Multiplex one-step blood direct asymmetric PCR and dual labelled probe mediated melting curve for MTHFR and MTRR polymorphisms genotyping

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Table S1. Sequences of the designed oligonucleotides for blood direct PCR and HRM genotyping

Name	Oligonucleotide Sequence (5'-3')	Size
C677T- Forward Primer	ATCCCTCGCCTTGAACAGGT	
C677T- Reverse Primer	ATGCCTTCACAAAGCGGAAG	199bp
C677T- Probe	HEX-TATGCGGGAG*C*CGATTTCATC-BHQ1	
A1298C- Forward Primer	CCCACTCCAGCATCACTCACTT	
A1298C- Reverse Primer	TACCTGAAGAGCAAGTCCCCC	149bp
A1298C- Probe	ROX-AAAGACACTT*T*CTTCACTGGT-BHQ2	
A66G- Forward Primer	ATGCCTTGAAGTGATGAGGAGG	
A66G- Reverse Primer	CCACTGTAACGGCTCTAACCTTAT	161bp
A66G- Probe	Cy5-CAGAAGAAAT*A*TGTGAGCAAG-BHQ2	

Note: Red letters represent dominant primers and blue letters represent inferior primers. Forward and reverse primers are also used for C677T, A1298C and A66G polymorphisms sequencing. Underline letters represent polymorphisms sites probe binding to target.

Table S2. Mean Tm values and SDs of homozygous genotypes

	Wild type		**Variant**		
	Tm, ℃	**SD, ℃**	**Tm, ℃**	**SD, ℃**	**△Tm, ℃**
C677T	66.1	0.43	56.1	0.33	10.0
A1298C	62.1	0.38	54.5	0.36	7.6
A66G	62.4	0.40	55.1	0.31	7.3

Table S3. Melting curves genotyping results of blood samples and gDNA samples

	Direct blood PCR and HRM			gDNA PCR and HRM		
No.	C677T	A1298C	A66G	C677T	A1298C	A66G
1	CC	AC	AG	CC	AC	AG
2	CC	AA	AG	CC	AA	AG
3	CT	AC	AA	CT	AC	AA
4	CT	AA	AG	CT	AA	AG
5	TT	AA	AG	TT	AA	AG
6	CC	AA	AG	CC	AA	AG
7	TT	AA	AA	TT	AA	AA
8	CC	AA	AA	CC	AA	AA
9	CT	AA	GG	CT	AA	GG
10	CT	AC	AA	CT	AC	AA

11	CC	AC	AA	CC	AC	AA
12	TT	AA	AA	TT	AA	AA
13	CC	AC	GG	CC	AC	GG
14	CC	AC	AA	CC	AC	AA
15	CC	AC	AG	CC	AC	AG
16	CT	AA	AG	CT	AA	AG
17	CT	AA	AA	CT	AA	AA
18	CT	AA	AA	CT	AA	AA
19	CT	AA	AA	CT	AA	AA
20	CC	AC	AA	CC	AC	AA
21	CT	AA	AG	CT	AA	AG
22	CC	AA	AA	CC	AA	AA
23	TT	AA	AA	TT	AA	AA
24	CT	AC	AA	CT	AC	AA
25	TT	AC	AA	TT	AC	AA
26	CT	AA	AA	CT	AA	AA
27	TT	AA	AG	TT	AA	AG
28	CC	AA	AG	CC	AA	AG
29	CC	AA	AA	CC	AA	AA
30	CC	AA	AA	CC	AA	AA
31	CT	AC	AG	CT	AC	AG
32	CT	AC	AA	CT	AC	AA
33	CT	AA	AG	CT	AA	AG
34	CC	CC	AA	CC	CC	AA
35	CC	AC	AG	CC	AC	AG
36	CC	AA	AA	CC	AA	AA
37	CT	AC	AA	CT	AC	AA
38	CT	AA	GG	CT	AA	GG
39	CT	AA	AA	CT	AA	AA
40	CT	AC	AA	CT	AC	AA
41	CT	AA	GG	CT	AA	GG
42	CT	AA	AG	CT	AA	AG
43	CT	AA	AG	CT	AA	AG
44	CC	AA	AA	CC	AA	AA
45	CT	AC	AG	CT	AC	AG
46	CT	AA	AA	CT	AA	AA
47	TT	AA	AA	TT	AA	AA
48	CC	AA	AA	CC	AA	AA
49	CC	AA	AG	CC	AA	AG
50	CT	AA	GG	CT	AA	GG

Table S4. WBC counts and cycle threshold (Ct) values in HEX, ROX, Cy5 channels

of 50 clinical samples

No.	WBC (*10E3/μL)	Direct blood PCR and HRM			gDNA PCR and HRM		
		C677T (HEX)	A1298C (ROX)	A66G (Cy5)	C677T (HEX)	A1298C (ROX)	A66G (Cy5)
1	6.01	28.24	29.89	31.84	30.21	30.94	31.14
2	6.79	27.38	28.15	31.55	30.94	30.52	32.01
3	4.87	30.22	32.47	32.64	32.28	32.59	31.13
4	3.45	29.26	29.73	33.06	32.58	31.34	32.64
5	5.43	NoCt	31.02	37.50	NoCt	31.92	31.02
6	5.97	26.25	27.91	31.99	30.38	30.95	31.20
7	4.12	NoCt	33.43	34.30	NoCt	31.65	30.96
8	8.64	28.16	28.93	31.82	30.60	31.04	29.52
9	7.01	31.15	29.68	NoCt	29.72	27.86	45.03
10	7.48	29.11	29.17	30.15	31.35	31.00	29.73
11	8.19	29.00	30.73	30.45	29.83	30.65	29.17
12	4.65	44.65	29.81	31.05	37.30	31.15	30.88
13	6.44	28.96	30.40	NoCt	30.53	31.70	NoCt
14	5.88	28.76	28.54	28.87	30.51	32.00	30.19
15	8.00	28.67	30.04	31.68	29.68	30.76	30.49
16	9.81	27.40	26.82	29.37	31.02	29.95	30.48
17	3.92	28.33	27.48	29.26	31.37	29.11	29.93
18	9.68	27.39	26.37	28.46	29.97	28.02	28.87
19	9.02	29.03	28.54	30.21	31.23	29.21	29.47
20	8.95	28.15	29.66	30.10	28.72	29.52	28.60
21	4.80	29.09	28.61	30.71	31.22	29.42	30.68
22	9.80	27.79	28.22	29.59	28.99	28.24	28.51
23	6.45	32.93	27.89	30.32	34.81	29.42	29.57
24	5.30	30.22	30.28	31.58	30.63	30.25	29.25
25	12.19	NoCt	27.94	28.73	NoCt	28.16	28.57
26	6.98	29.19	28.49	30.00	32.05	30.10	30.20
27	10.92	32.44	28.29	31.84	35.04	28.64	30.93
28	7.16	29.40	29.93	32.52	30.77	30.23	32.19
29	4.84	28.29	28.90	30.26	30.62	30.29	31.45
30	4.00	29.26	29.96	31.47	29.22	29.19	29.78
31	10.74	28.17	28.75	30.63	29.74	28.31	28.12
32	2.51	32.23	32.20	32.85	31.17	30.59	30.20
33	4.36	30.81	29.87	32.90	32.09	30.29	31.15
34	8.45	29.18	NoCt	31.21	29.59	NoCt	29.43
35	6.07	29.74	31.53	32.43	30.42	30.62	31.76
36	5.53	28.47	29.03	31.00	30.22	29.48	30.27
37	7.45	28.00	28.68	29.45	30.08	29.63	29.72

