1 Supporting Information

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3 **Universal probe-based SNP genotyping with visual readout: a robust**

4 **and versatile method**

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7 *Xie**
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S1 Tables

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45   Table S1. Rs671 related primer sequences.

Name	Oligonucleotide Sequence (5'-3')	Size (bp)
Sequencing-F	ACTTTGGGGCAATACAGGGG	20
Sequencing-R	CAGCAGGTCCTGAACTTCCA	20
FP-G	CCCACACTCACAGTTTTCACTTC	23
FP-A	CCCACACTCACAGTTTTCACTTT	23
RP	ACTTTGGGGCAATACAGGGG	20
LP-G	GGAGGATCACTCCAACTAGACTACCCACACTCACAGTTTTCACTTC	46
LP-A	ATCATCAGGTCTGGAAGATTACGCCCACACTCACAGTTTTCACTTT	46
LP-R	CTTCTTTCAGAGGTCAGGTGTATACTTTGGGGCAATACAGGGG	43
Wild-Probe	GGAGGATCACTCCAACTAGACTA-FAM	23
Mutant-Probe	ATCATCAGGTCTGGAAGATTACG-Digoxin	23
Shared-Probe	CTTCTTTCAGAGGTCAGGTGTAT-Biotin	23

46   FP: forward primer; RP: reverse primer; LP: long primer.

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49   Table S2. MTHFR A1298C related primer sequences.

Name	Oligonucleotide Sequence (5'-3')	Size(bp)
Sequencing-F	TCTCCCTTTGCCATGTCCAC	20
Sequencing-R	CAGGGGCAATTCCTCTTCCC	20
FP-A	GGGGAGGAGCTGACCAGTGAAGT	23
FP-C	GGGGAGGAGCTGACCAGTGAAGG	23
RP	TCTCCCTTTGCCATGTCCACAGC	23
LP-A	GGAGGATCACTCCAACTAGACTAGGGGAGGAGCTGACCAGTGAAGT	46
LP-C	ATCATCAGGTCTGGAAGATTACGGGGGAGGAGCTGACCAGTGAAGG	46
LP-R	CTTCTTTCAGAGGTCAGGTGTATTCTCCCTTTGCCATGTCCACAGC	46

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52   Table S3. MTHFR C677T related primer sequences.

Name	Oligonucleotide Sequence (5'-3')	Size(bp)
Sequencing-F	CCCTCACCTGGATGGGAAAG	20
Sequencing-R	AAGATCAGAGCCCCCAAAGC	20
FP-C	AAGCTGCGTGATGATGAAATCGC	23
FP-T	AAGCTGCGTGATGATGAAATCGT	23
RP	TGCAAGATCAGAGCCCCCAAAGC	23
LP-C	GGAGGATCACTCCAACTAGACTAAAGCTGCGTGATGATGAAATCGC	46
LP-T	ATCATCAGGTCTGGAAGATTACGAAGCTGCGTGATGATGAAATCGT	46
LP-R	CTTCTTTCAGAGGTCAGGTGTATTGCAAGATCAGAGCCCCCAAAGC	46

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55 Table S4. MTRR A66G related primer sequences.

Name	Oligonucleotide Sequence (5'-3')	Size(bp)
Sequencing-F	GTGTGGGTATTGTTGCATTGTTTC	24
Sequencing-R	GTGGTGGTATTAGTGTCCTTTTGTT	25
FP-A	CATGTACCACAGCTTGCTCACAT	23
FP-G	CATGTACCACAGCTTGCTCACAC	23
RP	GTGTGGGTATTGTTGCATTGTTT	23
LP-A	GGAGGATCACTCCAACTAGACTACATGTACCACAGCTTGCTCACAT	46
LP-G	ATCATCAGGTCTGGAAGATTACGCATGTACCACAGCTTGCTCACAC	46
LP-R	CTTCTTTCAGAGGTCAGGTGTATGTGTGGGTATTGTTGCATTGTTT	46

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58 Table S5. Comparison of typing formula and sequencing results of rs671 in 50 cases.

Sample Number	Genotype based on typing formula	Genotype based on Sequencing
1	GG	GG
2	GG	GG
3	GG	GG
4	GG	GG
5	GG	GG
6	GG	GG
7	GG	GG
8	GG	GG
9	GG	GG
10	GG	GG
11	GG	GG
12	GG	GG
13	GG	GG
14	GG	GG
15	GG	GG
16	GG	GG
17	GA	GA
18	GG	GG
19	GA	GA
20	GG	GG
21	GA	GA
22	GA	GA
23	GG	GG
24	GA	GA
25	GA	GA
26	GG	GG
27	GG	GG

28	GG	GG
29	GA	GA
30	GA	GA
31	GG	GG
32	GA	GA
33	GG	GG
34	GG	GG
35	GG	GG
36	GG	GG
37	GG	GG
38	AA	AA
39	GG	GG
40	GG	GG
41	GG	GG
42	GA	GA
43	GG	GG
44	GG	GG
45	GA	GA
46	GA	GA
47	GG	GG
48	GG	GG
49	GA	GA
50	GA	GA

