Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary materials for

A fully integrated nucleic acid analysis system for multiplex detection of genetic polymorphisms related to folic acid metabolism

This supplementary PDF file includes the following information:

Other Supplementary Material for this manuscript includes the following:

Table S1. List of primer sequences

Target	Primer	Sequences (5'-3')
λ-DNA (LAMP)	F3	ATACAAAAACACCAGTGTAGG
	B3	GCCGCCAGTTTGTTTCAG
	FIP	CGTTGAGATTTGCGAAGTACCAAGAATCAAATATGCTGCAAATGTG
	BIP	CAAACGCATAATAAGCAGGTGATTTCGGAGGTGATGTTTTCGGTC
	Loop F	TGCCCGGCCATC
	Loop B	ATCATATCGTTCGGCT
λ-DNA (PCR)	F	CAAGCTTTGCCACACCACGGTATT
	R	TAAGCACGAACTCAGCCAGAACGA
MTHFR677 (PCR)	F-W	GCTGCGTGATGATGAAATCGG
	F-M	GCTGCGTGATGATGAAATCGA
	R	GACTGTCATCCCTATTGGCA
	P	FAM-CCTTCTCCTTCAAGTGCTTCAGGT-BHQ1
MTHFR1298 (PCR)	F-W	GAACGAAGACTTCAAAGACACTTT
	F-M	ACGAAGACTTCAAAGACACTTG
	R	GGAGCTGAAGGACTACTACC
	P	FAM-CCACATCTTCAGCAGCTCCTCCT-BHQ
MTRR66 (PCR)	F-W	CAAAGGCCATCGCAGAAGAAATA
	F-M	AAGGCCATCGCAGAAGAAATG
	R	TTCTTCAAAGCACAAAACGGTAA
	P	FAM-GCTGTGGTACATGGATTTTCTGCAG-BHQ1

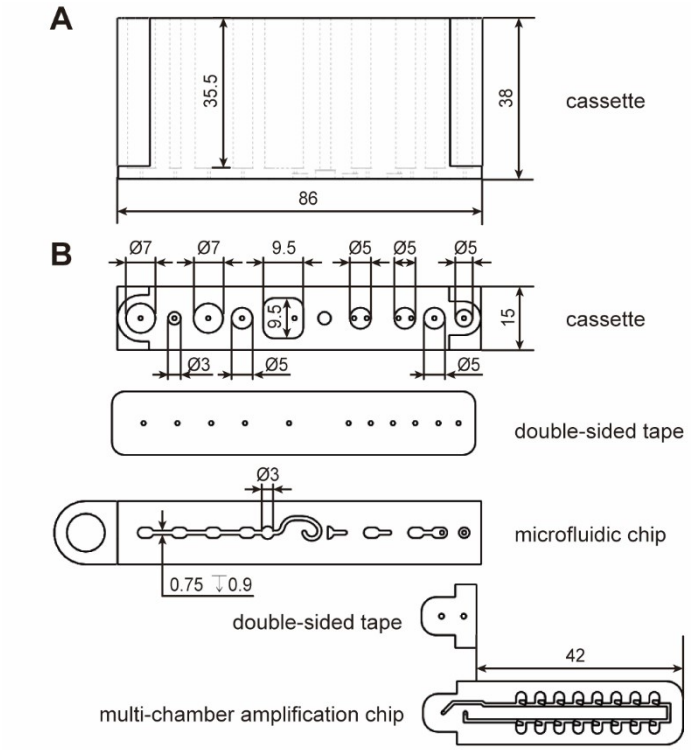


Figure S1. Schematic representation of the microfluidic cassette. All the dimensions are shown in millimeters. (**A**) Side view of the block. (**B**) Top views of different components of the microfluidic cassette.