38	8.95	29.53	28.84	NoCt	31.44	30.04	44.89
39	6.69	28.08	27.57	29.87	31.56	31.16	30.97
40	8.55	28.90	29.23	29.94	29.98	29.33	28.87
41	6.13	27.88	27.15	29.61	30.77	29.22	29.15
42	9.02	27.66	27.99	30.68	28.90	27.54	28.48
43	10.14	25.61	26.29	29.83	30.15	28.37	29.39
44	6.83	23.15	24.53	27.49	28.98	28.68	28.98
45	4.62	26.88	28.01	32.66	31.27	30.90	31.07
46	5.86	29.32	31.53	30.87	30.45	28.85	29.59
47	3.86	45.32	29.36	31.24	NoCt	30.23	30.31
48	5.50	23.39	26.26	30.27	30.42	30.00	29.77
49	6.42	27.60	27.97	31.28	29.27	28.49	29.98
50	10.10	28.14	27.51	NoCt	31.36	29.78	NoCt

Table S5. Genotype distribution in the blinded study

	Genotype		
	Wild	**Heterozygote**	**Homozygote**
C677T	19	24	7
A1298C	33	16	1
A66G	28	17	5

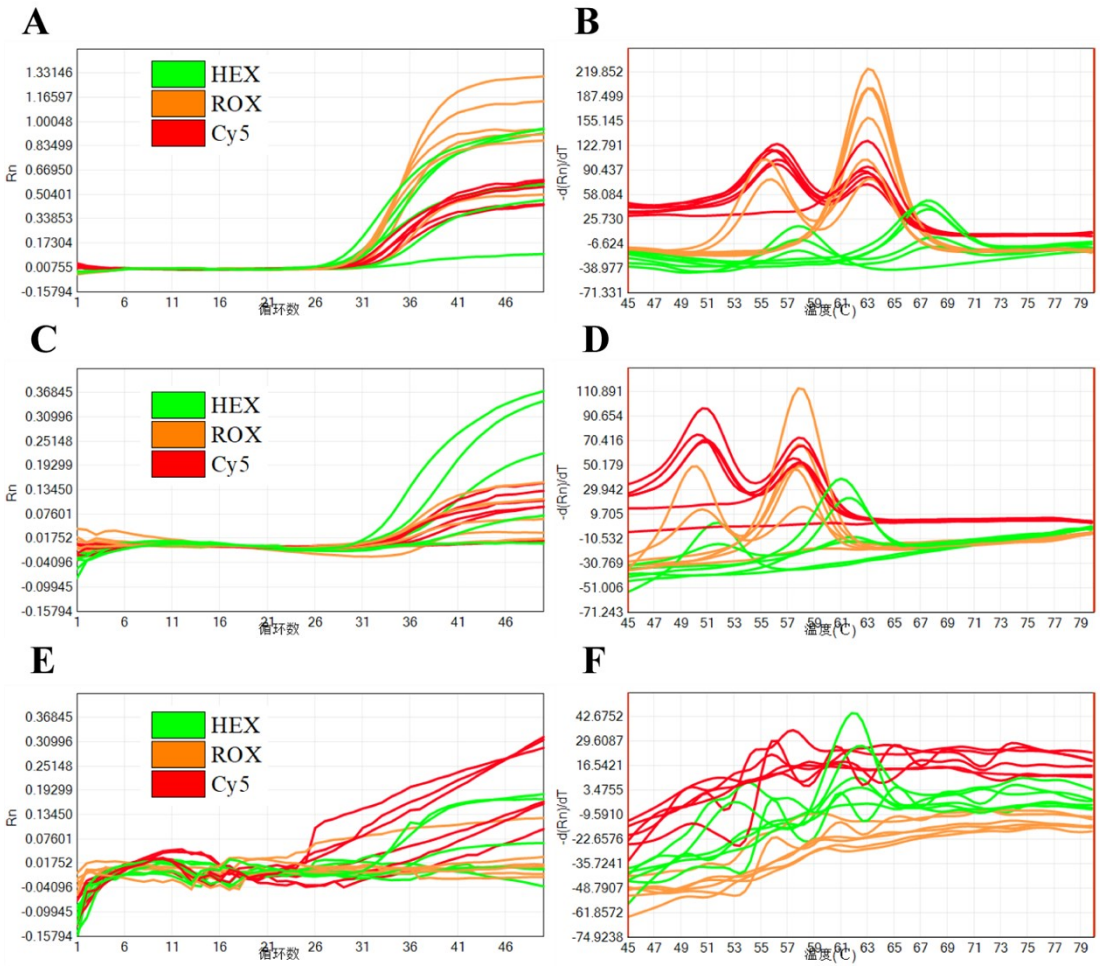

Figure S1 Original screenshots of amplification curves and melting curves for No.1 to 6 EDTA anticoagulated blood samples. PCR mix of Meridian Bioscience (A and B), Yeasen Biotech Co. (C and D) and FOREGENE Biotech Co. (E and F) were used.

