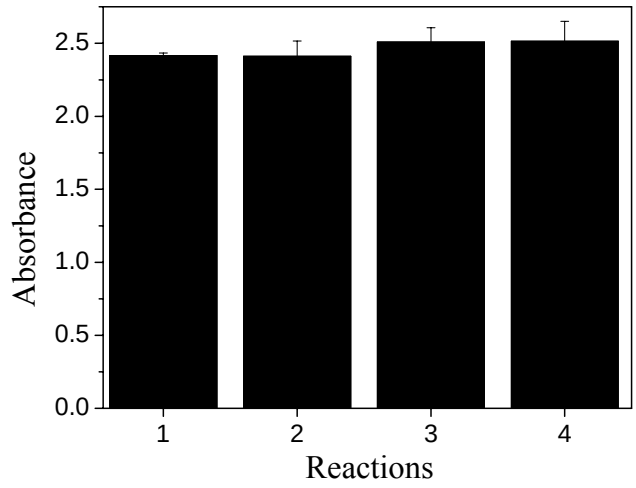


Fig. S1. The effect of PCR components on the ABTS-H_2O_2 system. **Reaction 1**: PCR-like temperature cycles were conducted but no dGTP and Taq DNA polymerase were added; **Reaction 2**: PCR-like temperature cycles were conducted and dGTP and Taq DNA polymerase were added; **Reaction 3**: no PCR-like temperature cycles were conducted and no dGTP and Taq DNA polymerase were added; **Reaction 4**: no PCR-like temperature cycles were conducted but dGTP and Taq DNA polymerase were added.

2.3 CD spectroscopy was used to demonstrate the G-quadruplex formation by the extended probe.

To further demonstrate the working mechanism in Scheme 1, the circular dichroism (CD) spectra of reaction mixtures in the absence or presence of the target were compared (Figure S2). When the PCR-like cycles were completed, the CD spectrum of the reaction mixture without target showed almost no positive peaks in the wavelength range of 220-320 nm, indicating that no G-quadruplexes were formed in the absence of target. However, in the presence of the target, the CD spectrum of the reaction mixture showed a positive peak at 267 nm, which is characteristic of parallel G-quadruplex structure,[1,2] suggesting that the probe had been extended along the target and folded into a parallel G-quadruplex. To preclude the possibility that the positive peak was caused by the target, the Pro-G probe was replaced by iPro-G, which had a sequence similar to Pro-G but no Domain I. Thus, even if iPro-G was extended, it had only one GGG repeat, and could not fold into a G-quadruplex. The absence of a positive peak in the iPro-G system demonstrated that the peak was not caused by the target but by the extended Pro-G probe.

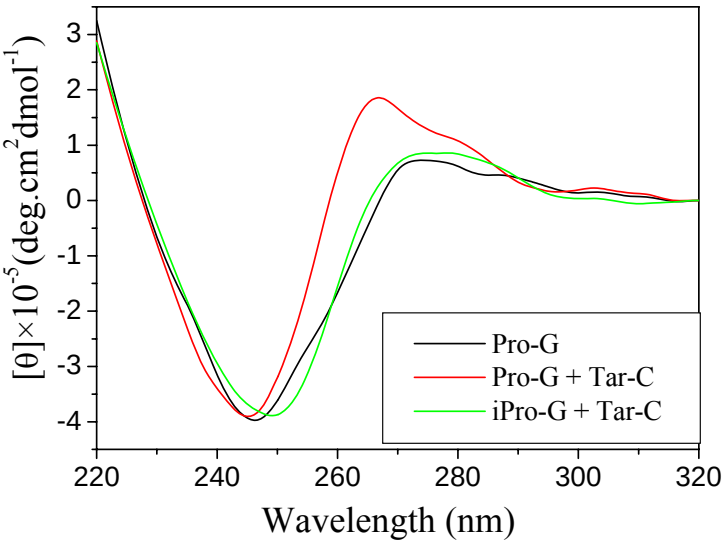


Fig. S2. The CD spectra of the PCR-like reaction mixtures in the presence of Pro-G, Pro-G + Tar-C or iPro-G + Tar-C.

2.4 UV-visible absorption spectroscopy was used to indirectly demonstrate the G-quadruplex formation by the extended probe.

UV-visible absorption spectroscopy also showed interaction between the extended probe and hemin, thus indirectly demonstrating G-quadruplex formation by the extended probe. When the PCR-like cycles were completed, hemin was added and the absorption spectrum of hemin was recorded (Figure S3). Compared with the absorption spectra of reaction mixtures containing Pro-G only, or iPro-G and Tar-C, the absorption spectrum of the reaction mixture containing Pro-G and Tar-C showed an obvious hyperchromicity and bathochromicity, indicating interaction between extended Pro-G and hemin. This result was perfectly consistent with other G-quadruplex DNAzyme reports,[3-7] suggesting the formation of G-quadruplex by extended Pro-G.

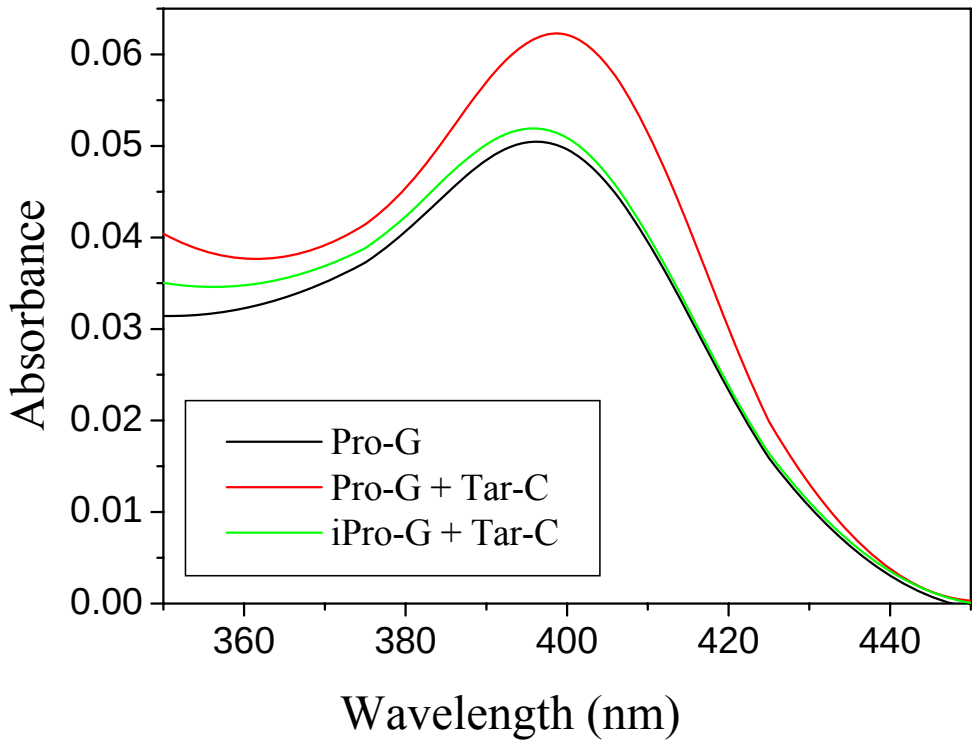


Fig. S3. The absorption signal of hemin in the PCR-like reaction mixtures containing Pro-G, Pro-G + Tar-C or iPro-G + Tar-C.

2.5 Specific nucleic acid detection using rapid temperature cycles

When rapid temperature cycles were used, the reaction mixtures were heated to 95 °C for 5 min, then were subjected to 99 cycles under following conditions: 95 °C for 1 s, 60 °C for 1 s.

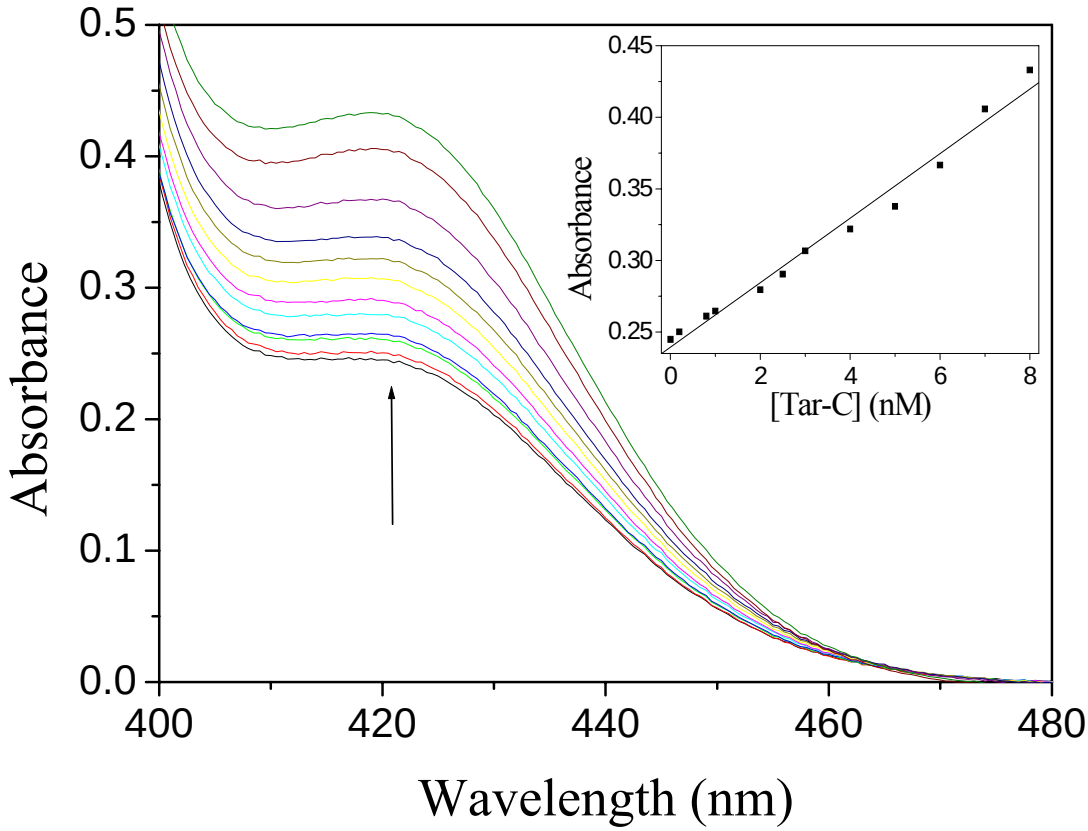
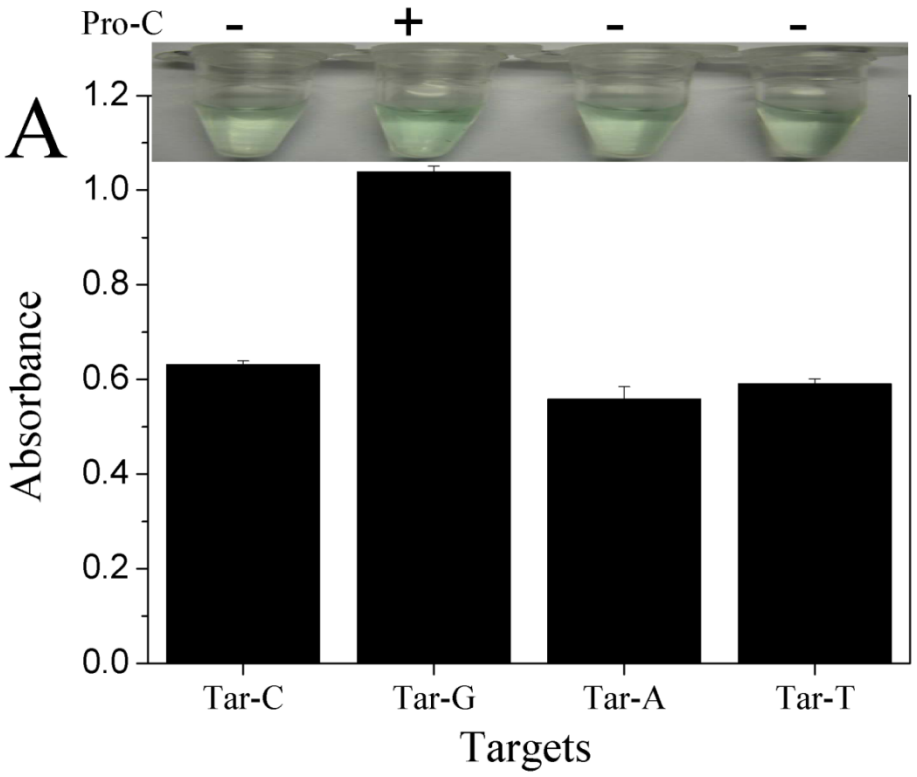
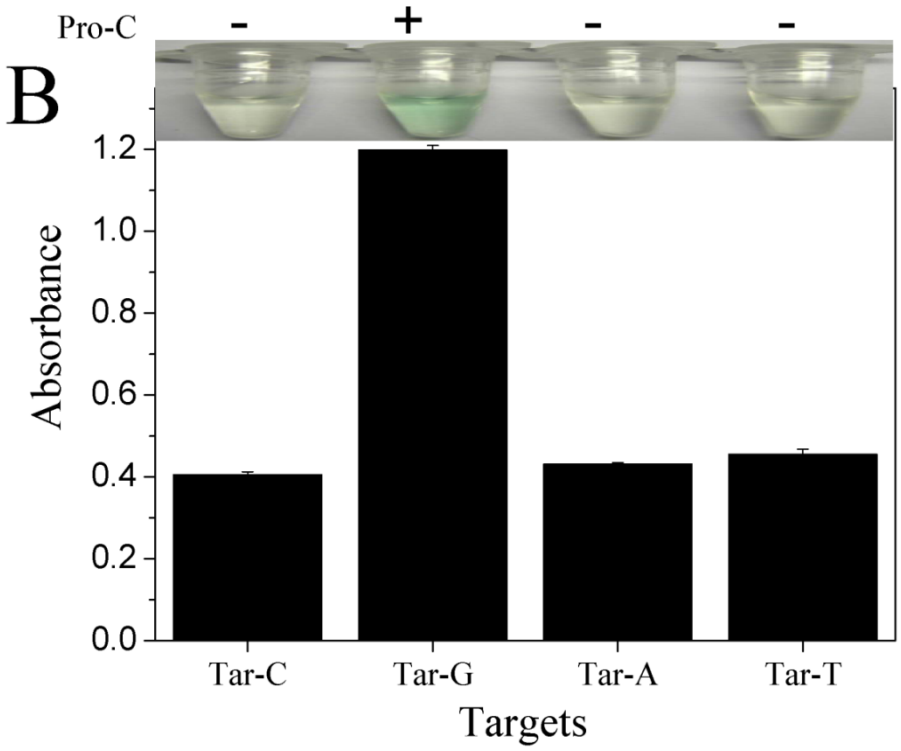


Fig. S4. Ulilization of rapid temperature cycles for spectroscopic analysis of different concentrations of nucleic acid target. The concetrations of Tar-C are (arrow direction): 0, 0.2, 0.8, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 6.0, 7.0, 8.0 nM. The insert shows the Tar-C concentration-dependent change of absorbance at 419 nm. The solid line represents linear fit to the data.