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61 Table S6. Comparison of MTHFR C677T, MTHFR A1298C and MTRR A66G typing results

Sample Number	Genotype based on typing formula			Genotype based on Sequencing		
	A1298C	C677T	A66G	A1298C	C677T	A66G
1	AC	CT	AA	AC	CT	AA
2	AA	CT	AA	AA	CT	AA
3	AA	CT	AA	AA	CT	AA
4	CC	TT	AA	CC	TT	AA
5	AC	CC	AG	AC	CC	AG
6	AC	CC	AG	AC	CC	AG
7	AC	CC	AG	AC	CC	AG
8	AC	CC	GG	AC	CC	GG

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S2 Figures
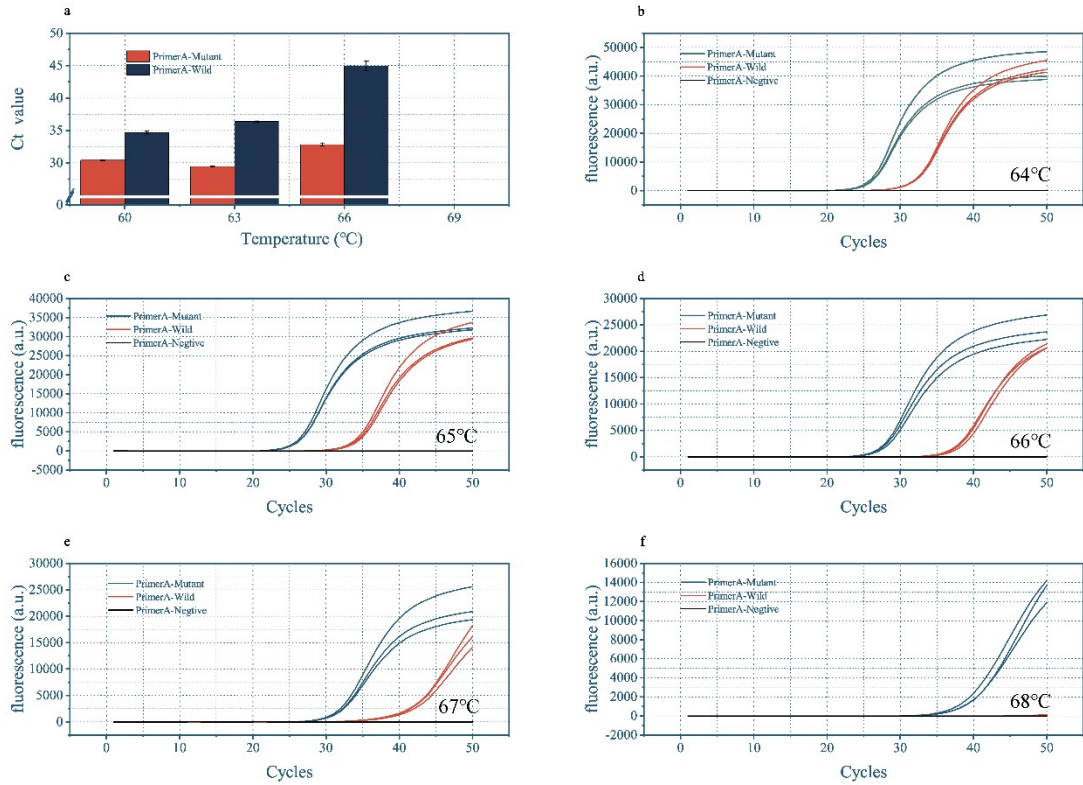
Fig S1. Effect of temperature on reaction specificity and efficiency: a: The comparison of specific (orange) and non-specific (dark blue) Ct values yielded the optimum temperature of approximately 64-68°C. b-f: Primer A amplified GG and AA at 64-68°C. 66°C was selected as the optimum temperature.

