Concentrating cell free DNA for donor derived cell free DNA analysis

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Introduction

Cell free DNA (cfDNA) is released into the bloodstream in large quantities at times of cell injury caused by transplant damage, rejection, malignancy, and inflammation. CfDNA can be analysed to identify donor derived cell free DNA (dd-cfDNA), which is a promising non-invasive and cost-effective marker of transplant injury.

10ng of cfDNA at a concentration of 0.6ng/µl is required for dd-cfDNA analysis with the CareDX® AlloSeq cfDNA kit. Isolating 10ng of cfDNA is challenging because cfDNA has a short half-life and a low concentration in peripheral blood.

The aim of this study was to investigate methods of concentrating cfDNA for dd-cfDNA analysis. Two approaches were investigated, evaporation and centrifugal filters.

Methods

16ml of peripheral blood was collected into 2 STRECK Cell-Free DNA BCT® tubes from patients who had consented to the KORAD study (IRAS 255433). Plasma was isolated in 2x 10-minute centrifugation steps at 1600g. Plasma was stored at -20^{0}C before cfDNA was isolated from 8ml of plasma using the Promega Maxwell® RSC ccfDNA LV Plasma Kit. 3 highly concentrated cfDNA samples were identified. These cfDNA samples were diluted to 0.1, 0.2, 0.3 and 0.4 ng/µl in 120µl of Promega NGS elution buffer. For the evaporation method, 60µl of each dilution was incubated at 56^{0}C for 24 hours. For the centrifugal filter method, the remaining 60µl was centrifuged in Amicon® Ultra-0.5 centrifugal filter devices following the manufacturer's instructions. The samples were resuspended in 20µl of NGS elution buffer. CfDNA concentrations were measured on a Qubit® fluorometer using a high sensitivity kit. The percentage of low molecular weight cfDNA in each sample was analysed on an Agilent TapeStation using Cell-free DNA Screening Tape (figure 1).

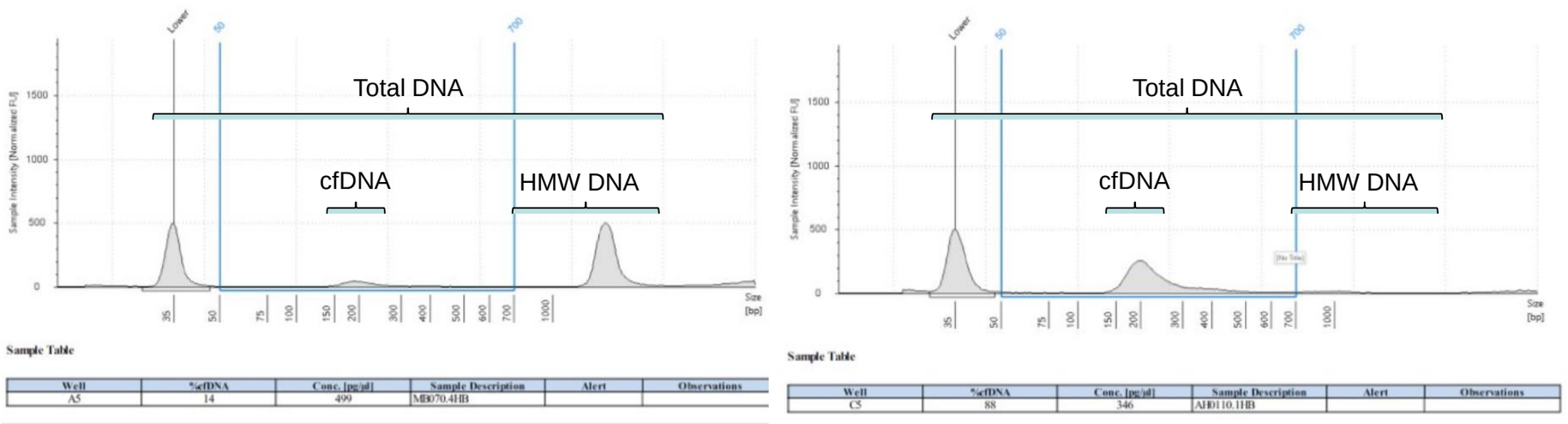

Figure 1. The Agilent TapeStation was used to assess the percentage of cfDNA in each sample. High Molecular Weight (HMW) DNA contamination can adversely affect dd-cfDNA analysis. CfDNA is 150-180 base pairs (BP). HMW DNA is >700BP. The sample on the right has a greater percentage of cfDNA

Results

Table 1. CfDNA values before and after concentration

Sample	Initial [cfDNA] ng/µl	CfDNA percentage	Evaporation method		Centrifugal filter method	
			[cf DNA] ng/µl	CfDNA percentage	[cf DNA] ng/µl	CfDNA percentage
1	0.1	90	0.355	88	0.342	93
	0.2	90	0.623	91	0.702	93
	0.3	90	1.180	93	1.070	94
2	0.1	29	0.247	15	0.208	45
	0.2	29	0.418	13	0.380	44
	0.3	29	0.607	15	0.493	36
	0.4	29	0.702	14	0.699	40
3	0.1	92	0.373	88	0.439	90
	0.2	92	0.750	89	0.489	92
	0.3	92	1.400	89	1.340	92
	0.4	92	1.200	89	1.470	91

- **None of the 0.1ng/µl dilutions were concentrated to ≥0.6ng/µl**
- **A two tailed t-test did not show a significant difference in concentrations of samples concentrated using the 2 different methods (n=18, standard deviation=0.37 and 0.35, P=0.15)**
- **The evaporation method concentrated 2/3 of the 0.2ng/µl dilutions, 3/3 0.3ng/µl dilutions and 2/2 of the 0.4ng/µl dilutions to ≥0.6ng/µl**
- **The centrifuge filter method concentrated 1/3 of the 0.2ng/µl dilutions, 2/3 0.3ng/µl dilutions and 2/2 of the 0.4ng/µl dilutions to ≥0.6ng/µl**
- **TapeStation analysis confirmed that the cfDNA percentage was not affected by the evaporation or centrifuge filtration methods**

Summary

Isolating 10ng of cfDNA from peripheral blood for dd-cfDNA analysis can be challenging because cfDNA has a short half life and a low concentration in peripheral blood.

This study shows that cfDNA can be concentrated with evaporation or centrifuge filtration methods to aid dd-cfDNA analysis.