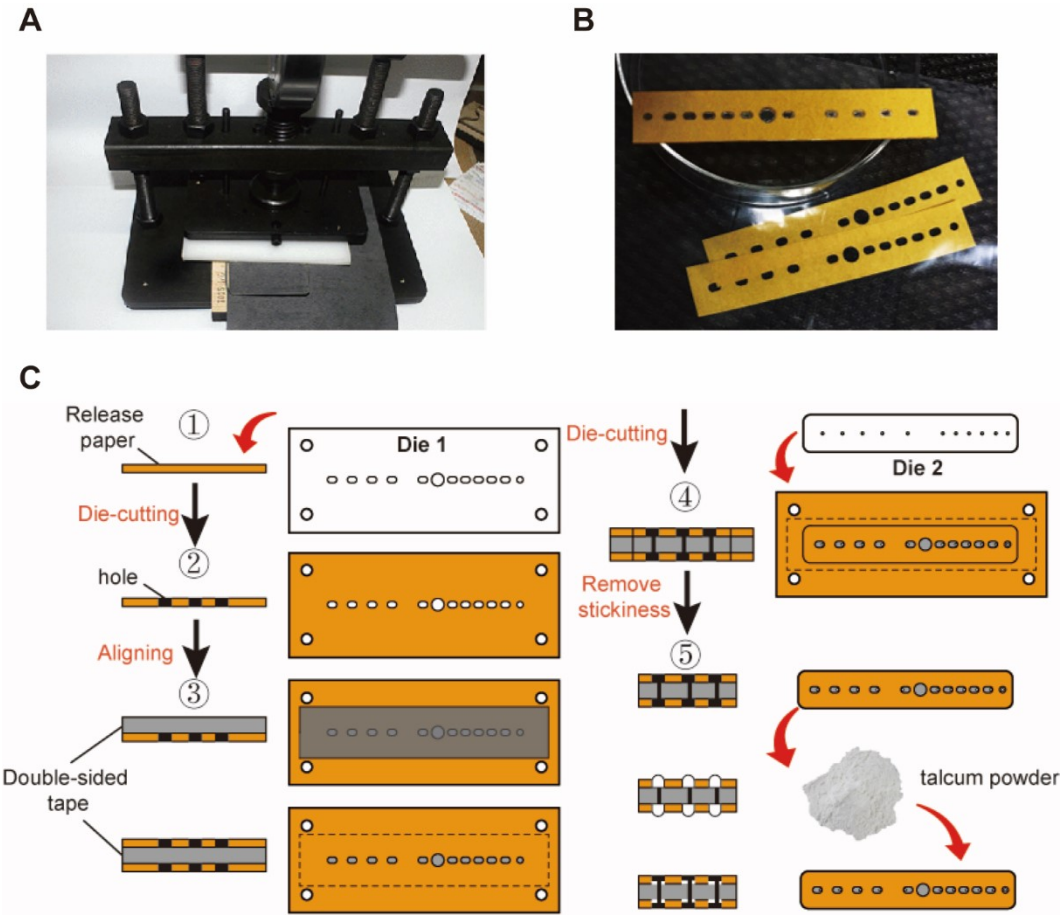


Figure S2. The production process of the DS tape. (**A**) Photo of the die-cutting tool. (**B**) DS tape with a mask made of release paper. **(C)** Non-adhesive patterning procedure of the DS tape.