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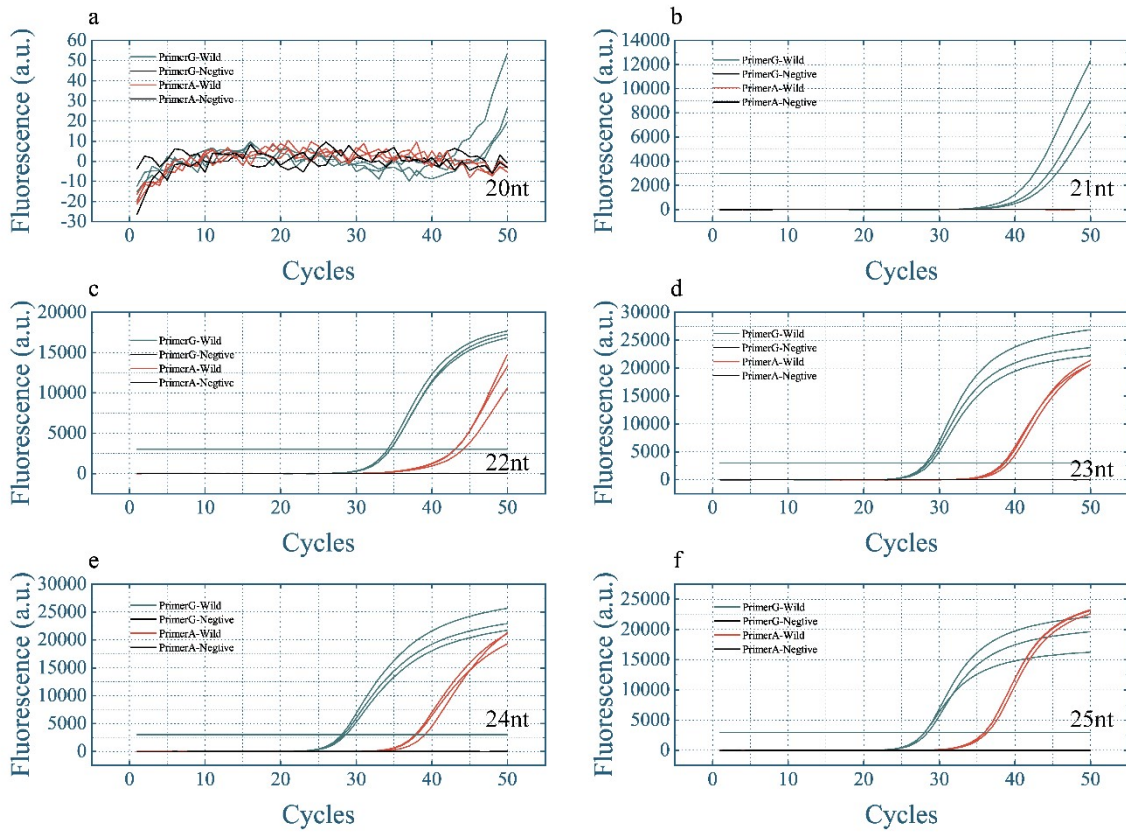
Fig S2. Effect of primer length on the reaction: a-f: reaction efficiency and specificity for primers of length 20-25nt. Among them, the efficiency and specificity of length 23nt is the most suitable.

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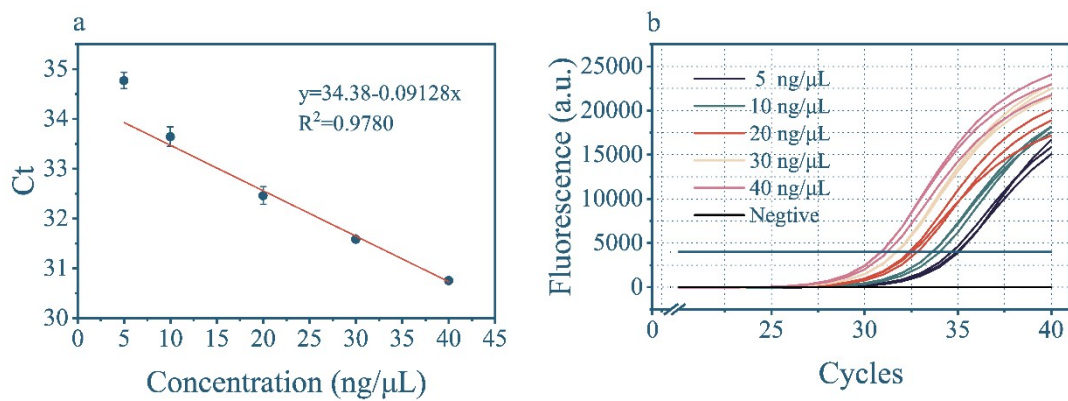
Fig S3. The resulting standard curve generated by plotting the target concentration versus threshold cycle(a); Amplification curves(b).
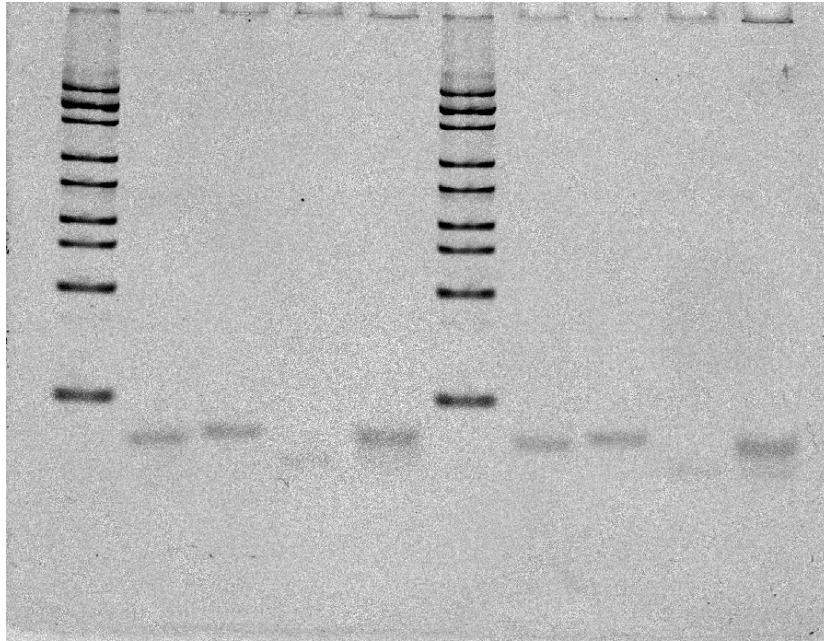
Fig S4. PAGE to verify probe dimerization (two parallel experiments). From left to right: biotin probe, digoxin probe, FAM probe, amplification of the three probes.

