Application Note

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Separation of cfDNA with the Agilent HS NGS Kit on the Agilent Fragment Analyzer Systems

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Abstract

Quality analysis of extracted circulating cell-free DNA (cfDNA) plays an important role in determining sizing and purity. The Agilent 5200 Fragment Analyzer system achieved excellent resolution and reliable sizing of cfDNA. Both the Agilent FA 12-Capillary Array Short, 33 cm, and the Agilent FA 12-Capillary Array Ultrashort, 22 cm, achieved reliable sizing with the Agilent HS NGS Fragment kit (1-6000 bp) on the 5200 Fragment Analyzer system, with the ability to visualize the presence of any genomic DNA.

Introduction

Circulating cell-free DNA (cfDNA) is gaining prevalence as a noninvasive, alternative approach for the detection of tumor mutations and screening for fetal abnormalities. Quantification and assessment of cfDNA and contaminating DNA is necessary for both preparation and quality control of cfDNA generated NGS libraries. The capability of the Agilent HS NGS Fragment kit (1-6000 bp) to separate cfDNA on the Agilent 5200 Fragment Analyzer system was investigated.

Experimental

The experiments in this study were done using an Agilent 5200 Fragment Analyzer system and can be replicated with comparable results on Agilent 5300 and 5400 Fragment Analyzer systems.

An Agilent 5200 Fragment Analyzer system equipped with an Agilent FA 12-Capillary Array Short, 33 cm (short array) (p/n A2300-1250-3355) or an Agilent FA 12-Capillary Array Ultrashort, 22 cm (ultrashort array) (p/n A2300-1250-2247) was used to analyze a 1.25 or 2.5 ng/µL cfDNA sample with an HS NGS Fragment kit (1-6000 bp) (p/n DNF-474).

cfDNA was extracted from four healthy human serum samples (Bioreclamation IVT, #HMSRM) using the QIAmp circulating nucleic acid kit (Qiagen, #55114) or the Quick-cfDNA serum and plasma kit (Zymo, #D4076). While carrier RNA was not used in the Quick-cfDNA kit, the QIAmp kit used carrier RNA for the extraction of cfDNA. All serum samples extracted with the QIAmp kit were split into two 5 mL samples and extracted with and without carrier RNA to address possible carrier RNA interference with cfDNA peak separation. Some cfDNA samples were also treated with DNase to further investigate the possible peak interference. A Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Inc.) was used for quantification of cfDNA.

Results and discussion

cfDNA separation profiles from healthy individuals display two or three distinct peaks. These peaks were often referred to as mononucleosome, dinucleosome and trinucleosome cfDNA[1]. The results in Figure 1 displays a typical separation profile from cfDNA extracted with the QIAmp kit with carrier RNA and separated using the HS NGS Fragment kit (1-6000 bp). Samples #1, #2 and #3 all showed similar separation profiles. cfDNA extracted without carrier RNA demonstrated a peak at 154 bp corresponding to