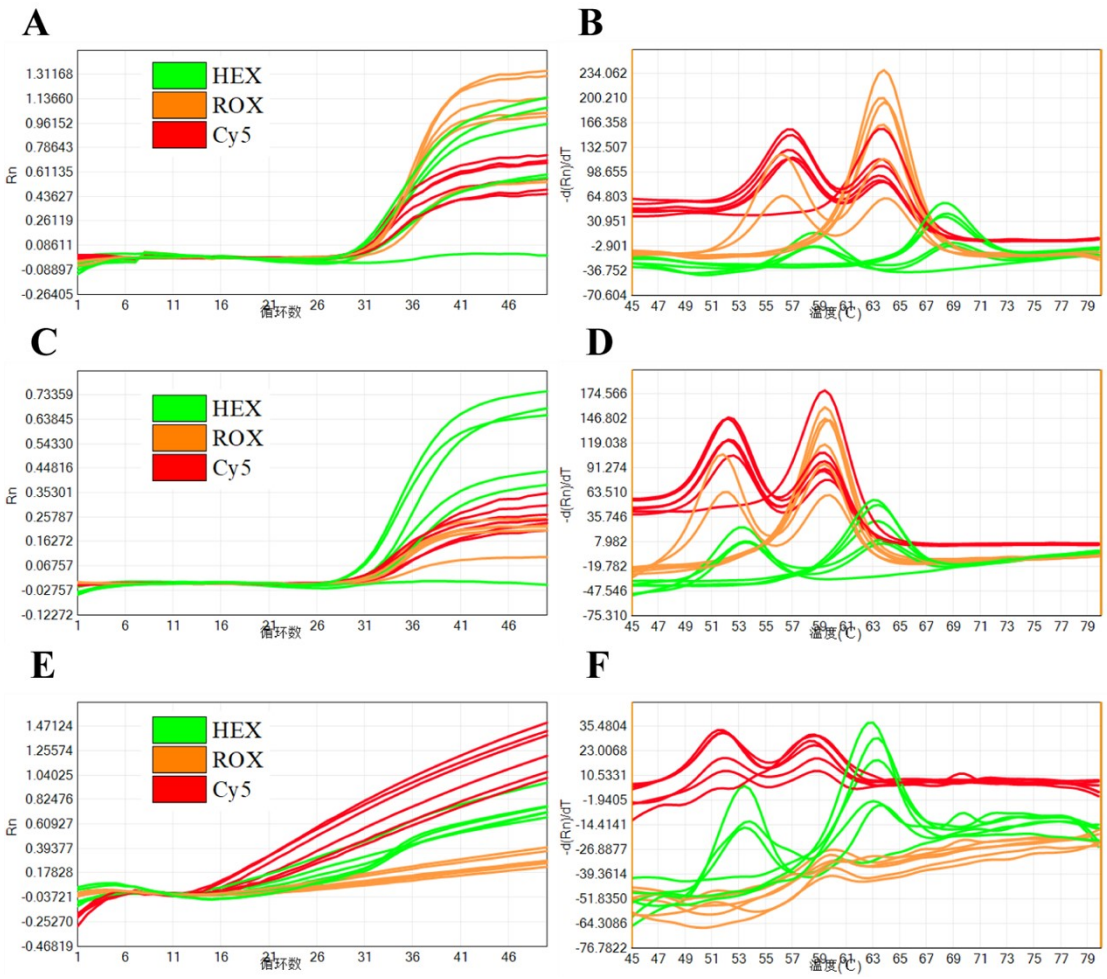

Figure S2 Original screenshots of amplification curves and melting curves for No.1 to 6 extracted gDNA samples. PCR mix of Meridian Bioscience (A and B), Yeasen Biotech Co. (C and D) and FOREGENE Biotech Co. (E and F) were used.

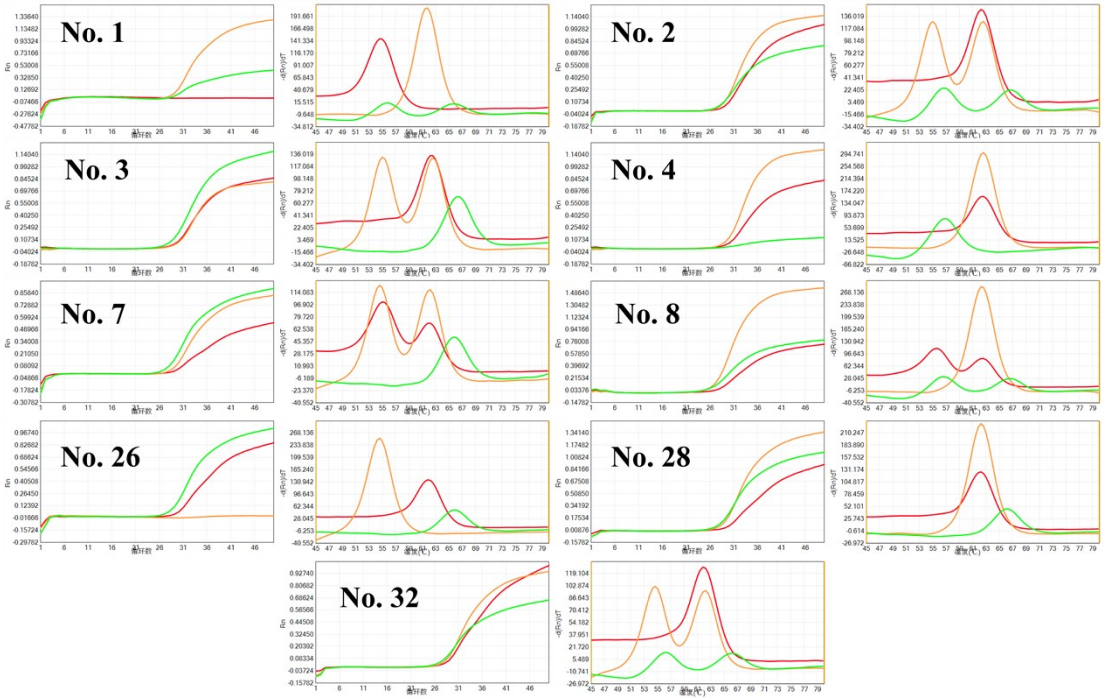

Figure S3 Original screenshots of amplification curves and derivate melting curves corresponding in Figure 2.