2.6 SNPs identification

2.6.1 SNPs identification by the Pro-C probe





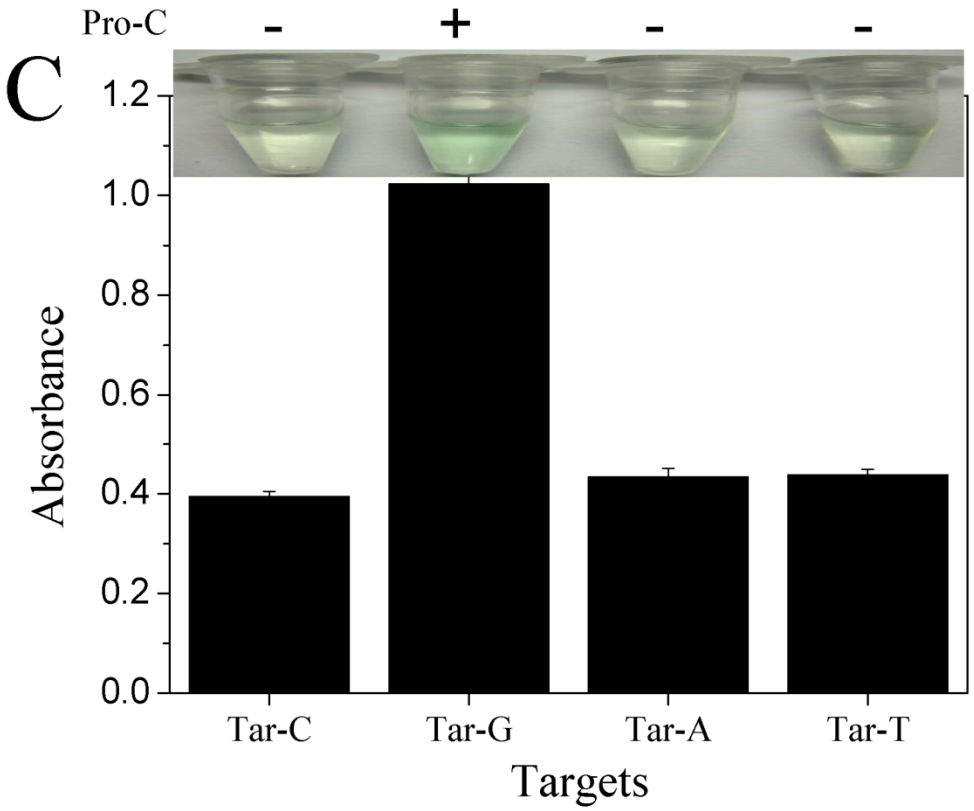
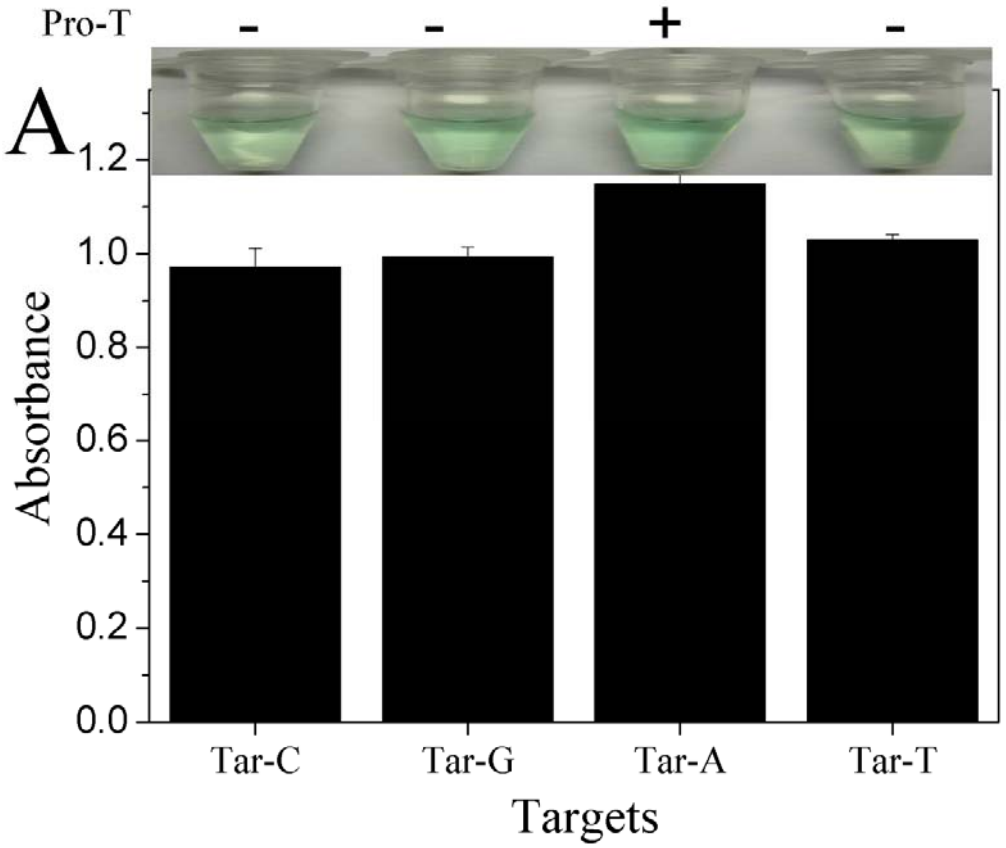
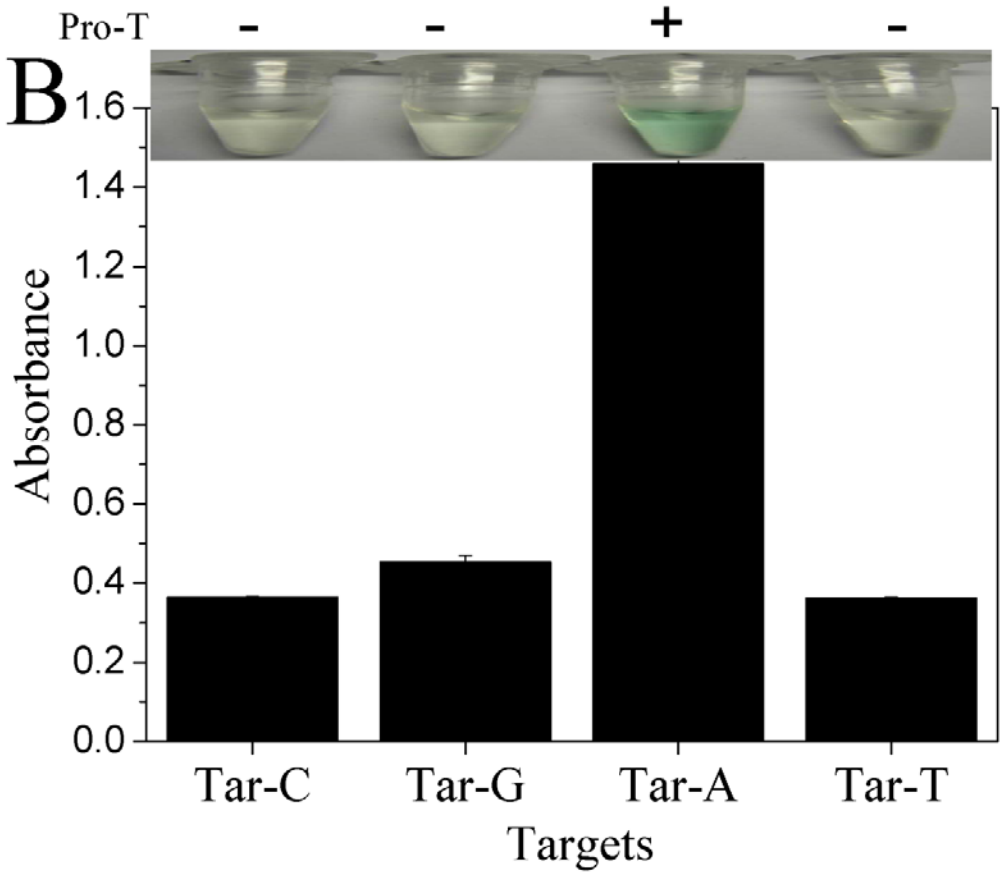


Fig. S5. The identification of SNPs by the Pro-C probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 68 ^{o}C and 25 cycles were conducted; (C) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro-C] = [iPro-G] = [iPro-T] = [iPro-A] = 0.6 μM. The photographed images of the reaction tubes are shown in the tops of the figures. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

2.6.2 SNPs identification by the Pro-T probe





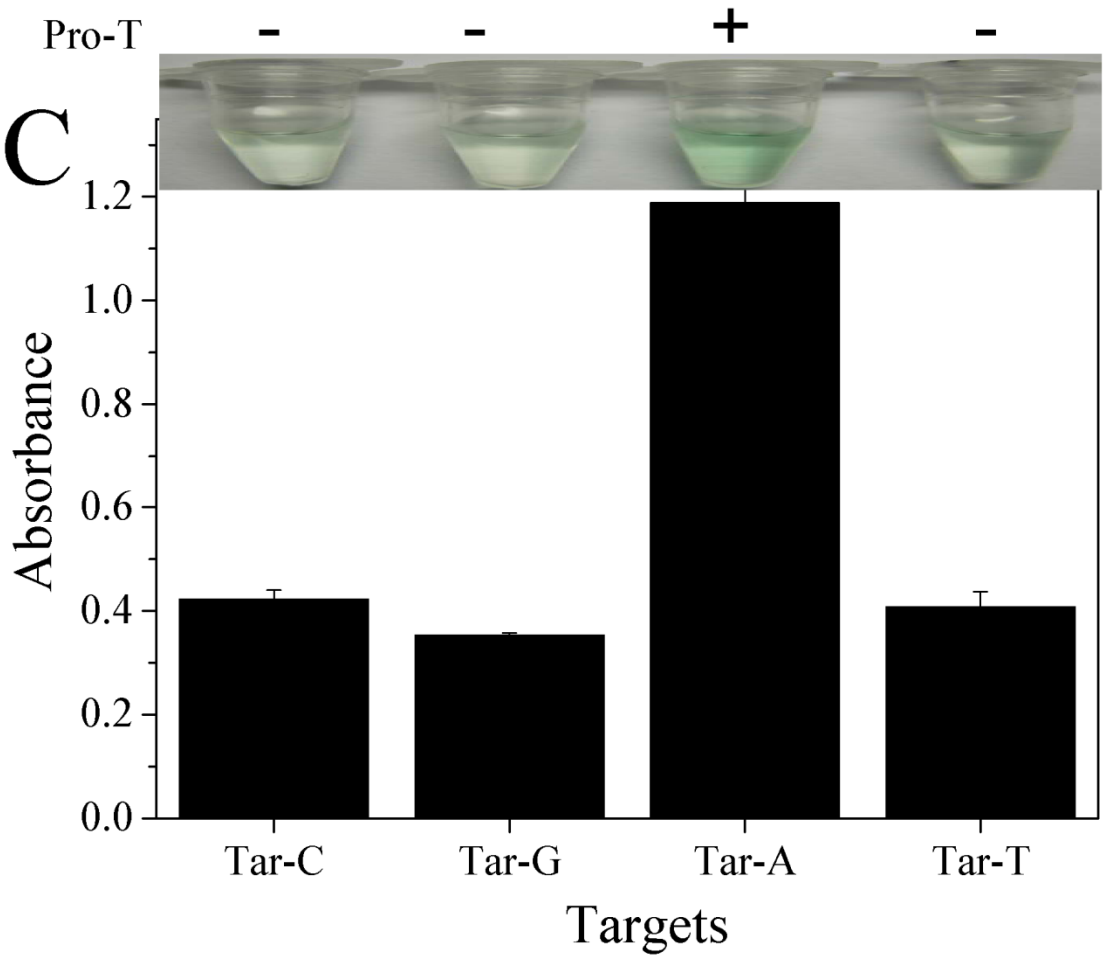
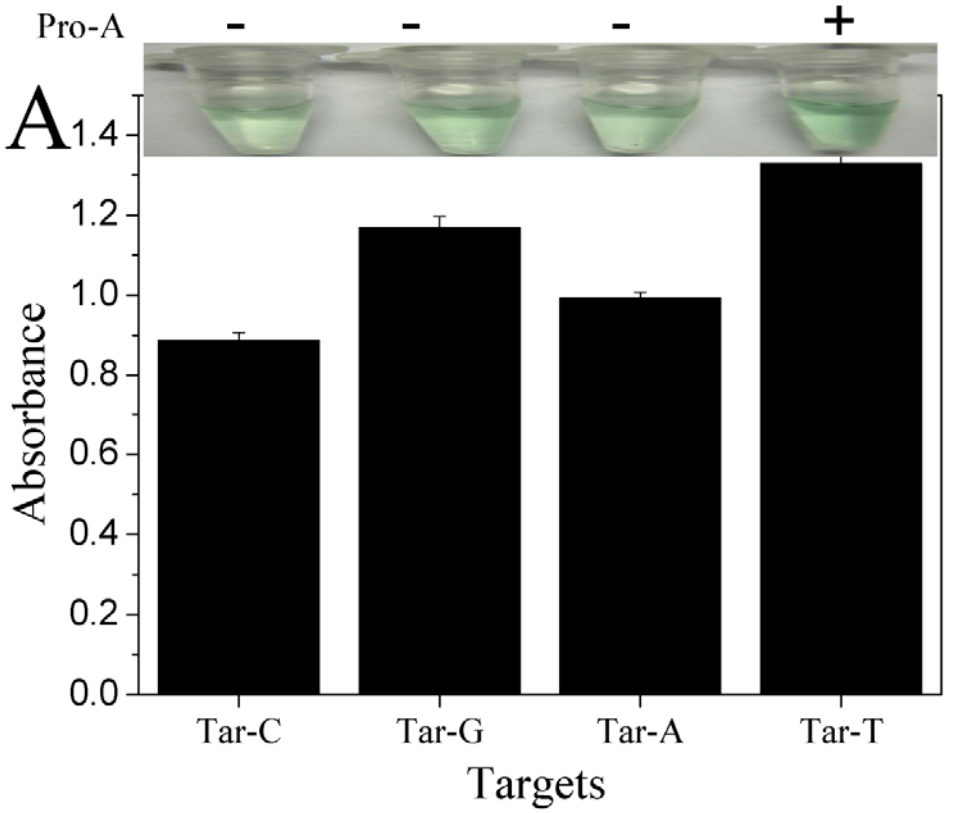
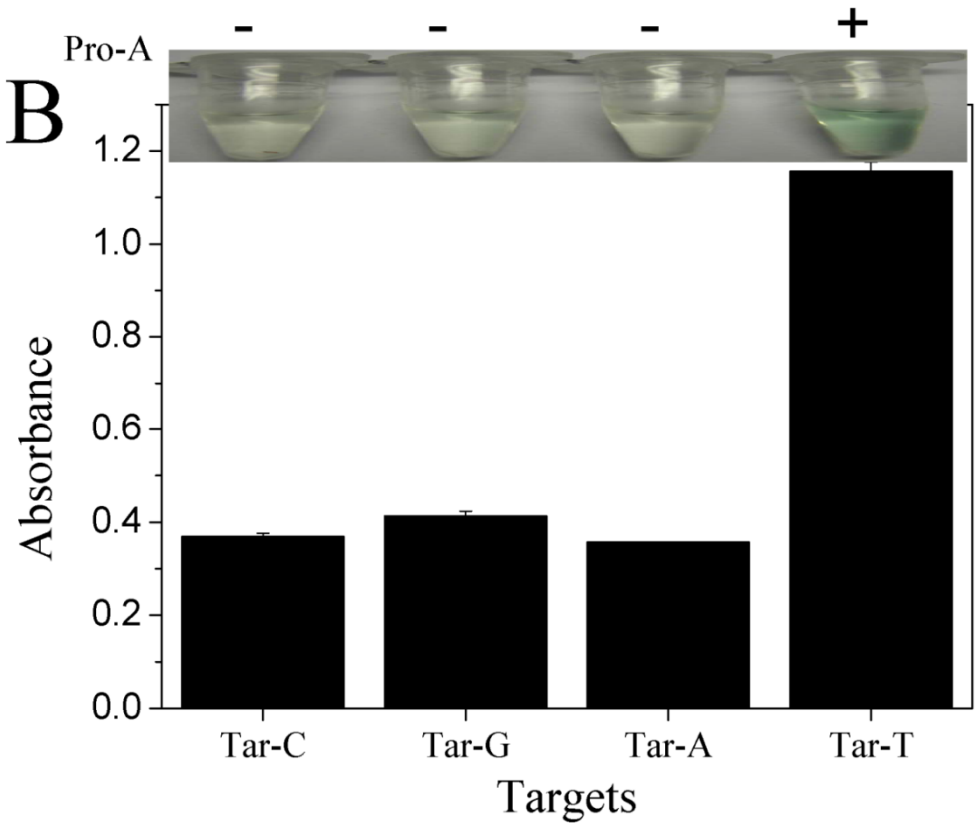


