

## **Optimized protocol for the extraction of RNA and DNA from frozen whole blood sample stored in a single EDTA tube**

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## Supplementary information

Supplementary Table S1. Characteristics of RNA purified from peripheral whole blood sample under different conditions after short-term storage (Participants1). Data are shown as mean  $\pm$  standard deviation. A260/280, absorbance ratio at 260 and 280 nm; A260/230, absorbance ratio at 260 and 230 nm; RIN, RNA integrity number; 28S/18S, 28S-to-18S rRNA ratio.

Supplementary Table S2. Characteristics of RNA purified from peripheral whole blood sample after long-term storage (Participants2). A260/280, absorbance ratio at 260 and 280 nm; A260/230, absorbance ratio at 260 and 230 nm; RIN, RNA integrity number; 28S/18S, 28S-to-18S rRNA ratio.

Supplementary Table S3. Characteristics of DNA purified from peripheral whole blood sample after long-term storage (Participants2). A260/280, absorbance ratio at 260 and 280 nm; A260/230, absorbance ratio at 260 and 230 nm.

Supplementary Table S4. Characteristics of RNA purified from peripheral whole blood sample (Participants3). RIN, RNA integrity number.

Supplementary Table S5. Expression of housekeeping genes in long-term storage samples (Participants2,  $n = 30$ ) and control samples of fresh blood (Participants3,  $n = 10$ ). SD, standard deviation; CV, coefficient of variation.

Supplementary Table S6. Demographic characteristics of the participants. Age is shown as mean  $\pm$  standard deviation.

Supplementary Table S7. Primer sequences used in this study and amplicon lengths.

Supplementary Table S8. Sequences of synthetic DNA.

Supplementary Table S9. Summary of statistical analyses.

Supplementary Fig. S1 Typical electropherograms of purified RNA. Thawing in a water bath increased the RNA degradation in samples P-37 and N-37 of participants 3 and 5.

Supplementary Fig. S2 Gel images of DNA. Agarose gel (1%) imaging was performed using ATTO printgraph and ATTO imageSaver AE-6905C. Five hundred nano grams of DNA was loaded in each lane. Five microliters of GeneRuler 100-bp Plus DNA Ladder (Thermo Fisher Scientific) was used for loading controls and markers.

Supplementary Fig. S3 Gel images of PCR products of GAPDH. Agarose gel (1.2%) imaging was performed using ATTO printgraph and ATTO imageSaver AE-6905C. Three microliters of GeneRuler 100-bp Plus DNA Ladder (Thermo Fisher Scientific) was used for loading controls and markers.

Supplementary Fig. S4 Gel images of PCR products of RPS18. Agarose gel (1.2%) imaging was performed using ATTO printgraph and ATTO imageSaver AE-6905C. Three microliters of GeneRuler 100-bp Plus DNA Ladder (Thermo Fisher Scientific) was used for loading controls and markers.

