Supporting Information

Terminal Protection of Small Molecule-Linked DNA : A Versatile Biosensor Platform for Protein Binding and Gene Typing Assay

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Labeling of FA to NH_2-modified oligonucleotides. The FA label was conjugated to the NH_2-moiety of the oligonucleotides using the succinimide coupling (EDC-NHS) method.[1,2] Briefly, 0.5 mL of 20 μM DNA with NH_2 modifier of nucleotide was mixed with 0.5 mL of 10 mM phosphate buffer (PB, pH 7.4) containing 10 mM FA, 1 mM EDC, and 5 mM Sulfo-NHS, and incubated for 2 h at 37 ºC in dark. The solution was then dialyzed against PB using a membrane with molecular weight cutoff of 1,000 Dalton to remove excessive FA. The dialysis was performed for 3 days with shield from light and changes of the fresh buffer every 4 h. The last time dialyzed was performed in sterile water.

Table S1. Synthesized oligonucleotides probes (5'→3') used in the experiments of supporting information.[a]

Probe **10**	Folate-GAC AAG ACA CTT GGA ATT CCA AGC GCG AAG TTT TCT TCG CGC TTG GAA TTC CAA GTG TCT TGT C
Probe **11**	GAA TTC CAA GCG CGA AGT TTT CTT CGC GCT TGG AAT TC-Folate
Probe **12**	Folate-CAC TGC CAA GAA TTC CAA GCG CGA AGT TTT TT
Probe **13**	TTC TTT TTC ACC ATT CTA AAG AAT AAC AGT AAT TTC TGG GTT AAG GT-Folate
Probe **14**	NH_2-GAC AAG ACA CTT GGA ATT CCA AGC GCG AAG TTT TCT TCG CGC TTG GAA TTC CAA GTG TCT TGT C
Probe **15**	NH_2-CAC TGC CAA GAA TTC CAA GCG CGA AGT TTT TT
Probe **16**	TTC TTT TTC ACC ATT CTA AAG AAT AAC AGT AAT TTC TGG GTT AAG GT-NH_2
Probe **17**	FITC-GAC AAG ACA CTT GGA ATT CCA AGC GCG AAG TTT TCT TCG CGC TTG GAA TTC CAA GTG TCT TGT C
Probe **18**	GAA TTC CAA GCG CGA AGT TTT CTT CGC GCT TGG AAT TC-FITC
Probe **19**	FITC-CAC TGC CAA GAA TTC CAA GCG CGA AGT TTT TT
Probe **20**	TTC TTT TTC ACC ATT CTA AAG AAT AAC AGT AAT TTC TGG GTT AAG GT-FITC

[a] Probes **10**, **11**, **12, 13, 17, 18, 19** and **20** were used in gel electrophoresis analysis for validating terminal protection of DNA–small molecule chimeras against double-strand or single-strand selective exonucleases. Probes **10, 11, 12** and **13** were obtained by conjugating probes **14, 5, 15** and **16**, respectively, with folate via -NH_2 moiety at the 3' terminus.

