

PCR-Free cfDNA Profiling Using the LabChip Gel Xpress (GX) Touch

Introduction

Circulating cell-free DNA (cfDNA) is being widely researched for use as a biomarker for various types of cancer, autoimmune diseases, and chromosomal disorders. All cells shed small DNA fragments into the blood stream as they undergo apoptosis or necrosis. Tumor cells shed DNA at higher concentrations than non-tumor cells, and the fragments contain somatic mutations that can be detected via sequencing. One of the advantages of analyzing cfDNA is that it can be easily obtained from a blood draw, providing a “liquid biopsy” option that is less invasive than traditional biopsy techniques. The cfDNA is then isolated from the blood sample, quantitated, sequenced, and analyzed.

cfDNA profiling encompasses numerous steps and sample transfers that can propagate sample initial concentration errors; thus, necessitating the requirement for accurate quantitation and sizing of the cfDNA. PCR amplification is usually a necessary step in sequencing workflows to ensure there is adequate sample for quality control and downstream sequencing analysis. However, PCR-related problems, such as uneven amplification bias and nucleotide mis-incorporation have proven difficult when interpreting genome assembly and variation analyses. While PCR-free sequencing has generated great interest in recent years, the resulting low concentrations make PCR-free library preparation quality control quite challenging.

The PerkinElmer LabChip® Gel Xpress (GX) Touch automates and replaces the multiple, manual steps of slab gel electrophoresis with high levels of accuracy, precision and throughput. It quantifies DNA libraries at concentrations as low as 2 pg/μL with accurate fragment size determination. It can analyze up to 384 samples per run and provide the results within 28 seconds per sample, which is a great improvement over the hours or days required by traditional slab gel electrophoresis methods. The LabChip Reviewer software provides the data in electropherogram format or virtual gel view, as well as in tabular format for easy evaluation or export.

Here we present PCR-free cfDNA quantitation and fragment size distribution performance results using the LabChip instrument. Eleven PCR-free samples from healthy donors were processed using the LabChip DNA NGS 3K Assay, with each sample run in triplicate at a 1:10 sample-to-marker dilution.

Results and Discussion

Results for sample quantitation and fragment sizing of the cfDNA samples are shown in Table 1. Within the triplicate sets, the mean concentration ranged from 0.007 to 0.079 ng/μL. The LabChip platform provided precision ranging from 4.02 to 18.61% CV (the standard deviation as the percent of the mean concentration). The median fragment size for the samples ranged from 170.63 to 181.09 base pairs. The LabChip provided fragment size precision ranging from 0.31 to 2.80% CV.

The data demonstrate the high-level of reproducibility and sensitivity achieved by the LabChip instrument.

Table 1. cfDNA Quantitation and Fragment Sizing Results.

Sample	cfDNA Concentration, Mean+1SD (ng/μL)	Concentration Precision (%CV)	Fragment Size, Median+1SD (bp)	Size Precision (%CV)
cfDNA1	0.070±0.0044	6.19%	176.55±0.60	0.34%
cfDNA2	0.017±0.0020	12.25%	171.72±3.85	2.24%
cfDNA3	0.072±0.0080	11.15%	171.84±2.28	1.33%
cfDNA4	0.028±0.0044	15.92%	173.96±2.02	1.16%
cfDNA5	0.072±0.0072	10.01%	178.94±1.48	0.83%
cfDNA6	0.041±0.0030	7.19%	180.06±2.90	1.61%
cfDNA7	0.040±0.0024	6.03%	173.83±2.86	1.65%
cfDNA8	0.034±0.0014	4.02%	170.63±4.78	2.80%
cfDNA9	0.079±0.0048	6.06%	174.79±0.55	0.31%
cfDNA10	0.057±0.0105	18.61%	181.09±1.83	1.01%
cfDNA11	0.007±0.0007	9.19%	179.56±1.19	0.66%

To evaluate the LabChip NGS 3K Assay's sensitivity, we show electropherograms of three representative PCR-free cfDNA samples. Figures 1 and 2 show the electropherograms for two PCR-free cfDNA samples, each showing the tight, monolithic peak typical in electropherograms of cfDNA samples. These data demonstrate the sensitivity of the NGS 3K assay to provide high resolution data for PCR-free samples at low concentrations.

The data provided by the LabChip can also be valuable for the identification of sample contamination. The electropherogram for Sample 5 (Figure 3) illustrates the monolithic cfDNA peak along with a wide band of other peaks of varying concentration and fragment size, indicating possible sample contamination (e.g. genomic DNA).

Conclusion

The results, demonstrate that the DNA NGS 3K Assay on the LabChip Gel Xpress (GX) Touch can be used to quantitate PCR-free cfDNA samples with accuracy and reproducibility. The LabChip and its assay portfolio enable researchers to quantitate and analyze data more efficiently, accurately and with a higher degree of reproducibility for genomic research applications.

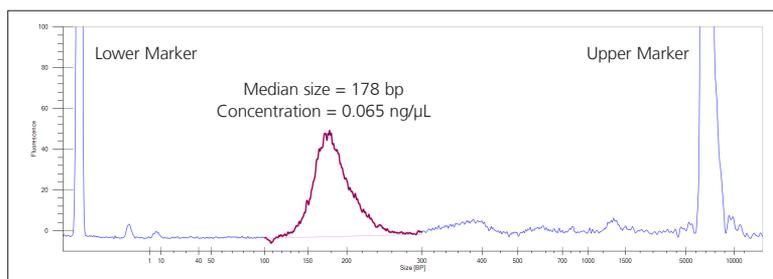


Figure 1. PCR-free cfDNA Sample.

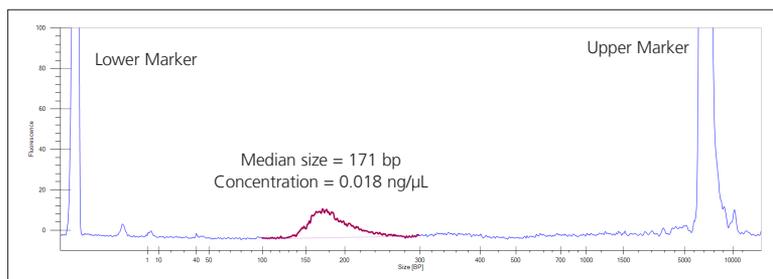


Figure 2. PCR-Free cfDNA Sample 2.

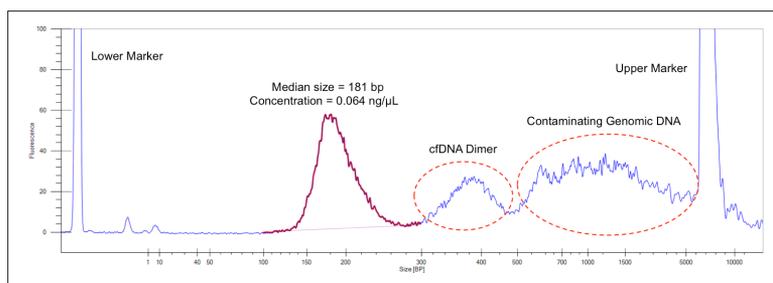


Figure 3. PCR-free cfDNA Sample 5.

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