# Table S1

protocol	ID	time for thawing (minutes)	A260/A280	A260/A230	yeild (ug/mL) by A260	yeild (ug/mL) by Qubit	RIN	28S/18S
P-Con	1	—	2.09	1.87	2.03	2.13	8.4	1.3
	2	—	2.14	1.71	0.81	0.89	7.9	1.9
	3	—	2.14	1.51	1.91	2.00	6.9	1.8
	4	—	2.16	1.58	0.84	0.98	7.7	1.7
	5	—	2.07	2.08	2.11	1.73	7.3	2.0
	6	—	2.12	2.02	2.02	2.22	8	1.9
	7	—	2.11	1.56	0.86	0.94	9.2	1.6
P-37	1	4.7	2.17	1.76	1.36	1.48	6.7	1.5
	2	4.7	2.22	1.52	0.90	1.05	6.4	1.4
	3	5.2	2.42	0.24	0.91	0.92	4.2	1.0
	4	5.2	3.63	0.07	0.27	0.16	2.1	0.4
	5	5.3	2.05	1.48	0.73	0.79	4.5	1.1
	6	5.3	2.11	1.89	1.08	1.16	6	1.3
	7	5.3	2.15	1.32	0.60	0.65	6.1	1.0
P-AI	1	18.0	2.2	1.44	1.22	1.34	7	1.5
	2	18.0	2.17	1.89	1.68	1.77	7.5	1.8
	3	16.8	2.16	1.95	2.02	3.59	7.2	0.9
	4	16.8	2.22	1.45	0.71	0.77	5.3	1.4
	5	18.3	2.22	1.75	1.24	1.35	6.6	1.1
	6	18.3	2.18	1.94	1.19	1.18	6.8	1.1
	7	18.3	2.27	1.95	0.85	0.95	6.1	1.3
N-37	1	4.5	2.04	1.64	6.93	6.30	6.4	0.8
	2	4.5	2.01	1.59	5.88	5.40	6.4	0.8
	3	5.3	2	1.62	6.93	7.06	4.7	0.8
	4	5.3	1.42	0.69	1.04	0.43	4.2	1.6
	5	4.8	2	1.39	5.25	4.73	4.6	0.9
	6	4.8	2.08	1.79	8.26	8.04	6.2	0.8
	7	4.8	1.98	1.32	6.52	5.82	5.2	0.7
N-AI	1	16.5	2.01	1.71	7.50	6.47	6.5	0.9
	2	16.5	2.02	1.5	6.45	5.54	6.2	0.9
	3	17.7	2.01	1.72	8.36	8.18	6.2	0.6
	4	17.7	1.81	1.23	2.73	2.60	3.1	1.0
	5	18.5	2	1.44	6.05	5.60	5.6	1.0
	6	18.5	2	1.47	9.02	7.87	6.7	0.9
	7	18.5	1.96	1.36	6.73	5.80	6.3	0.6
N-Ice	1	120	2.05	1.68	7.17	6.58	6.9	1.3
	2	120	2.03	1.5	5.70	5.35	6.5	0.9
	3	120	2.04	1.7	8.41	8.12	6.6	1.1
	4	120	1.64	0.82	1.54	0.87	2.6	1.8
	5	120	2.02	1.42	6.55	5.54	5.8	1.0
	6	120	2.11	1.79	8.06	7.36	6.5	0.7
	7	120	2.05	1.54	6.20	5.32	5.8	0.9

## Table S2

ID	sex	age	A260/A280	A260/A230	Yield ( $\mu\text{g/ml}$ )	RIN	28S/18S	Preservation period (years)
Dep007	F	25	2.01	1.49	6.67	5.9	1	8.5
Dep008	F	28	2.09	1.67	8.43	5.8	1.3	8.5
Dep029	M	39	2.08	1.62	9.58	5.4	1.4	8.2
Dep053	F	36	2.10	1.45	6.46	5.9	1	7.9
Dep060	F	39	2.01	1.58	6.95	5.6	1.4	7.8
Dep064	M	51	2.05	1.47	5.13	5.1	1.3	7.7
Dep077	M	44	2.08	1.94	11.90	6	0.9	7.6
Dep085	F	42	2.06	1.33	8.50	5.8	1.5	7.5
Dep093	M	41	2.10	1.83	13.96	6.4	1.7	7.4
Dep103	F	28	2.02	1.72	9.50	6.6	1.1	7.3
Dep130	M	47	2.05	1.77	8.00	5.3	1.4	6.7
Dep135	F	46	2.09	1.86	10.60	4.7	0.9	6.6
Dep137	M	33	2.06	1.96	16.98	4.7	0.7	6.5
Dep140	F	33	2.04	1.76	10.19	7	1.5	6.5
Dep142	M	55	2.05	1.48	9.33	7.1	1.2	6.5
Dep147	F	27	2.05	1.65	6.82	5.6	1.1	6.4
Dep153	F	41	2.03	1.70	8.71	7	1.3	6.3
Dep166	F	41	2.07	1.22	6.98	6.7	0.6	6.0
Dep169	F	27	2.06	1.40	5.42	5.3	1	6.0
Dep172	M	29	2.10	1.92	15.59	5	0.9	5.9
Dep173	M	43	2.06	1.60	6.73	5.5	1	5.8
Dep176	M	40	2.06	1.86	10.46	6.5	1	5.8
Dep180	M	28	2.02	1.55	6.35	7.1	1.8	5.6
Dep183	F	43	2.05	1.78	10.64	4.9	0.9	5.5
Dep211	F	39	2.05	1.45	7.84	5.7	1.1	5.1
Dep215	M	34	2.04	1.76	10.42	7.4	1.3	5.0
FD010	F	39	2.01	1.57	6.16	7.3	1.8	2.6
FD014	M	25	2.10	1.61	10.97	5.5	1	2.5
FD016	F	33	2.04	1.75	8.87	6.9	1.3	2.5
FD020	M	31	2.04	1.71	7.87	4.7	1	2.4