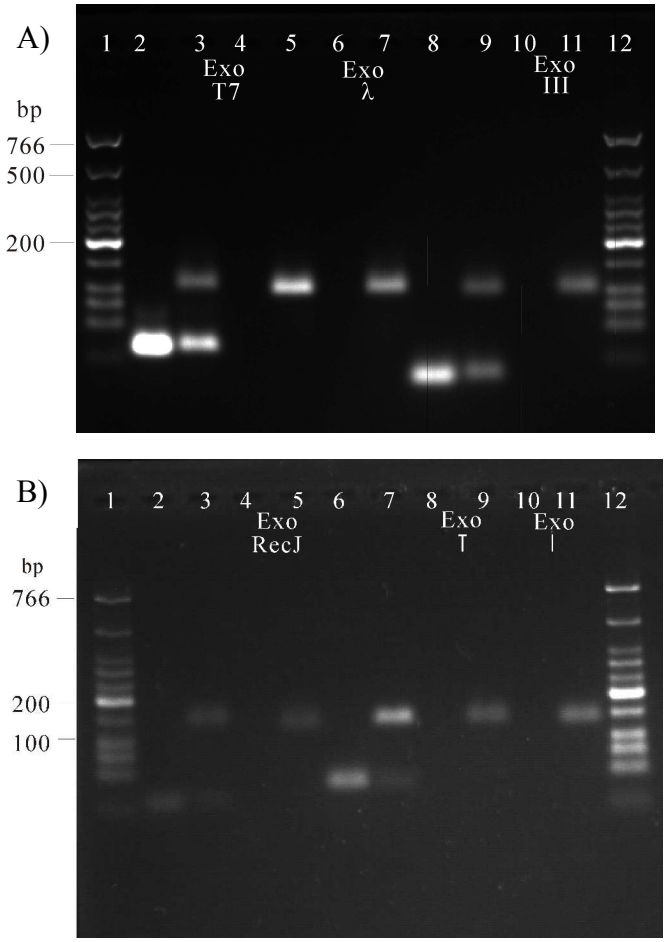

Figure S1. Gel electrophoresis image for terminal protection against double-strand exonucleases. Lanes 1 and 12, DNA size marker; lane 2, folate-linked probe **10**; lane 3, probe **10** plus FR; lane 4, probe **10** digested by Exo T7; lane 5, probe **10** plus FR digested by Exo T7; lane 6, probe **10** digested by Exo λ; lane 7, probe **10** plus FR digested by Exo λ; lane 8, folate-linked probe **11**; lane 9, probe **11** plus FR; lane 10, probe **11** digested by Exo III; lane 11, probe **11** plus FR digested by Exo III; (B) Gel electrophoresis image for terminal protection against single-strand exonucleases. Lanes 1 and 12, DNA size marker; lane 2, folate-linked probe **12**; lane 3, probe **12** plus FR; lane 4, probe **12** digested by Exo RecJ; lane 5, probe **12** plus FR digested by Exo RecJ; lane 6, folate-linked probe **13**; lane 7, probe **13** plus FR; lane 8, probe **13** digested by Exo T; lane 9, probe **13** plus FR digested by Exo T; lane 10, probe **13** digested by Exo I; lane 11, probe **13** plus FR digested by Exo I. The single-stranded DNA was stained with SYBR Green II. The DNA size marker was stained with SYBR Green I.