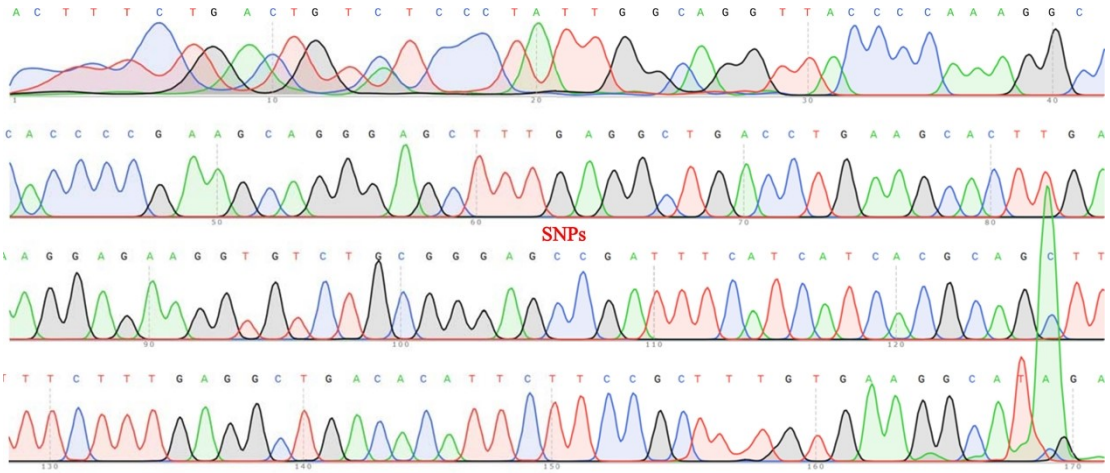

Figure S4 Sanger sequencing results of CC genotype of C677T loci

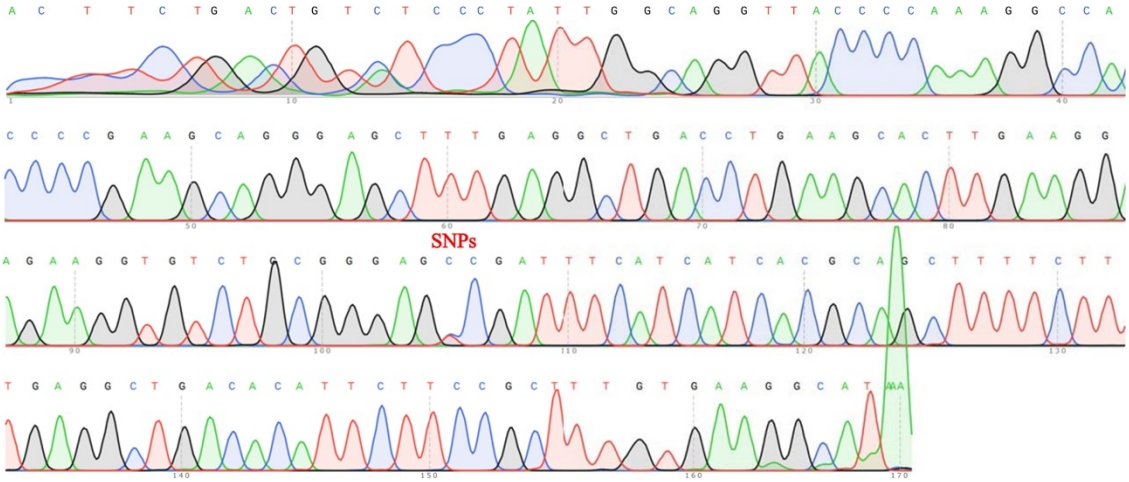

Figure S5 Sanger sequencing results of CT genotype of C677T loci

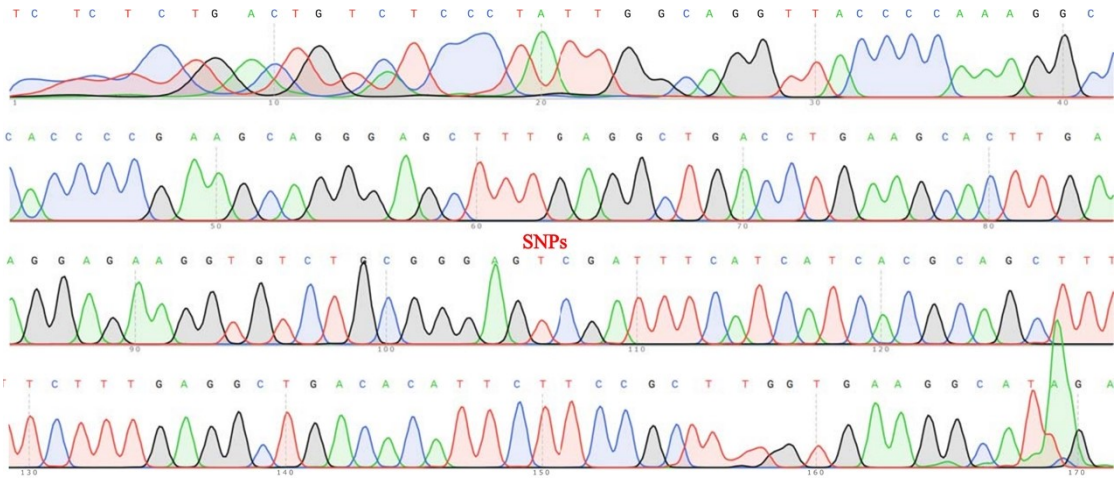

Figure S6 Sanger sequencing results of TT genotype of C677T loci

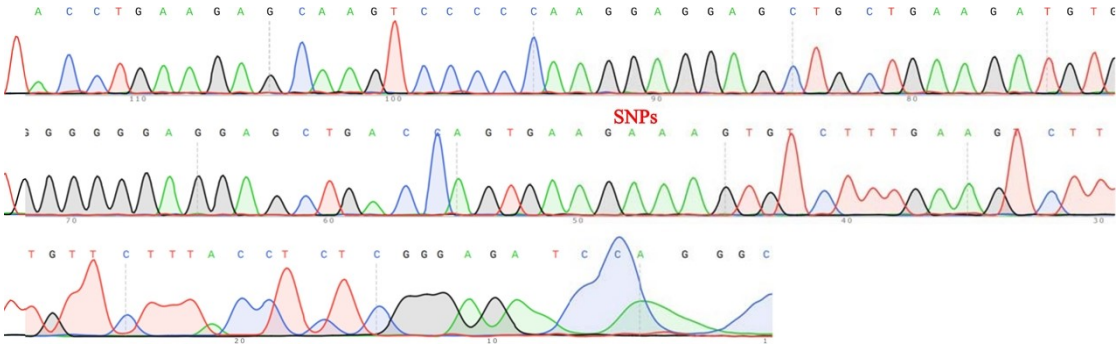

Figure S7 Sanger sequencing results of AA genotype of A1298C loci

