Electronic Supplementary Material (ESI) for Analytical Methods

A simplified pyrosequencing protocol based on linear-after-the-exponential (LATE)-PCR using whole blood as starting material directly

Yunlong Liu,†[a, c] Haiping Wu,†[b] Hui Ye,[a, c] Zhiyao Chen,[a] Qingxin Song,[a, c] Bingjie Zou,[a] Jianzhong Rui[a] and Guohua Zhou[a, b, c, d *]

[a] *Department of Pharmacology, Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, China.*

[b] *Huadong Research Institute for Medicine and Biotechnics, Nanjing 210002, China.*

[c] *China Pharmaceutical University, Nanjing 210009, China.*

[d] *State Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, China.*

* Corresponding author at: No. 305 Zhongshan East Road, Nanjing 210002, China. Fax: +8625 8451 4223.
E-mail address: ghzhou@nju.edu.cn (Guohua Zhou)
† These authors contributed equally to this work.

Supplementary Table

Supplementary Table S1. Sequences of LATE-PCR primers

Name [a]	Sequence (5'-3')	Conc. (μM)	Tm value (°C)	Tm^L- Tm^X [b] (°C)	Tm^A [c] (°C)
677-PX	TGTCATCCCTATTGGCAGGTTAC	1	65.9	5.8	77.0
677-PL	AGATCCCGGGGACGATGGGGCAAG	0.1	71.7		
1298-PX	GGACTACTACCTCTTCTACCTGA	1	63.5	6.5	75.7
1298-PL	GGGTCCCCACTCCAGCATCACTCAC	0.1	70.0		
DPYD-PX	GGG [d]TATAAGCCTATGAATTGGATG	1	62.7	7.2	73.8
DPYD-PL	TGGCCCTGGACAAAGCTCCTTTCTGA	0.1	69.9		

[a] PX mean excess primer, PL mean limiting primer; [b] Tm^L and Tm^X mean the Tm value of limiting primer and excess primer, respectively; [c] Tm^A mean the Tm value of amplicons; d: underlined letters were artificially mismatched bases.