Fig. S6. The identification of SNPs by the Pro-T probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 68 ^{o}C and 25 cycles were conducted; (C) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro-T] = [iPro-G] = [iPro-C] = [iPro-A] = 0.6 μM. The photographed images of the reaction tubes are shown in the tops of the figures. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

2.6.3 SNPs identification by the Pro-A probe





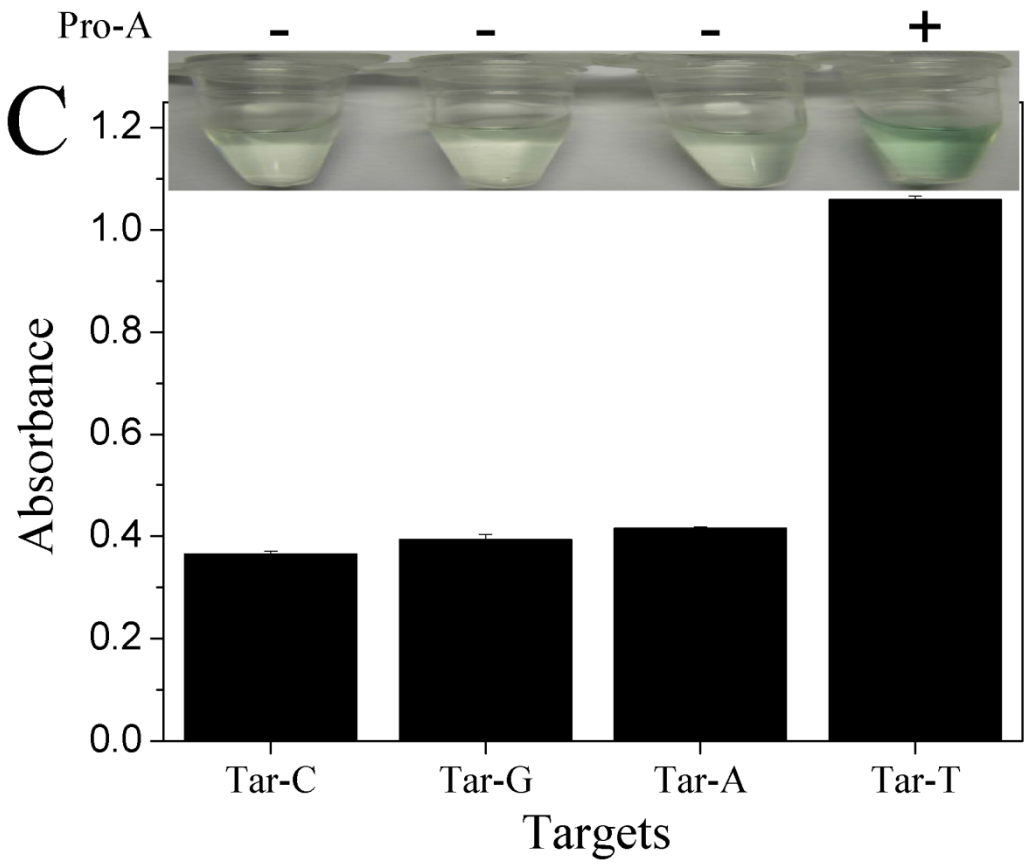


Fig. S7. The identification of SNPs by the Pro-A probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 68 ^{o}C and 25 cycles were conducted; (C) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro-A] = [iPro-G] = [iPro-C] = [iPro-T] = 0.6 μM. The photographed images of the reaction tubes are shown in the tops of the figures. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

2.6.4 SNPs identification by the Pro′-G probe

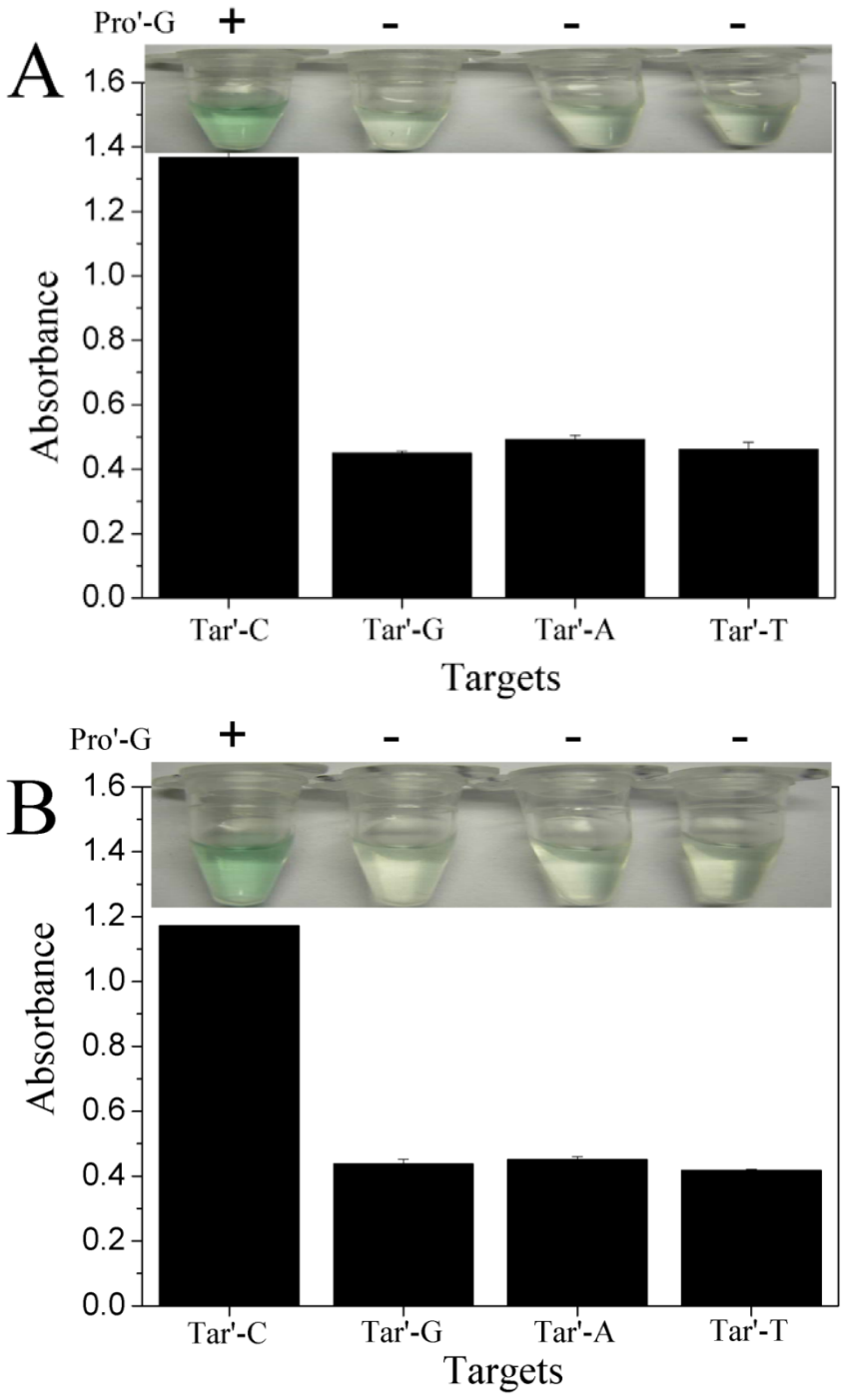


Fig. S8. The identification of SNPs by the Pro′-G probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro′-G] = [iPro′-C] = [iPro′-T] = [iPro′-A] = 0.6 μM. The photographed images of the reaction tubes are shown in the tops of the figures. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

2.6.5 SNPs identification by the Pro′-C probe

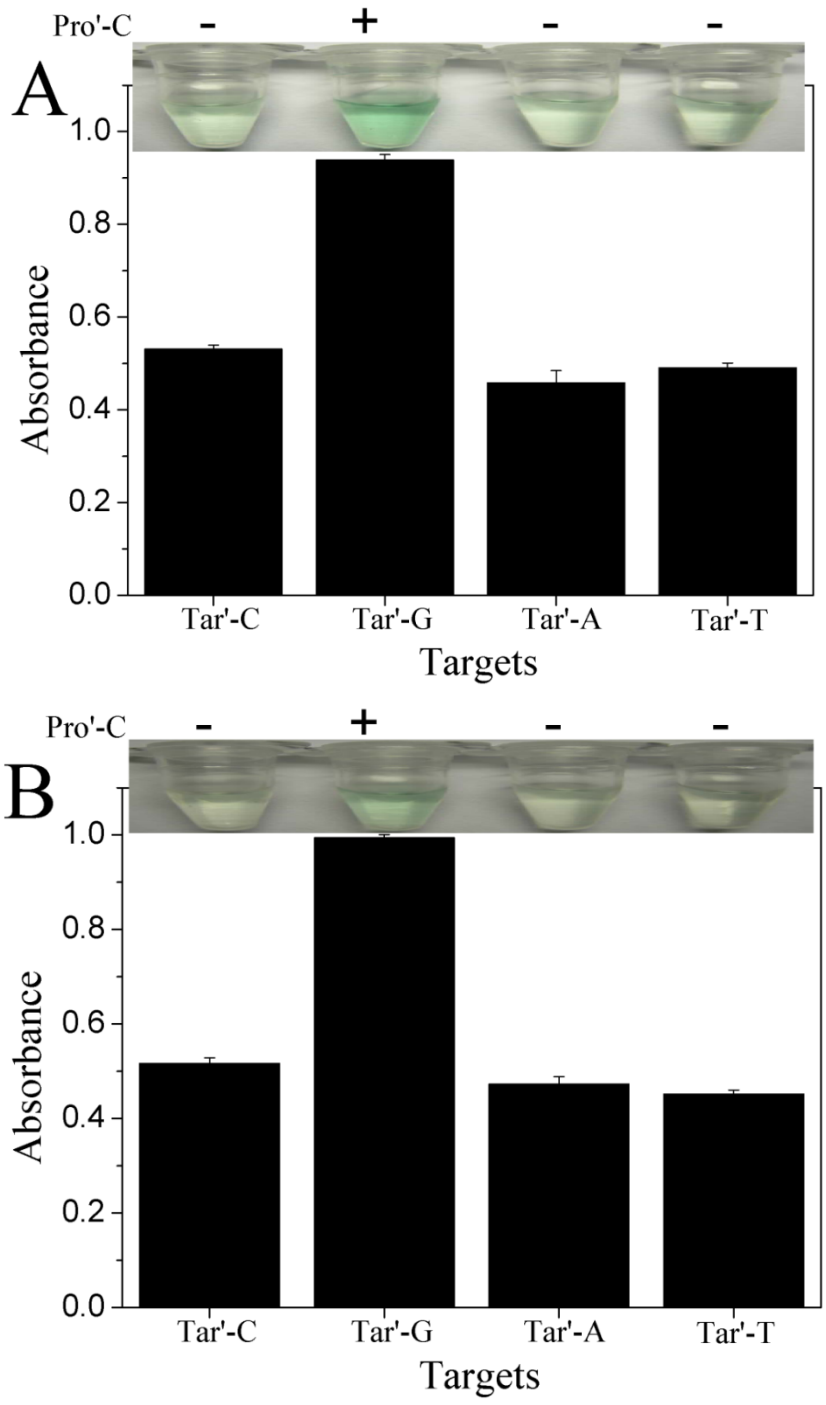


Fig. S9. The identification of SNPs by the Pro′-C probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro′-C] = [iPro′-G] = [iPro′-T] = [iPro′-A] = 0.6 μM. The photographed images of the reaction tubes are shown in the tops of the figures. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

