

SUPPLEMENTAL DATA

Pre-analytical storage effects on ALU- and LINE1-derived cell-free DNA biomarkers in whole blood and plasma

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Full article is available at the following link: [Pre-analytical storage effects on ALU- and LINE1-derived cell-free DNA biomarkers in whole blood and plasma](#)

Table S1. Primers utilized for the amplification of short and long ALU and LINE1 fragments.

Target	Forward Primer Sequence	Reverse Primer Sequence	Amplicon Size (bp)	Tm (°C)	PCR efficiency	Concentration calculation (ng/mL plasma)
ALU-115	CCTGAGGTC AGGAGTTC GAG	CCCGAGTAG CTGGGATTA CA	115	65	94.8%	$C=130 \times 10^{(11.97-Y)/3.453}$
ALU-247	GTGGCTCAC GCCTGTAAT C	CAGGCTGG AGTGCAGT GG	247	65	91.7%	$C=130 \times 10^{(10.58-Y)/3.538}$
LINE1-97	TGGCACATA TACACCATG GAA	TGAGAATGA TGGTTTCCA ATTTC	97	60	100.8%	$C=130 \times 10^{(15.229-Y)/3.302}$
LINE1-266	ACTTGGAAC CAACCCAAA TG	CACCACAGT CCCCAGAGT G	266	60	95.3%	$C=130 \times 10^{(15.635-Y)/3.356}$

The plasma input volume is 500 µL, while the elution volume of cfDNA is 65 µL. In this context, Y represents the cycle threshold (Ct) value. Abbreviations: ALU: *Alu*; LINE1: Long interspersed nuclear element 1; bp: Base pairs; Tm: Melting temperature; PCR: Polymerase chain reaction; cfDNA: Circulating cell-free DNA; Ct: Cycle threshold.

Table S2. Concentrations of cfDNA biomarkers in plasma derived from whole blood samples stored at room temperature for varying durations.

Biomarker	0 h	3 h	6 h	<i>P</i>	Effect size (η^2_p) [95%CI]
ALU-115 (ng/mL)	13.14 [5.85, 14.48]	13.13[5.94, 15.92]*	13.28 [6.85, 16.02]*	0.035	0.32 [0.04, 1.00]
ALU-247 (ng/mL)	4.12±2.00	4.58±2.16	5.05±2.03**	0.002	0.48 [0.17, 1.00]
ALU-247/115	0.40±0.074	0.41±0.073	0.45±0.10*	0.001	0.49 [0.19, 1.00]
LINE1-97 (ng/mL)	2.79±1.55	2.71±1.60	2.67±1.43	0.220	0.14 [0.00, 1.00]
LINE1-266 (ng/mL)	0.37±0.19	0.48±0.22***	0.54±0.25***	<0.00 1	0.75 [0.56, 1.00]
LINE1-266/97	0.14±0.032	0.19±0.042***	0.22±0.06***##	<0.00 1	0.84 [0.70, 1.00]

Normally distributed values are expressed as mean ± SD, while non-normally distributed values are reported as median with IQR ($n = 11$). Statistical significance is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to 0 hours; ## $P < 0.01$, compared to 3 hours. Abbreviations: cfDNA: Circulating cell-free DNA; ALU: *Arthrobacter luteus*; LINE1: Long interspersed nuclear element 1; CI: Confidence interval; SD: Standard deviation; IQR: Interquartile range.

Table S3. Concentrations of cfDNA biomarkers in plasma derived from whole blood samples stored at 4°C for varying durations.

Biomarker	0 h	3 h	6 h	<i>P</i>	Effect size (η^2_p) [95%CI]
ALU-115 (ng/mL)	6.79±3.46	6.83±3.55	8.03±4.13 ^{***###}	<0.001	0.75 [0.58, 1.00]
ALU-247 (ng/mL)	2.57±0.83	2.63±0.81	2.72±0.85	0.018	0.31 [0.04, 1.00]
ALU-247/115	0.43±0.12	0.44±0.12	0.38±0.11 ^{***###}	<0.001	0.87 [0.76, 1.00]
LINE1-97 (ng/mL)	2.80±1.06	2.81±1.08	2.82±1.08	0.888	0.01 [0.00, 1.00]
LINE1-266 (ng/mL)	0.25±0.09	0.27±0.09	0.27±0.10	0.024	0.29 [0.03, 1.00]
LINE1-266/97	0.09±0.02	0.10±0.024 [*]	0.10±0.03 [*]	0.005	0.38 [0.10, 1.00]

All values are normally distributed and are presented as mean ± standard deviation (SD) ($n = 12$). Statistical significance is indicated as follows: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (vs. 0 h); $###P < 0.001$ (vs. 3 h). Abbreviations: cfDNA: Circulating cell-free DNA; ALU: *Arthrobacter luteus*; LINE1: Long interspersed nuclear element 1; CI: Confidence interval; SD: Standard deviation.

