Isolation of cfDNA from various liquid biopsy samples and characterization of its DNA fragment sizes with capillary gel electrophoresis

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Introduction

cfDNA (cell-free DNA) refers to DNA fragments found in the bloodstream or other bodily fluids after removing cells and cell debris. These fragments are present in both healthy and diseased individuals. Cell-free DNA serves as a biomarker in various medical contexts and is widely used in many research fields.

Besides blood, other body fluids are increasingly used for isolating and analyzing cfDNA (Figure 1). Among these, lung pleural effusion fluids, bronchoalveolar lavage fluids, and ascites fluids (3) are becoming more popular (1–3).

Independently of the sample type, proper isolation and quality assessment of extracted cfDNA is important for downstream analysis of the samples. This application note focuses on the cfDNA isolation from lung pleural effusion fluids, bronchoalveolar lavage fluids and ascites fluids using the QIAamp® Circulating Nucleic Acid Kit. In addition, cfDNA was isolated from blood collected from transplant patients using the QIAsymphony® DSP Circulating DNA Maxi Kit.

DNA fragment sizes of all cfDNA samples were evaluated on the QIAxcel® Connect capillary gel electrophoresis system.

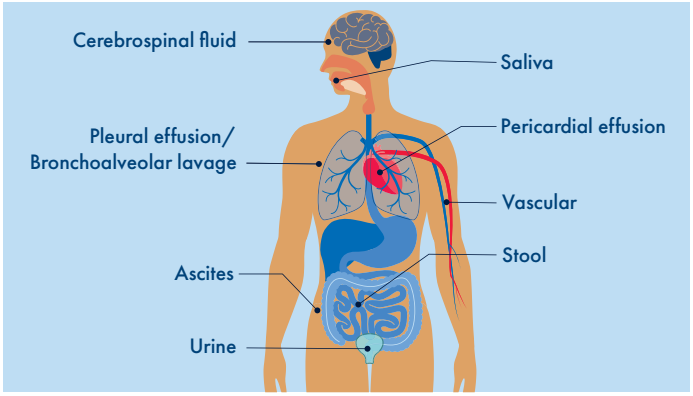

Figure 1. Overview of liquid biopsy sources

Materials and methods

cfDNA isolation from lung pleural effusion, bronchoalveolar lavage and ascites fluid

cfDNA from all samples was isolated manually with the QIAamp Circulating Nucleic Acid Kit. Sample input volumes ranged between 4 and 13 mL. cfDNA was isolated with a customer-validated method with adapted buffer volumes for lysis and an adapted binding step for large sample volumes of up to 13 mL. To test isolation efficiency, a non-human analogue synthetic construct was spiked-in before isolation with a volume equal to the elution volume of 110 µL. DNA concentrations were determined by fluorometric quantification.

cfDNA isolation from blood

Plasma was obtained from human blood collected in EDTA blood collection tubes. To test isolation efficiency, a non-human analogue synthetic construct was spiked-in before isolation with a volume equal to the elution volume. cfDNA from 7–10 mL sample volumes was automatically isolated with the QIAsymphony DSP Circulating DNA Maxi Kit on the QIAsymphony SP instrument. DNA was eluted in 60 µL and concentrations were determined by fluorometric quantification.

Capillary gel electrophoresis

DNA fragment sizes were analyzed on the QIAxcel Connect system with ScreenGel® SW 2.1 software. For the analysis of lung pleural effusion, bronchoalveolar lavage and ascites fluid samples, the QIAxcel DNA Screening Kit with run method AM1800 (available as a customized method), Alignment Marker QX 15 bp and Size Marker QX DNA Large-Fragment 1–20 kb were used.

For the analysis of plasma samples, the QIAxcel DNA High Sensitivity Kit with run method DNA High-Sensitivity_V2, Alignment Marker QX 15 bp HS (diluted QX RNA Alignment Marker) and Size Marker QX DNA HS 100 bp – 1kb (provided with QIAxcel DNA High Sensitivity Kit) were used. For the QIAxcel run, the plasma samples were diluted 1:2 with QX DNA HS Dilution buffer.

Digital PCR

Isolation efficiency was determined with custom primers and probes for a synthetic construct on 5 µL, 1.3x diluted DNA.