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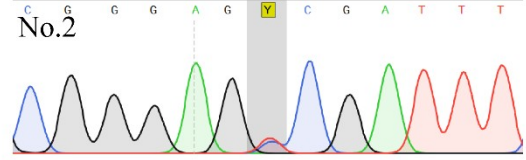
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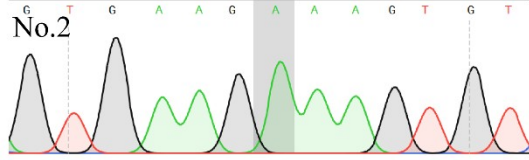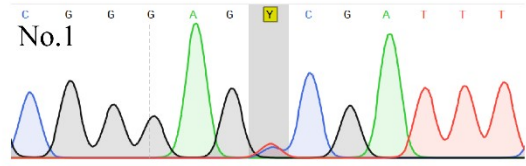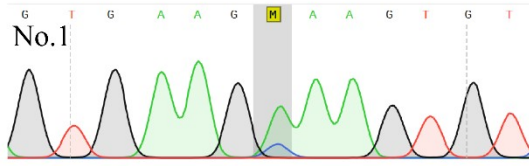
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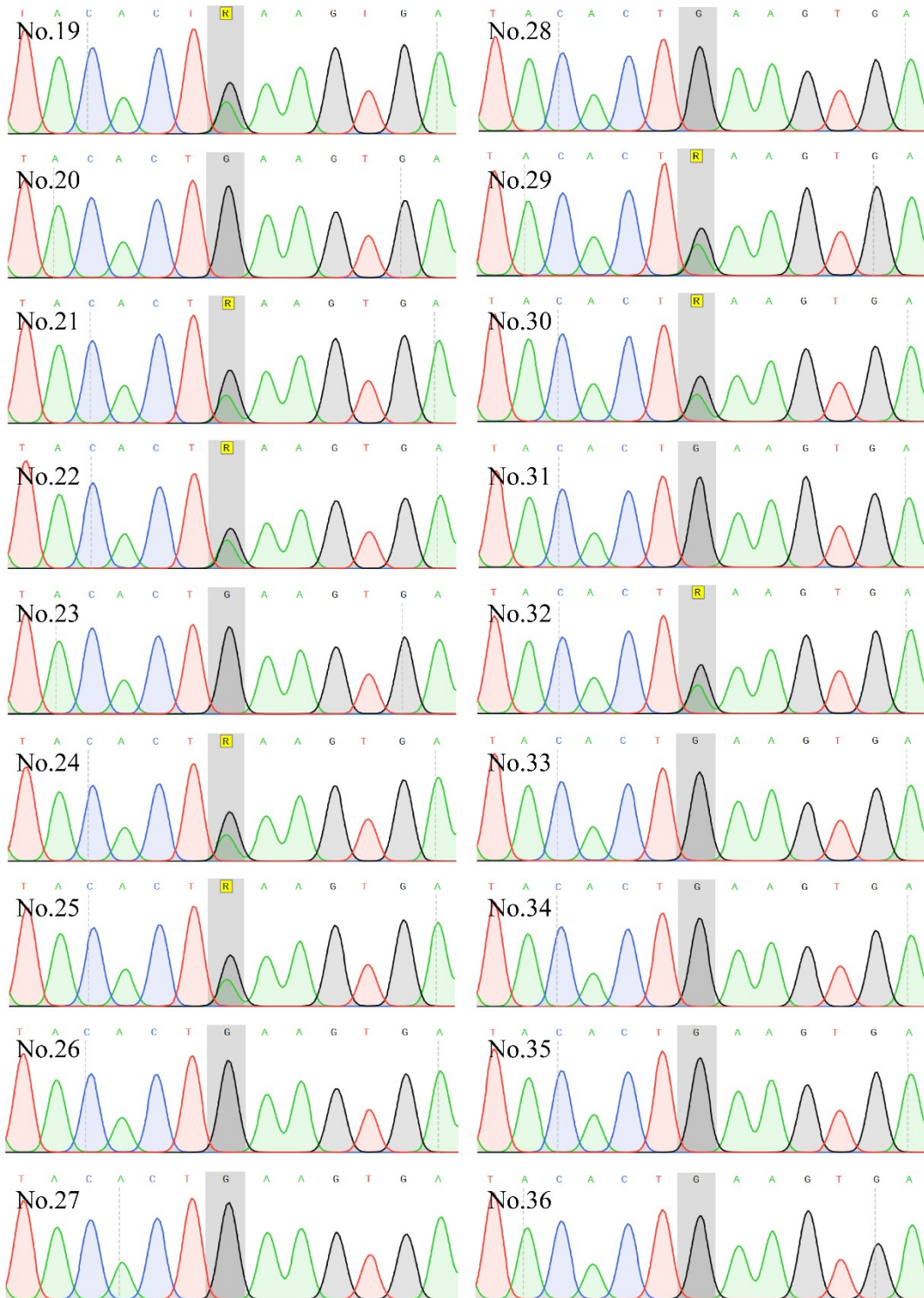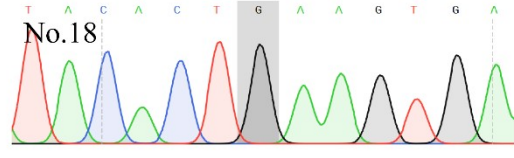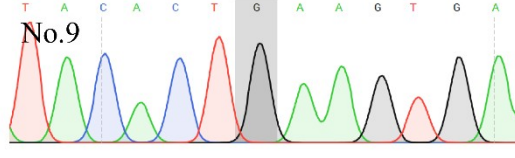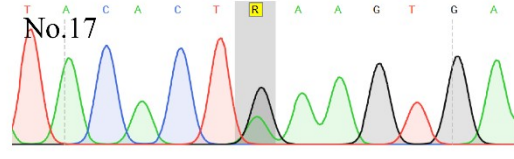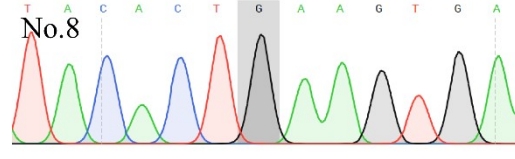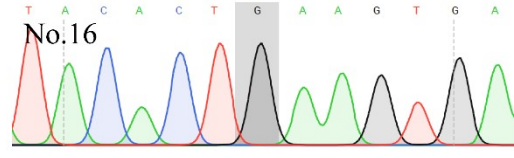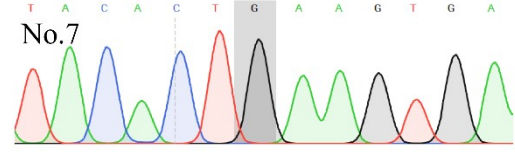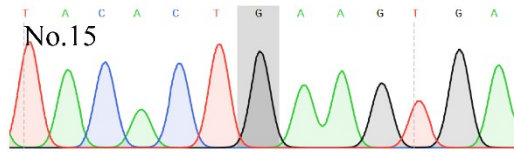