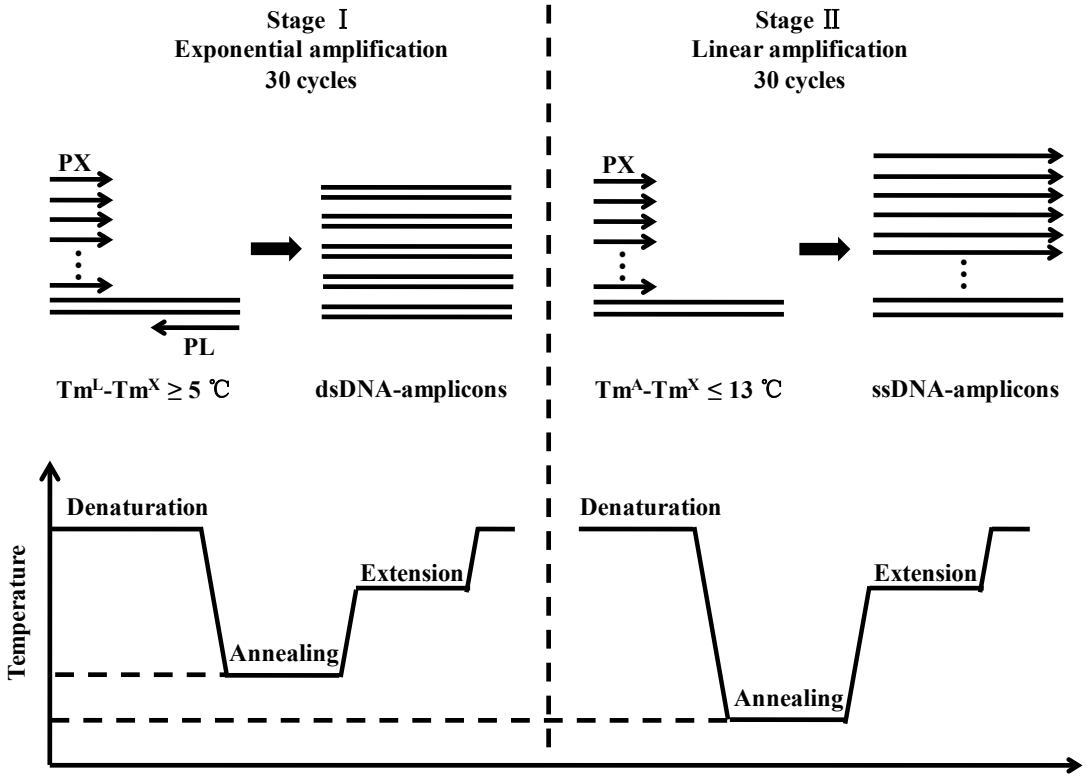
Supplementary Figure Captions

Supplementary Fig. S1. The principle of linear-after-the-exponential (LATE)-PCR.

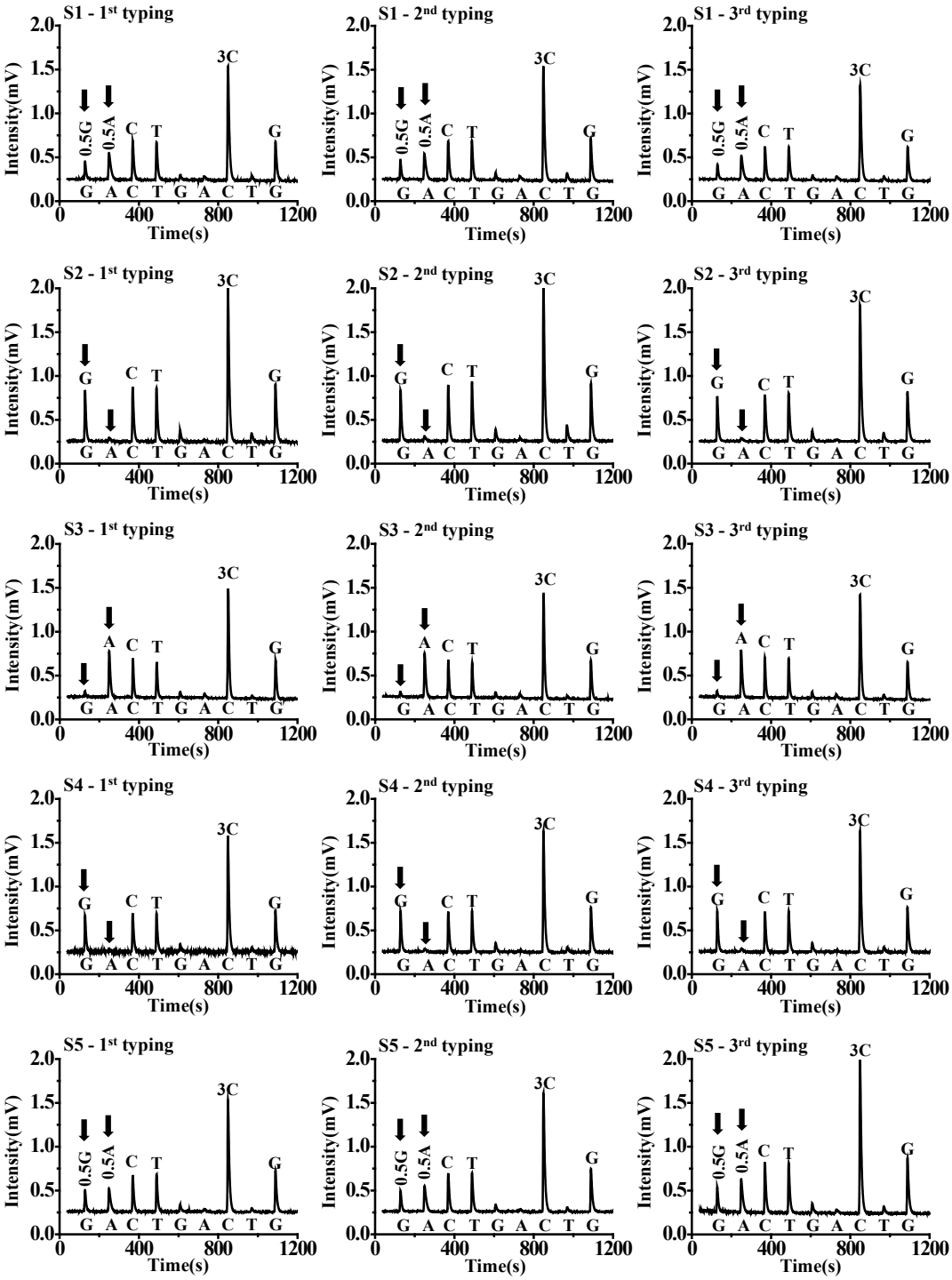
Supplementary Fig. S2. Pyrograms for genotyping MTHFR C677T in a set of five typical samples in triplicate.