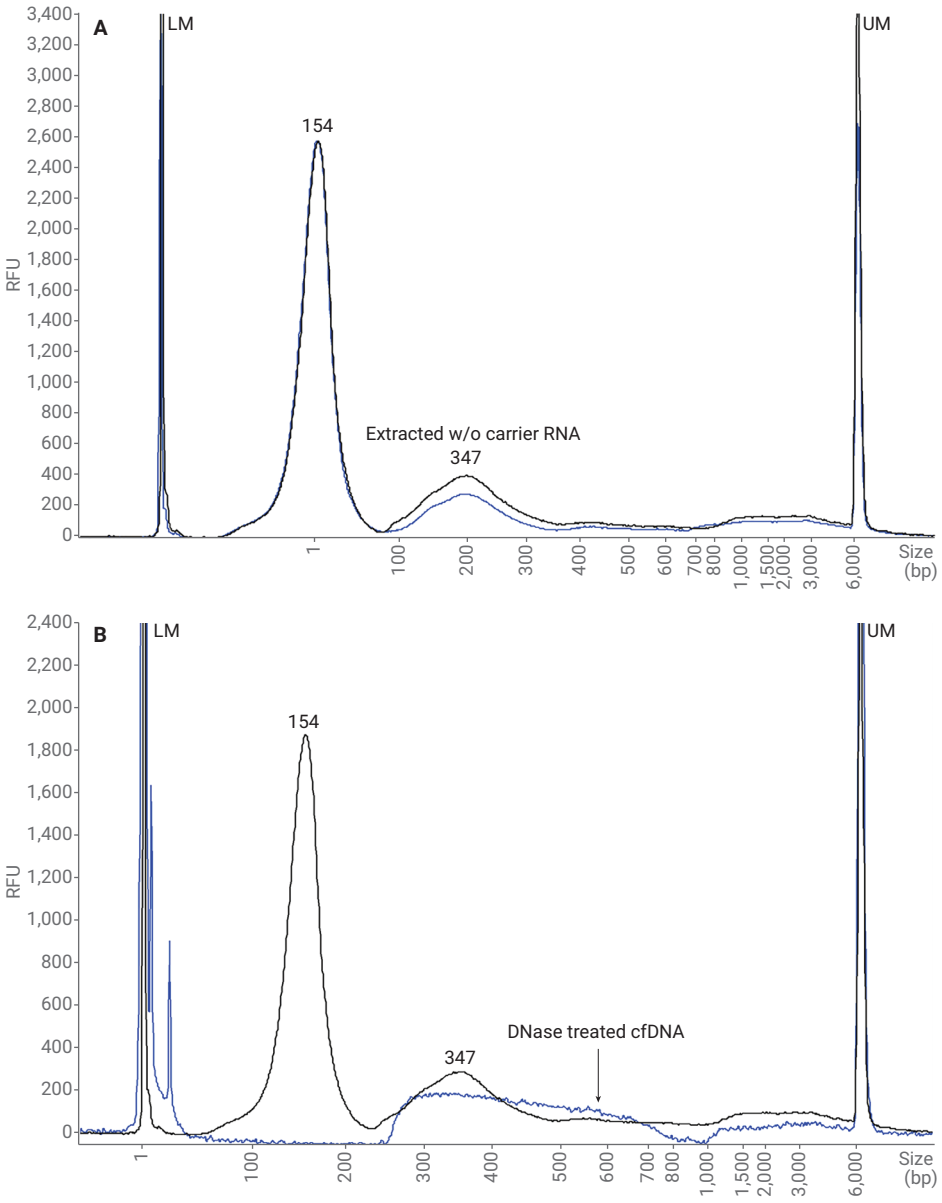

Figure 1. cfDNA extracted by the QIAmp kit and separated on the Agilent 5200 Fragment Analyzer system short array using the Agilent HS NGS Fragment kit (1-6000 bp). (A) cfDNA extracted with carrier RNA (black trace) and without carrier RNA (blue trace). (B) cfDNA extracted with carrier RNA (black trace) and treated with DNase (blue trace). LM = lower marker; UM = upper marker.

mononucleosome cfDNA, followed by a broad peak at 347 bp corresponding to dinucleosome cfDNA (Figure 1A, blue trace). Comparison of cfDNA extraction with carrier RNA (black trace) showed the same two peaks but revealed an increased concentration of the dinucleosome peak compared to extraction without carrier RNA (blue trace). This increased concentration indicated that something else, possibly carrier RNA, separated out with the dinucleosome cfDNA peak. To further investigate carrier RNA interference, cfDNA was treated with DNase to degrade all DNA in the sample. The DNase-treated sample (Figure 1B, blue trace) displayed a carrier RNA smear from 250 to 700 bp, overlapping the dinucleosome cfDNA peak. These results confirmed the findings from Figure 1A that carrier RNA comigrated with the dinucleosome cfDNA on the HS NGS Fragment kit (1-6000 bp).

To avoid carrier RNA interference, cfDNA was extracted with the Quick-cfDNA kit, which does not use carrier RNA. Figure 2 displays a similar cfDNA profile to Figure 1A (black trace), with three nucleosome cfDNA peaks seen at 165, 350, and 581 bp.