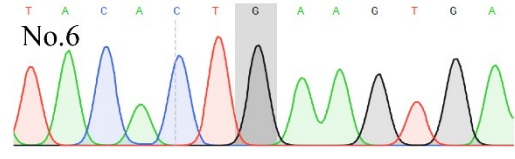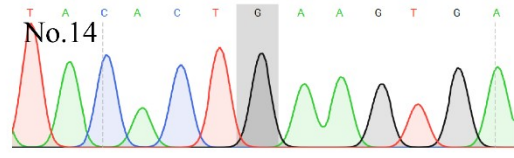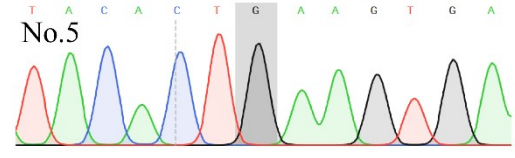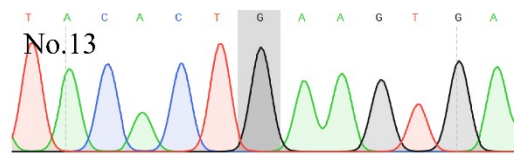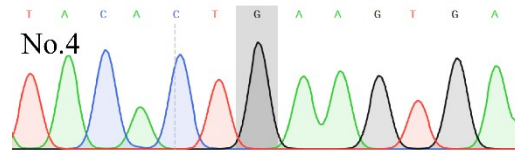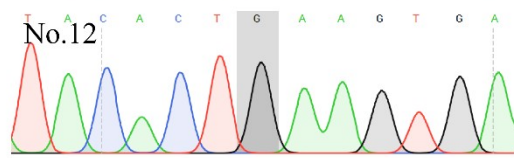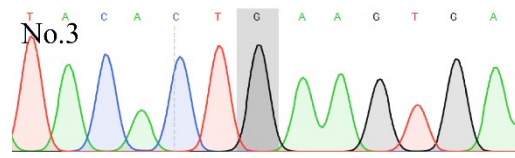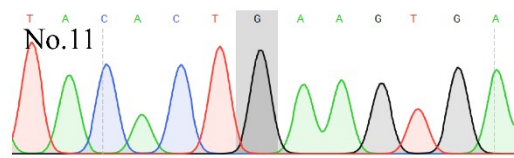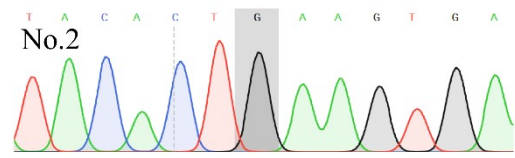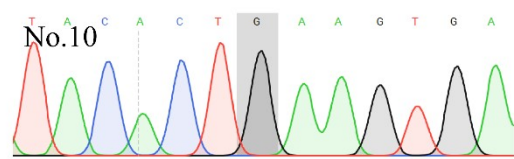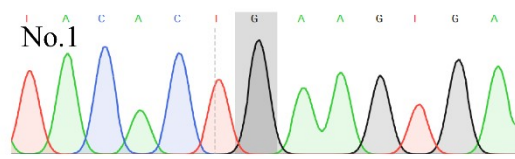
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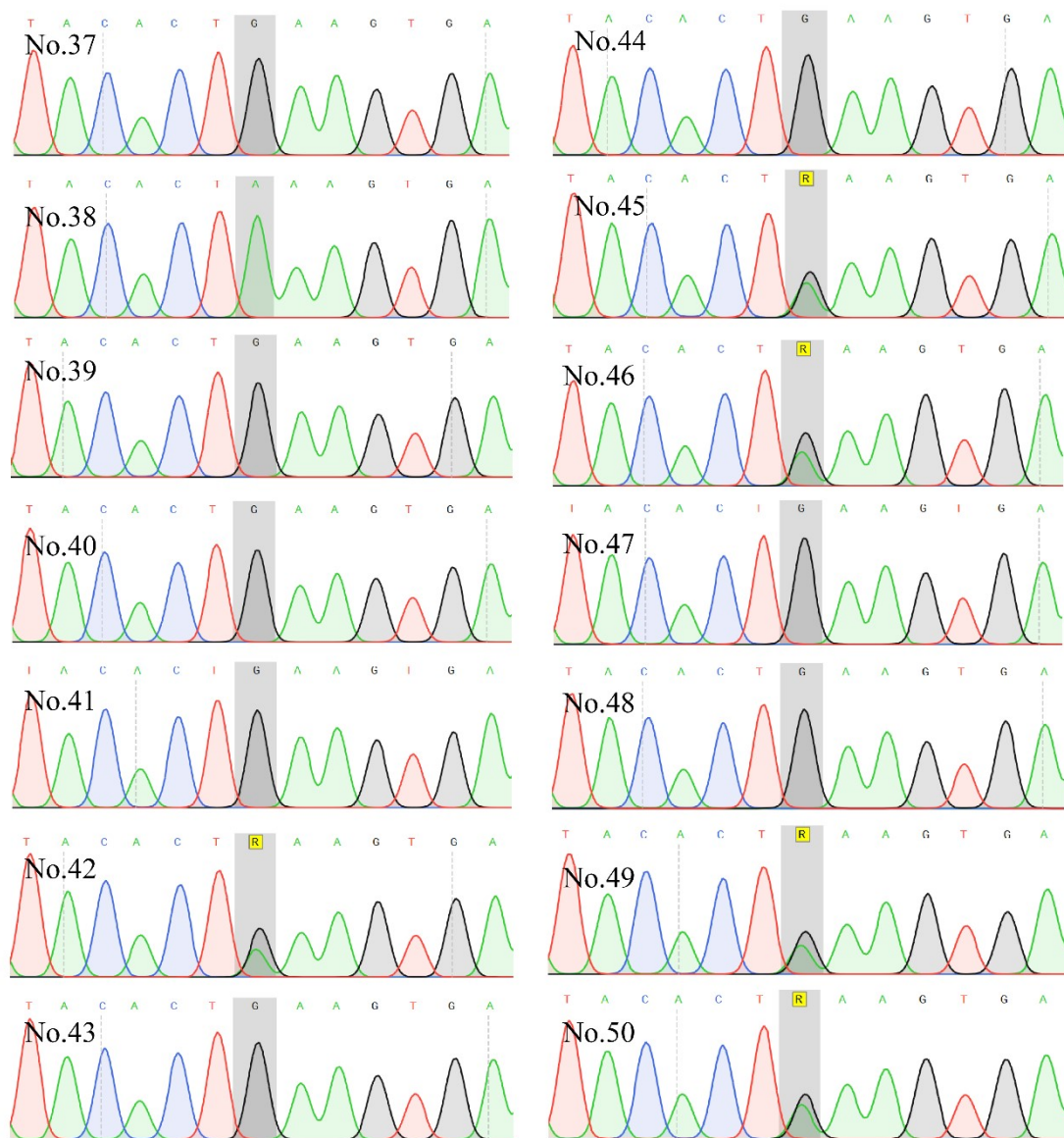
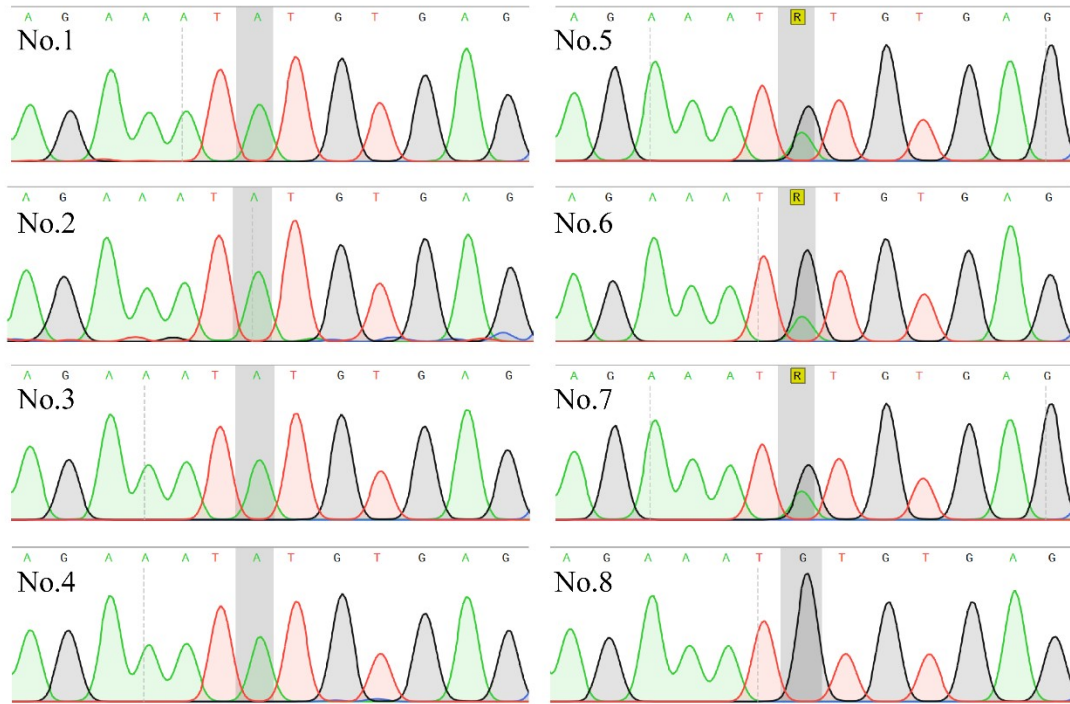**Fig S5.** Sequencing results of rs671 blood gDNA in 50 cases. (The degenerate base R represents A/G)

