Extraction & Quality Analysis of Circulating, Cell-Free DNA from Serum Samples Using Omega Bio-tek Kits

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Introduction

Circulating, cell-free DNA (cfDNA) holds great clinical significance for non-invasive disease detection, diagnosis, and monitoring. cfDNA are usually small fragments of DNA (size distribution peak at ~170 bp) found circulating in plasma, serum, or other bodily fluids and offers a tremendous potential as a screening method for tumor, cancer, as well as in fetal DNA studies. Circulating DNA extraction is often challenging as they are found in low quantities and accurate, consistent methods are needed to isolate the less abundant cfDNA with higher sensitivity. In this context, Omega Bio-tek has developed cfDNA extraction kits for both manual processing using silica spin columns (E.Z.N.A.® Circulating DNA Kit, D3091) and for automated, high throughput workflows using magnetic beads (Mag-BIND® cfDNA Kit, M3298). Omega Bio-tek's kits allow flexible sample inputs (1-4 mL for column-based; 0.5-4 mL for magnetic bead-based) and low elution volumes for isolating concentrated cfDNA suitable for a variety of downstream applications such as qPCR, next-generation sequencing etc. In this study, we elucidate the key features of the Omega Bio-tek circulating DNA kits and compare their performance to that of Company Q's column-based cfDNA kit in terms of DNA quality.

Materials & Methods

Circulating, cell-free DNA was extracted from 1 mL of unspiked serum using Omega Bio-tek's Mag-BIND® cfDNA Kit and a comparable column-based kit from Company Q following manufacturer's recommended protocols. The extraction workflow using the Mag-BIND® cfDNA Kit is outlined in Figure 1. The Omega protocol features reduced lysis and binding buffer volumes compared to Company Q (Table 1), making it automation-friendly not only for larger input sample volumes, but also for rapid processing of 96 1 mL samples in a 96-well deep well plate. The DNA isolations were performed in triplicate and eluted in 60 µL volume. cfDNA purified from all the experiments was analyzed on Agilent's TapeStation® 2200 to assess its fraction relative to the total DNA extracted.

Table 1. Protocol comparison for a 1 mL and 4 mL sample.

	Omega Bio-tek		Company Q	
Sample volume	1 mL	4 mL	1 mL	4 mL
Volume of lysis buffer required	67 µL	270 µL	0.8 mL	3.2 mL
Volume of binding buffer required	1 mL	4 mL	1.8 mL	7.2 mL
Total volume	2.067 mL	8.27 mL	3.6 mL	14.4 mL

Preparing high quality libraries is a critical component for the success of any next-generation sequencing application. In order to ensure the integrity of the purified cfDNA and to test its downstream application suitability, we prepared next-generation sequencing libraries using KAPA Biosystems' HyperPrep Kit following the recommended protocol. The same number of PCR cycles was used for cfDNA extracted from both the Omega Bio-tek kit and the Company Q kit. Amplified DNA was analyzed on Agilent's TapeStation® 2200 following library construction.

Results & Discussion

Figure 2 shows the electropherogram overlay of 1 mL unspiked serum sample purified using the Mag-BIND® cfDNA Kit and a comparable kit from Company Q. The TapeStation® results indicate that the Mag-BIND® cfDNA Kit could capture the circulating, cell-free DNA with little to no genomic DNA contamination. In contrast, Company Q's eluate contained high molecular weight fragments indicating the presence of genomic DNA in the circulating DNA isolation.

To arrive at the cfDNA concentration without any interference from the genomic DNA, we utilized the regional analysis functionality of the TapeStation® 2200 analysis software. The DNA concentration within the 100-300 bp region where cfDNA is most likely to be present was quantified using the software

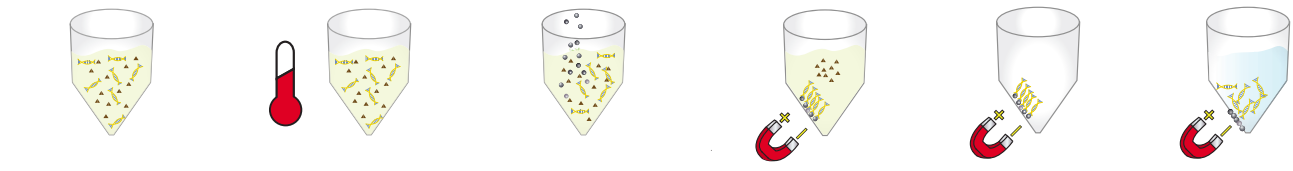

Figure 1. Magnetic bead-based extraction workflow using Omega Bio-tek's Mag-BIND® cfDNA Kit (M3298).

and shown in Table 2. The cfDNA extracted from the Mag-BIND® cfDNA Kit within the 100-300 bp region was determined to be 45.2 pg/µL and was significantly higher than that of Company Q's as 25.1 ph/µL (Table 2).