2.6.6 SNPs identification by the Pro′-T probe

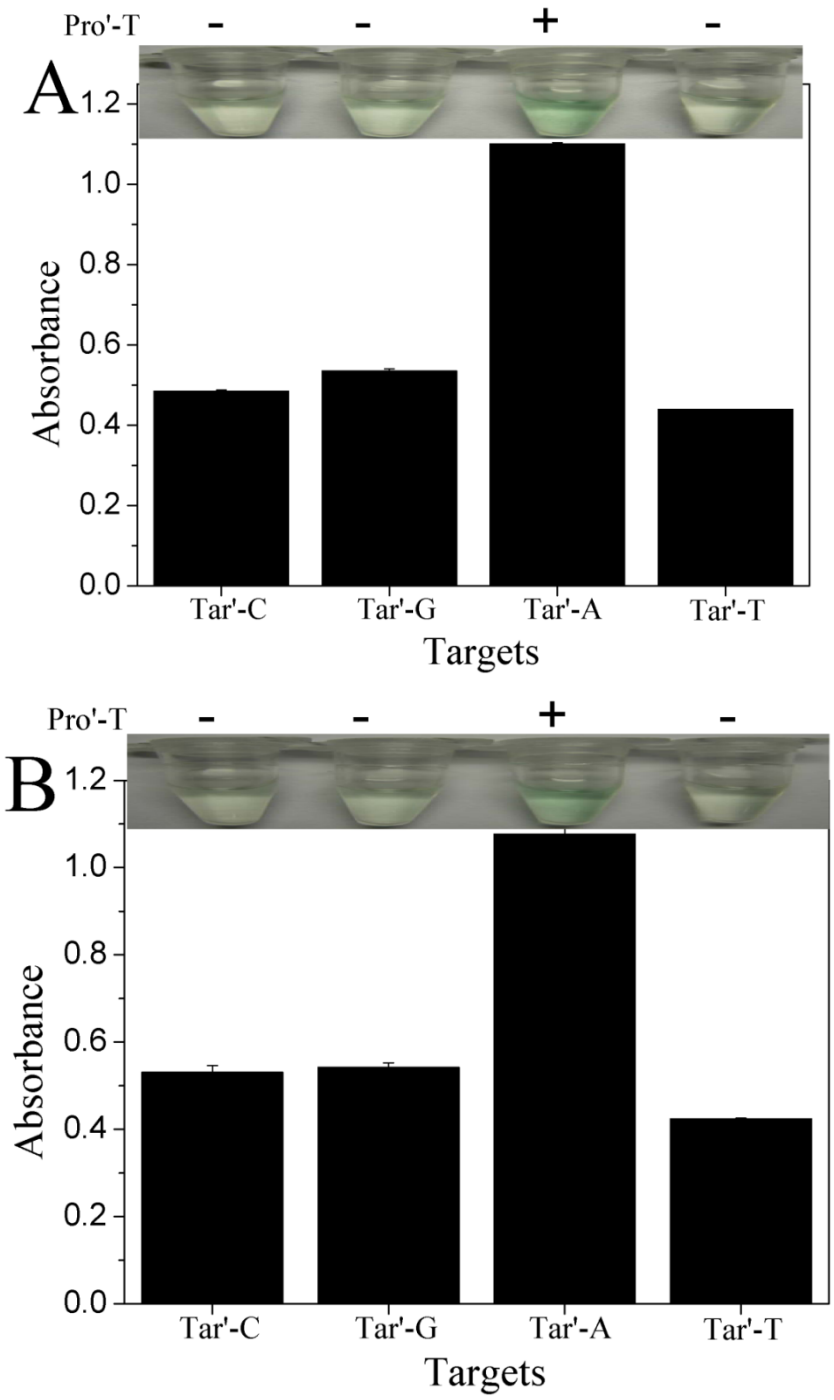


Fig. S10. The identification of SNPs by the Pro′-T probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro′-T] = [iPro′-G] = [iPro′-C] = [iPro′-A] = 0.6 μM. The photographed images of the reaction tubes are shown in the tops of the figures. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

2.6.7 SNPs identification by the Pro′-A probe

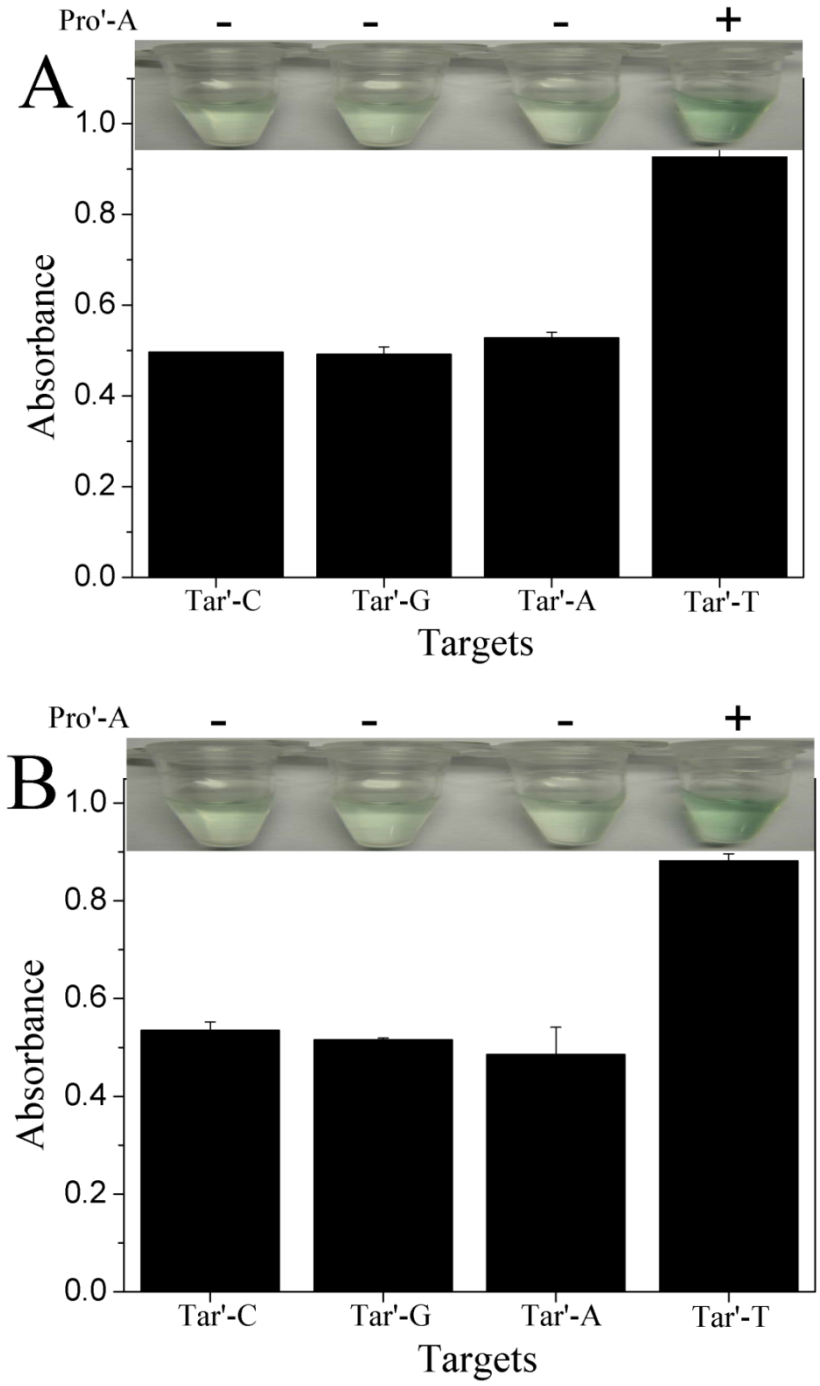


Fig. S11. The identification of SNPs by the Pro′-A probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro′-A] = [iPro′-G] = [iPro′-C] = [iPro′-T] = 0.6 μM. The photographed images of the reaction tubes are shown in the tops of the figures. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

2.6.8 SNPs identification in the mixed target sample

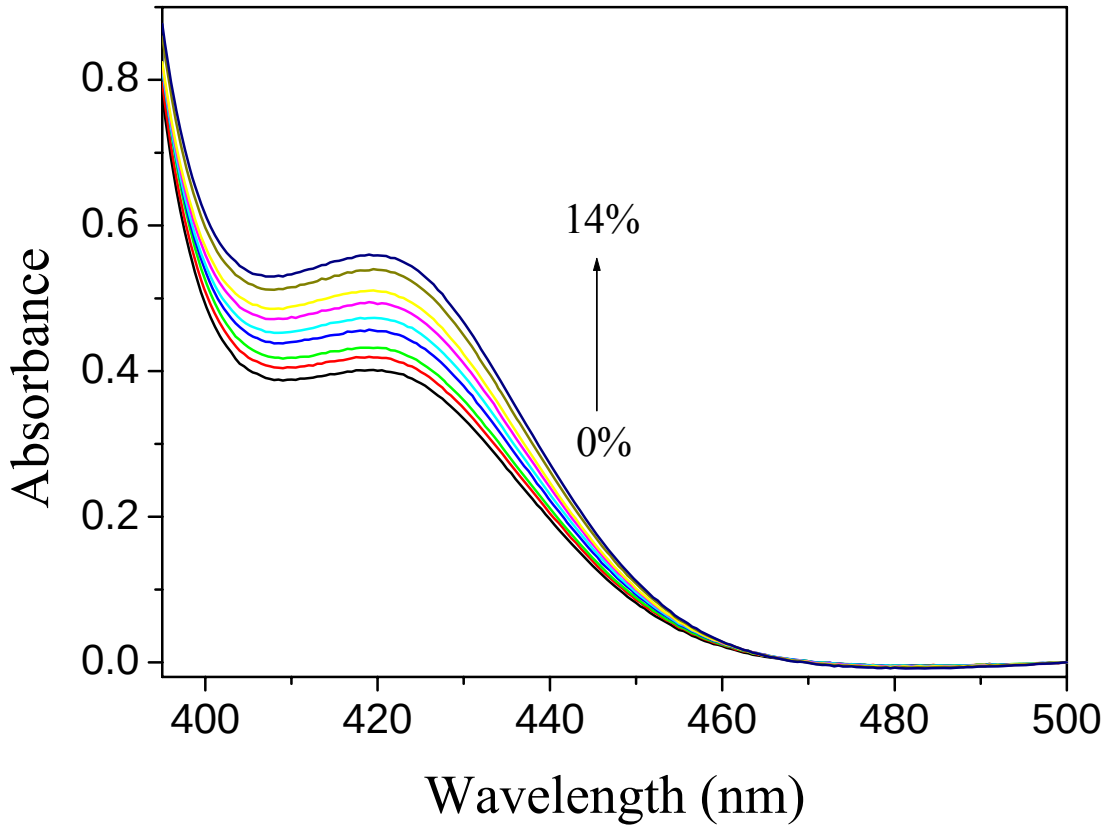


Fig. S12. SNP identification in the mixed target sample. The total concentration of Tar′-C and Tar′-G was 200 nM and the percentage of Tar′-C was (arrow direction) 0, 1, 2, 4, 6, 8, 10, 12, 14% respectively. The probe of Pro′-G was used and no competition strategy was adopted. The absorbance spectrum of the oxygenation product $ABTS^{\cdot+}$ was recorded after the reaction had run for 4 min.

