Electronic Supplementary Material (ESI) for Analytical Methods.

Specificity Improvement of Invader Assay by Introducing an Artificially Mismatched Base into the Probe

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Supplementary

Table S1 Comparison between Invader assay and other close-tube techniques for SNP detection.

Methods	Experiment set-up *	Specificity	Dye-labelled reporter
Invader assay	++	+++++	Universal
Real-time PCR (TaqMan™)	+++++	+++	SNP-specific
Allele-specific amplification	++++	++++	SNP-specific
Allele-specific ligation	+++++	+++++	SNP-specific

*The number of "+" means the difficulty for setting up an experiment for detecting SNPs.

Table S2 Oligonucleotides used for PCR or Invader reaction.

Name	Sequence (5'-3')	Tm (°C)
FRET-1	VIC-TCT T (BHQ1) AG CCG GTT TTC CGG CTA AGA CCT CGG CGC G-C6-NH_2	
FRET-2	FAM-TCT T (BHQ1) AG CCG GTT TTC CGG CTA AGA CTC CGC GTC CGT-C6-NH_2	
*ALDH2**2-F	CCG GGA GTT GGG CGA GTA C	70.1
*ALDH2**2-R	GCC CCC AGC AGG TCC C	71.6
*ALDH2**2-UP	GGG CTG CAG GCA TAC ACT T	69.0
*ALDH2**2-WP-1	CGC GCC GAG GGA AGT GAA AAC TGT GAG TG-PO_3	62.3
*ALDH2**2-WP-2	CGC GCC GAG GG*g* AGT GAA AAC TGT GAG TG-PO_3	59.6
*ALDH2**2-WP-3	CGC GCC GAG GGA *g*GT GAA AAC TGT GAG TG-PO_3	58.8
*ALDH2**2-WP-4	CGC GCC GAG GGA A*a*T GAA AAC TGT GAG TG-PO_3	56.9
*ALDH2**2-WP-5	CGC GCC GAG GGA AGT GAA AAC TGT GAG *g*G -PO_3	60.1
*ALDH2**2-WP-6	CGC GCC GAG GG*c* AGT GAA AAC TGT GAG TG-PO_3	57.4
*ALDH2**2-WP-7	CGC GCC GAG GG*t* AGT GAA AAC TGT GAG TG-PO_3	57.2
*ALDH2**2-MP	ACG GAC GCG GAG AAA GTG AAA ACT GTG AGT G-PO_3	62.6

Notes：FRET-1, 2 are fluorescence resonance energy transfer (FRET) probes used for reporting wild-type and mutant in Invader reactions, respectively. ALDH2*2-F, R are paired PCR primers. ALDH2*2-UP is an invasive oligo used for Invader reactions. ALDH2*2-WP (Wild Probe), MP (Mutant Probe) are detection probes for wild-type and mutant respectively. Underlined nucleotides are sequence-tags for different alleles. Italic lowercase are artificially mismatched bases.

Supplementary Figure Captions

Fig. S1 Fluorescence signals of *ALDH2**2 polymorphisms assay. A: Fluorescence intensities of three genotypes detected by end-point Invader Plus assay. The signal of VIC was specific to allele *1 and the signal of FAM was specific to allele *2. B: Fluorescence intensity curves of an *ALDH2* *2*2 homozygote by real-time Invader Plus assay.