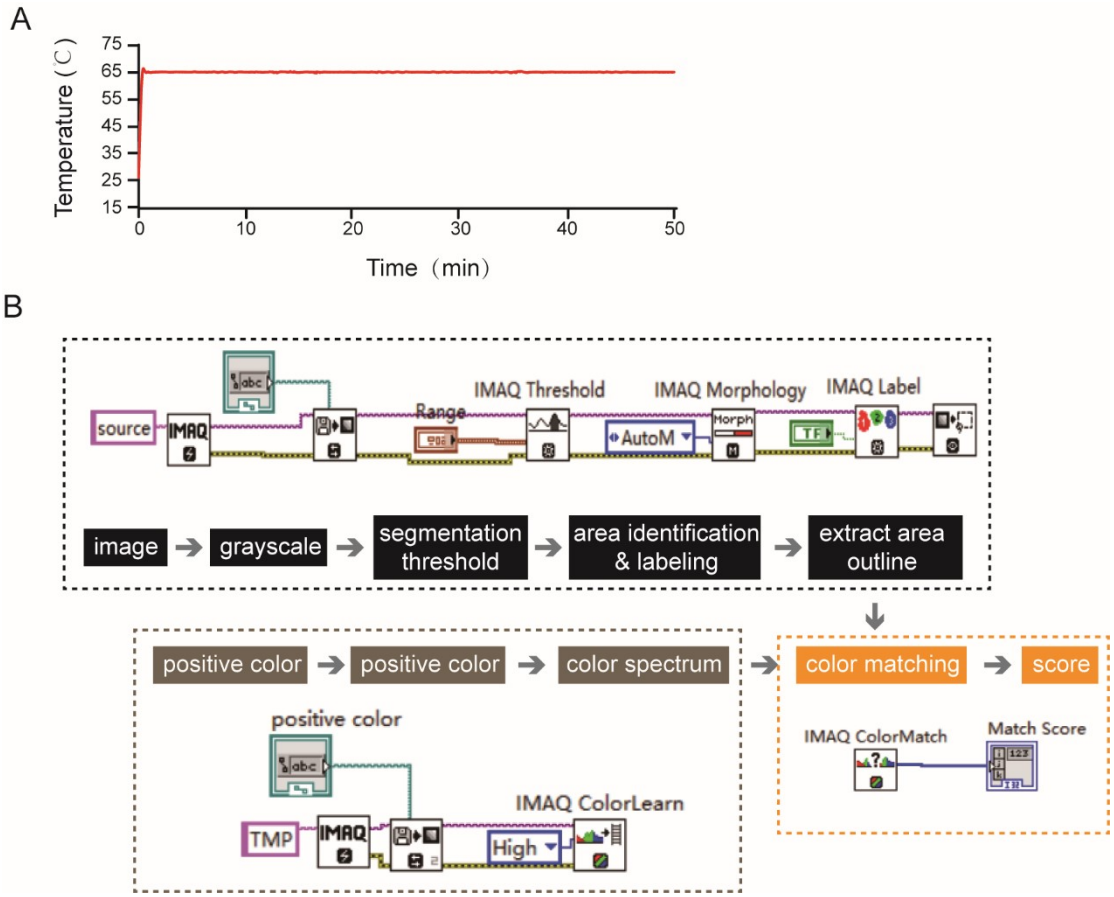


Figure S3. Modules used in the nucleic acid analyzer. (**A**) Temperature calibration of the cassette analyzer. (**B**) Photo processing of the reaction results using the LabVIEW software.