Extraction of fragmented genomic DNA (gDNA) with cfDNA can occur. Figure 3 demonstrates that cfDNA sample #4 contained fragmented gDNA, which was distinctively separated from the three nucleosome cfDNA peaks at 158, 367, and 576 bp and merged with the upper marker. The results showed that the HS NGS Fragment kit (1-6000 bp) was able to effectively separate three nucleosome cfDNA peaks and visualize gDNA with a slightly longer run time to allow the gDNA to separate. The increased concentration of the trinucleosome cfDNA peak may be due to a combination of cfDNA and gDNA.

The ultrashort array, used with the HS

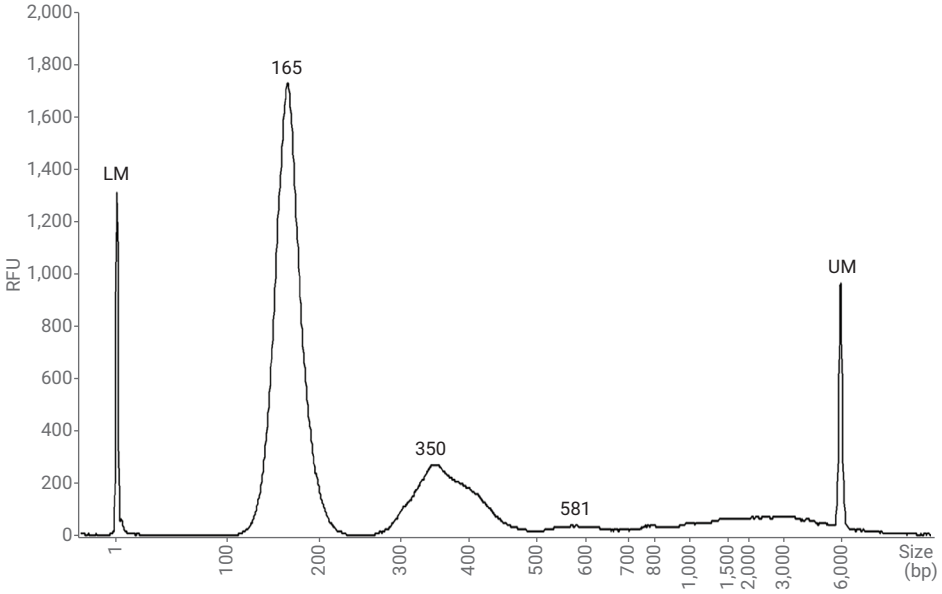

Figure 2. cfDNA sample #1 extracted by Quick-cfDNA kit was separated on the Agilent 5200 Fragment Analyzer system short array with the Agilent HS NGS Fragment kit (1-6000 bp). LM = lower marker; UM = upper marker.

