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SHORT COMMUNICATION

Development of an Effective Method to Enrich Cell-Free Nucleic Acids in Liquid Samples

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SUMMARY

Background: **We aimed to establish a method to extract cell-free DNA (cfDNA) from liquid samples by combining protamine solution with nucleic acid extraction reagents.**
Methods: **Samples comprised 1 mL EDTA/2K plasma from healthy individuals (n = 22) and 5, 10, 20, and 40 mL K562 cell culture supernatants. After adding protamine solution and NaCl, the samples were incubated and subsequently centrifuged. Genomic DNA (gDNA) was extracted using a High Pure PCR Template Preparation Kit. For comparison, plasma was extracted using a cobas® cfDNA Sample Preparation Kit. The gDNA was subjected to real-time quantitative PCR.**
Results: **Our method yielded results comparable to those obtained using a cfDNA-specific kit. Culture supernatants showed volume-dependent yield variations, and gDNA was successfully extracted from samples of all volumes following enrichment.**
Conclusions: **Our method enhanced cfDNA enrichment, highlighting its potential for seamless integration with commonly available nucleic acid extraction reagents to process large sample volumes.**
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Supplementary Data

Table S1.

No.	71 bp, pg/μL		150 bp, pg/μL		255 bp, pg/μL	
	a	b	a	b	a	b
1	42.8	26.6	11.2	7.1	5.2	0.9
2	34.1	33.5	2.7	1.0	2.0	4.5
3	24.5	41.8	4.1	9.1	2.9	2.0
4	40.9	43.9	11.8	5.9	3.8	10.0
5	35.1	43.2	8.8	2.3	3.1	4.0
6	53.7	53.4	6.4	11.9	2.7	2.5
7	56.4	37.8	10.9	9.5	4.4	3.6
8	36.1	45.8	10.4	16.0	2.3	2.2
9	47.4	58.1	13.7	9.5	2.1	4.3
10	40.5	53.2	6.0	14.1	7.2	1.5
11	49.8	60.4	16.0	15.5	7.5	6.8
12	66.4	59.0	10.1	12.9	6.8	3.7
13	57.4	56.0	28.4	12.7	11.0	10.2
14	25.6	43.8	10.0	11.3	4.5	3.9
15	43.9	44.4	16.2	8.4	6.6	3.4
16	25.2	39.5	15.0	9.7	10.2	2.8
17	66.9	46.6	14.9	13.3	12.2	7.8
18	46.9	45.9	13.2	9.4	10.7	0.7
19	42.2	50.7	20.9	16.1	6.7	3.2
20	57.0	40.2	16.4	11.4	5.6	8.1
21	42.7	43.1	13.7	8.6	8.2	6.9
22	35.1	44.7	16.1	7.2	9.4	5.9
Mean	44.1	46.0	12.6	10.1	6.1	4.5
SD	12.1	8.4	5.7	4.0	3.2	2.8

Table S2.

Sample volume	No.	71 bp, pg/μL	150 bp, pg/μL	255 bp, pg/μL
5 mL	1	659	132	92
	2	618	204	89
	3	672	152	88
	Mean	650	162	90
	SD	28	37	2
10 mL	1	1,022	281	128
	2	1,210	260	195
	3	1,155	305	135
	Mean	1,129	282	153
	SD	97	23	37
20 mL	1	1,848	649	241
	2	1,914	583	218

Table S2 (continued).

Sample volume	No.	71 bp, pg/µL	150 bp, pg/µL	255 bp, pg/µL
20 mL	3	2,167	497	227
	Mean	1,976	576	228
	SD	168	76	12
40 mL	1	3,443	1,166	438
	2	3,641	1,010	343
	3	3,839	985	424
	Mean	3,641	1,053	402
	SD	198	98	51