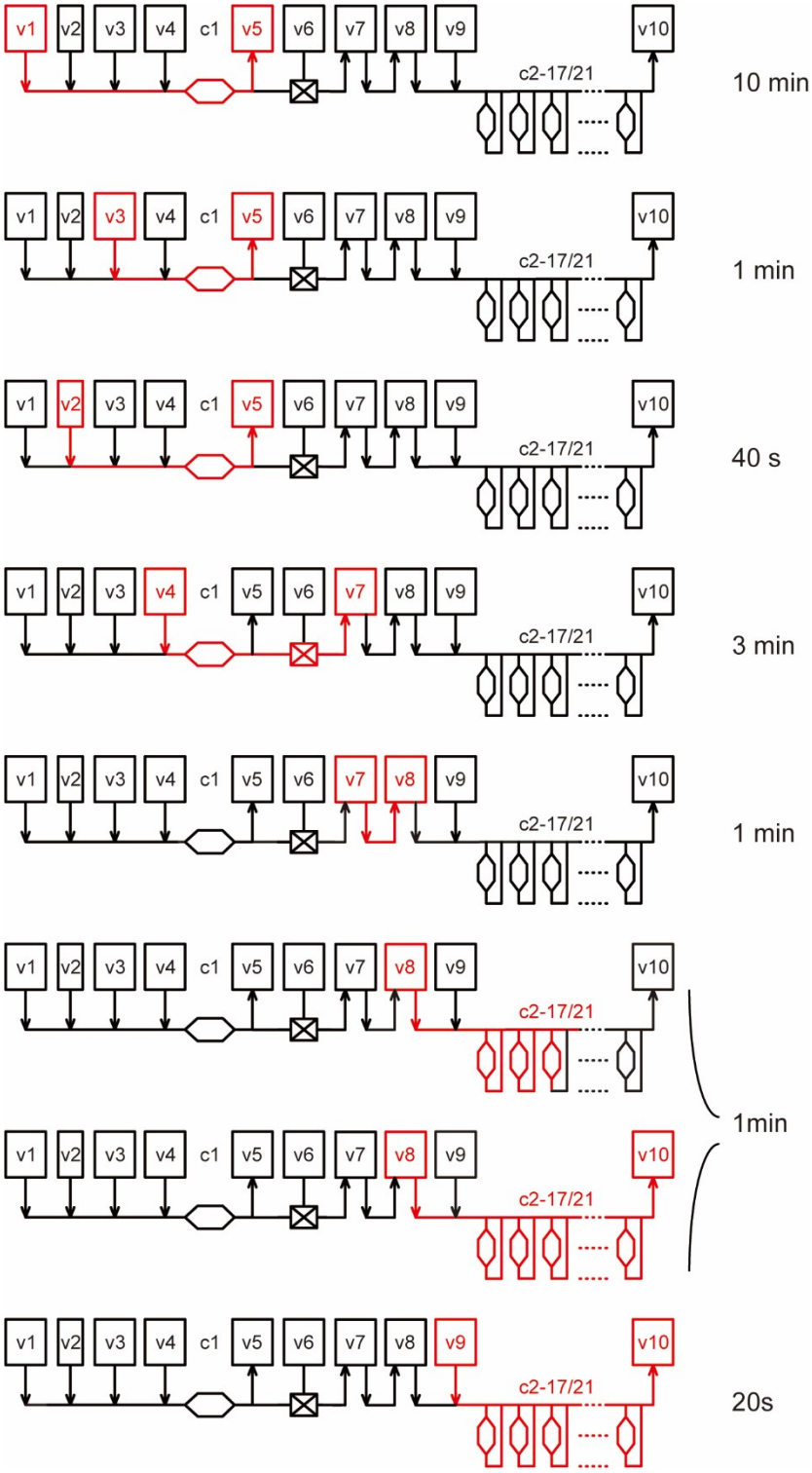


Figure S4. Schematic representation of the fluid operation process of the cassette.

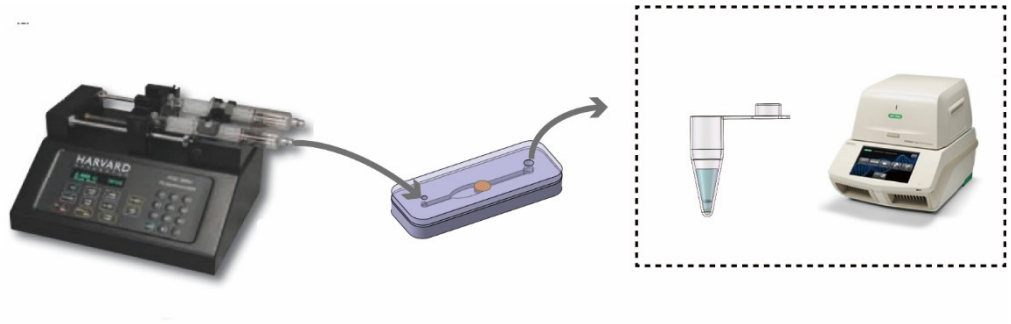


Figure S5. Schematic representation of the fluid for nucleic acid extraction with the silica membrane. The syringe pump is connected to the chip through a Teflon tube. The lysis solution, washing solution, air, and eluent flow sequentially through the chip. The eluted DNA is amplified by qPCR.

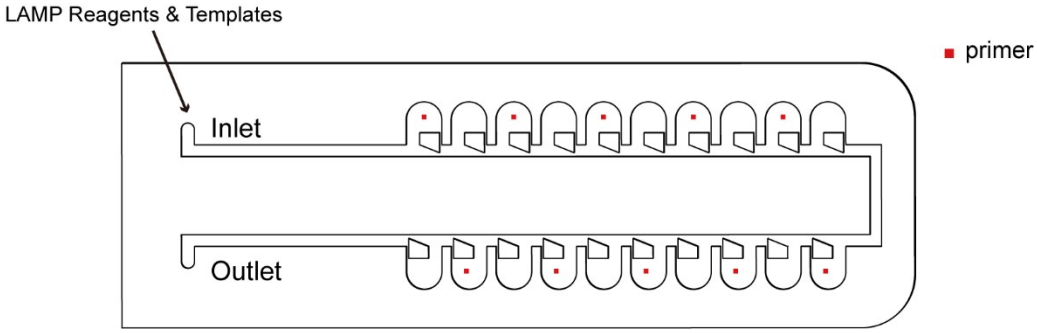


Figure S6. Pre-embedding of primers on amplification chips in cross-contamination experiments.