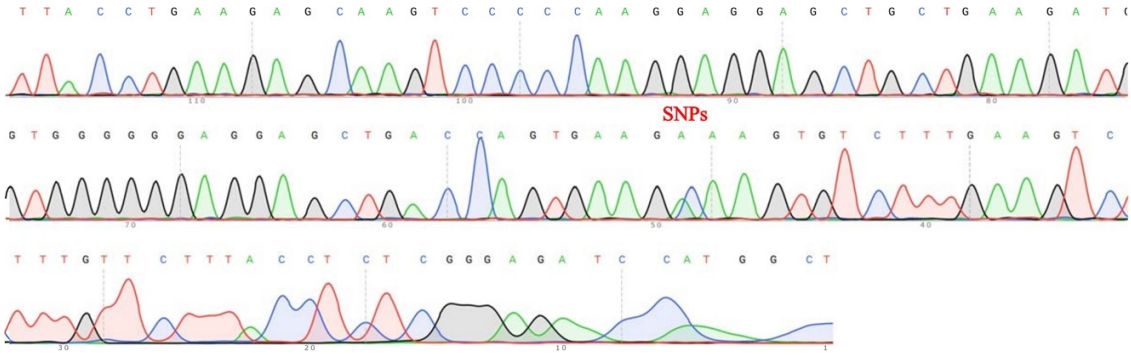

Figure S8 Sanger sequencing results of AC genotype of A1298C loci

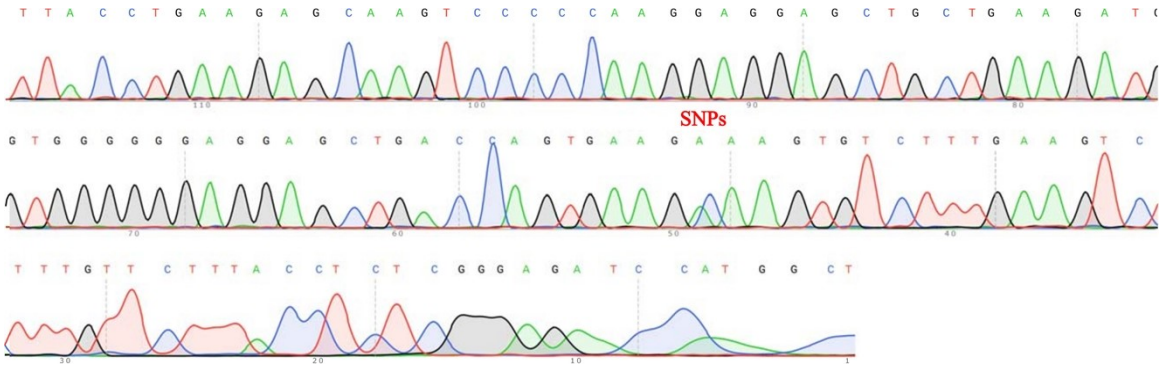

Figure S9 Sanger sequencing results of CC genotype of A1298C loci

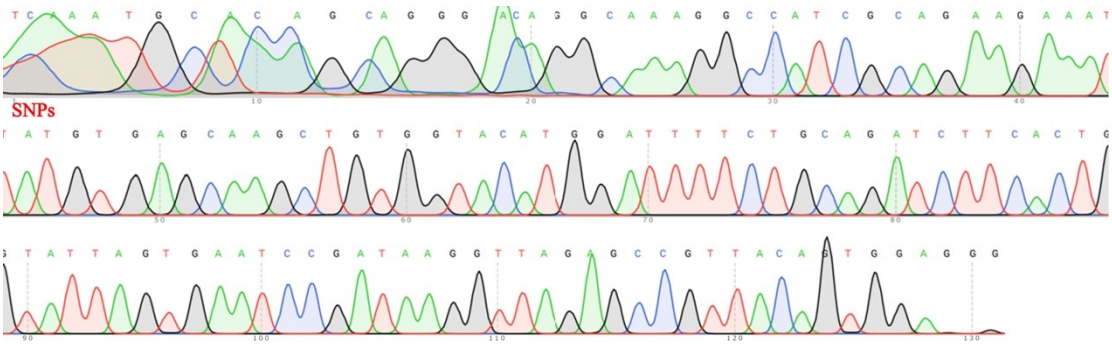

Figure S10 Sanger sequencing results of AA genotype of A66G loci

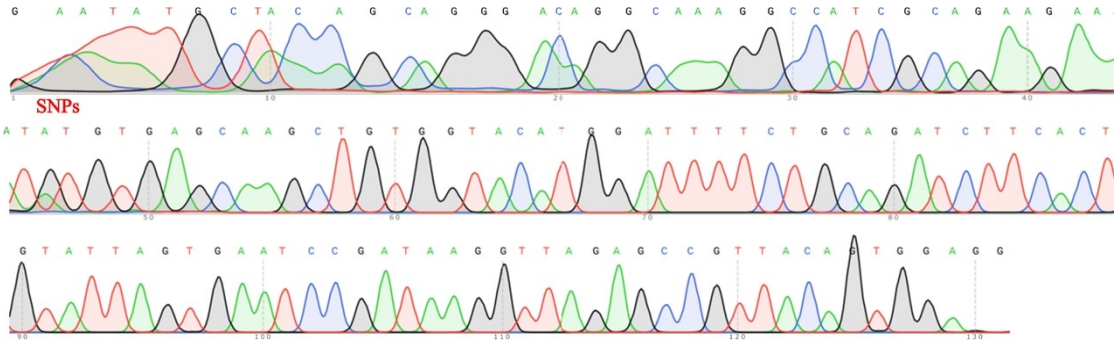

Figure S11 Sanger sequencing results of AG genotype of A66G loci

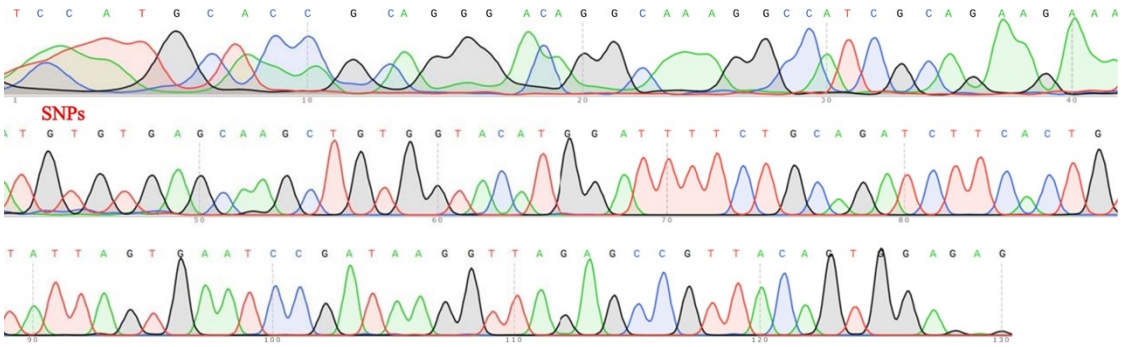