Supplementary Fig. S3. The genotyping results of the 3 polymorphisms from the same sample using the whole blood LATE-PCR based pyrosequencing(A), conventional PCR based pyrosequencing(B) and commercial Sanger sequencing(C).

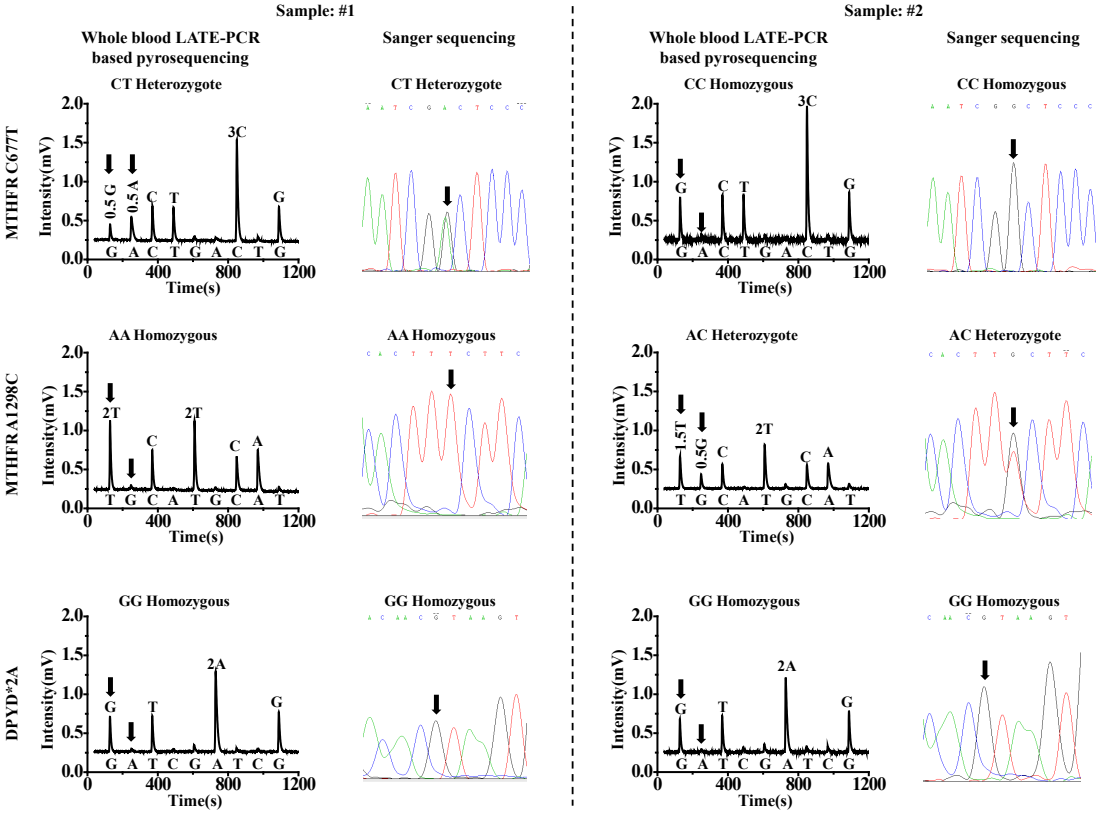
Supplementary Figures



Supplementary Fig. S1. The principle of linear-after-the-exponential (LATE)-PCR.



Supplementary Fig. S2. Pyrograms for genotyping MTHFR C677T in a set of five typical samples in triplicate.



Supplementary Fig. S3. The genotyping results of the 3 polymorphisms from the same sample using the whole blood LATE-PCR based pyrosequencing and commercial Sanger sequencing.