# Table S3

ID	sex	age	A260/A280	A260/A230	Yield ( $\mu$ g/ml)	Preservation period (years)
Dep007	F	25	1.94	2.62	14.8	8.5
Dep008	F	28	1.93	2.40	18.7	8.5
Dep029	M	39	1.93	2.45	24.0	8.2
Dep053	F	36	1.92	2.37	15.9	7.9
Dep060	F	39	1.94	2.47	13.8	7.8
Dep064	M	51	1.91	2.45	15.3	7.7
Dep077	M	44	1.96	2.65	15.1	7.6
Dep085	F	42	1.91	2.41	21.6	7.5
Dep093	M	41	1.93	2.45	15.3	7.4
Dep103	F	28	1.94	2.54	18.5	7.3
Dep130	M	47	1.95	2.64	22.7	6.7
Dep135	F	46	1.91	2.38	16.6	6.6
Dep137	M	33	1.94	2.53	17.1	6.5
Dep140	F	33	1.91	2.36	25.6	6.5
Dep142	M	55	1.93	2.45	15.4	6.5
Dep147	F	27	1.93	2.43	16.6	6.4
Dep153	F	41	1.91	2.34	25.9	6.3
Dep166	F	41	1.91	2.45	18.9	6.0
Dep169	F	27	1.90	2.39	11.8	6.0
Dep172	M	29	1.93	2.49	13.8	5.9
Dep173	M	43	1.90	2.49	17.3	5.8
Dep176	M	40	1.90	2.41	25.1	5.8
Dep180	M	28	1.91	2.47	14.7	5.6
Dep183	F	43	1.91	2.38	22.0	5.5
Dep211	F	39	1.90	2.45	22.2	5.1
Dep215	M	34	1.92	2.54	12.2	5.0
FD010	F	39	1.94	2.49	13.8	2.6
FD014	M	25	1.93	2.54	20.7	2.5
FD016	F	33	1.96	2.51	12.6	2.5
FD020	M	31	1.94	2.43	26.8	2.4

# Table S4

ID	sex	age	Concentration ( $\mu\text{g/mL}$ )	RIN
t1	F	25	100.0	8.5
t2	M	28	79.8	8.7
t3	M	39	92.0	8.4
t4	M	36	70.4	8.7
t5	F	39	94.2	8.6
t6	M	51	108.6	8.6
t7	F	44	83.6	8.8
t8	F	42	50.2	8.3
t9	F	41	43.0	7.8
t10	M	28	80.4	7.2