Figure S12 Sanger sequencing results of GG genotype of A66G loci

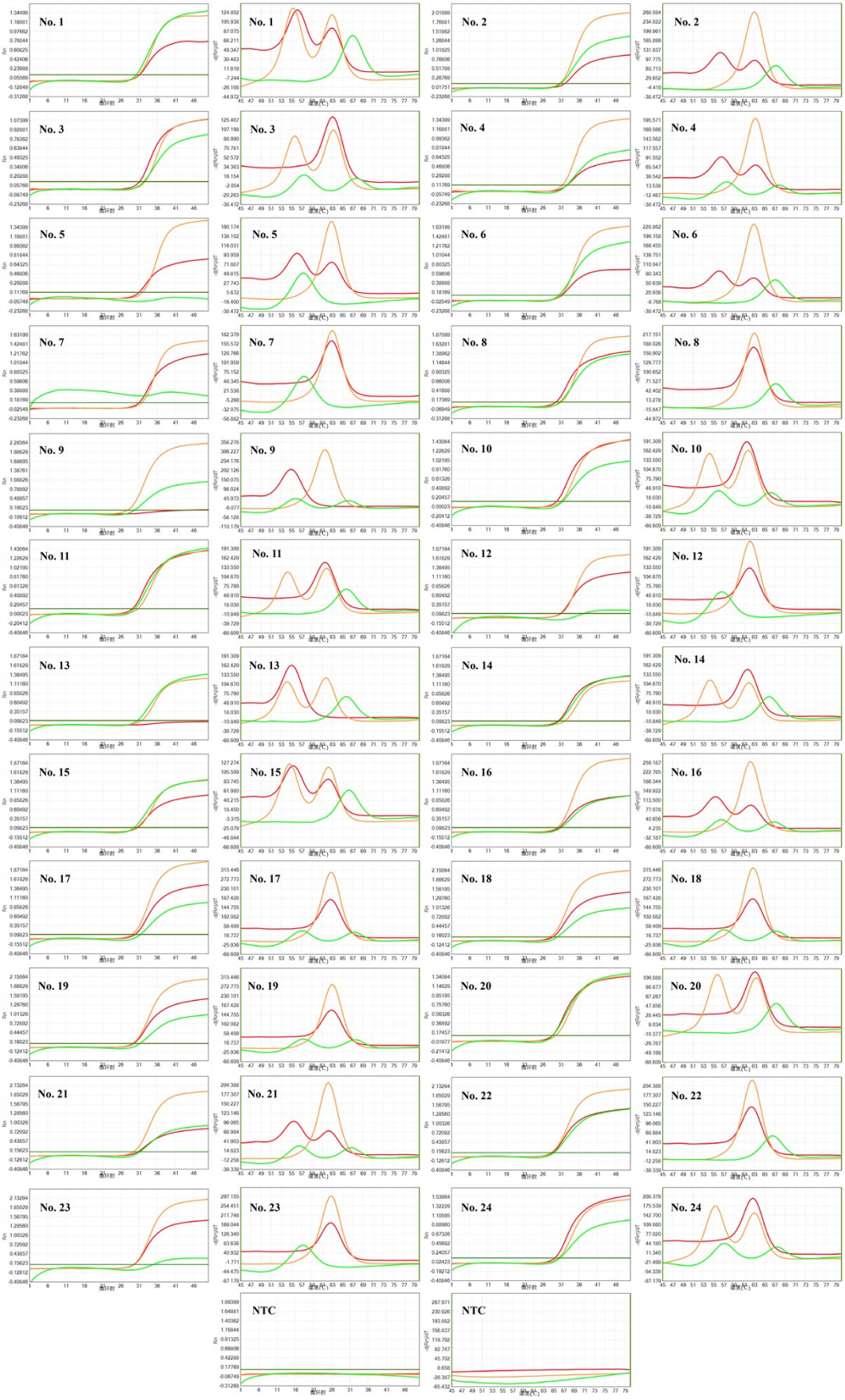

Figure S13 Amplifications and melting curves for gDNA samples of No. 1 to 24

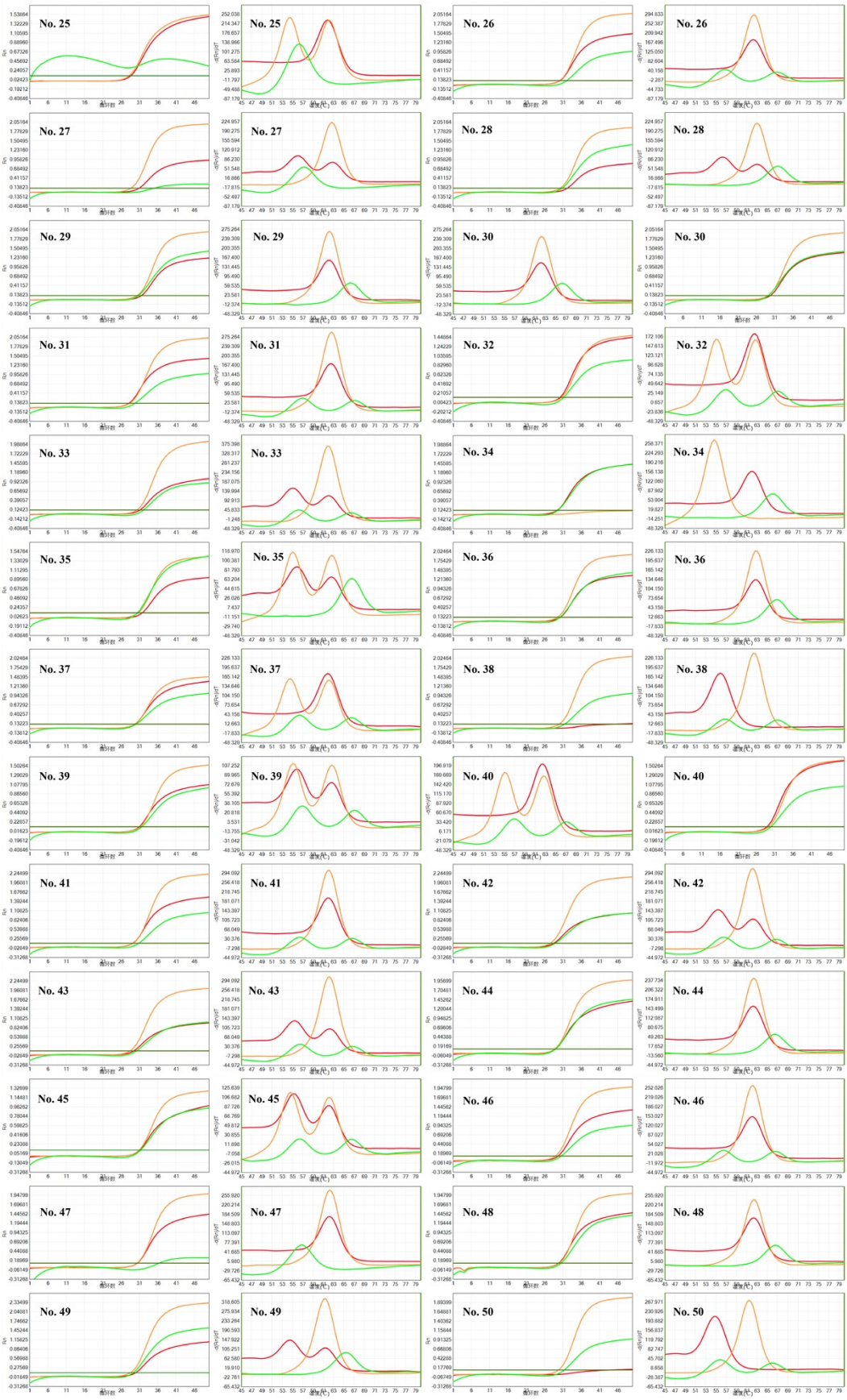

Figure S14 Amplifications and melting curves for gDNA samples of No. 25 to 50

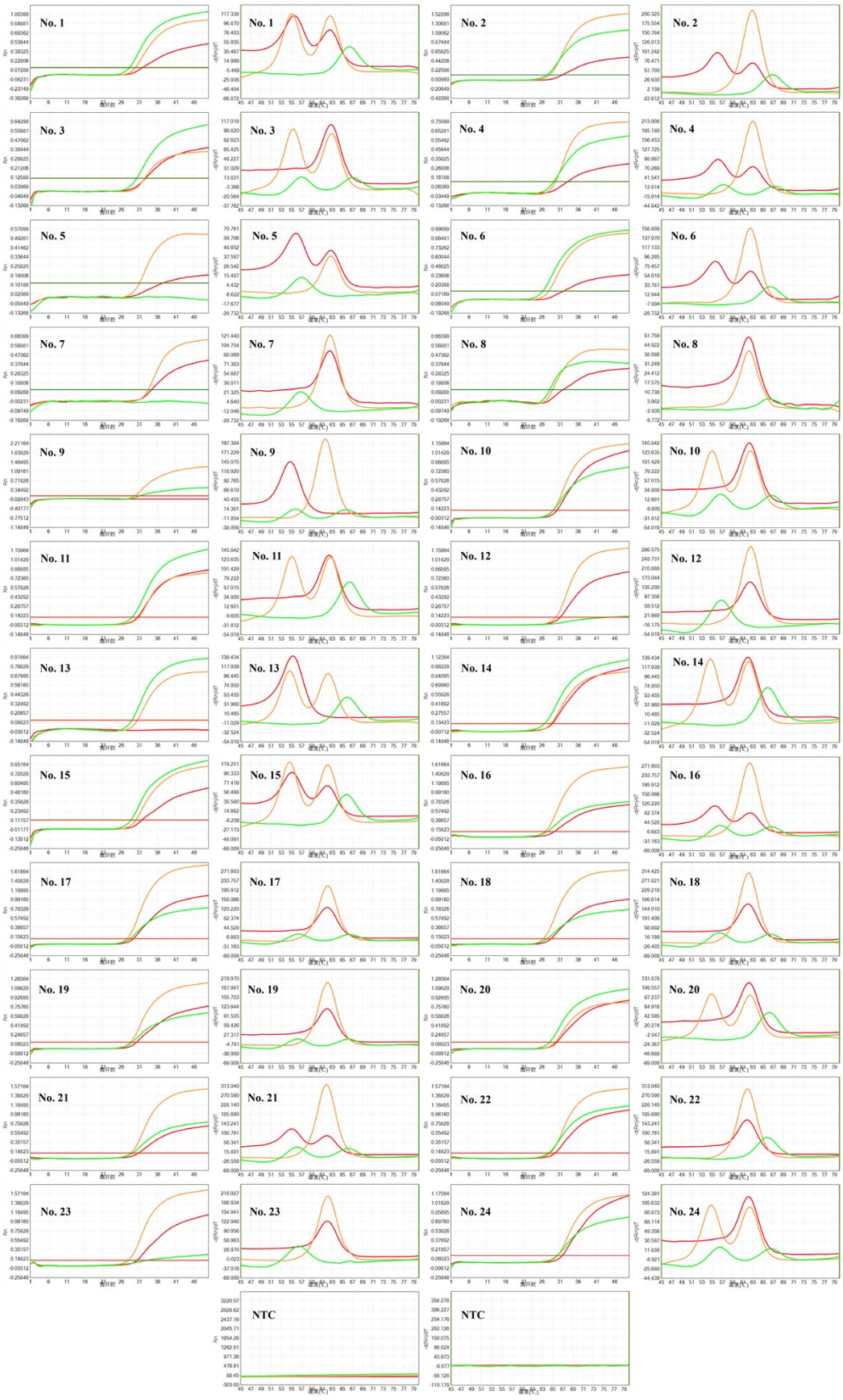

Figure S15 Amplifications and melting curves for whole blood samples of No. 1 to 24

