Strategies for retrieving cfDNA of short fragment lengths using Mag-Bind® cfDNA Kit from Omega Bio-tek

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Introduction

The landscape of cfDNA research is constantly evolving with new evidence pointing to the existence of shorter fragment sizes and their importance in terms of tumor detection, early disease diagnosis, and genomic profiling. Circulating tumor DNA is often fragmented and is of shorter length than the nucleosome bound cfDNA from normal healthy cells[1, 2]. The difference in size can be exploited to enrich shorter DNA fragments to enhance the sensitivity of detecting genomic level alterations, point mutations, and copy number alterations, specifically in tumor and cancer diagnostics. Circulating tumor DNA is a subset of cfDNA and its isolation is even more technically challenging since it is present in such low quantities in an already less abundant sample type. To answer this unmet need, Omega Bio-tek has expanded the capability of Mag-Bind® cfDNA kit (M3298) to be able to recover fragment sizes as short as 50bp through certain protocol modifications. This kit provides the customers different protocols to fit their different application needs. Thus, this kit offers researchers and scientists the flexibility to use the same kit for not only large volume sample processing but also for recovering short cfDNA fragments.

Materials and Methods

The standard M3298 protocol can be modified by either addition of supplemental binding buffer (JSB Buffer) or by addition of isopropanol depending on the fragment size of interest. Table 1 elucidates the protocol modifications needed to extract fragments >50bp or >75bp and compared to the standard protocol.

Protocol	Recovers Fragment Size (bp)	Reagent Volume Used	
		JSB Buffer	Isopropanol
Standard	> 150	1 volume	-
Modified # 1	> 50	1 volume	1 volume
Modified # 2	> 75	2 volumes	-

Table 1. Protocol modifications for recovery of shorter cfDNA fragment lengths with respect to 1 volume of sample used.

An equal mix of ultra-low range ladder (10 – 300 bp) and 50bp ladder (50 – 500 bp) were spiked into 1mL of pooled normal human plasma and purified using the standard protocol and the two modifications from Table 1. The DNA was eluted in 50 µL volume and 10 µL of that was run on a 3% agarose gel for 1 hour at 100V to analyze the size fragments recovered with each of those protocols.

Results and Discussion

Figure 1 shows all the DNA fragments recovered using the different protocol modifications. The results indicate that with the modified protocol # 1, there is purification of fragments as short as 50bp but with reduced recovery of fragments >150bp compared to the standard protocol. With modified protocol # 2 by doubling the JSB buffer volume, fragments as short as 75bp were isolated. The recovery of fragments >150 bp seems to be comparable to that of the standard protocol. The choice of the modified protocol depends on the application need. For example, if enrichment of shorter fragment size is of more interest, modified protocol # 1 would be a better choice.

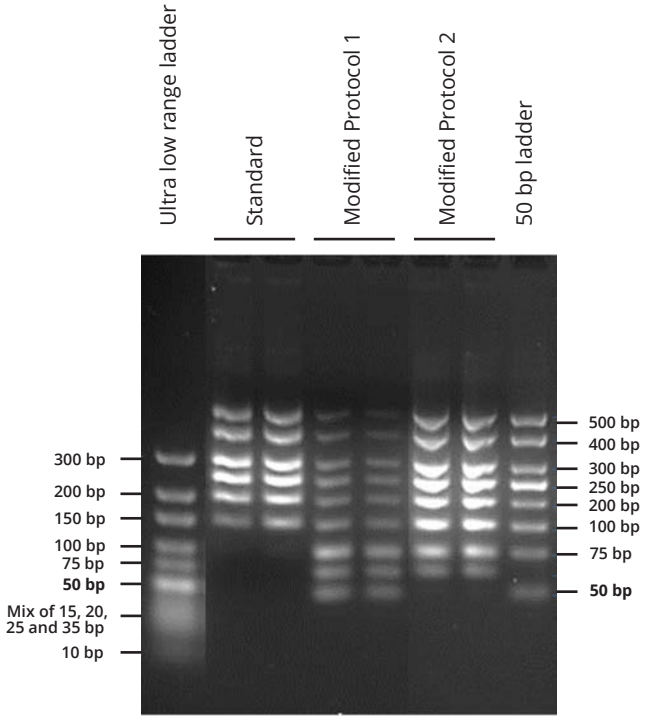

Figure 1. Gel image depicting the recovery of short DNA fragments using different protocol modifications.

Conclusions

Omega Bio-tek's Mag-Bind® cfDNA kit offers a flexible solution for extracting short circulating DNA fragments from input sample volumes ranging from 1 – 8 mL. While the protocol modifications may require extra buffers that are not provided with the standard product, Omega Bio-tek offers the flexibility to purchase the individual components separately. The magnetic bead-based chemistry can be automated on most open-ended

liquid handling platforms to provide customers with a high through-put solution.

References

1. Zhang, R., Nakahira, K., Guo, X., Choi, A. M. K., & Gu, Z. (2016). Very Short Mitochondrial DNA Fragments and Heteroplasmy in Human Plasma. Scientific Reports, 6, 36097. http://doi.org/10.1038/srep36097
2. Underhill HR, Kitzman JO, Hellwig S, Welker NC, Daza R, Baker DN, et al. (2016) Fragment Length of Circulating Tumor DNA. PLoS Genet 12(7): e1006162. https://doi.org/10.1371/journal.pgen.1006162.

Product Information

Company	Product No.	Description
Omega Bio-tek	M3298-00	Mag-bind® cfDNA Kit (5 Preps)
	M3298-01	Mag-bind® cfDNA Kit (50 Preps)
	M3298-02	Mag-bind® cfDNA Kit (500 Preps)

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