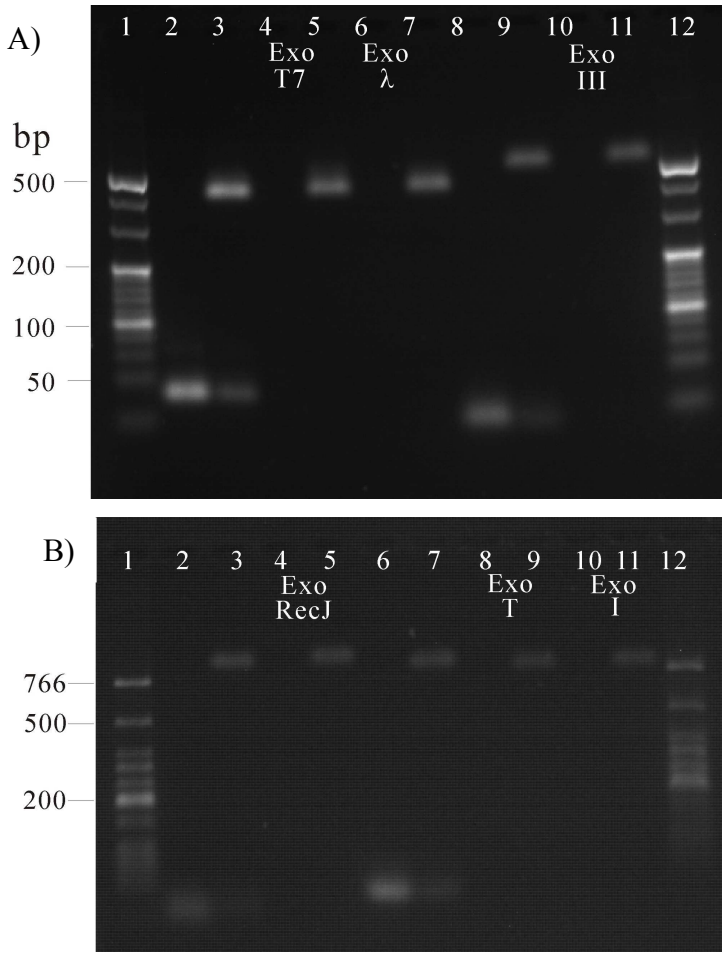

Figure S2. Gel electrophoresis image for terminal protection against double-strand exonucleases. Lanes 1 and 12, DNA size marker; lane 2, FITC-linked probe **17**; lane 3, probe **17** plus FITC antibody (Ab); lane 4, probe **17** digested by Exo T7; lane 5, probe **17** plus FITC Ab digested by Exo T7; lane 6, probe **17** digested by Exo λ; lane 7, probe **17** plus FITC Ab digested by Exo λ; lane 8, FITC-linked probe **18**; lane 9, probe **18** plus FITC Ab; lane 10, probe **18** digested by Exo III; lane 11, probe **18** plus FITC Ab digested by Exo III; (B) Gel electrophoresis image for terminal protection against single-strand exonucleases. Lanes 1 and 12, DNA size marker; lane 2, FITC-linked probe **19**; lane 3, probe **19** plus FITC Ab; lane 4, probe **19** digested by Exo RecJ; lane 5, probe **19** plus FITC Ab digested by Exo RecJ; lane 6, FITC-linked probe **20**; lane 7, probe **20** plus FITC Ab; lane 8, probe **20** digested by Exo T; lane 9, probe **20** plus FITC Ab digested by Exo T; lane 10, probe **20** digested by Exo I; lane 11, probe **20** plus Anti-FITC Ab digested by Exo I. The single-stranded DNA was stained with SYBR Green II. The DNA size marker was stained with SYBR Green I.

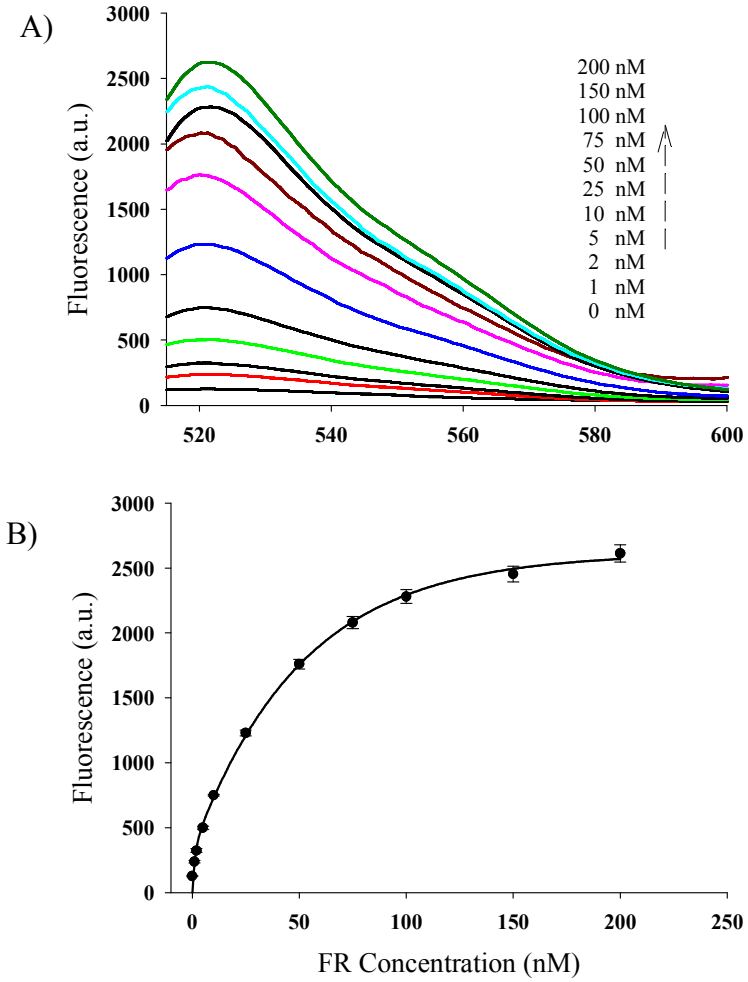

Figure S3. (A) Typical fluorescence spectral responses of small molecule–protein interaction assay to folate receptor FR of varying concentrations. (B) Fluorescence response at 522 nm of small molecule–protein interaction assay versus FR concentration. Error bars are standard deviation (SD) across five repetitive experiments.