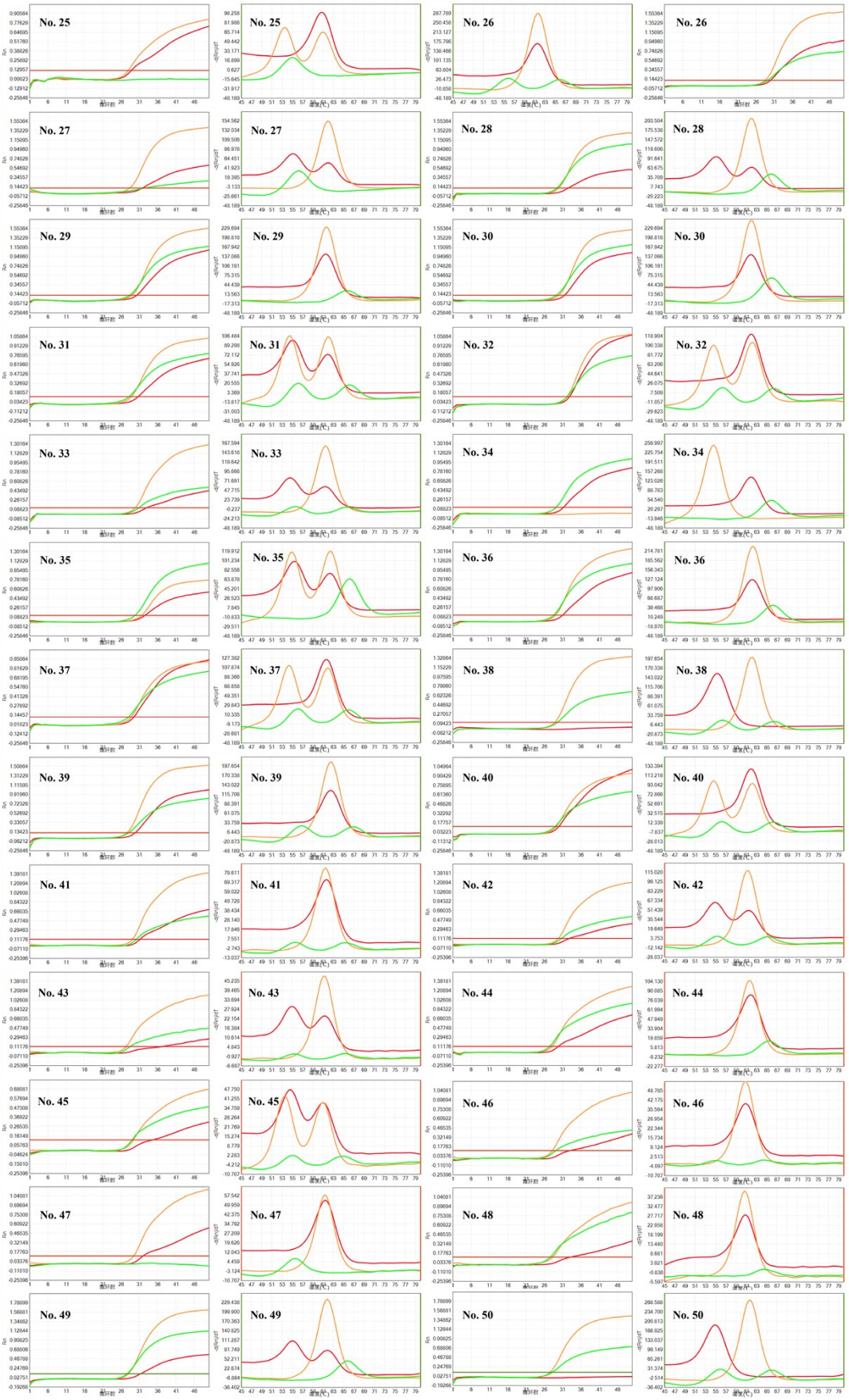
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Figure S16 Amplifications and melting curves for whole blood samples of No. 25 to 50

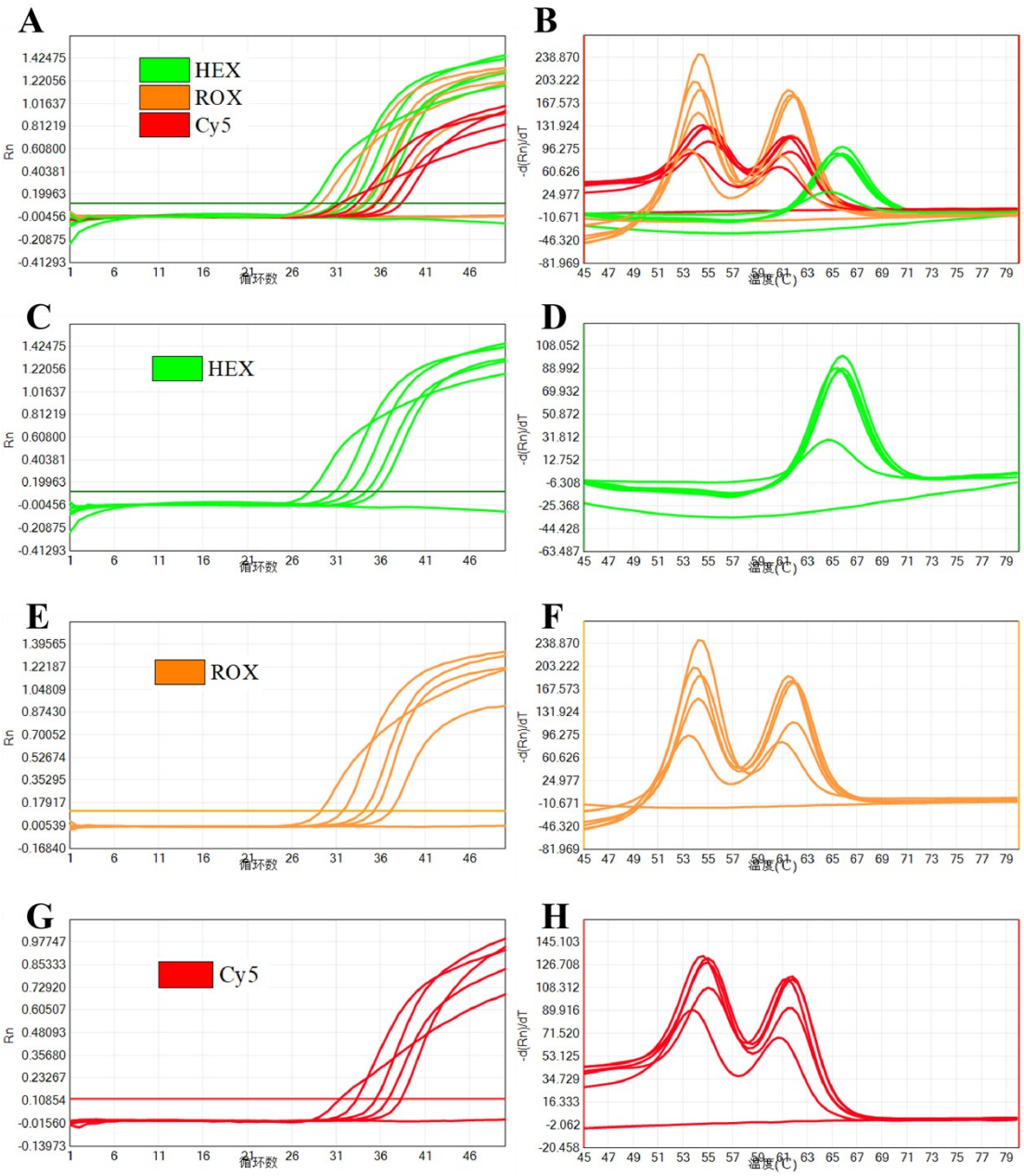

Figure S17 Original screenshots of multiplex and individual amplifications curves (A, C, E, G), melting curves (B, D, F, H) of EDTA-K_2 anti-coagulated blood, gradient 1:10, 1:40, 1:160 and 1:320 dilutions of EDTA-K_2 treated blood.