Table S5

genes	Participants2			Participants3			corrected p-values of F-test
	Mean (TPM)	SD	CV	Mean (TPM)	SD	CV	
<i>ACTB</i>	2530.19	596.71	0.24	774.78	596.57	0.77	2.0.E-05
<i>ALAS1</i>	9.34	2.80	0.30	10.83	4.42	0.41	1.000
<i>ATP5F1</i>	30.77	9.44	0.31	32.98	12.41	0.38	1.000
<i>B2M</i>	4287.65	1188.12	0.28	4330.86	1372.86	0.32	1.000
<i>G6PD</i>	25.33	6.37	0.25	11.81	7.29	0.62	4.4.E-03
<i>GAPDH</i>	345.33	91.27	0.26	288.59	122.27	0.42	1.000
<i>GUSB</i>	11.15	2.96	0.27	6.62	2.85	0.43	1.000
<i>HMBS</i>	6.24	1.06	0.17	9.53	2.11	0.22	1.000
<i>HPRT1</i>	6.58	2.01	0.31	5.20	2.64	0.51	0.838
<i>IPO8</i>	11.89	3.60	0.30	10.00	4.53	0.45	1.000
<i>PGK1</i>	81.94	22.28	0.27	136.64	55.84	0.41	1.000
<i>POLR2A</i>	22.11	7.31	0.33	7.23	6.65	0.92	4.7.E-04
<i>PPIA</i>	68.97	21.35	0.31	58.57	21.25	0.36	1.000
<i>RPL13A</i>	898.94	240.66	0.27	535.97	184.30	0.34	1.000
<i>RPLP0</i>	370.98	103.78	0.28	319.62	99.42	0.31	1.000
<i>RPLP1</i>	487.64	157.09	0.32	101.21	106.98	1.06	1.8.E-05
<i>RPLP2</i>	1190.20	257.46	0.22	816.98	280.86	0.34	1.000
<i>RPS18</i>	1254.58	365.46	0.29	550.45	186.51	0.34	1.000
<i>SDHA</i>	17.93	4.69	0.26	14.82	8.96	0.60	0.012
<i>TBP</i>	5.66	1.85	0.33	3.72	1.86	0.50	1.000
<i>TFRC</i>	50.36	10.93	0.22	13.30	3.85	0.29	1.000
<i>UBC</i>	101.02	29.35	0.29	184.46	63.82	0.35	1.000
<i>YWHAZ</i>	249.88	76.32	0.31	139.48	65.28	0.47	1.000

## Table S6

	number	sex (Male/Female)	age
Participants1 for RNA (short-term storage)	n = 7	3/4	38.6 ± 8.3
Participants1 for DNA (short-term storage)	n = 6	2/4	37.5 ± 8.5
Participants2 (long-term storage)	n = 30	14/16	36.9 ± 7.9
Participants3 (Fresh blood)	n = 10	5/5	53.7 ± 8.9

## Table S7

<i>GAPDH</i>	Forward	CAGCCTCAAGATCATCAGCA
	Reverse	TGTGGTCATGAGTCCTTCCA
	amplicon lengths	106 base pairs
<i>RPS18</i>	Forward	CACTTCTGGGGCCTTCGTG
	Reverse	TTCTTGGACACACCCACGGT
	amplicon lengths	80 base pairs

## Table S8

<i>GAPDH</i>	TGTTTCGTCATGGGTGTGAACCATGAGAAGTATGACAACAGCCTCAAGATCATCAGCAATGC CTCCTGCACCACCAACTGCTTAGCACCCCTGGCCAAGGTCATCCATGACAACCTTTGGTATC GTGGAAGGACTCATGACCACAGTCCATGCCATCACTGCCACCCAGAAGACTGTGGATGGC CCCTCCGGGAAACTGTGGCGTGATGGCCGCGGGGCTCTCCAGAACATCATCCCTGCCTCT ACTGGCGC
<i>RPS18</i>	ACAGACAGAAGGATGTAAAGGATGGAAAATACAGCCAGGTCCTAGCCAATGGTCTGGACAA CAAGCTCCGTGAAGACCTGGAGCGACTGAAGAAGATTTCGGGCCCATAGAGGGCTGCGTCA CTTCTGGGGCCTTCGTGTCCGAGGCCAGCACACCAAGACCACTGGCCGCCGTGGCCGCAC CGTGGGTGTGTCCAAGAAGAAATAAGTCTGTAGGCCTTGTCTGTTAATAAATAGTTTATAT ACCTATGG