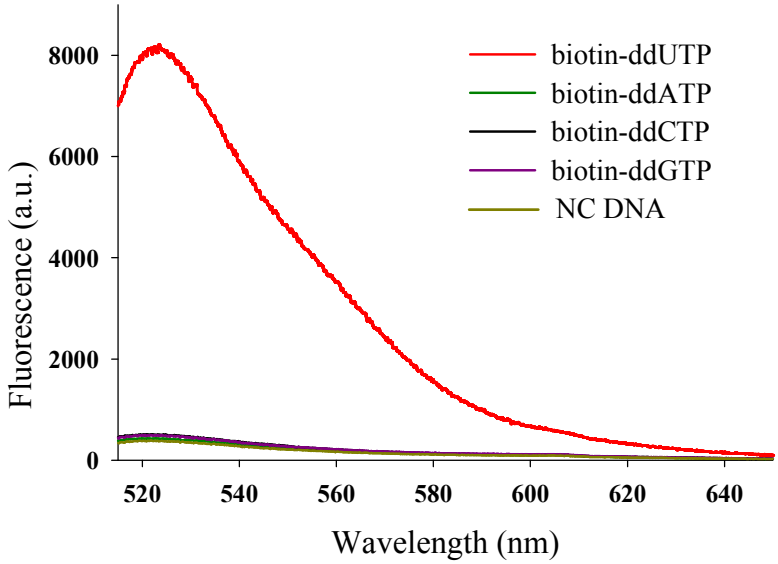

Figure S4. Typical fluorescence responses of genotyping assay to 100 nM mutant DNA target **8** in the presence of one of four biotinylated nucleotides and to 100 nM non-complementary DNA target **9** (NC DNA).

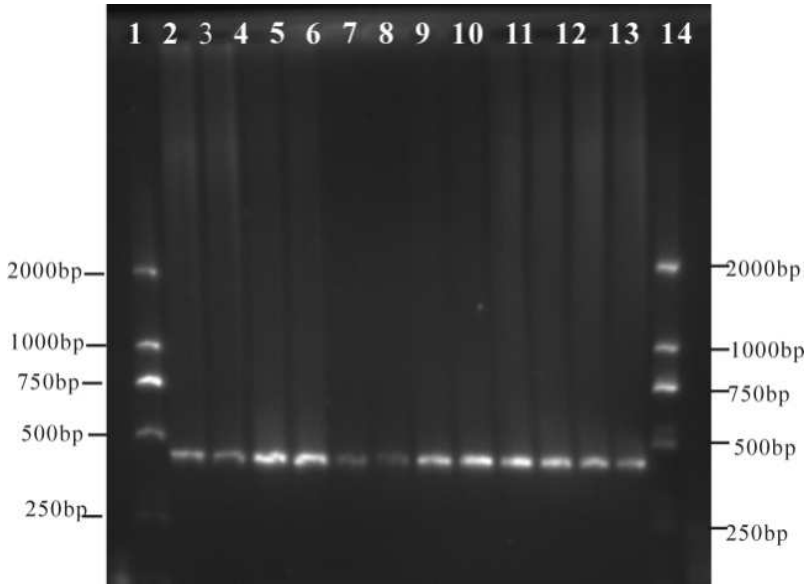

Figure S5. Image of 1% agarose gel electrophoresis of PCR amplicons. Lanes 1 and 14 are DNA size marker, the other lanes are the PCR products. Bright bands were observed for the PCR amplicons near the position of DNA marker with a length 500 base-pairs. This is consistent with the length of target amplicons with predicted 423 base-pairs.

