

Supplemental Information

Table S1. Primer pairs designed for *MTHFR* C677T genotyping.

Primer pairs	Introduced mismatch	Sequences (5'→3')	
M primers for T allele	F(M)	No mismatch introduced	AGTCCCTGTGGTCTCTTCATCC
	R(M0)	No mismatch introduced	GCTGCGTGATGATGAAATCGA
	R(M1)	Medium mismatch at the -2 position	GCTGCGTGATGATGAAATCCA
	R(M2)*	Strong mismatch at the -2 position	GCTGCGTGATGATGAAATCTA
WT primers for C allele	F(WT0)	No mismatch introduced	GAGAAGGTGTCTGCGGGAGC
	F(WT1)*	Weak mismatch at the -2 position	GAGAAGGTGTCTGCGGGAAC
	F(WT2)	Strong mismatch at the -2 position	GAGAAGGTGTCTGCGGGATC
	R(WT)	No mismatch nucleotide introduced	CCTCACCTGGATGGGAAAGAT

* means the selected allele-specific primer. Allele-specific nucleotides are bold. Mismatch nucleotides are underlined.

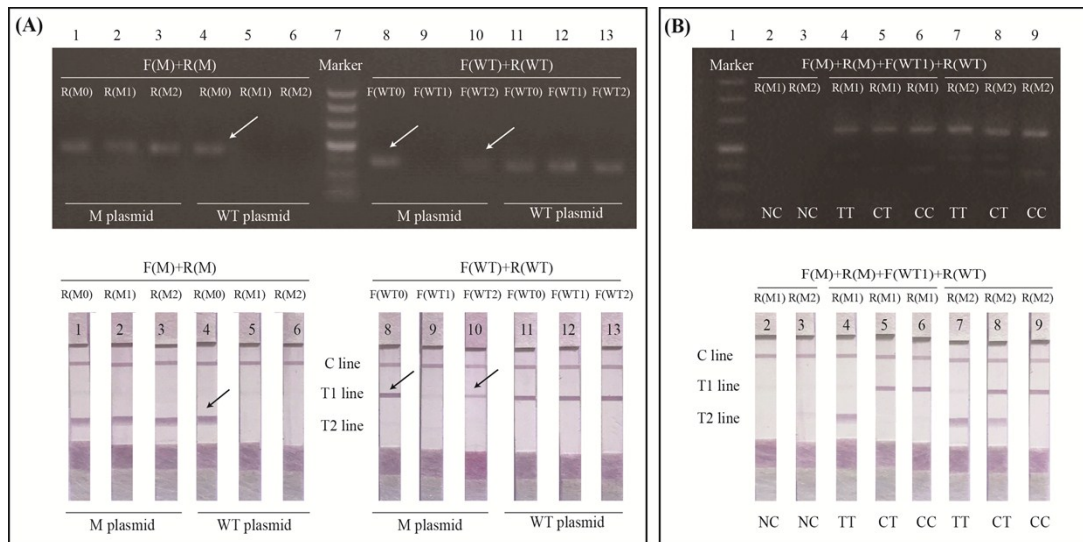


Figure S1. Related to Figure 1 and Table S1. Primer selecting of T-ARMS-LFA method.

(A) The primer sets were designed according to the principle of ARMS-PCR (as showed in Table S1). We tested three sets of mismatched primers respectively by agarose gel and LFA strips to select the most specific primer sets. The results indicated that, except for primer R(M1), R(M2) and F(WT1), the other allele-specific primers result in non-specific bands (arrow).

(B) Four primers were added into the same PCR reaction. Using DNA samples with different genotypes to evaluated the sensitivity and specificity of primers. The results indicated the combination of F(M), R(M2), F(WT1) and R(WT) primers showed a higher sensitivity and specificity for genotyping.