3. Specific nucleic acid and SNP detection using a probe to which a GG repeat needs to be added

3.1 Sequences of the oligonucleotides used in this part

Table S2. Sequences of the oligonucleotides used in this part

DNAs		Sequence (from 5′ to 3′)
Probes	Pro2-G	GTTGGGTAGGGCGGGAATCTTACCGGAAGT**G**TTGATAAGATA
	Pro2-C	GTTGGGTAGGGCGGGAATCTTACCGGAAGT**C**TTGATAAGATA
	Pro2-T	GTTGGGTAGGGCGGGAATCTTACCGGAAGT**T**TTGATAAGATA
	Pro2-A	GTTGGGTAGGGCGGGAATCTTACCGGAAGT**A**TTGATAAGATA
	Pro2′-G	GTTGGGTAGGGCGGGAATCTTAGAAGTGTT**G**ATAAgATA
	Pro2′-C	GTTGGGTAGGGCGGGAATCTTAGAAGTGTT**C**ATAAgATA
	Pro2′-T	GTTGGGTAGGGCGGGAATCTTAGAAGTGTT**T**ATAAgATA
	Pro2′-A	GTTGGGTAGGGCGGGAATCTTAGAAGTGTT**A**ATAAgATA
Inhibition Probes	iPro-G	AATCTTACCGGAAGT**G**TTGATAAGATA
	iPro-C	AATCTTACCGGAAGT**C**TTGATAAGATA
	iPro-T	AATCTTACCGGAAGT**T**TTGATAAGATA
	iPro-A	AATCTTACCGGAAGT**A**TTGATAAGATA
	iPro′-G	AATCTTAGAAGTGTT**G**ATAAgATA
	iPro′-C	AATCTTAGAAGTGTT**C**ATAAgATA
	iPro′-T	AATCTTAGAAGTGTT**T**ATAAgATA
	iPro′-A	AATCTTAGAAGTGTT**A**ATAAgATA
Targets	Tar2-C	AAATGCCTATCTTATCAA**C**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar2-G	AAATGCCTATCTTATCAA**G**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar2-T	AAATGCCTATCTTATCAA**T**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar2-A	AAATGCCTATCTTATCAA**A**ACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar2′-C	AAATGCCTATCTTAT**C**AACACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar2′-G	AAATGCCTATCTTAT**G**AACACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar2′-T	AAATGCCTATCTTAT**T**AACACTTCCGGAAACTACTGTTGTTAGACGACG
	Tar2′-A	AAATGCCTATCTTAT**A**AACACTTCCGGAAACTACTGTTGTTAGACGACG

3.2 CD spectroscopy was used to demonstrate the G-quadruplex formation by the extended probe.

CD spectroscopy was used to demonstrate that even if the Pro2-G probe was extended a GG repeat along its target, the extended probe can also fold into G-quadruplex structure. When the PCR-like cycles completed, the CD spectrum of the reaction mixture without target nearly showed no positive peaks in the wavelength range of 220 ~ 320 nm, indicating that no G-quadruplexes were formed in the absence of the target. However, in the presence of the target, the CD spectrum of the reaction mixture showed a positive peak at around of 271 nm, suggesting that the probe had been extended along the target and the extended probe folded into a parallel G-quadruplex. Compared with the Pro-G probe, the positive peak of the Pro2-G probe was red-shifted by about 4 nm to 271 nm. The reason may be that the G-quadruplex formed by extended Pro2-G has less G-quartets (perhaps 2 G-quartets) and less stability than that formed by extended Pro-G. Thus, the presence of the double-stranded hairpin structure, which shows a positive peak at around 277 nm,[8] has more obvious effect on the CD spectrum.

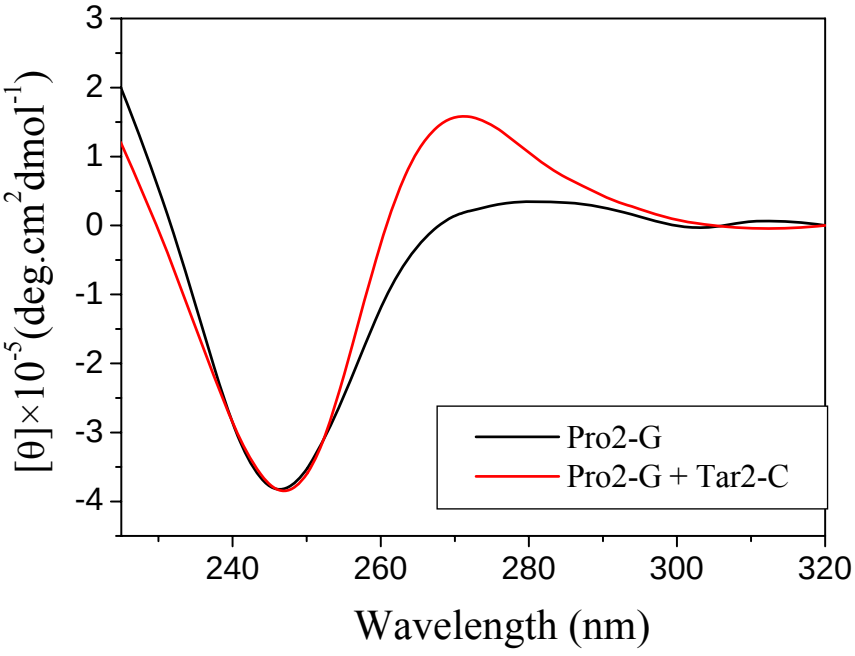
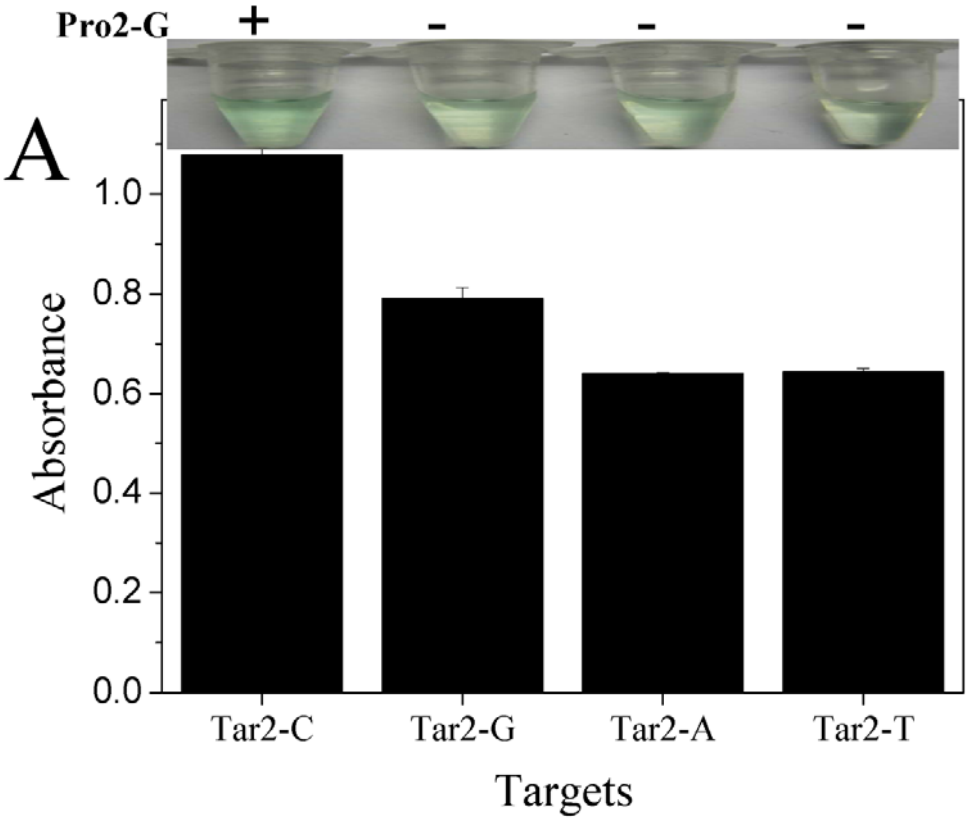
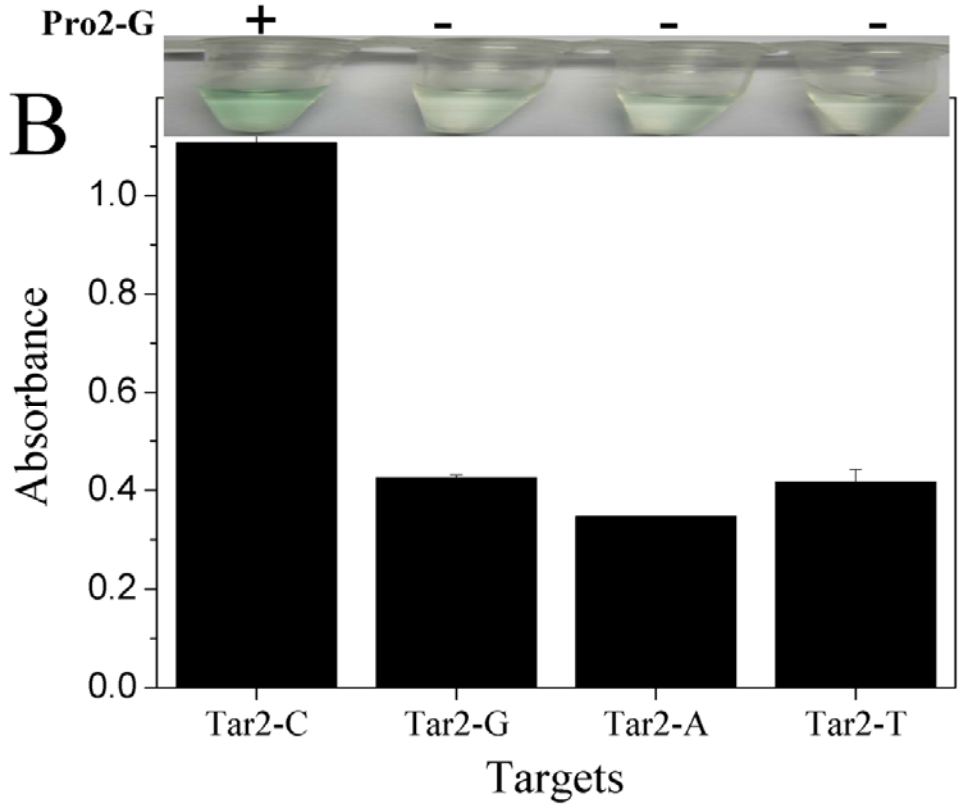


Fig. S13. The CD spectra of the PCR-like reaction mixtures in the presence of Pro2-G or Pro2-G + Tar2-C.

3.3 SNPs identification

3.3.1 SNPs identification by the Pro2-G probe





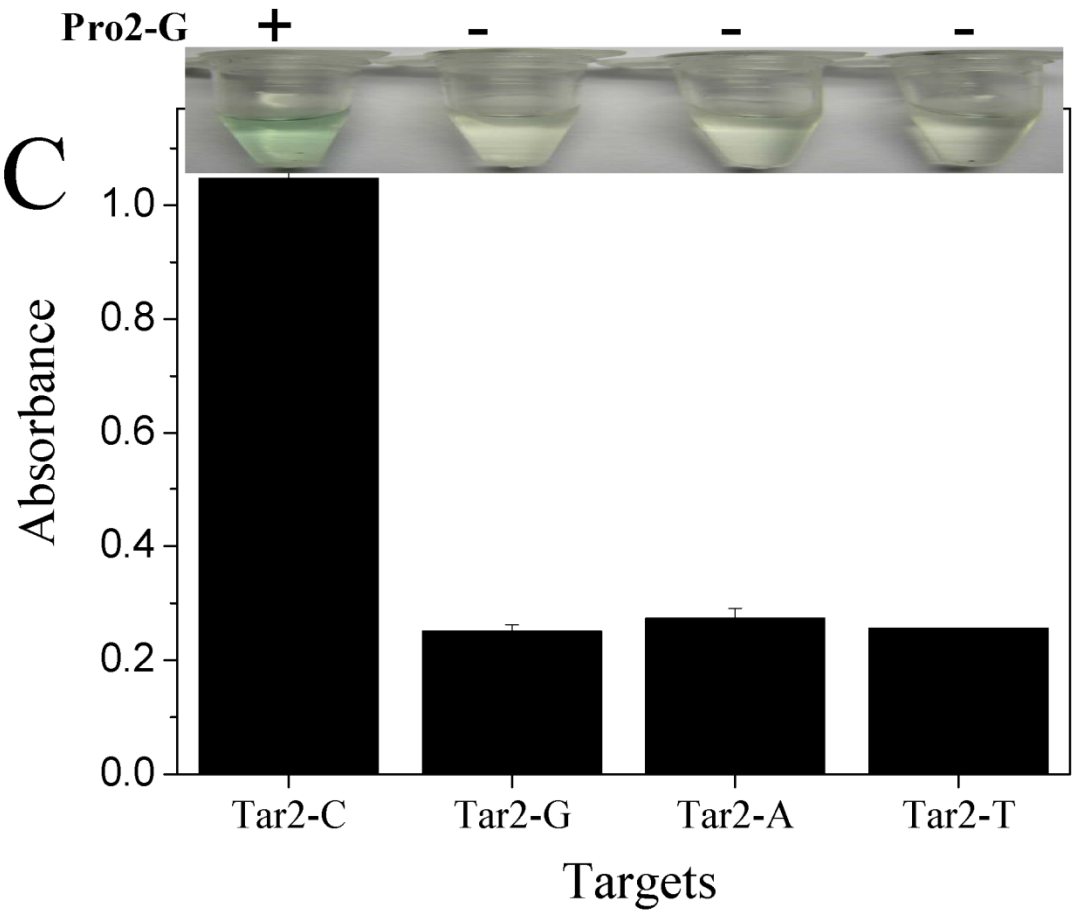
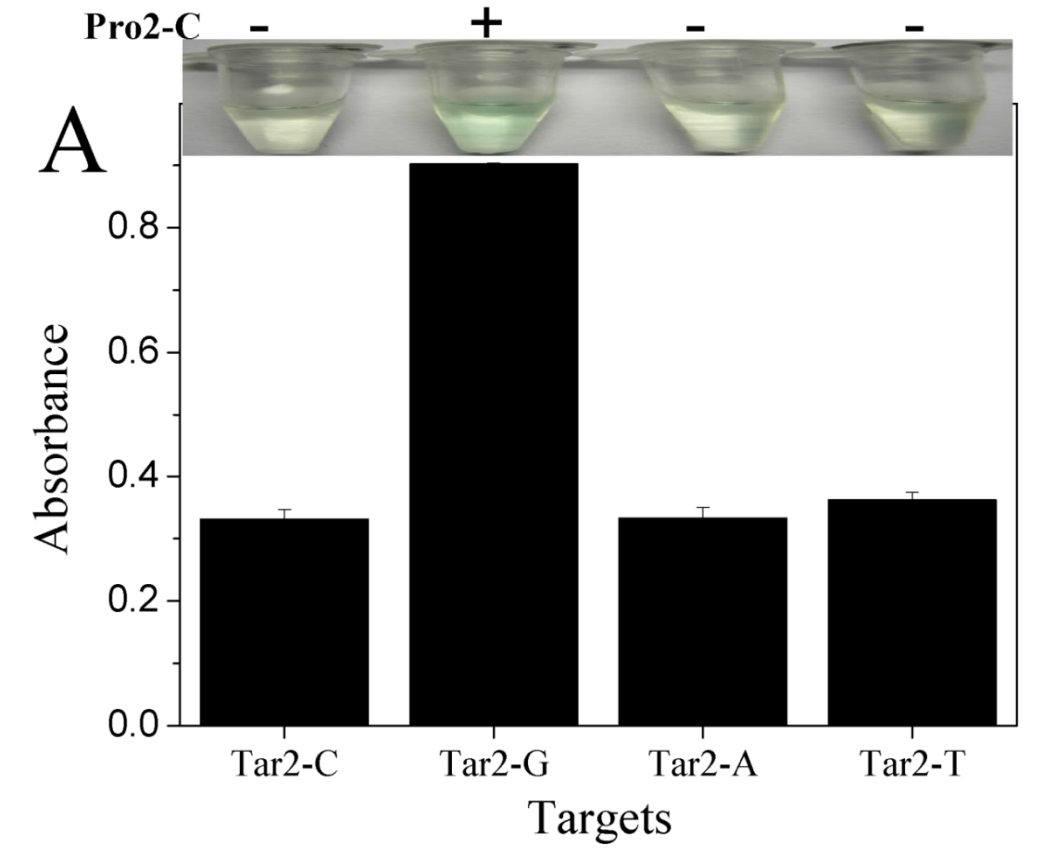
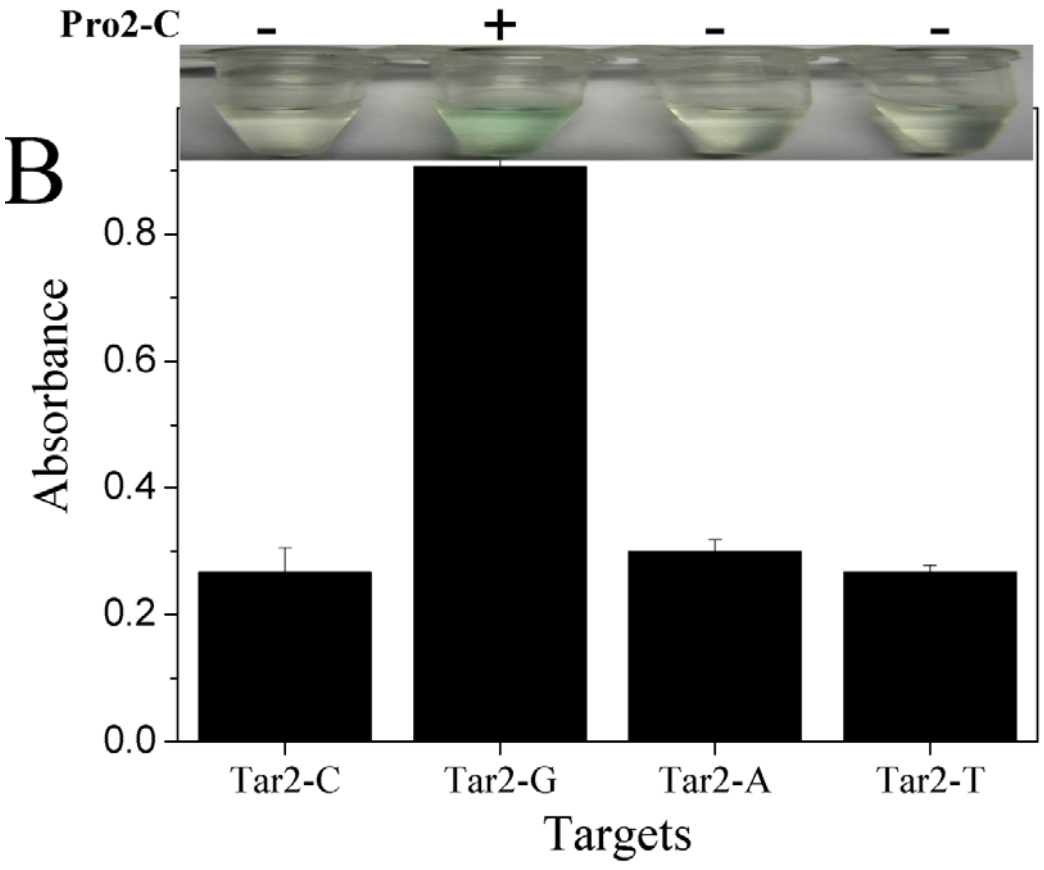