Results and Discussion

cfDNA isolation with the QIAamp Circulating Nucleic Acid Kit

cfDNA yields and quality of eluates

With the QIAamp Circulating Nucleic Acid Kit, cfDNA could be isolated from all three sample types, as described in section 2. DNA concentrations showed a high variability within samples of one sample type, due to the high variability of the source material. DNA concentrations are shown in Tables 1–3.

Table 1. Overview of cfDNA samples from lung pleural effusion

Sample Name	Concentration (ng/µL)
Sample 1	2.75
Sample 2	18.43
Sample 3	2.75
Sample 4	30.25
Sample 5	2.75

Table 2. Overview of cfDNA samples from bronchoalveolar lavage

Sample Name	Concentration (ng/µL)
Sample 6	> 60.00
Sample 7	4.43
Sample 8	39.88
Sample 9	1.53
Sample 10	40.15

Table 3. Overview of cfDNA samples from ascites

Sample Name	Concentration (ng/µL)
Sample 11	10.31
Sample 12	20.90
Sample 13	5.78
Sample 14	11.28
Sample 15	22.83

With digitial PCR, the extraction efficiency of spike-in DNA construct was evaluated as 70.4% on average over all samples. The extraction efficiency for each sample is summarized in Table 4.

Table 4. Extraction efficiency of spike-in DNA construct evaluated with dPCR

Sample type	Sample name	Extraction efficiency of spike-in DNA construct (%)
Lung pleural effusion	Sample 1	77
Lung pleural effusion	Sample 2	64
Lung pleural effusion	Sample 3	65
Lung pleural effusion	Sample 4	66
Lung pleural effusion	Sample 5	62
Bronchoalveolar lavage	Sample 6	81
Bronchoalveolar lavage	Sample 7	80
Bronchoalveolar lavage	Sample 8	79
Bronchoalveolar lavage	Sample 9	71
Bronchoalveolar lavage	Sample 10	82
Ascites	Sample 11	77
Ascites	Sample 12	67
Ascites	Sample 13	68
Ascites	Sample 14	66
Ascites	Sample 15	51

DNA fragment sizes from lung pleural effusion fluid

Evaluation of DNA fragment sizes of five lung pleural effusion samples showed a mixed pattern between samples. Both higher molecular weight DNA as well as lower molecular weight DNA are present in the samples (Figure 2). This is in agreement with findings from a previous study (1).

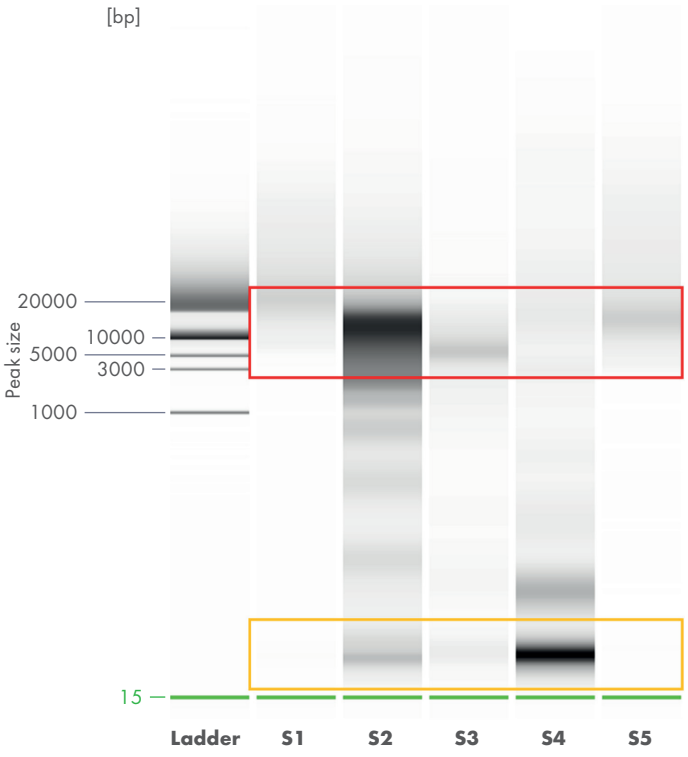

Figure 2. Gel image of samples (S) 1 to 5. DNA in the red box is higher molecular weight DNA and DNA in the yellow box is lower molecular weight DNA.