# Table S9

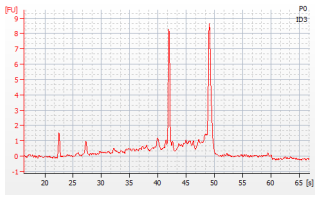
Measurement	Statistical test	Comparison	Statistics	P value	Figures and Tables
Yield of RNA	2-way ANOVA	Factor1: thawing	$F(1,24) = 1.11$	0.302	Fig. 2a
		Factor2: kit	$F(1,24) = 53.75$	1.40E-07	
		Interaction (F1 x F2)	$F(1,24) = 0.003$	0.957	
RIN	2-way ANOVA	Factor1: thawing	$F(1,24) = 4.53$	0.044	Fig. 2b
		Factor2: kit	$F(1,24) = 0.45$	0.511	
		Interaction (F1 x F2)	$F(1,24) = 1.46$	0.239	
Yield of RNA	Repeated measures ANOVA		$F(2,12) = 6.76$	0.011	Fig. 2c
	<i>post-hoc</i> test	N-37 vs N-AI		0.014	
		N-37 vs N-Ice		0.588	
		N-AI vs N-Ice		0.256	
RIN	Repeated measures ANOVA		$F(2,12) = 1.16$	0.107	Fig. 2d
	<i>post-hoc</i> test	N-37 vs N-AI		0.310	
		N-37 vs N-Ice		1	
		N-AI vs N-Ice		0.390	

<i>GAPDH</i>	Repeated measures ANOVA		$F(5,30) = 8.57$	3.90E-05	Fig. 3a
	<i>post-hoc</i> test	P-Con vs P-37		0.001	
		P-Con vs P-AI		1	
		P-Con vs N-37		0.171	
		P-Con vs N-AI		1	
		P-Con vs N-Ice		0.596	
		P-37 vs P-AI		0.036	
		P-37 vs N-37		0.495	
		P-37 vs N-AI		0.495	
		P-37 vs N-Ice		0.391	
		P-AI vs N-37		0.484	
		P-AI vs N-AI		1	
		P-AI vs N-Ice		1	
		N-37 vs N-AI		0.807	
		N-37 vs N-Ice		1	
N-AI vs N-Ice		1			
<i>RPS18</i>	Repeated measures ANOVA		$F(5,30) = 14.91$	2.30E-07	Fig. 3b
	<i>post-hoc</i> test	P-Con vs P-37		8.90E-04	
		P-Con vs P-AI		0.260	
		P-Con vs N-37		1	
		P-Con vs N-AI		0.448	
		P-Con vs N-Ice		0.495	
		P-37 vs P-AI		0.068	
		P-37 vs N-37		0.027	
		P-37 vs N-AI		0.033	
		P-37 vs N-Ice		0.052	
		P-AI vs N-37		0.156	
		P-AI vs N-AI		0.066	
		P-AI vs N-Ice		0.122	
		N-37 vs N-AI		1	
		N-37 vs N-Ice		0.540	
N-AI vs N-Ice		1			
Yield of DNA	Repeated measures ANOVA		$F(2,10) = 14.20$	0.001	Fig. 4
	<i>post-hoc</i> test	Q-37 vs Q-AI		0.023	
		Q-37 vs Q-Ice		0.353	
		Q-AI vs Q-Ice		0.021	

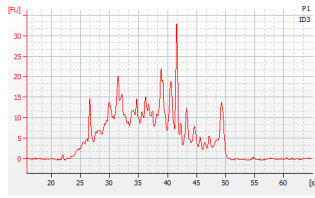
<i>ACTB</i>	F-test	Participants2 vs Participants3	F(9,29) = 10.66	2.04E-05	Table S4
<i>ALAS1</i>			F(9,29) = 1.85	1	
<i>ATP5F1</i>			F(9,29) = 1.50	1	
<i>B2M</i>			F(9,29) = 1.31	1	
<i>G6PD</i>			F(9,29) = 6.02	4.45.E-03	
<i>GAPDH</i>			F(9,29) = 2.57	1	
<i>GUSB</i>			F(9,29) = 2.62	1	
<i>HMBS</i>			F(9,29) = 1.70	1	
<i>HPRT1</i>			F(9,29) = 2.76	0.838	
<i>IPO8</i>			F(9,29) = 2.24	1	
<i>PGK1</i>			F(9,29) = 2.26	1	
<i>POLR2A</i>			F(9,29) = 7.75	4.73.E-04	
<i>PPIA</i>			F(9,29) = 1.37	1	
<i>RPL13A</i>			F(9,29) = 1.65	1	
<i>RPLP0</i>			F(9,29) = 1.24	1	
<i>RPLP1</i>			F(9,29) = 10.77	1.84E-05	
<i>RPLP2</i>			F(9,29) = 2.53	1	
<i>RPS18</i>			F(9,29) = 1.36	1	
<i>SDHA</i>			F(9,29) = 5.34	0.012	
<i>TBP</i>			F(9,29) = 2.32	1	
<i>TFRC</i>			F(9,29) = 1.78	1	
<i>UBC</i>	F(9,29) = 1.42	1			
<i>YWHAZ</i>	F(9,29) = 2.35	1			