Fig. S14. The identification of SNPs by the Pro2-G probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 65 ^{o}C and 25 cycles were conducted; (C) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2-G] = [iPro-C] = [iPro-T] = [iPro-A] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

3.3.2 SNPs identification by the Pro2-C probe





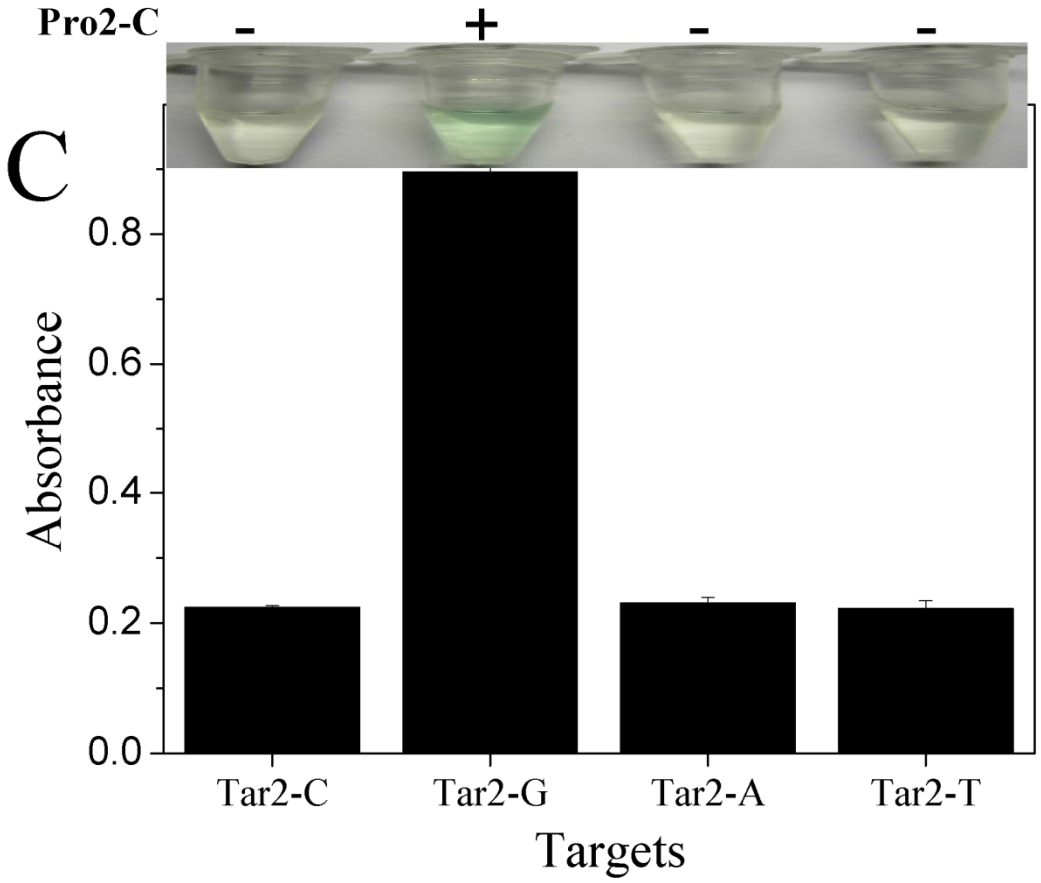
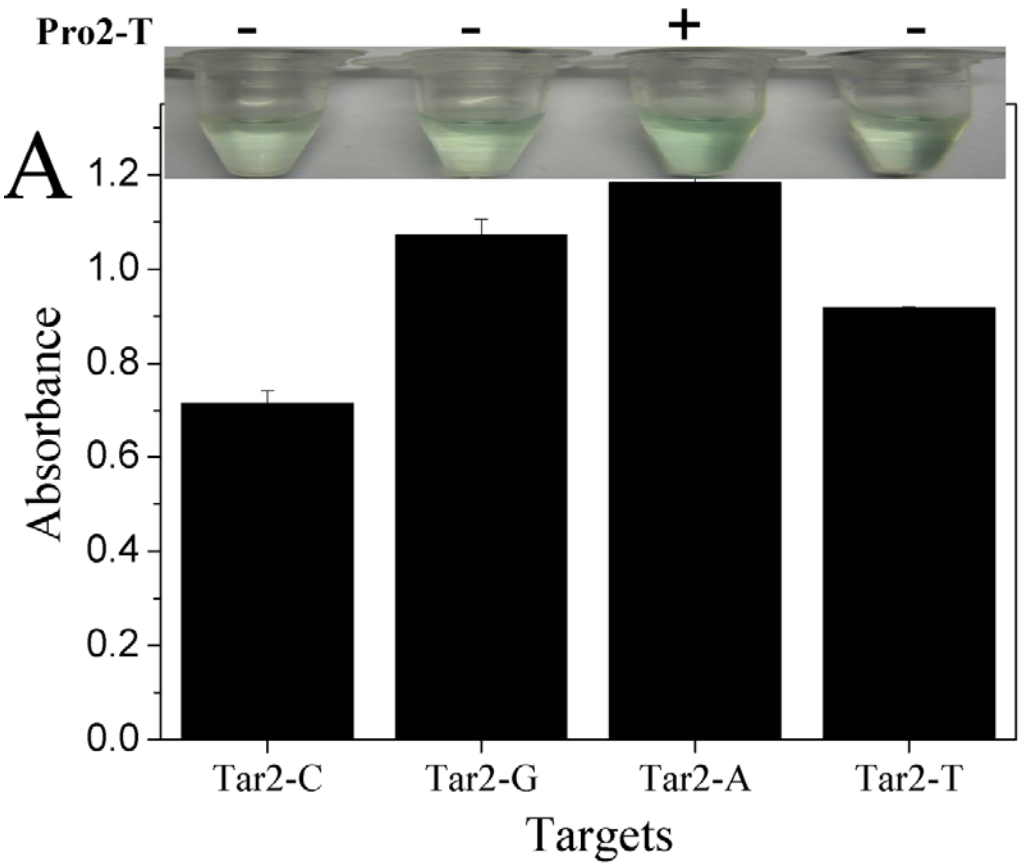
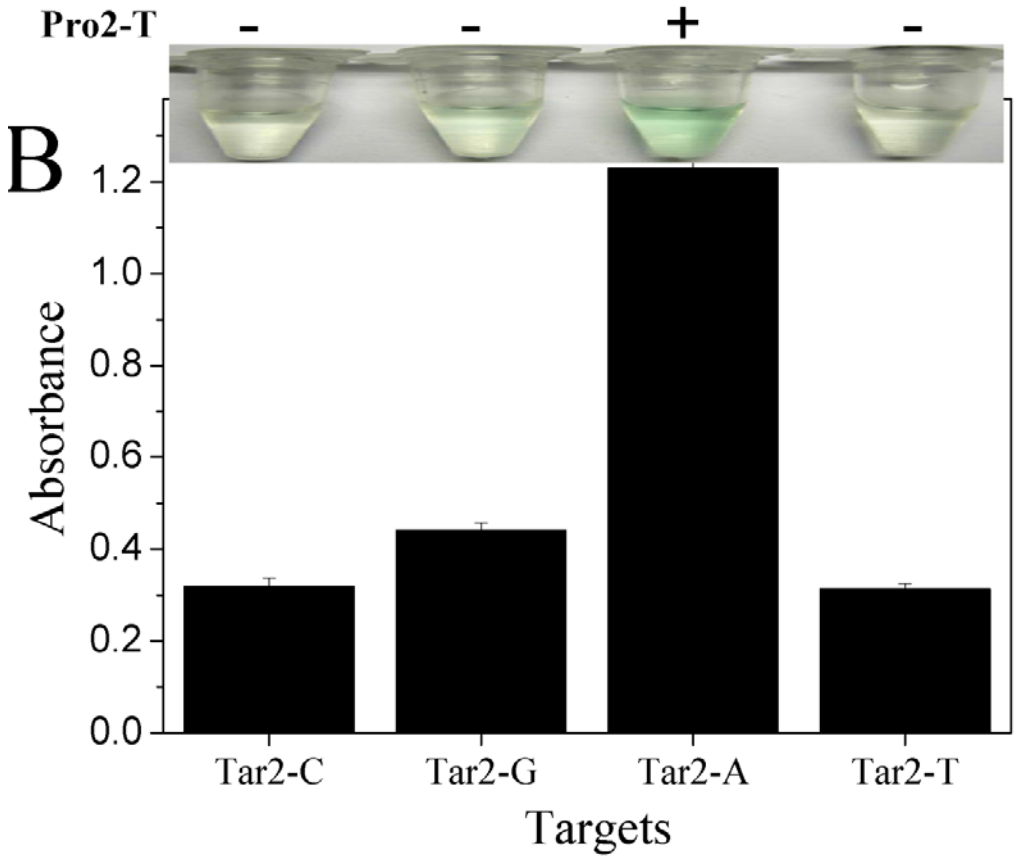


Fig. S15. The identification of SNPs by the Pro2-C probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 65 ^{o}C and 25 cycles were conducted; (C) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2-C] = [iPro-G] = [iPro-T] = [iPro-A] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