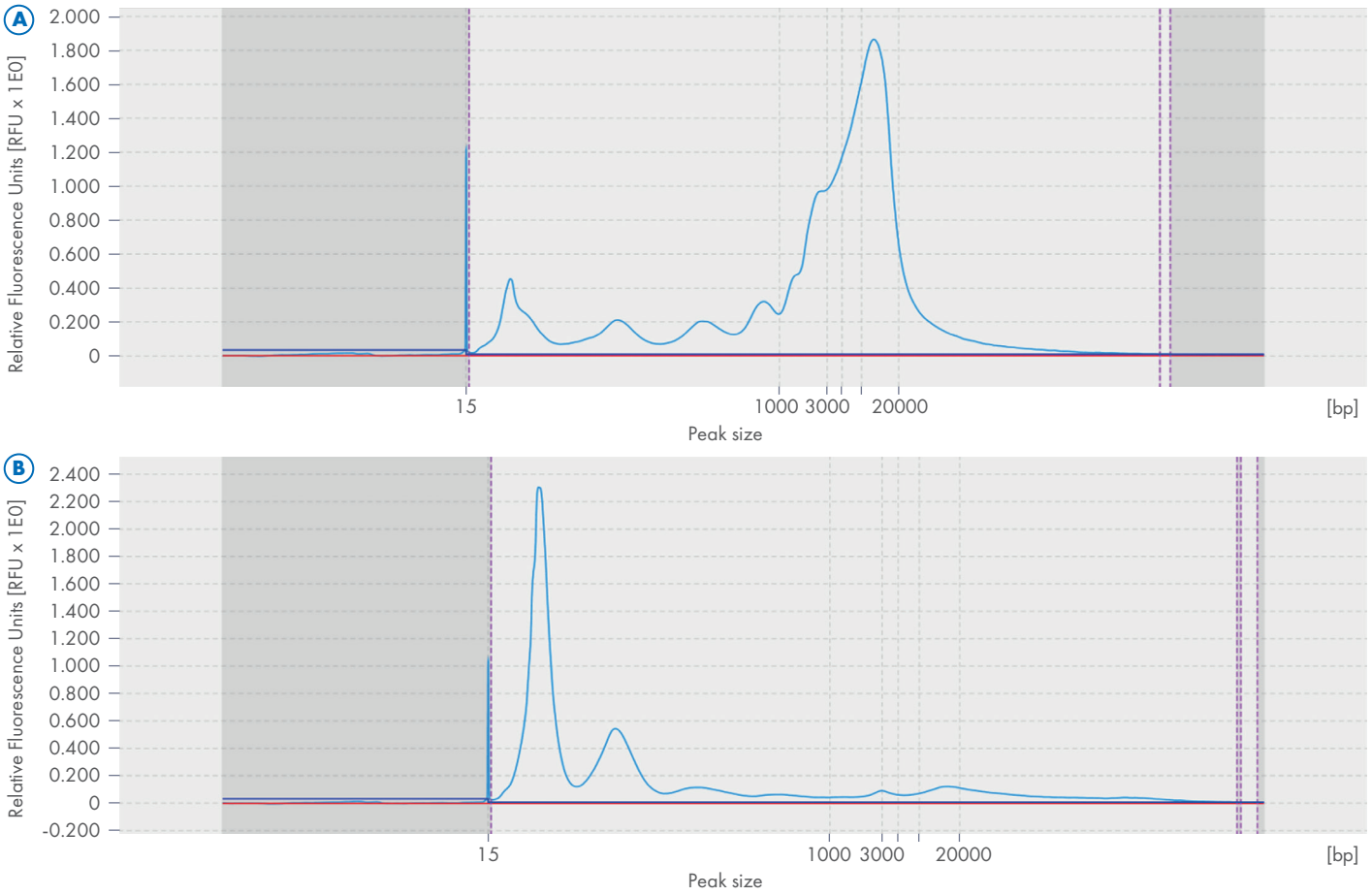

Figure 3. Electropherograms of sample 2 [A] and sample 4 [B].