Figure S1

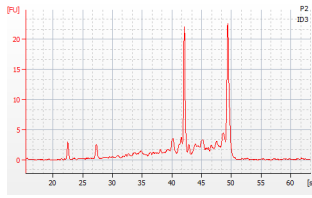
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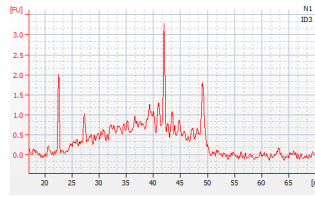
P-37 ID3



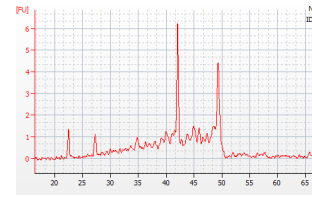
P-AI ID3



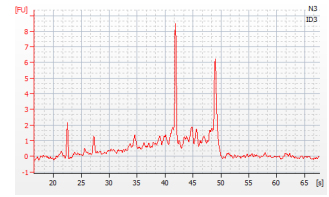
N-37 ID3



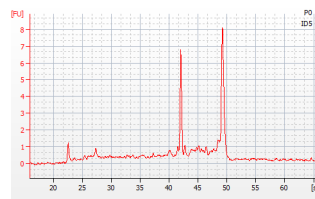
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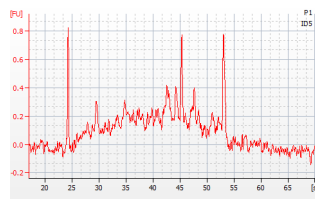
N-Ice ID3