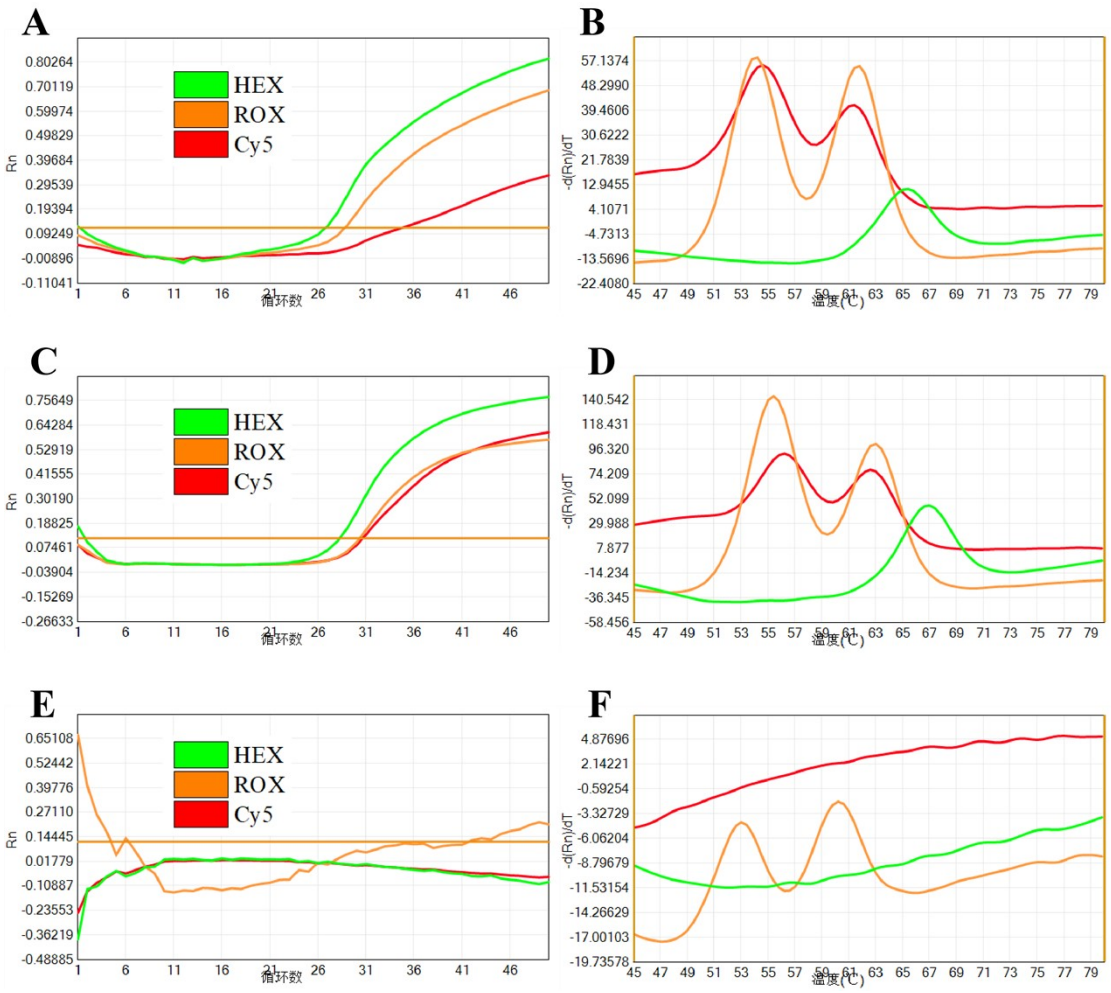

Figure S18 Original screenshots of PCR amplification curves and melting curves for 1 μL (A, B), 2 μL (C, D) and 4 μL (E, F) blood added. Total reaction volume is 20 μL.

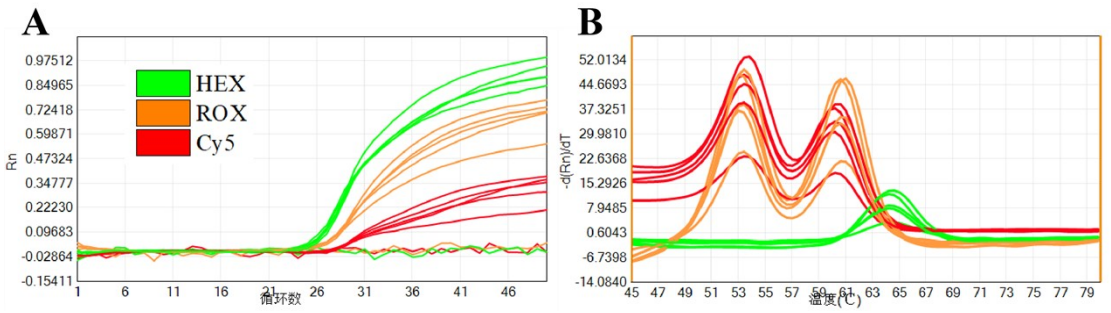

Figure S19 Amplification curves (A) and melting curves (B) of fresh blood and frozen specimen storage at -20 ℃ (sample No.1) for 1, 2, 3 and 4 weeks

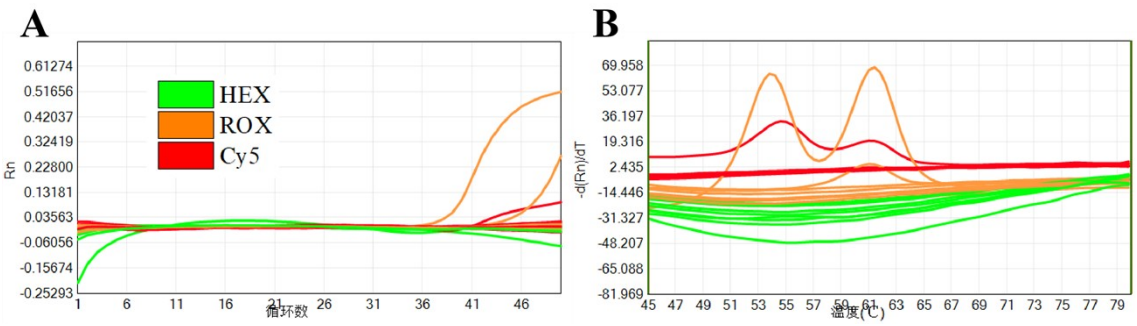

Figure S20 Amplification curves (A) and melting curves (B) of No.1 to 8 plasma samples