The distribution between lower and higher molecular weight DNA differs between the samples, independent of each sample's overall DNA concentration. Figure 3 shows the electropherograms of sample 2, mainly containing higher molecular weight DNA (Figure 3A) and sample 4, mainly containing lower molecular weight DNA (Figure 3B).

DNA fragment sizes from bronchoalveolar lavage fluid

In bronchoalveolar lavage samples, mainly DNA of higher molecular weight is present in the five samples (Figure 4). This is in line with findings from a previous study (4).

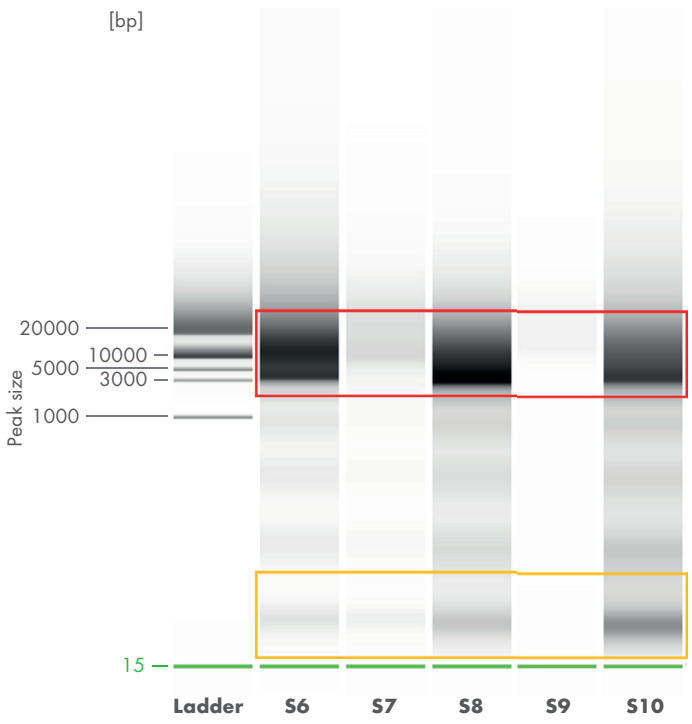

Figure 4. Gel image of samples (S) 6 to 10. DNA in the red box is higher molecular weight DNA and DNA in the yellow box is lower molecular weight DNA.