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Fig S6. LFTS results of rs671 blood gDNA in 50 cases. (The first 15 results were left for too long, more than four months, and the strips had varying degrees of discoloration.)
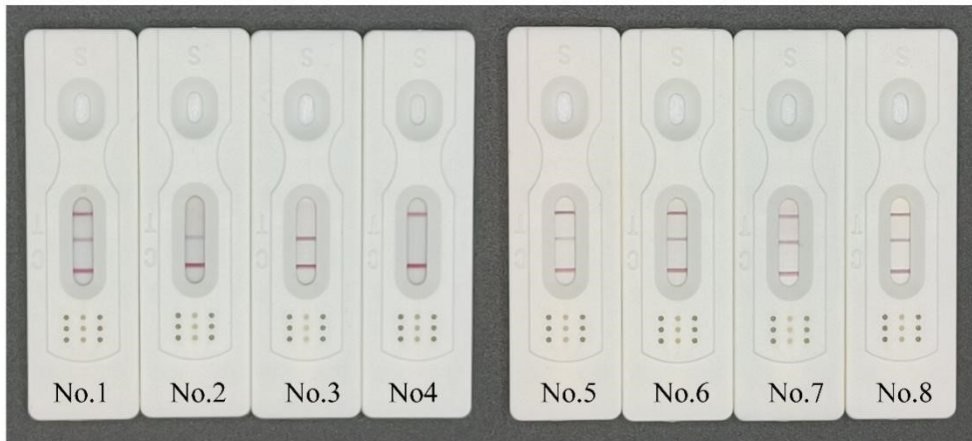
MTHFR A1298C