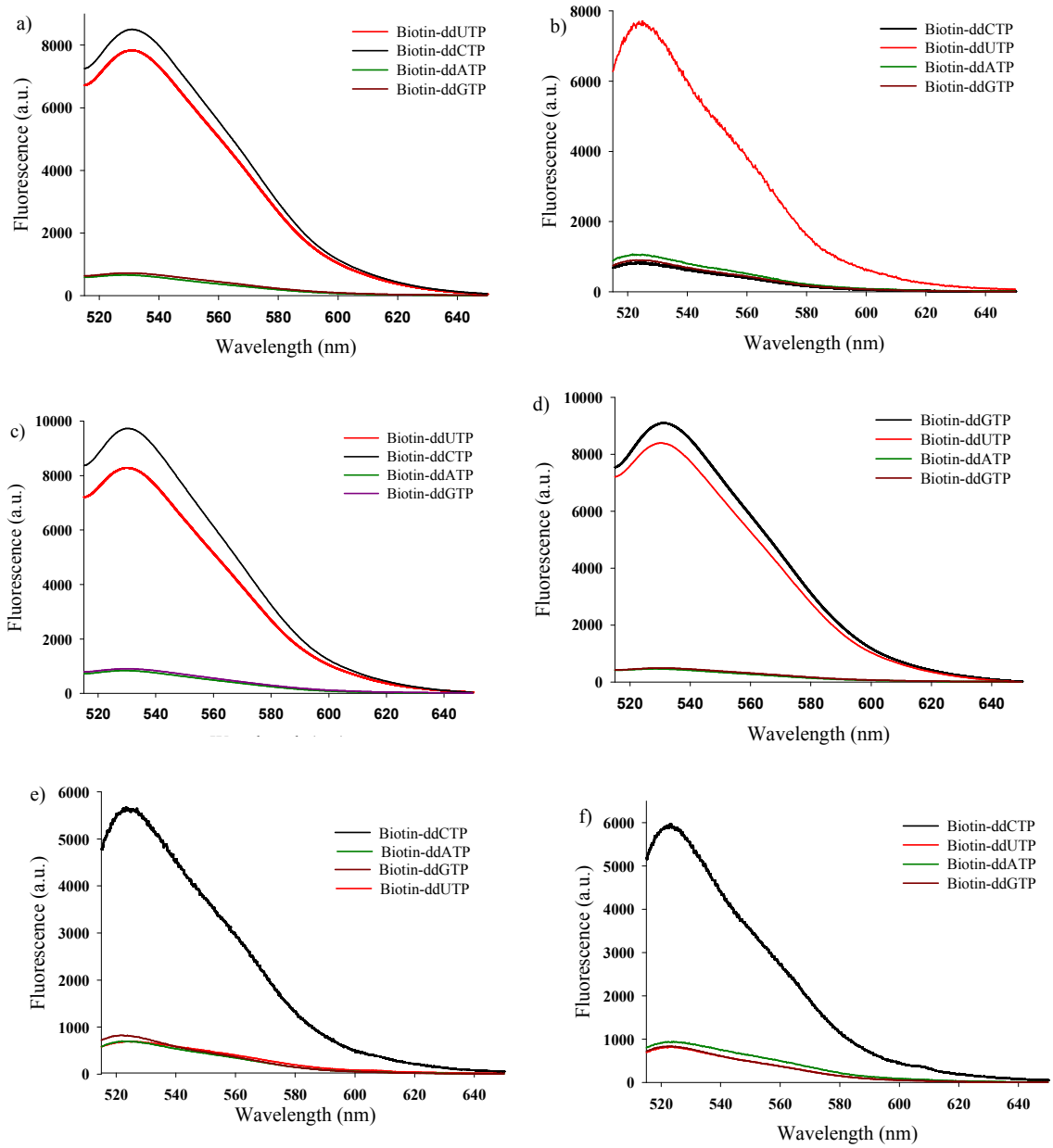

Figure S6. Typical fluorescence responses of genotyping assay to genomic samples with one of four biotinylated nucleotides. a) Addition of biotin-ddCTP and biotin-ddUTP both yield strong fluorescence signals, indicating both the wildtype and the mutant targets are present in the sample, i.e., the sample is heterozygous (gene is mutant on one chromosome, and not on the other chromosome); `b) Addition of biotin-ddUTP yields a strong fluorescence signal, indicating only the mutant target is present in the sample, i.e., the sample is homozygous mutant (gene is mutant on both chromosomes); c-d) Addition of biotin-ddCTP and biotin-ddUTP both yield strong fluorescence signals, identifying a heterozygous sample; e-f) Addition of biotin-ddCTP yields a strong fluorescence signal, indicating only the wildtype target is present in the sample, i.e., the sample is in normal state (gene is not mutant on both chromosomes);

(To be continued)