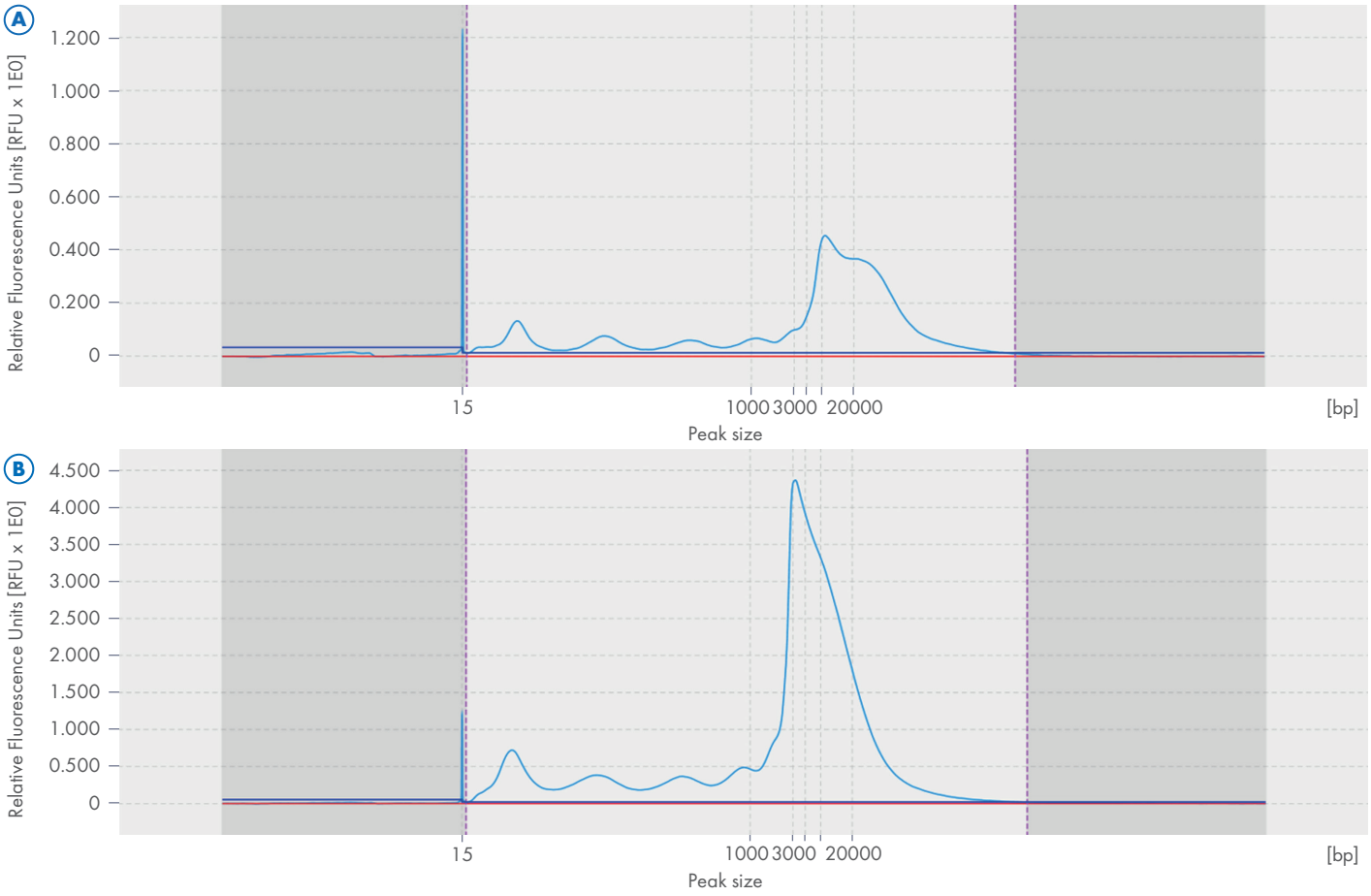

Figure 5. Electropherograms of samples 7 [A] and 8 [B].

The electropherograms of samples 7 and 8 show that the distribution between lower and higher molecular weight DNA is independent of the overall DNA concentration (Figure 5).

DNA fragment sizes from ascites fluid

In ascites samples, a mixed pattern of DNA fragment sizes was observed for the five samples (Figure 6). The samples consist of either mainly higher molecular weight DNA or show a nearly equal distribution between lower and higher molecular weight DNA. The presence of higher molecular weight DNA was also observed in a previous study (5). Other studies have shown that the cfDNA fragment sizes in ascites fluid resemble the cfDNA fragment sizes in plasma (3, 5). These findings could not be confirmed in this study.

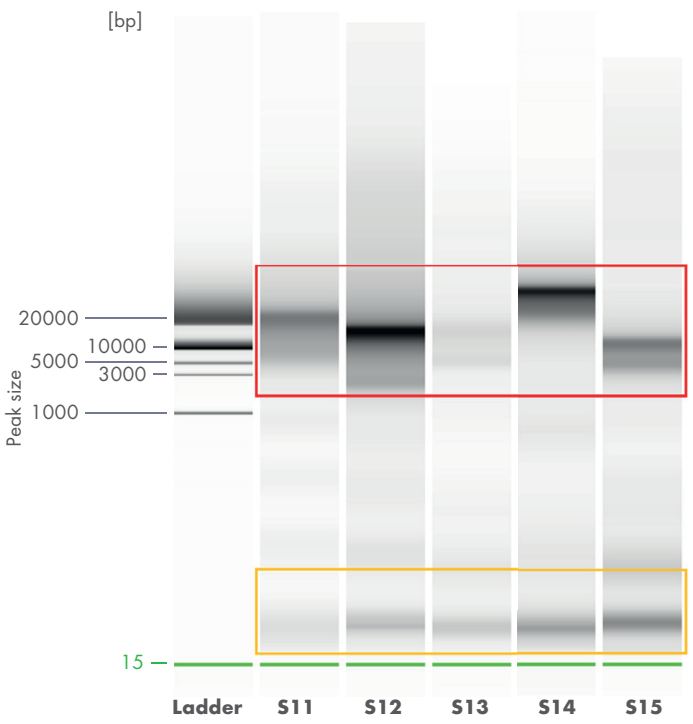

Figure 6. Gel image of samples (S) 11 to 15. DNA in the red box is higher molecular weight DNA and DNA in the yellow box is lower molecular weight DNA.