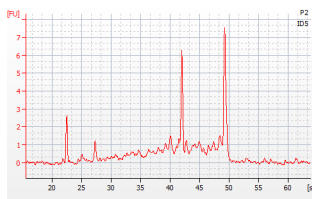
P-Con ID5



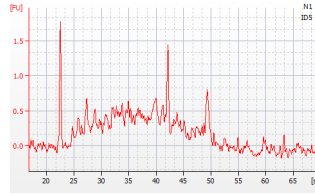
P-37 ID5



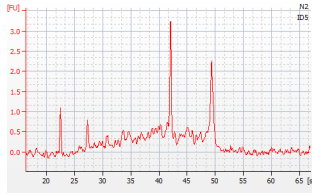
P-AI ID5



N-37 ID5



N-AI ID5



N-Ice ID5

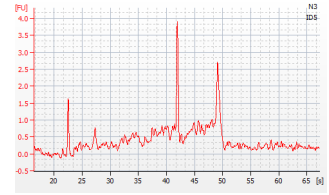




Figure S2

Gel images of DNA

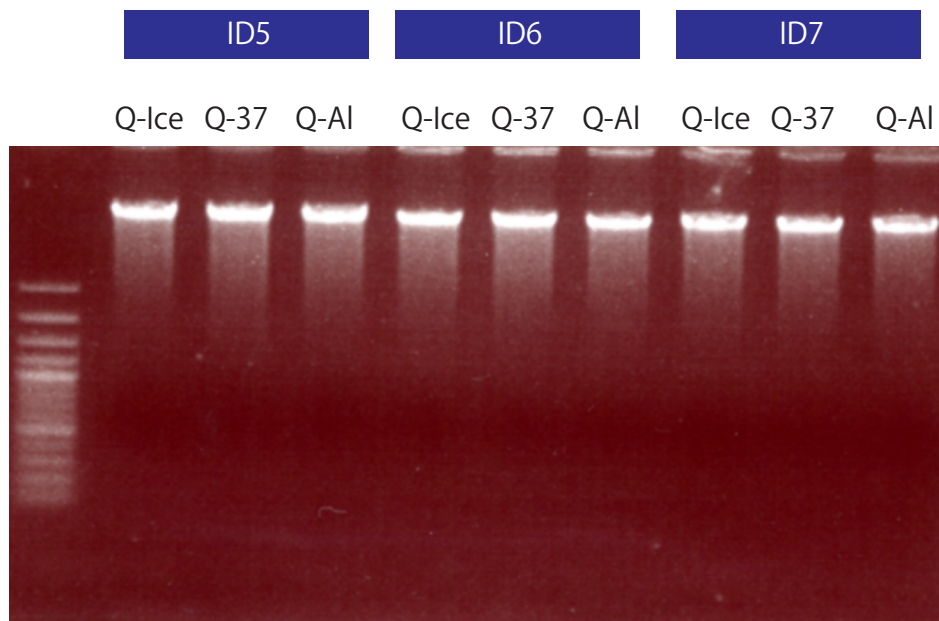
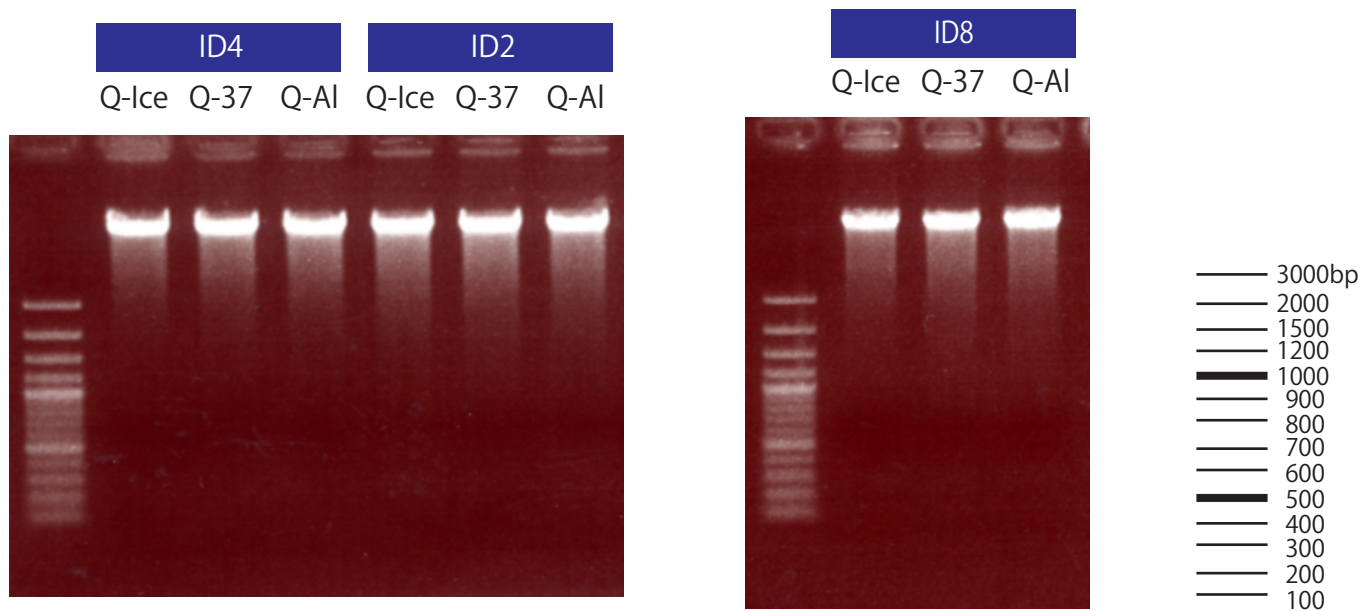