MTHFR C677T

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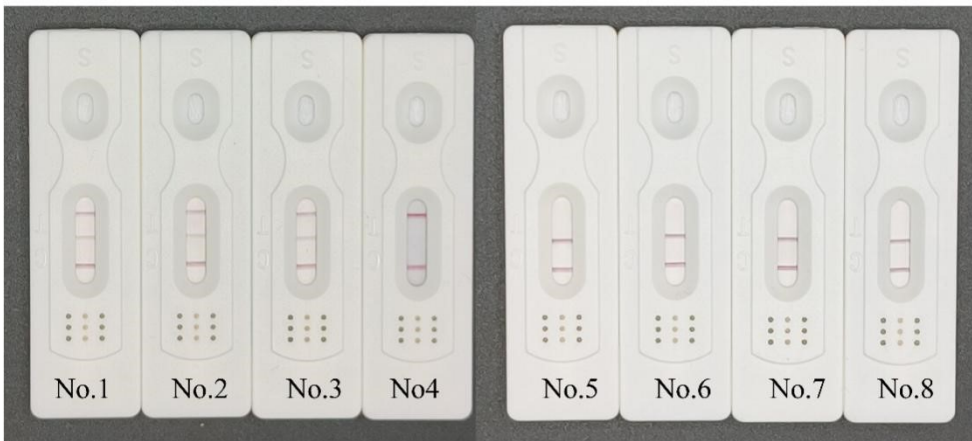No.3