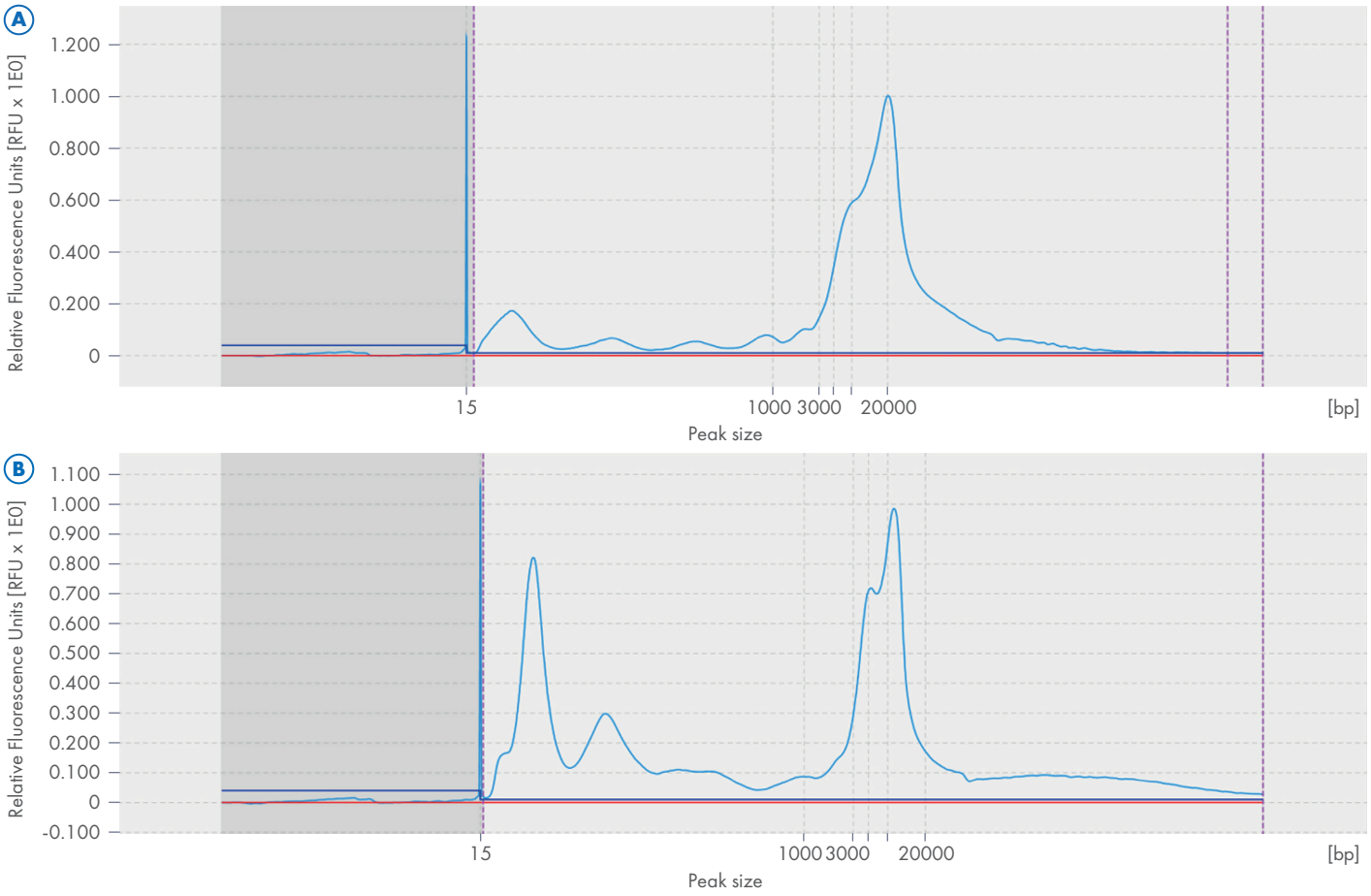

Figure 7. Electropherograms of samples 11 [A] and 15 [B].