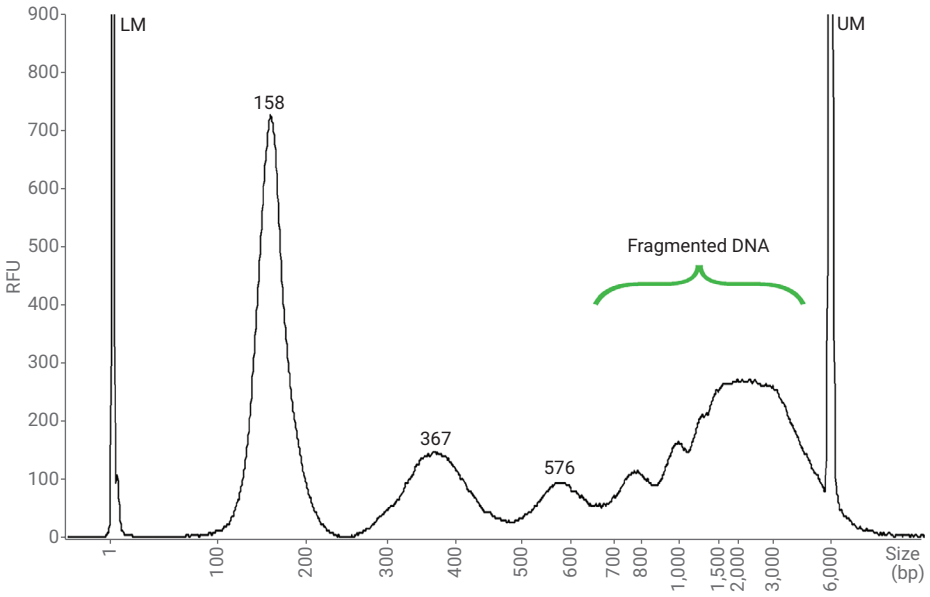

Figure 3. cfDNA sample #4 with fragmented genomic DNA extracted by Quick-cfDNA kit was separated on the Agilent 5200 Fragment Analyzer system short array with the Agilent HS NGS Fragment kit (1-6000 bp). LM = lower marker; UM = upper marker.

NGS Fragment kit (1-6000 bp), shortened the separation time by 20 minutes and allowed for complete separation of the first two nucleosome cfDNA peaks at 169 and 356 bp as shown in Figure 4.

Conclusions

Table 1 summarizes the HS NGS Fragment kit (1-6000 bp) analysis of cfDNA on the 5200 Fragment Analyzer system. The HS NGS fragment kit separated the nucleosome cfDNA peaks with both the short and ultrashort array, but detected interference from the carrier RNA, which comigrated with the dinucleosome cfDNA peak. Fragmented genomic DNA merged with the upper marker and required an extended run time to complete the separation. When possible, cfDNA extracted without carrier RNA is recommended to avoid carrier RNA interference.

Reference

1. Suzuki, N. *et al.*, Characterization of circulating DNA in healthy human plasma, *Clinica Chimica Acta* **2008**, *387*, 55–58.

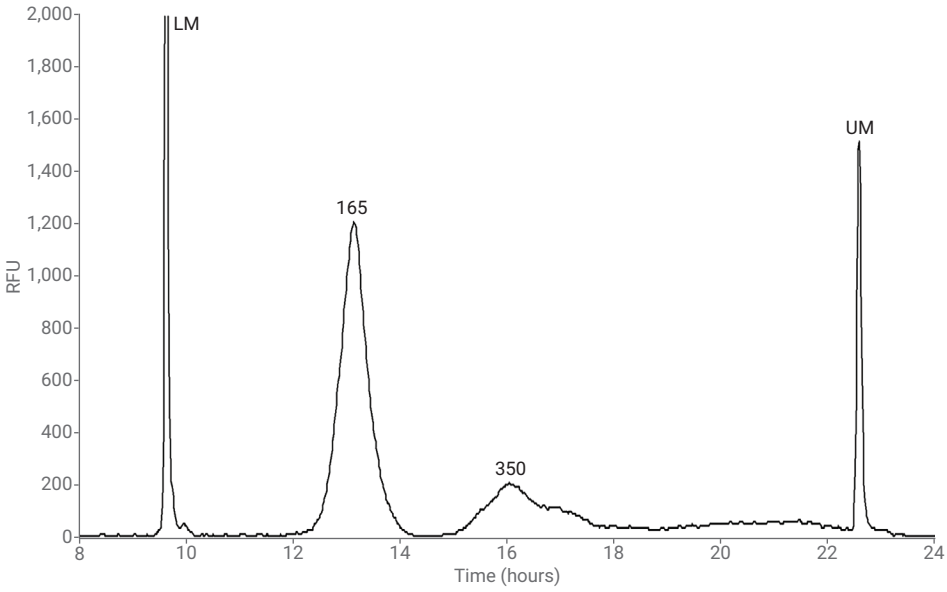

Figure 4. cfDNA extracted by Quick-cfDNA kit was separated on the Agilent 5200 Fragment Analyzer system ultrashort array with the Agilent HS NGS Fragment kit (1-6000 bp). The run time was shortened by 20 minutes. LM = lower marker; UM = upper marker.

Table 1. Summary of cfDNA analysis with the Agilent HS NGS Fragment kit (1-6000 bp).

	5200 Fragment Analyzer system		
	Short Array Run Time	Ultra-Short Array Run Time	Comments
HS NGS Fragment kit (1-6000 bp)	45 min	25 min	- Good separation of cfDNA peaks - Interference from carrier RNA - Fragmented genomic DNA merged slightly with upper marker

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