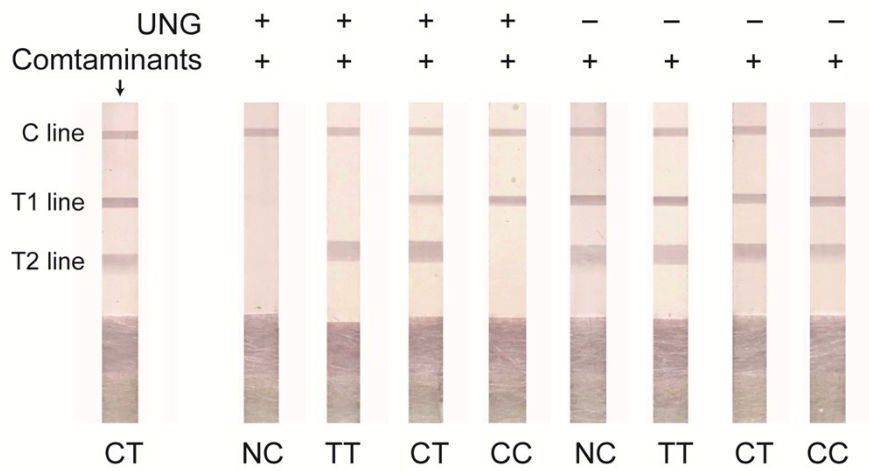


Figure S2. The efficacy of the UNG system to prevent contamination in the T-ARMS-LFA method. NC = Negative control, TT = *MTHFR* 677 TT genotype, CT = *MTHFR* 677 CT genotype, CC = *MTHFR* 677 CC genotype.

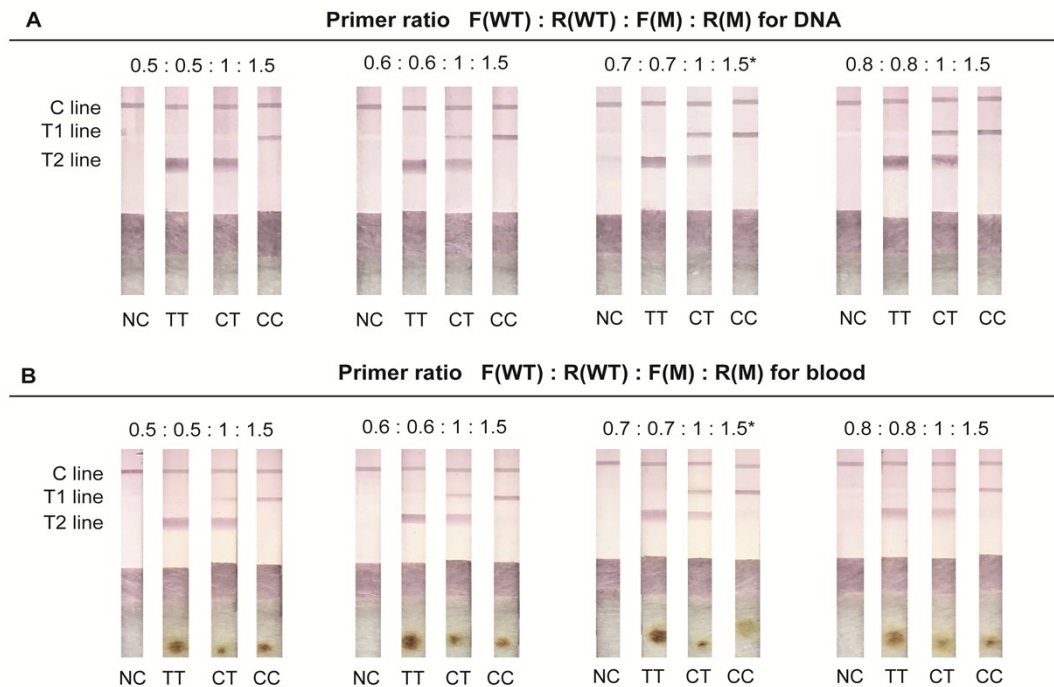


Figure S3. Related to figure 3 and figure 4. The effect of the primers volume ratio. NC = Negative control, TT = *MTHFR* 677 TT genotype, CT = *MTHFR* 677 CT genotype, CC = *MTHFR* 677 CC genotype, and * indicates the selected conditions for each parameter.