The electropherograms of sample 11 (Figure 7A) and sample 15 (Figure 7B) demonstrate that the distribution between lower and higher molecular weight DNA is independent of the overall DNA concentration.

cfDNA isolation from large plasma volumes with the QIAsymphony DSP Circulating DNA Maxi Kit

cfDNA yield

A large volume of plasma (up to 10 mL) from transplant patients is needed to isolate sufficient cfDNA to achieve high sensitivity in downstream applications. The QIAsymphony DSP Circulating DNA Maxi Kit can meet this need as shown in Table 5, which includes examples of five plasma samples with DNA concentrations ranging from 1.50 to 13.40 ng/µL.

Table 5. Overview of cfDNA samples from plasma

Sample Name	Concentration (ng/µL)
Sample 16	8.95
Sample 17	11.60
Sample 18	13.40
Sample 19	1.50
Sample 20	1.54

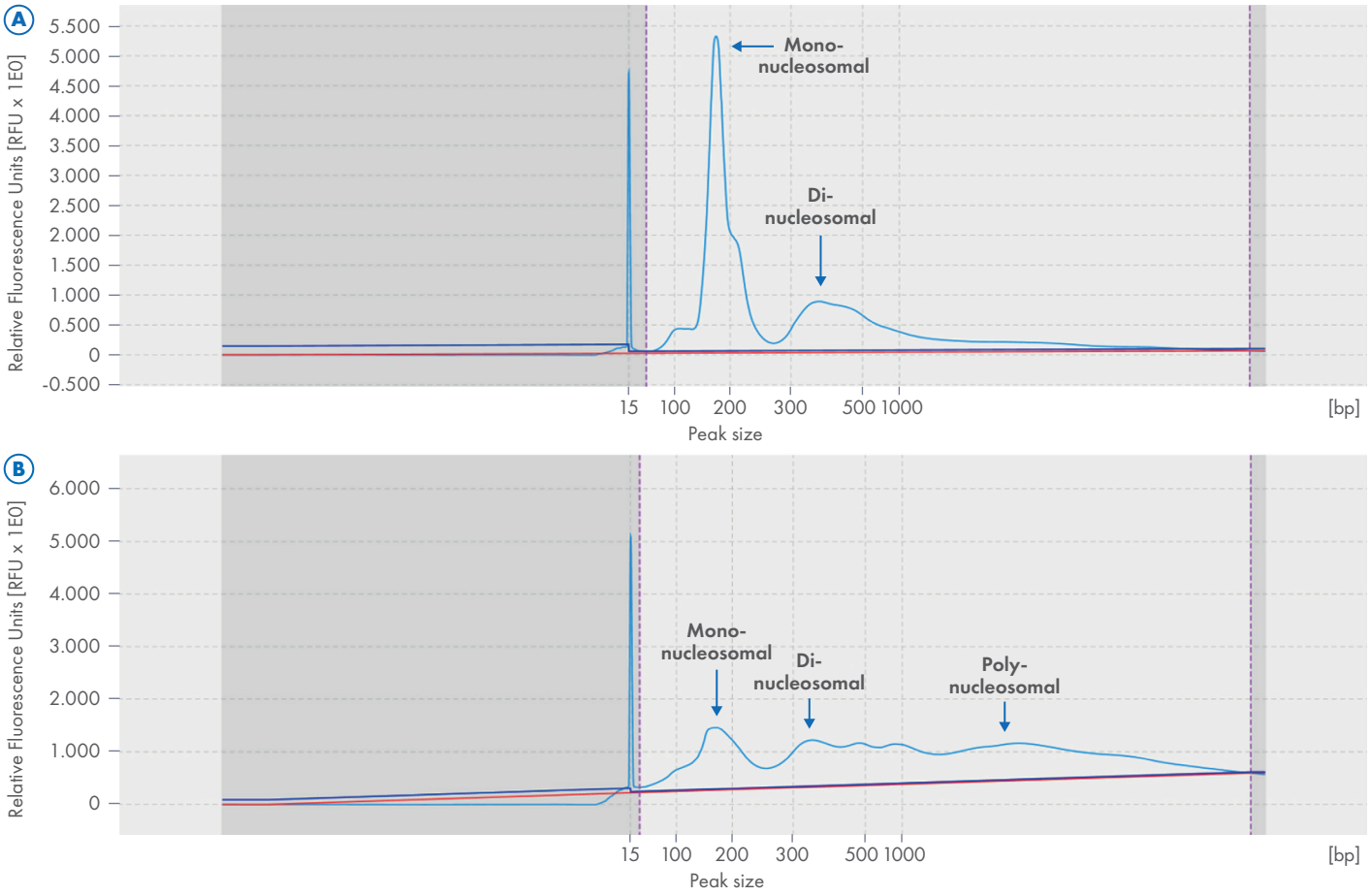

Figure 8. Electropherograms of samples 16 [A] and 17 [B].

DNA fragment sizes in plasma from transplant patients

Typically, cfDNA has a fragment size range of around 166–170 bp in plasma samples. This corresponds to the length of DNA that can wrap around a nucleosome (147 bp), plus an additional stretch to link two nucleosome cores. Apoptosis can also produce longer cfDNA fragments that correspond to di- (~350 bp), tri- (~565 bp) or poly-nucleosomes (6).

Plasma samples from transplant patients showed a mixed pattern of DNA fragment sizes. The electropherogram of sample 16, which represents the majority of the analyzed samples, shows the typical cfDNA size distribution with mainly a mono-nucleosomal and di-nucleosomal peak (Figure 8A). The electropherogram of sample 17, which represents a smaller portion of patient samples, also includes DNA of higher molecular weight (Figure 8B).

Conclusion

cfDNA-based testing is a less-invasive approach for detecting biomarkers in cancer and other research fields. Besides blood, various body fluids are increasingly used for the isolation and analysis of cfDNA in applications like dPCR and next-generation sequencing. For reliable analysis of cfDNA, efficient extraction and proper quality assessment of the samples is crucial.

