Supporting information

Allele specific DNAzyme assembly for fast and convenient SNP colorimetric genotyping directly from noninvasive crude sample

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Contents:

Supplement of Experimental section

DNA purification. Total genomic DNA was extracted from 200 μL of blood samples using a TIANamp genomic DNA kit from TianGen Biotech Co., Ltd (Beijing, China) according to the manufacturer's instructions. The quantity of DNA was measured by a NanoDrop 1000 ultraviolet spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The DNA yield per buccal swab was calculated by multiplying the DNA concentration by the final volume of DNA extract and the epithelial cell amount was calculated by the DNA yield divided by the amount of DNA per cell (~6pg).[1]

Supplement of Figures and Tables

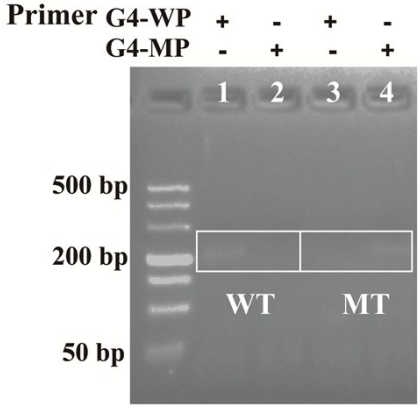

Fig. S1. The gel electrophoresis analysis of the amplification products of G4-AS primers with 68 °C annealing temperature.

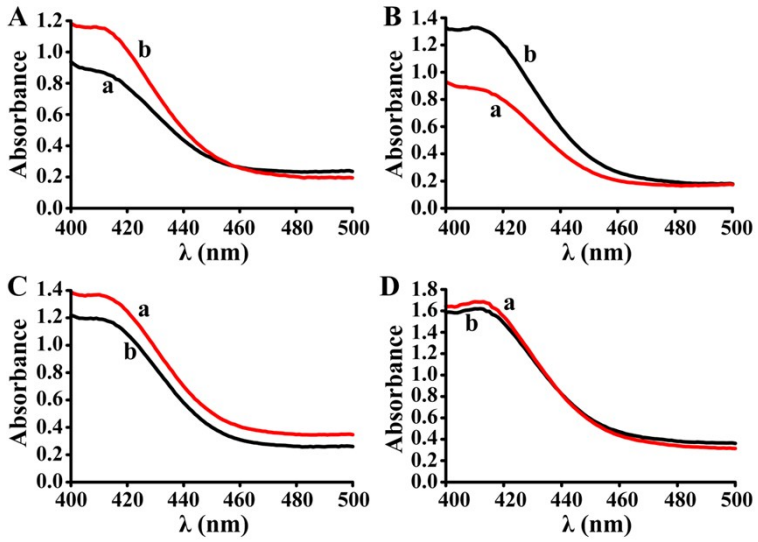

Fig. S2. The UV-vis absorbance spectra of the ASDA-based colorimetric assay for (A) negative control (without DNA polymerase), (B) wild, (C) heterozygous, (D) mutation whole blood sample. (a) WP tube, (b) MP tube.

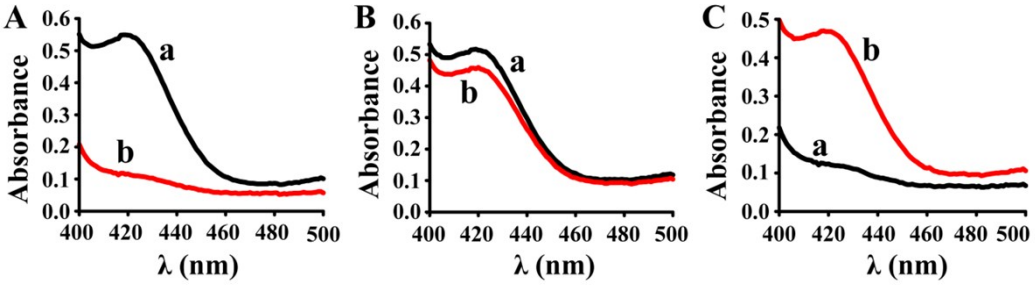

Fig. S3. The UV-vis absorbance spectra of the ASDA-based colorimetric assay of (A) wild, (B) heterozygous, (C) mutation leukocyte sample. (a) WP tube, (b) MP tube.

Table S1. Primer sequences used in this study (WP, wild specific primer, MP, mutation specific primer, The red and italics letters stand for an additional mismatch at the third base of the 3' terminus of forward primers. The stem regions of G4-containing hairpin are underlined).

Application	Primer	Sequence (from 5' to 3')
AS PCR	WP (Match)	AGG AGA AGG TGT CTG CGG G**A**G C
	MP (Match)	AGG AGA AGG TGT CTG CGG G**A**G T
	WP (TT mismatch)	AGG AGA AGG TGT CTG CGG G*T*G C
	MP (TT mismatch)	AGG AGA AGG TGT CTG CGG G*T*G T
	WP (TC mismatch)	AGG AGA AGG TGT CTG CGG G*C*G C
	MP (TC mismatch)	AGG AGA AGG TGT CTG CGG G*C*G T
	WP (TG mismatch)	AGG AGA AGG TGT CTG CGG G*G*G C
	MP (TG mismatch)	AGG AGA AGG TGT CTG CGG G*G*G T
	Reverse primer	GCC CCT CAC CTG GAT GGG AAA G
	G4 -WP	<u>GGG TAG GGC GGG</u> TTG GGT T spacer18 AA<u>C</u> <u>CCG CCC TAC CC</u>A AAA GGA GAA GGT GTC TGC GGG *T*GC
	G4-MP	<u>GGG TAG GGC GGG</u> TTG GGT T spacer18 AA<u>C</u> <u>CCG CCC TAC CC</u>A AAA GGA GAA GGT GTC TGC GGG *T*GT
	G4-RP	<u>GGG TAG GGC GGG</u> TTG GGT T spacer18 AA<u>C</u> <u>CCG CCC TAC CC</u>A AAG CCC CTC ACC TGG ATG GGA AAG
PCR-RFLP	Forward primer	CAT CCC TAT TGG CAG GTT AC
	Reverse primer	GAC GGT GCG GTG AGA GTG

Table S2. Concentration and yield of DNA extracted from buccal swab samples

Sample	1	2	3	4	5
DNA concentration (ng/μL)	4.0	18.1	7.5	6.0	9.2
Total DNA yield (ng)	200	543	216	180	276
The calculated epithelial cell amount ($\times 10^4$)	3.3	9.1	3.6	3.0	4.6

Table S3. Genotyping results of MTHFR C667T polymorphism of 30 buccal swab samples and their frequencies.

		ASDA (n = 30)						
		CC	CT	TT	Discrepant	Total	Agreement	Frequency
PCR-RFLP (n = 30)	CC	8	0	0	0	8	100%	27%
	CT	0	18	0	0	18	100%	60%
	TT	0	0	4	0	4	100%	13%
	Total	8	18	4	0	30	100%	100%

Reference

1. V. J. Bailey, H. Easwaran, Y. Zhang, E Griffiths, S. A. Belinsky, J. G Herman, S. B. Baylin, H. E. Carraway and T. H. Wang, *Genome Res*, 2009, **19**, 1455-1461.