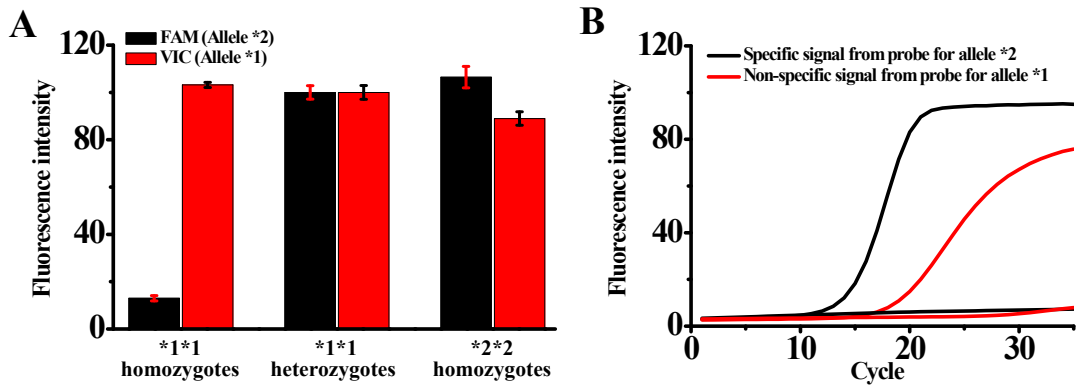
Fig. S2 The real-time fluorescence intensity curves of *ALDH2* *2*2 homozygotes by using different concentrations of *Afu* flap endonuclease. a-e were specific signals detected by using 6.68, 2.67, 1.34, 0.89, 0.67 ng/μL *Afu* flap endonuclease, respectively. a'-e' were non-specific signals detected by allele*1-specific probes when using 6.68, 2.67, 1.34, 0.89, 0.67 ng/μL *Afu* flap endonuclease, respectively. f and f' were blank controls.

Fig. S3 The real-time fluorescence signal curves of an *ALDH2* *1*1 homozygotes and a *2*2 homozygotes with different concentrations of probe 1 for allele *1. a-c were specific signals of a *1*1 homozygotes with 500, 250, 125 nmol/L probe 1, respectively. a'-c' were non-specific signals of a *2*2 homozygotes with 500, 250, 125 nmol/L probe 1, respectively.

Fig. S4 The distribution of Allele*1 : Allele*2 ratios using the complementary probe of WP-1 (A) and the mismatched probe of WP-2 (B). The FOZ value and the ratio can be calculated as following formulas: $FOZ = \frac{Raw\ counts\ from\ sample}{Raw\ counts\ from\ No\ Template\ Control}$, $Ratio = \frac{(Net\ Allele * 1\ FOZ)}{(Net\ Allele * 2\ FOZ)}$. The Net Allele FOZ=FOZ-1.

Fig. S5 The typical results of the three genotypes using commercial Sanger sequencing (Left) and the real-time improved-Invader Plus method (Right). The arrows show the *ALDH2**2 polymorphism site in Sanger sequencing.

Supplementary Figures



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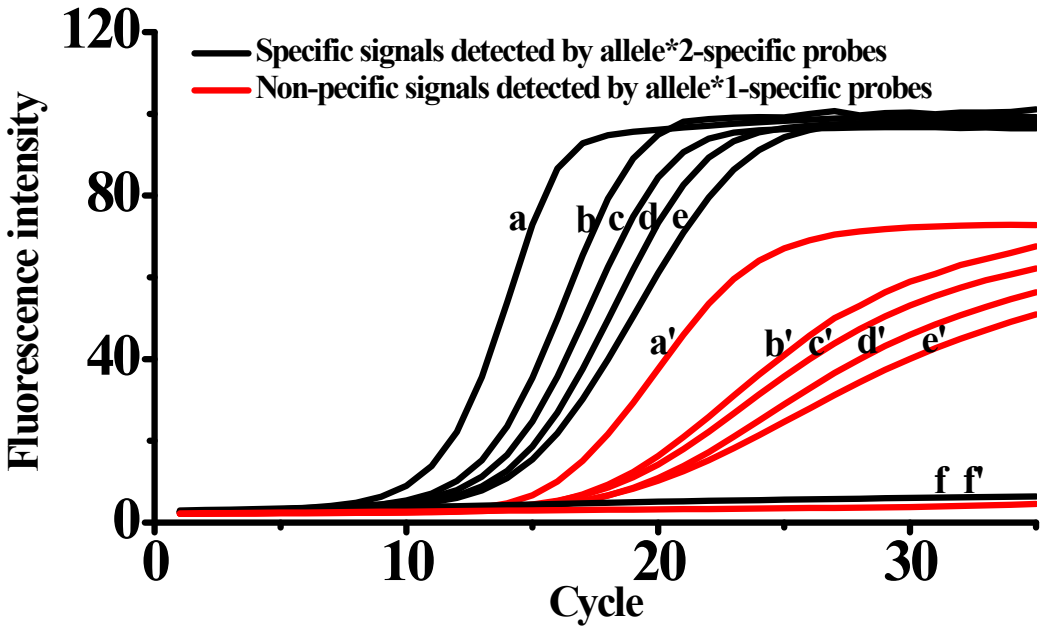