3.3.3 SNPs identification by the Pro2-T probe





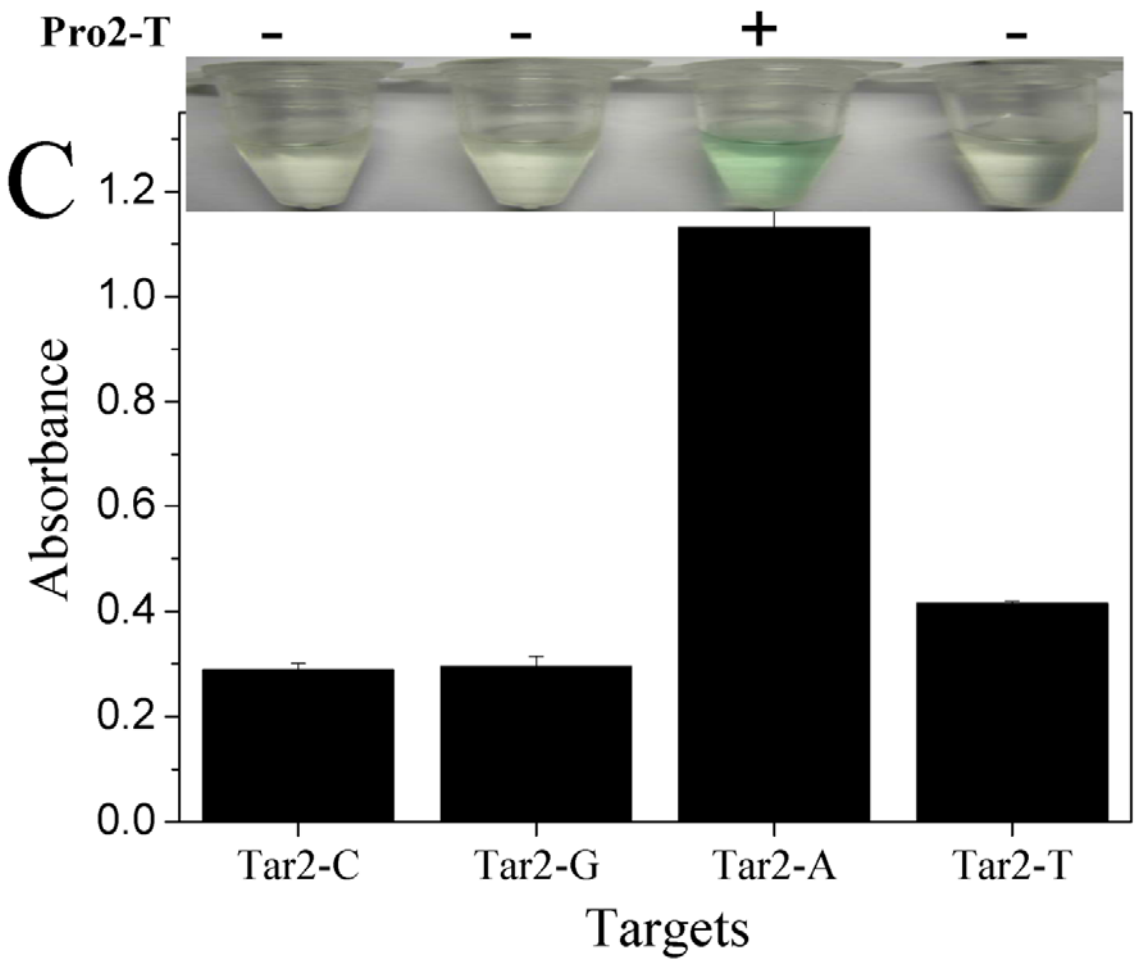
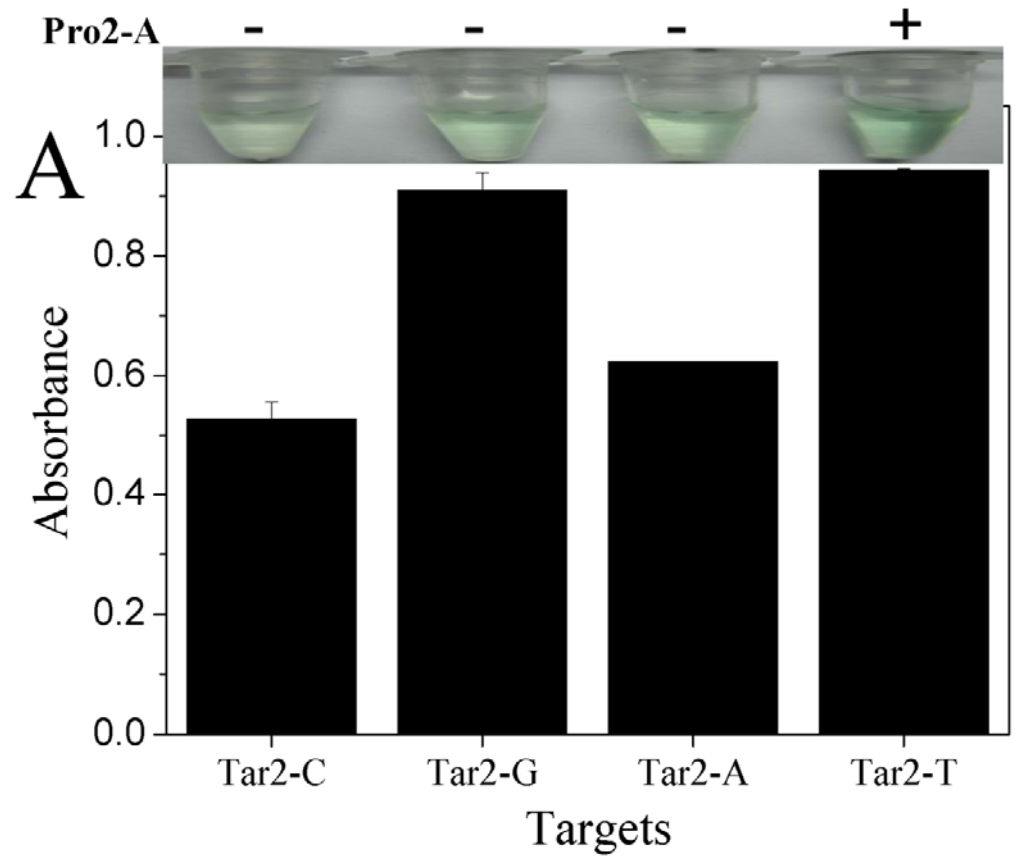
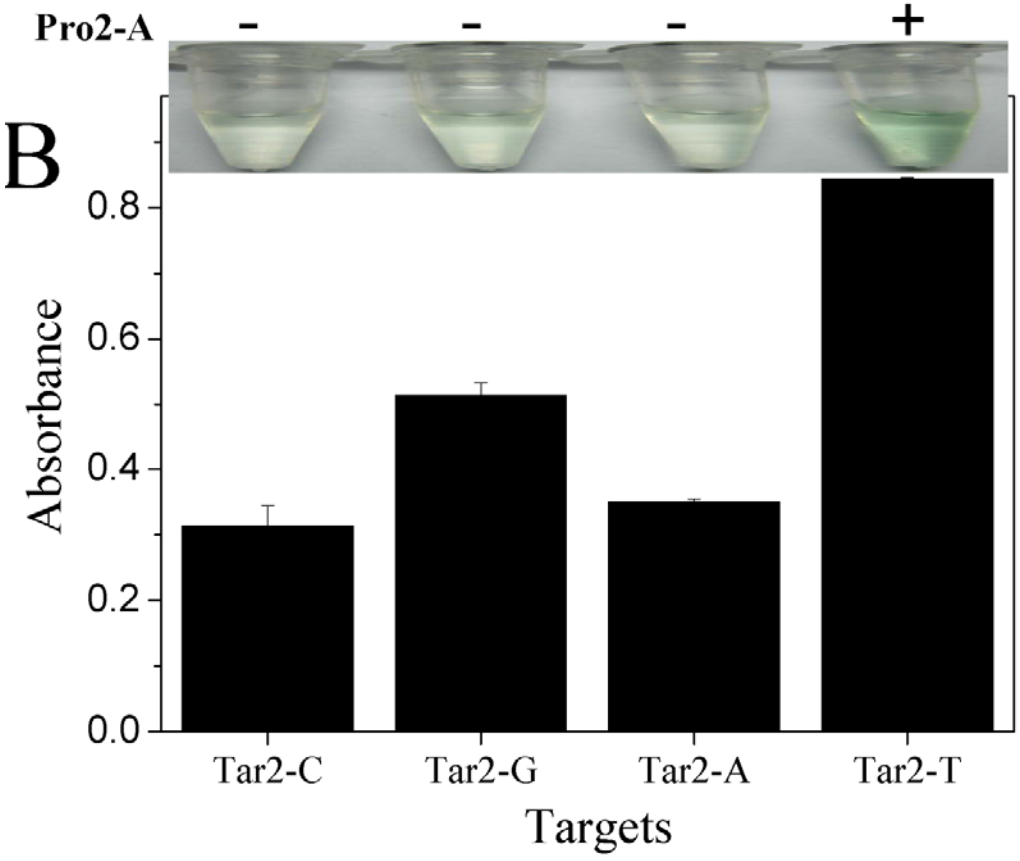


Fig. S16. The identification of SNPs by the Pro2-T probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 65 ^{o}C and 25 cycles were conducted; (C) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2-T] = [iPro-G] = [iPro-C] = [iPro-A] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

3.3.4 SNPs identification by the Pro2-A probe





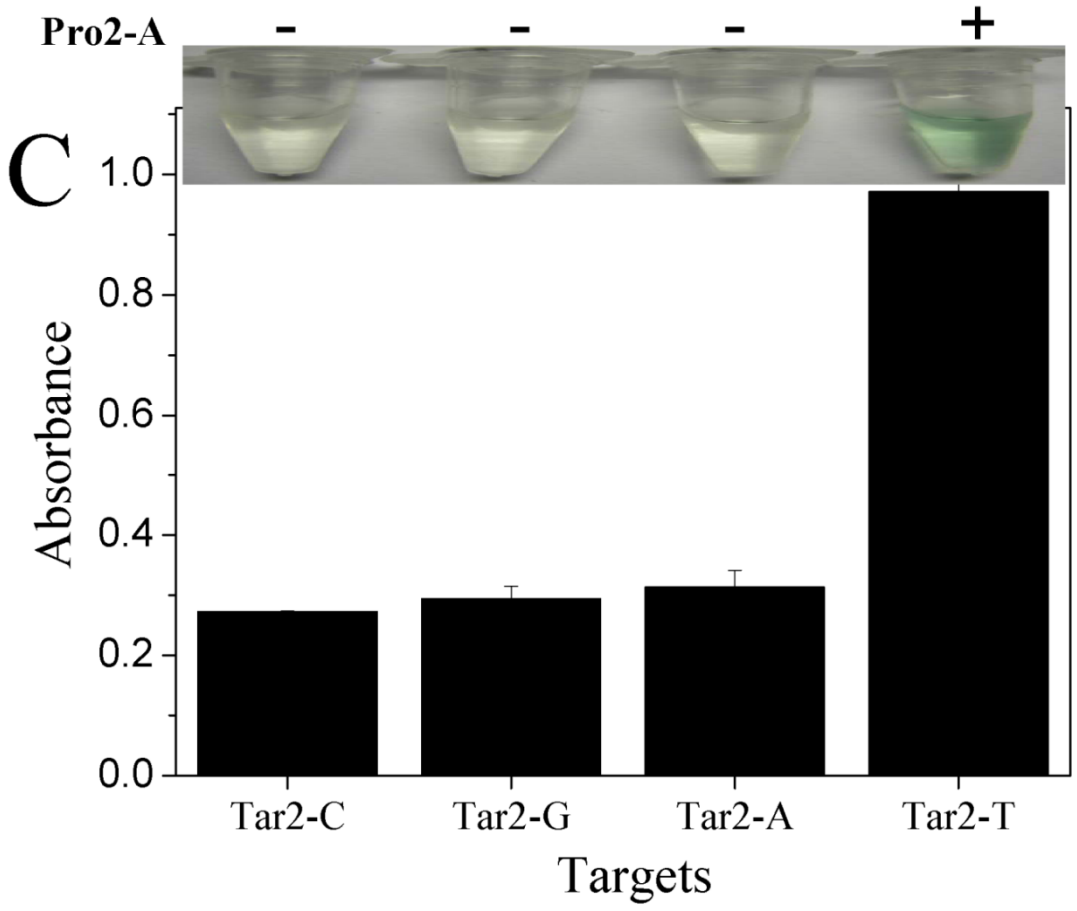


Fig. S17. The identification of SNPs by the Pro2-A probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 65 ^{o}C and 25 cycles were conducted; (C) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2-A] = [iPro-G] = [iPro-C] = [iPro-T] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

3.3.5 SNPs identification by the Pro2′-G probe

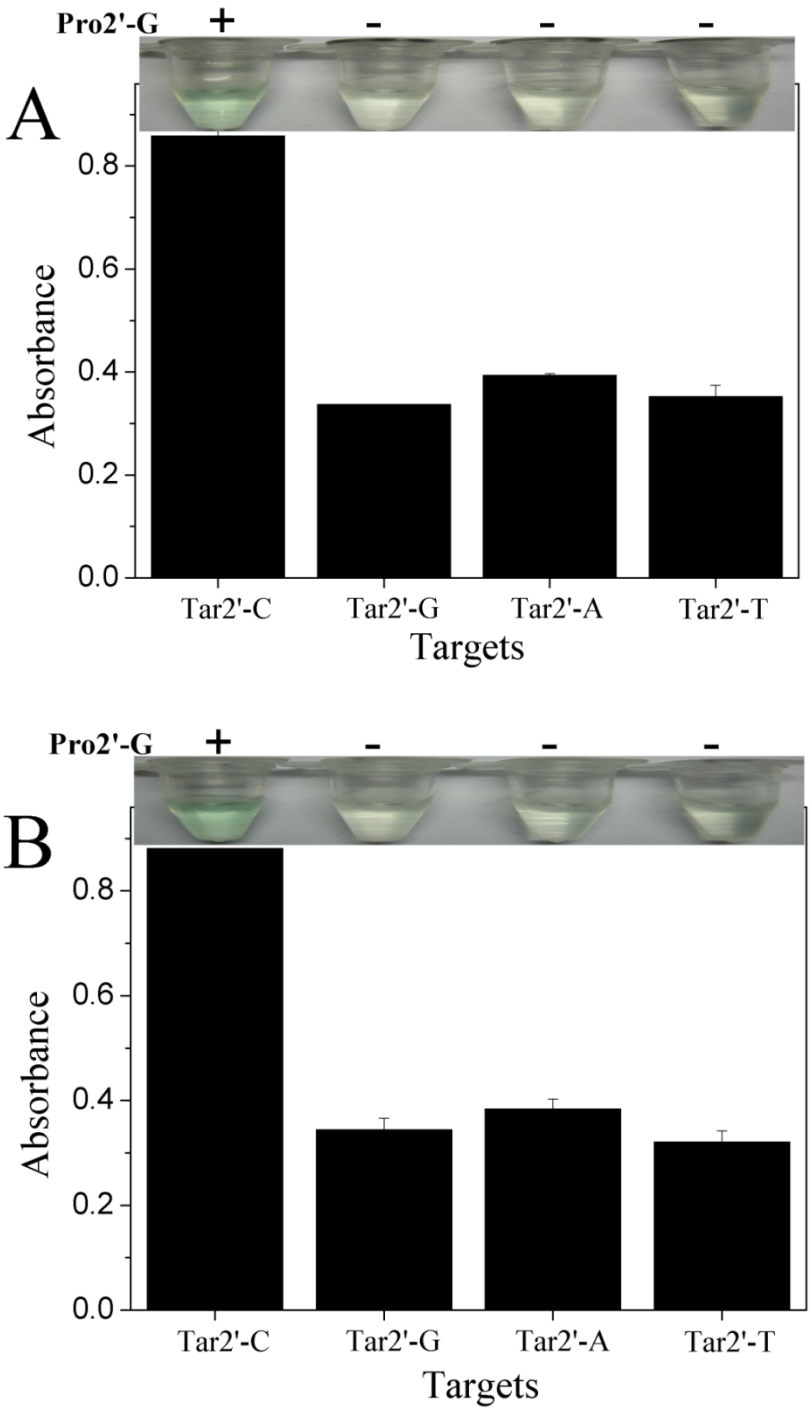


Fig. S18. The identification of SNPs by the Pro2′-G probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2′-G] = [iPro′-C] = [iPro′-T] = [iPro′-A] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