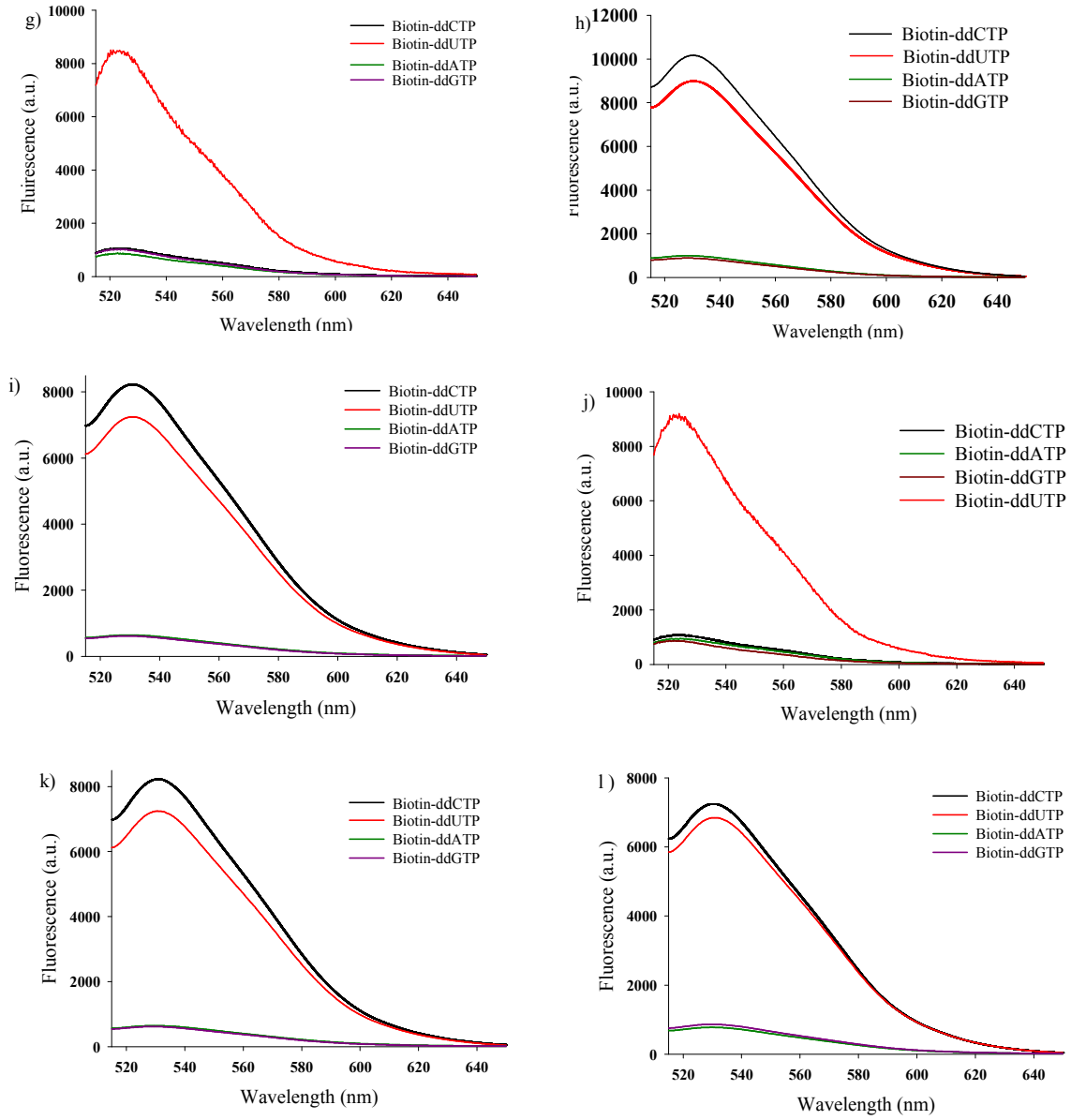

Figure S6. (Continued). g) Addition of biotin-ddCTP yields a strong fluorescence signal, identifying a normal sample; h-i) Addition of biotin-ddCTP and biotin-ddUTP both yield strong fluorescence signals, identifying a heterozygous sample; j) Addition of biotin-ddCTP yields a strong fluorescence signal, identifying a normal sample; k-l) Addition of biotin-ddCTP and biotin-ddUTP both yield strong fluorescence signals, identifying a heterozygous sample. The identified SNP types are in good agreement with sequencing analysis.

References:

(1) Zuker. M. *Nucleic Acids Res*. **2003**, *3*1, 3406-3415.

(2) Fahlman, R. P.; Sen, D. *J. Am. Chem. Soc.* **2002**, *124*, 4610-4616.