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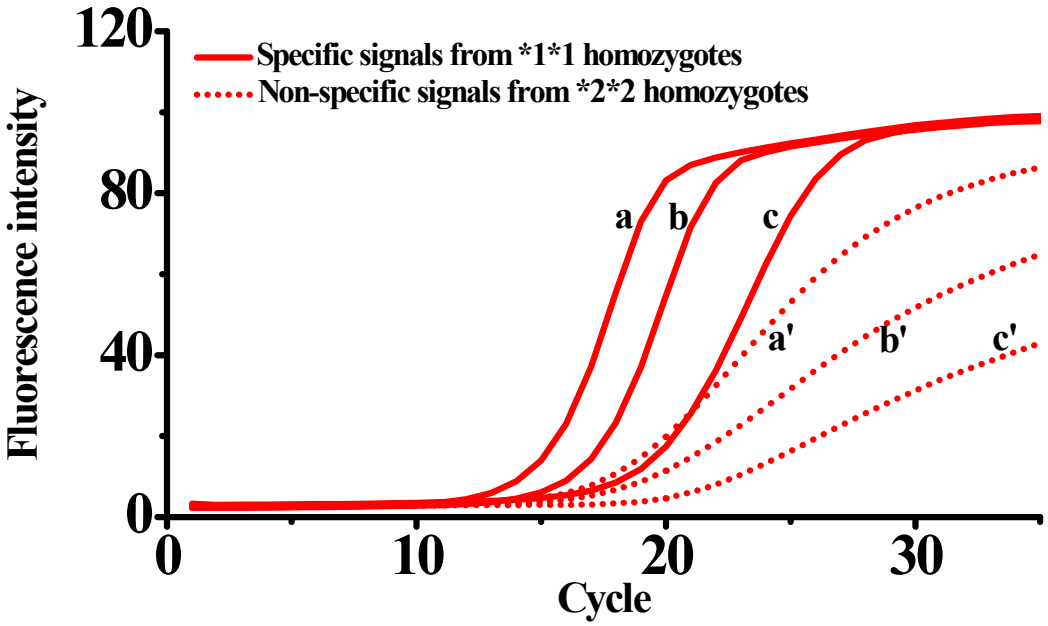

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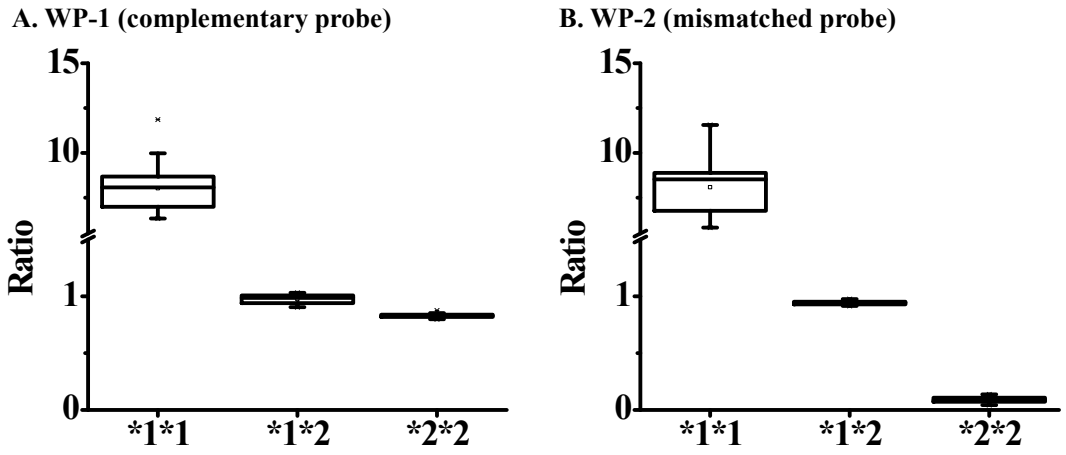