Figure S3 Gel images of PCR products of GAPDH

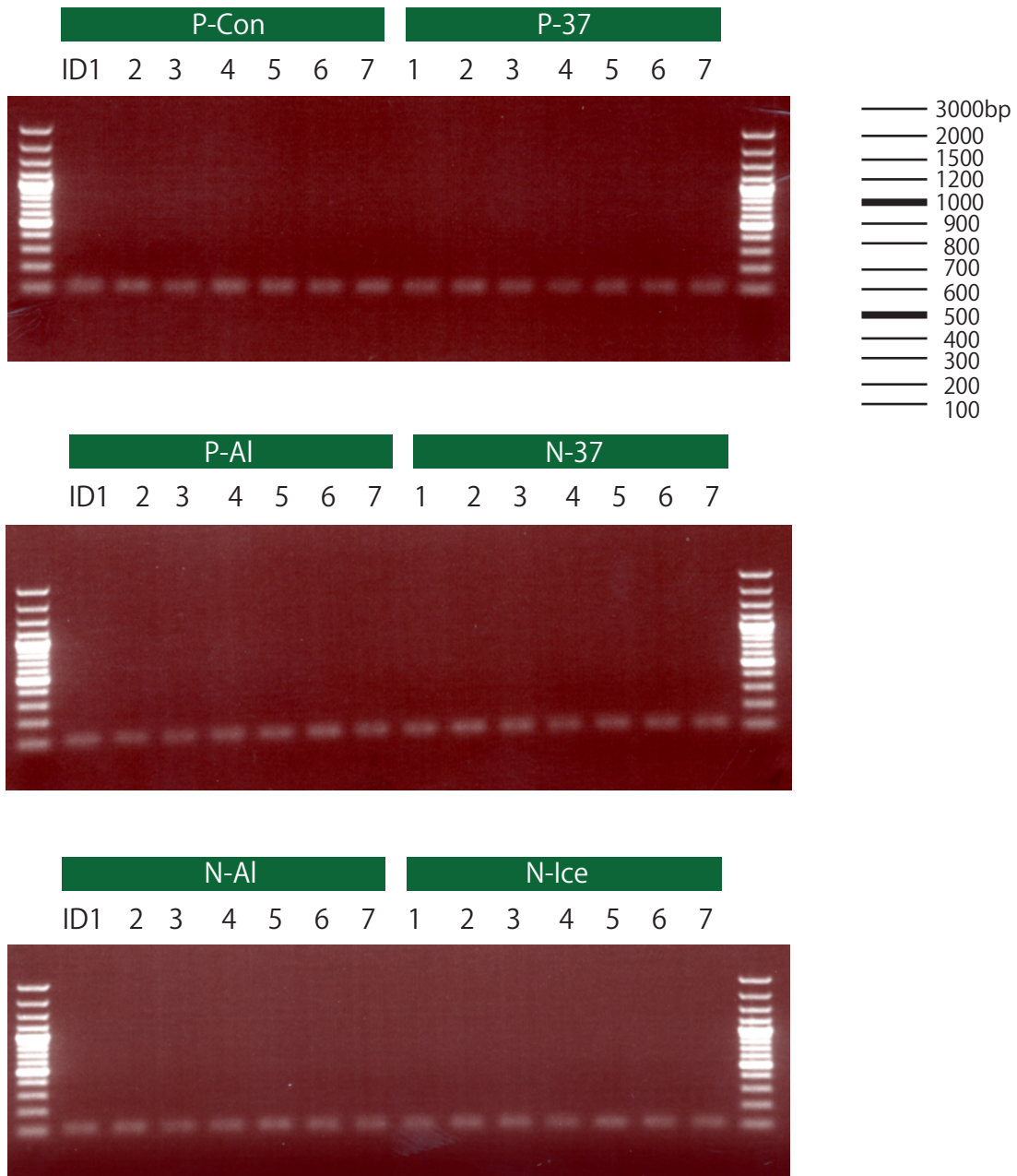


Figure S4 Gel images of PCR products of RPS18