Table S4. Levels of cfDNA biomarkers measured in plasma stored at -80°C for up to 12 months.

Biomarker	0 m	1 m	3 m	6 m	9 m	12 m	P	Effect size (η^2_p) [95%CI]
ALU-115 (ng/mL)	13.14 [5.85, 14.48]	13.18 [5.48, 14.76]	12.71 [5.61, 14.79]	12.61 [5.56, 14.70]	12.16 [5.65, 15.30]	11.61 [5.70, 14.43]	0.787	0.07 [0.00, 1.00]
ALU-247 (ng/mL)	3.99[2.2 7,6.08]	4.10[2.1 0,6.24]	4.75[2.1 0,5.82]	4.67 [2.03, 5.97]	4.91 [2.09, 6.03]	4.93[2.0 8, 5.74]	0.188	0.15 [0.00, 1.00]
ALU-247/115	0.40±0.0 7	0.39±0.0 8	0.40±0.0 8	0.40±0.0 8	0.41±0.0 8	0.42±0.0 9	0.001	0.32 [0.10, 1.00]
LINE1-97 (ng/mL)	2.79±1.5 5	2.46±1.4 1*	2.44±1.3 6*	2.55±1.4 1*	2.54±1.4 6*	2.55±1.4 0*	<0.00 1	0.47 [0.26, 1.00]
LINE1-266 (ng/mL)	0.37±0.1 9	0.39±0.2 3	0.39±0.2 2	0.38±0.2 1	0.39±0.2 3	0.39±0.2 2	0.679	0.06 [0.00, 1.00]
LINE1-266/97	0.12±0.0 3	0.14±0.0 3*	0.14±0.0 3*	0.14±0.0 3*	0.14±0.0 3*	0.14±0.0 2*	<0.00 1	0.44 [0.23, 1.00]

Values that are normally distributed are presented as mean ± SD, while non-normally distributed values are reported as median with IQR ($n = 11$). * $P < 0.05$ vs. 0 months.

Abbreviations: cfDNA: Circulating cell-free DNA; ALU: Arthrobacter luteus; LINE1: Long interspersed nuclear element 1; CI: Confidence interval; SD: Standard deviation; IQR: Interquartile range.

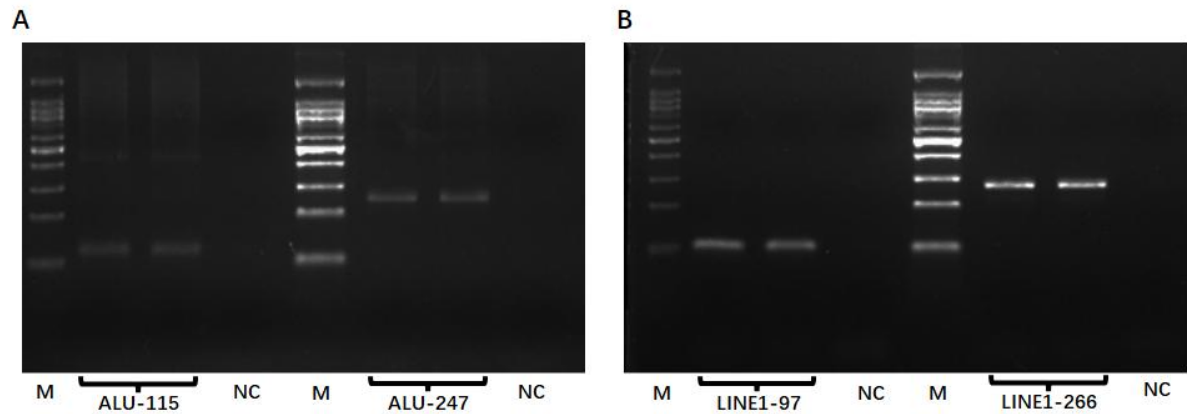


Figure S1. Agarose gel validation of ALU and LINE1 qPCR amplicons.

Representative 2% agarose gels showing amplification products of **(A)** ALU-115 (115 bp) and ALU-247 (247 bp) and **(B)** LINE1-97 (97 bp) and LINE1-266 (266 bp).

For each target, the short amplicon is nested within the corresponding long amplicon at the same locus. Abbreviations: ALU: *Arthrobacter luteus*; LINE1: Long interspersed nuclear element 1; qPCR: Quantitative polymerase chain reaction; bp: Base pairs; M: 100-bp DNA ladder; NC: No-template negative control (H₂O).