Electropherogram Overlay of Purified DNA from 1 mL Serum

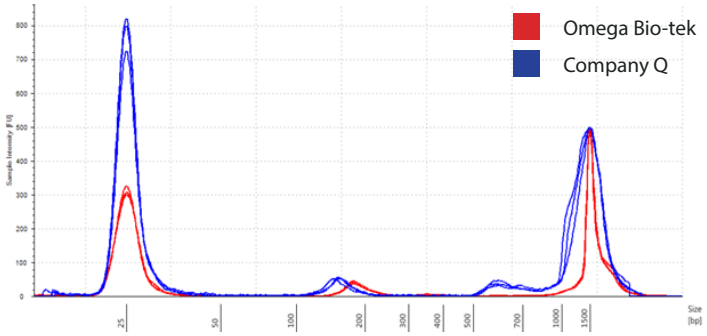

Figure 2. 1 mL of unspiked serum was purified using kits from Omega Bio-tek and Company Q following manufacturer's recommended protocols. Purified DNA was analyzed on Agilent's TapeStation® 2200.

Table 2. The cfDNA region (100-300 bp) was examined using the regional analysis functionality of the TapeStation® 2200 analysis software to arrive at the cfDNA concentration excluding any genomic DNA that might be present.

Extraction Method	100-300 bp region concentration (pg/µL)
Omega Bio-tek	45.2 ± 4.4
Company Q	25.1 ± 2.2

Using the cfDNA extracted from the Mag-BIND® cfDNA Kit and Company Q kits as the input, we could successfully generate libraries using KAPA's HyperPrep kit and from the electropherogram overlay amplified libraries seem comparable (Figure 3). This data indicates that the purified DNA was of sufficiently high quality fortifying its suitability for various downstream applications.

Electropherogram Overlay of Amplified Libraries Prepared from cfDNA Purified from 1 mL Serum

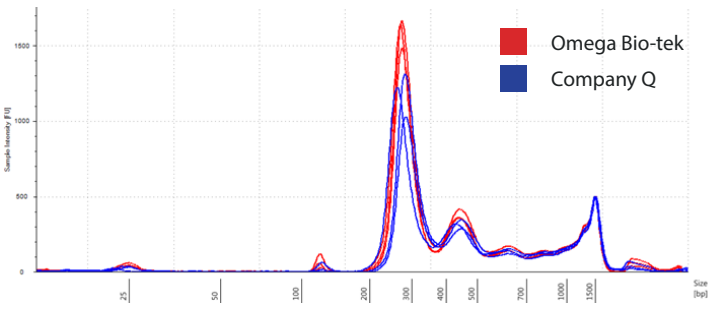

Figure 3. TapeStation® analysis of amplified libraries from cfDNA purified from 1 mL of serum using kits from Omega Bio-tek and Company Q. KAPA's HyperPrep kit was used for library generation.

Conclusions

Omega Bio-tek's circulating DNA kits offer a better solution to isolate the cfDNA with little to no genomic DNA contamination compared to the kit from Company Q. Results from the TapeStation® showed that only DNA around 170 bp was found, and high molecular weight DNA was not detected. We could successfully prepare libraries from low amounts of cfDNA as the input and demonstrate its suitability for NGS and other downstream applications.

For high throughput applications, Omega Bio-tek's Mag-BIND® cfDNA Kit using magnetic bead-based purification technology can be automated on most open-ended liquid handling platforms and provides a solution for processing 192 (2 mL) in 2 hours from sample to tube to extracted DNA.

Product Information

Description	Product No.	Preps
Mag-BIND® cfDNA Kit	M3298-00	5
	M3298-01	50
	M3298-02	200
E.Z.N.A.® Circulating DNA Kit	D3091-00	5
	D3091-01	20

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