In this application note, the QIAamp Circulating Nucleic Acid Kit was shown to efficiently isolate cfDNA from pleural effusion, bronchoalveolar lavage and ascites fluid samples. DNA fragment size evaluation with the QIAxcel Connect System showed that these sample types include higher molecular weight DNA as well as lower molecular weight DNA. These findings differ from the known size distribution profile of plasma samples.

For cfDNA isolation from up to 10 mL plasma, the QIAsymphony DSP Circulating DNA Maxi Kit was used to achieve a high cfDNA yield. Interestingly, the cfDNA size distribution in plasma from transplant patients showed a broader range of fragmented cfDNA from 50 bp to more than 1000 bp, in addition to the typical mono- and di-nucleosomal peaks.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Connect	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9003110
QIAsymphony SP	For fully automated DNA/RNA purification from a broad range of samples with varying input volumes	9001297
QIAamp Circulating Nucleic Acid Kit	For 50 preps: includes buffers and columns for isolation of free-circulating DNA and RNA	55114
QIAsymphony DSP Circulating DNA Maxi Kit	For 192 preps: includes reagents and consumables for automated purification of circulating DNA using the QIAsymphony SP	937566
QIAxcel DNA High Sensitivity Kit	QIAxcel DNA High Sensitivity Cartridge, QIAxcel DNA High Sensitivity Marker Set, Buffers, Mineral Oil, 12-Tube Strips	929012
QIAxcel DNA Screening Kit	QIAxcel DNA Screening Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QX DNA Size Marker Large-Fragment	Ready-to-use DNA size marker with fragments of 1, 3, 5, 10 and 20 kb; concentration 22.75 ng/µL	929710

References

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6. Suzuki N, et al. Characterization of circulating DNA in healthy human plasma. *Clin Chim Acta.* 2008;**378**(1-2).

For more information on the QIAxcel Connect System, visit:
www.qiagen.com/qiaxcel-connect