3.3.6 SNPs identification by the Pro2′-C probe

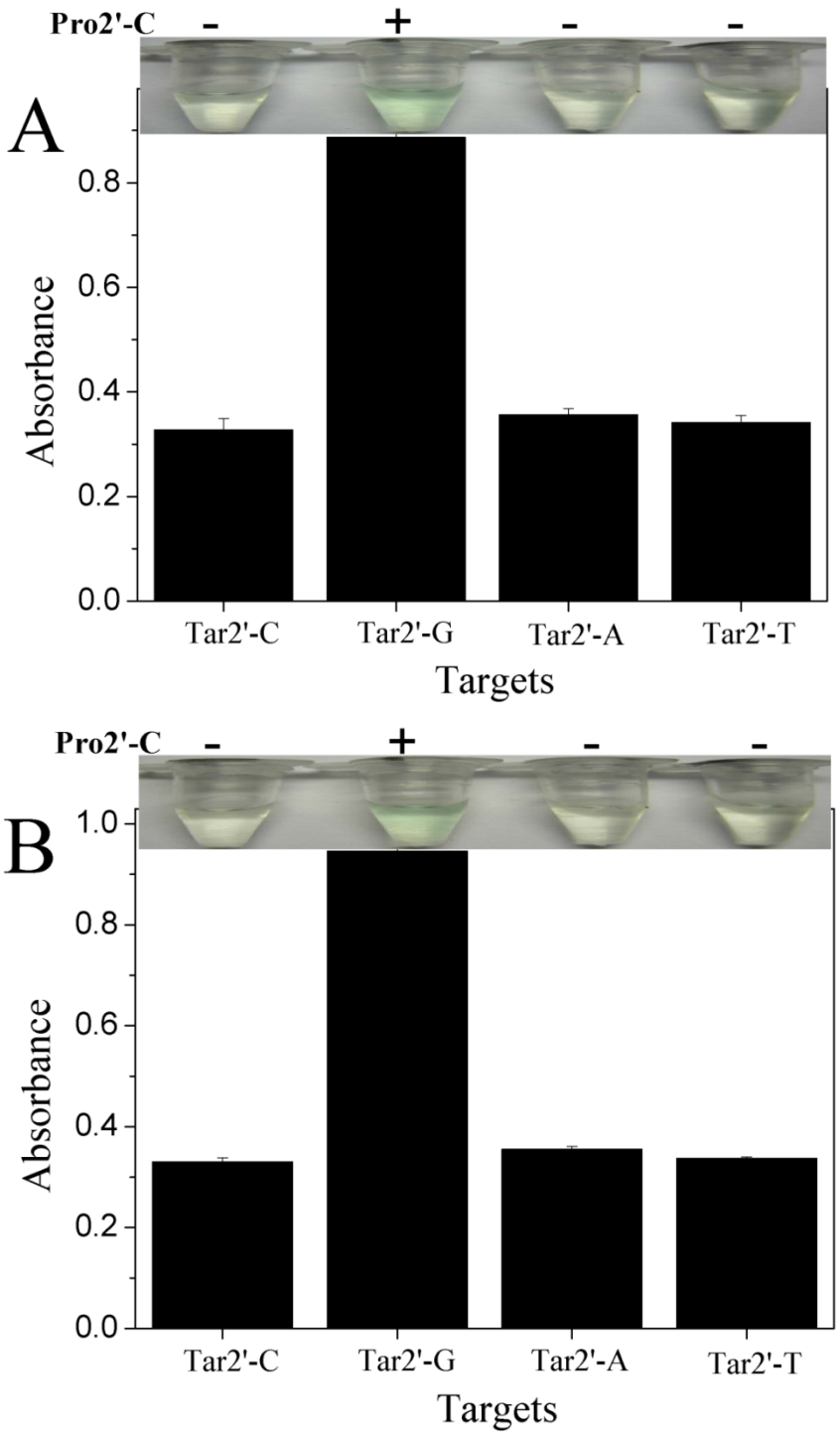


Fig. S19. The identification of SNPs by the Pro2′-C probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2′-C] = [iPro′-G] = [iPro′-T] = [iPro′-A] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

3.3.7 SNPs identification by the Pro2′-T probe

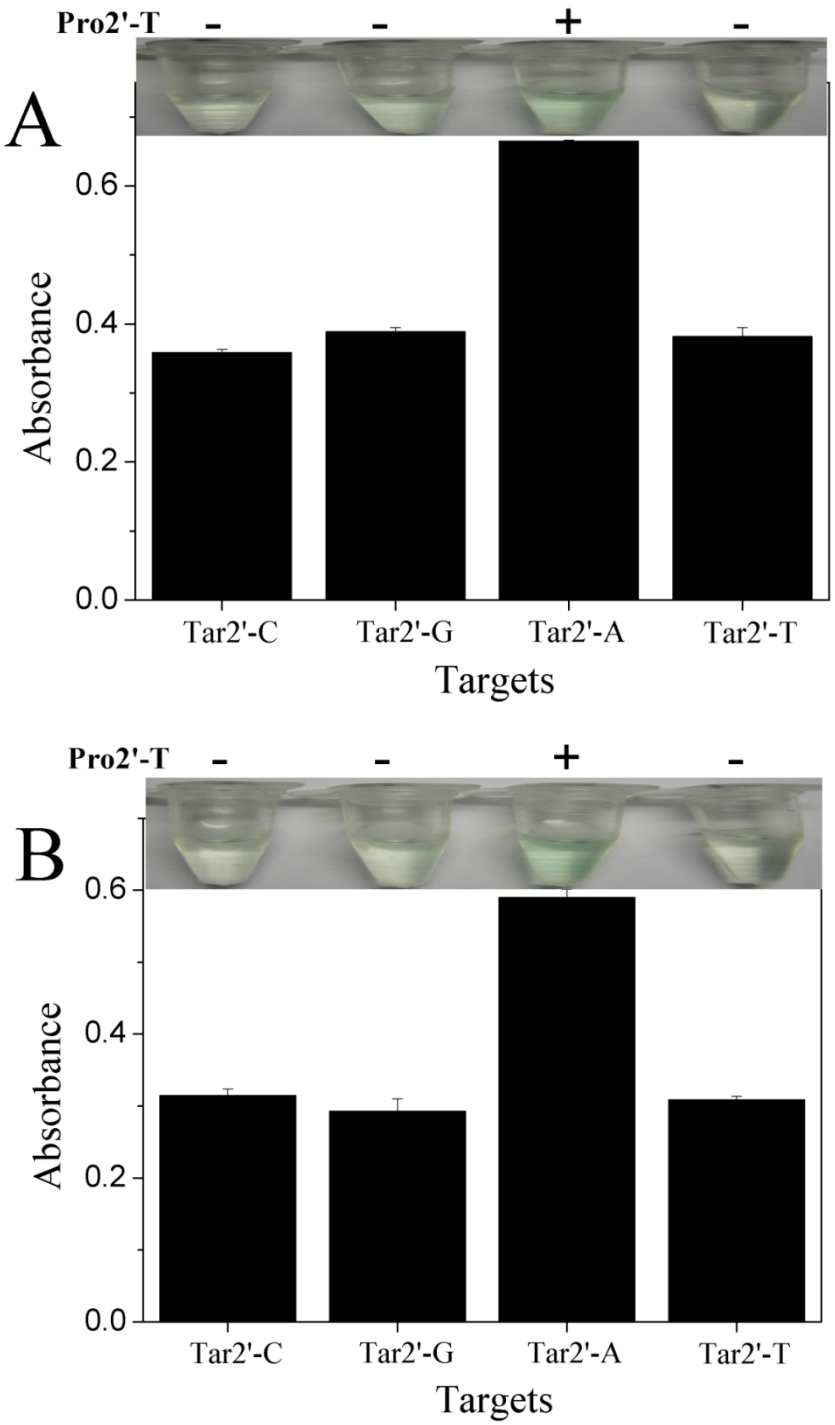


Fig. S20. The identification of SNPs by the Pro2′-T probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2′-T] = [iPro′-G] = [iPro′-C] = [iPro′-A] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

3.3.8 SNPs identification by the Pro2′-A probe

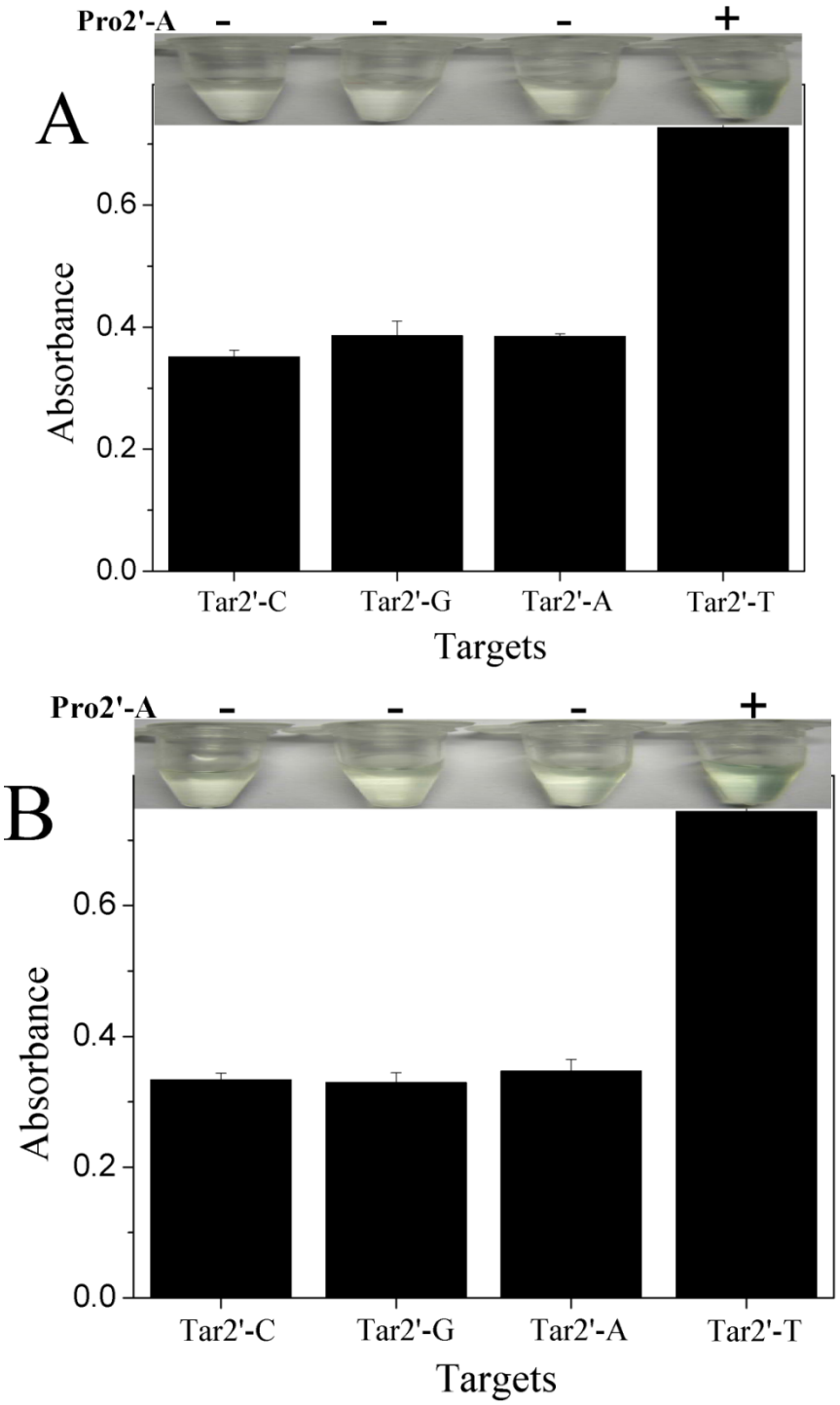


Fig. S21. The identification of SNPs by the Pro2′-A probe. (A) The anneling temperature was 60 ^{o}C; (B) The anneling temperature was 60 ^{o}C and competition strategy was used. [Pro2′-T] = [iPro′-G] = [iPro′-C] = [iPro′-A] = 0.6 μM. The symbol of "+" represents that the probe is perfectly complementary to the target, "-" represents that there is a single base mismatch between the probe and the target.

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