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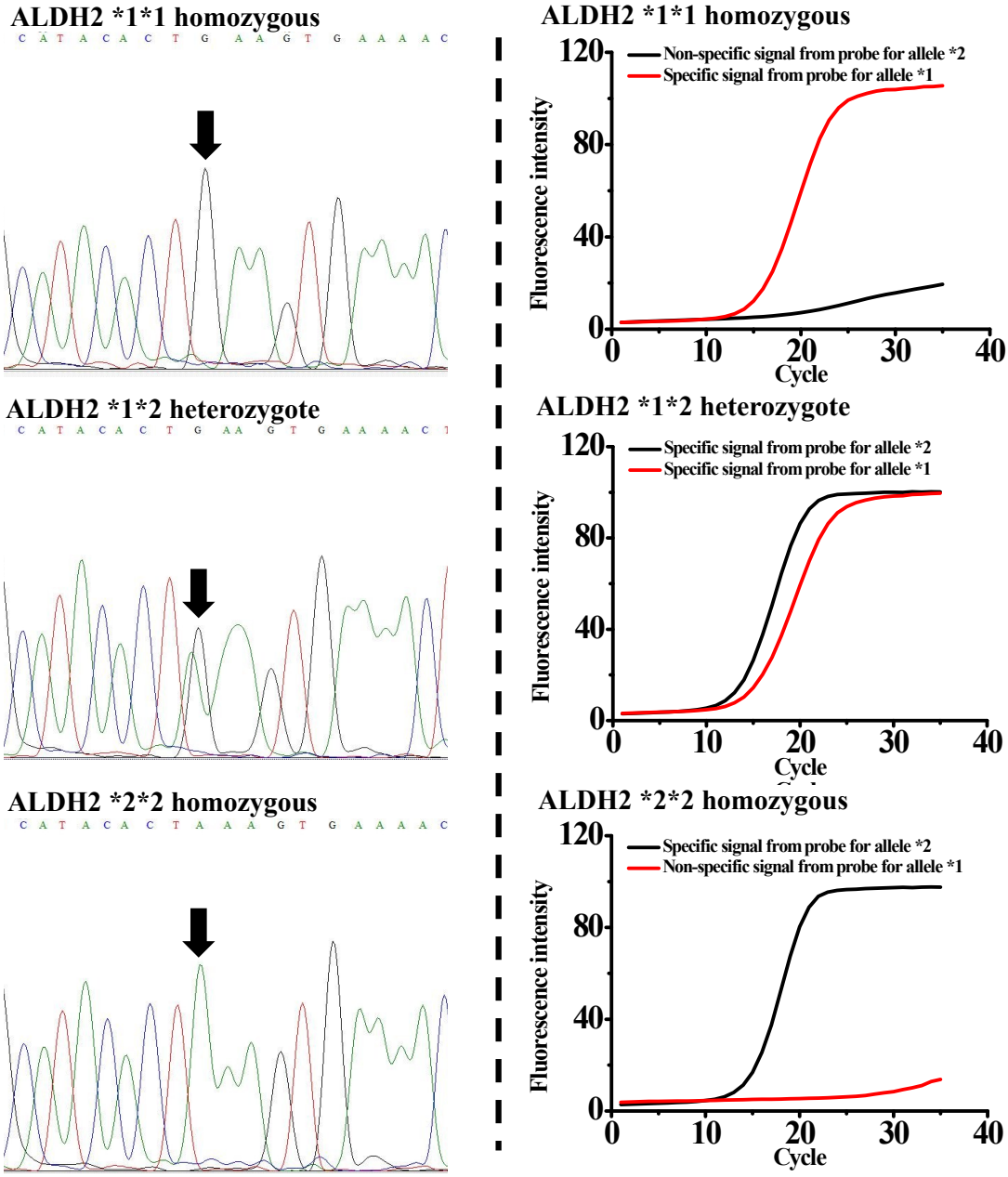

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