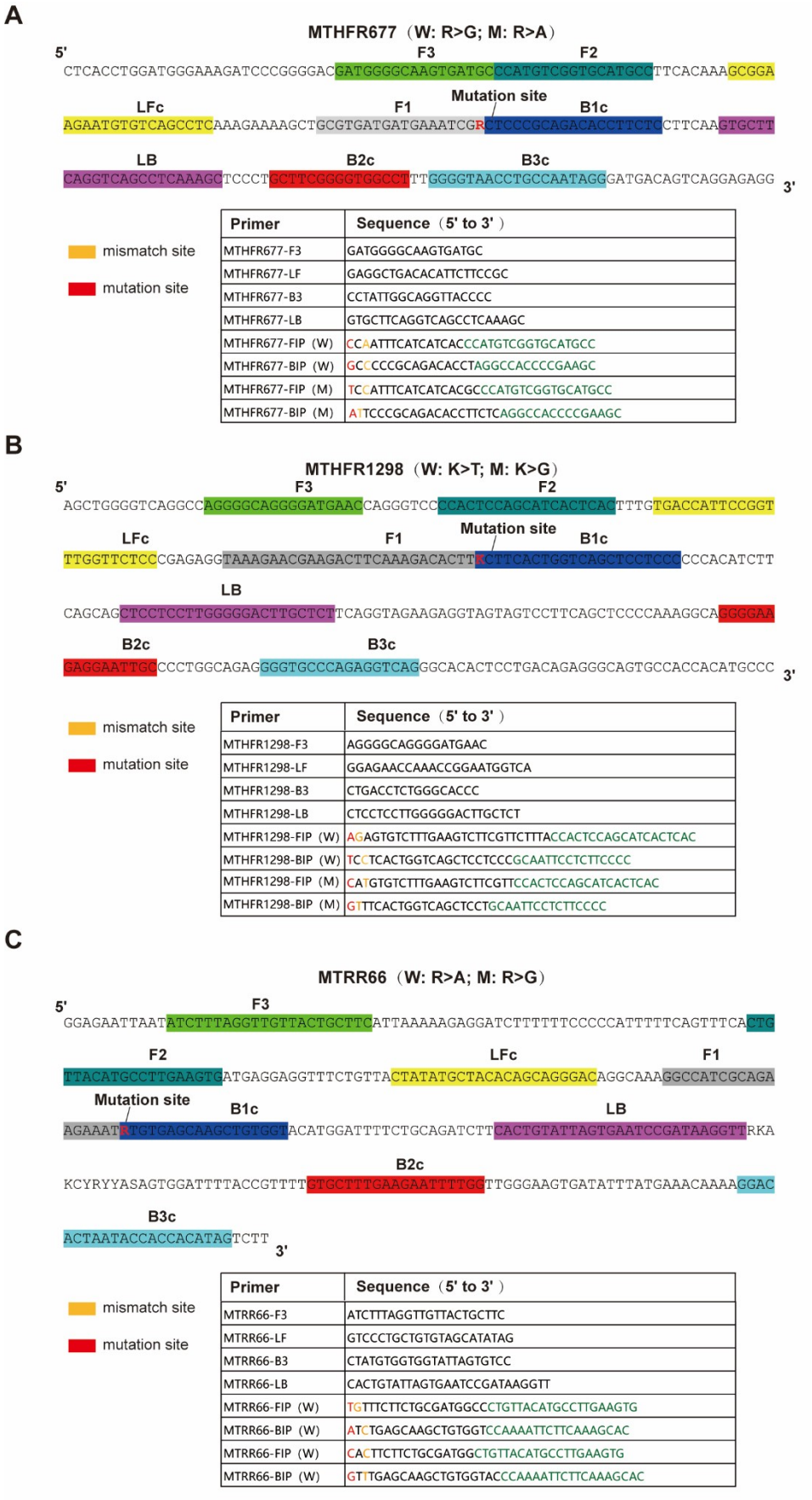
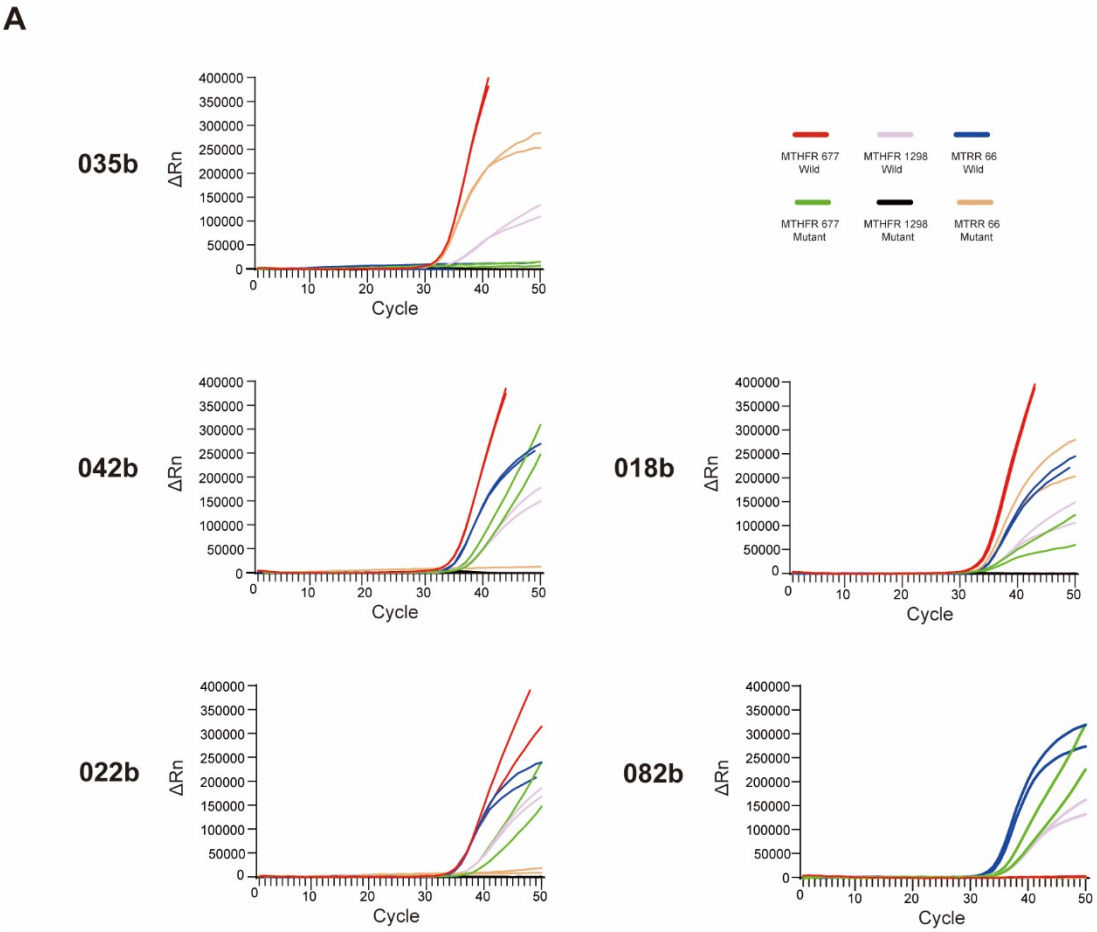