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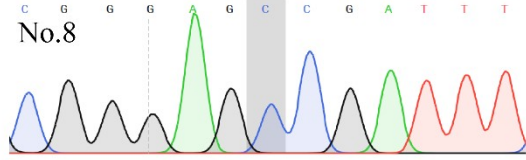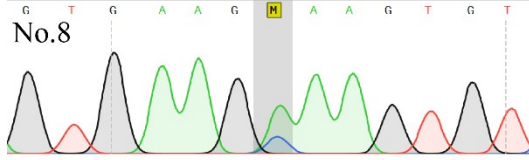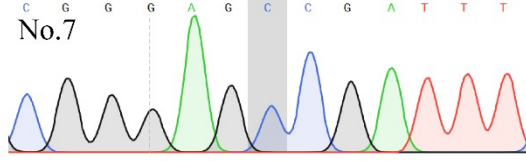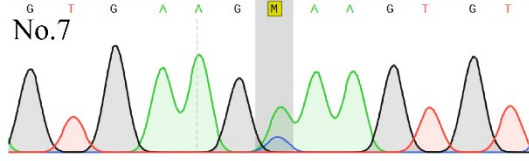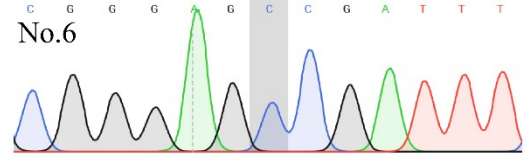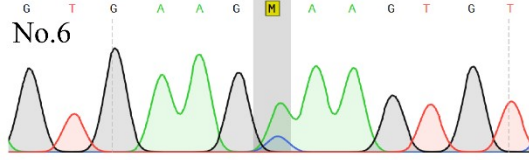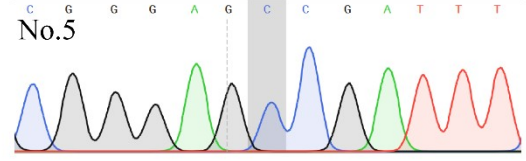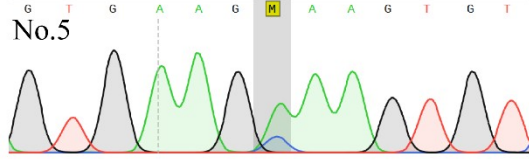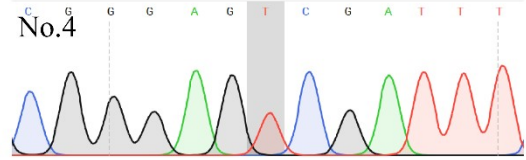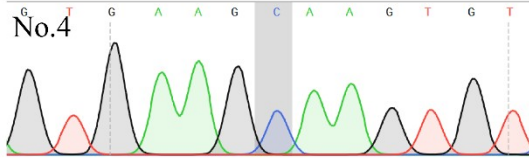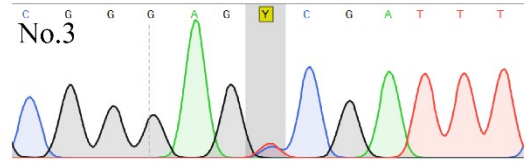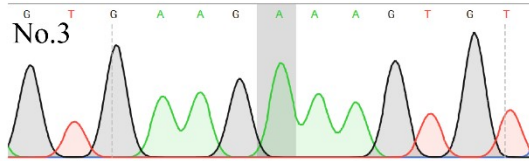
MTRR A66G

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Fig S7. Sequencing results of MTHFR A1298C, MTHFR C677T, MTRR A66G blood gDNA in 8 cases each. (The degenerate base R represents A/G; Y represents C/T; M represents A/C)
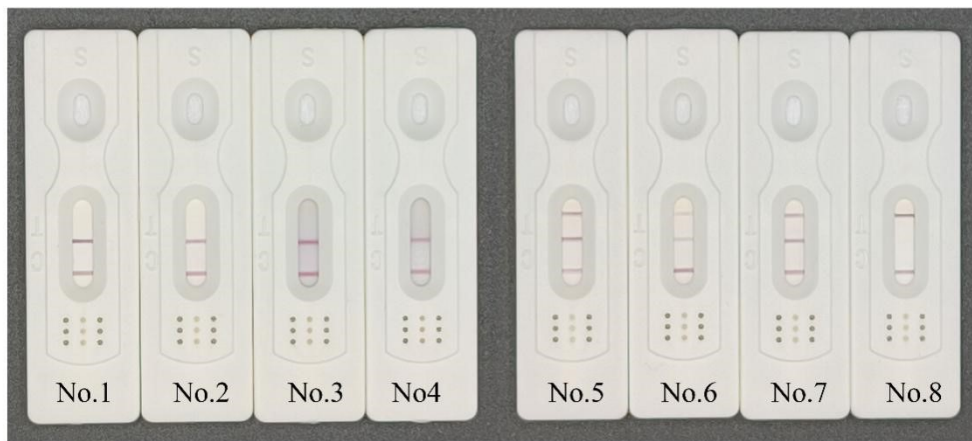
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A1298C

C677T

A66G

Fig S8. LFTS results of MTHFR A1298C, MTHFR C677T, MTRR A66G blood gDNA.