Figure S7. Sequences and design principles of the primers used for detecting single nucleotide polymorphisms of genes in folate metabolism. (A) MTHFR677（W: R>G; M: R>A）(B) MTHFR1298（W: K>T; M: K>G）(C) MTRR66（W: R>A; M: R>G）

A



B

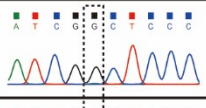
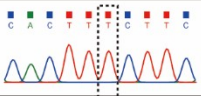
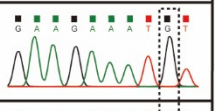
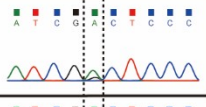
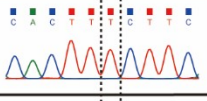
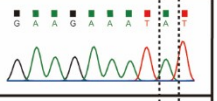
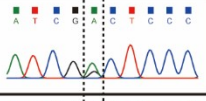
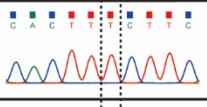
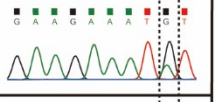
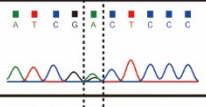
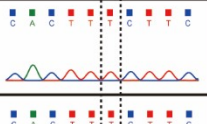
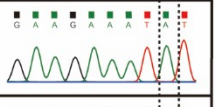
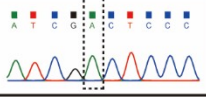
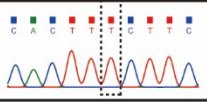
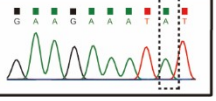
Sample ID	MTHFR 677 Wild: G Mutant: A	MTHFR 1298 Wild: T Mutant: G	MTRR 66 Wild: A Mutant: G
035b			
042b			
018b			
022b			
082b			

Figure S8. A) qPCR and (B) Sequencing results of the 5 clinical samples analyzed.