Sample Quality Control of Cell-free DNA

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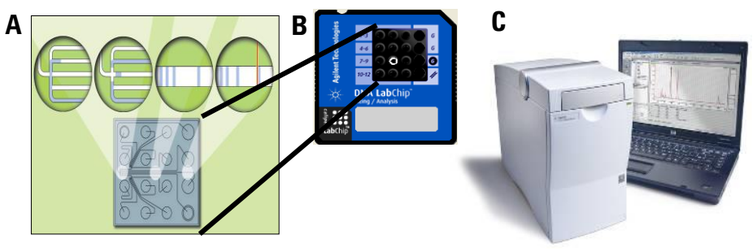
Introduction

Sequencing of cell-free DNA (cfDNA) as a marker for liquid biopsies is possible due to the establishment of low input library protocols for next-generation sequencing (NGS) workflows. Accurate quantitation of cfDNA samples is essential to determine suitable input amounts for cfDNA library preparation. cfDNA samples show a variety of characteristic peaks and may contain high molecular weight material which can negatively influence library preparation and subsequently result in lower sequencing depth. Therefore, reliable quantitation of cfDNA requires a method that separates DNA fragments by size, such as automated electrophoresis with the Agilent 2100 Bioanalyzer system.

Agilent 2100 Bioanalyzer System



Components of the Agilent 2100 Bioanalyzer system: A) Electrophoretic analysis of samples within a microfabricated glass chip, B) Plastic caddy housing the glass chip and providing wells for sample loading, and C) Bioanalyzer system for control and analysis of data.

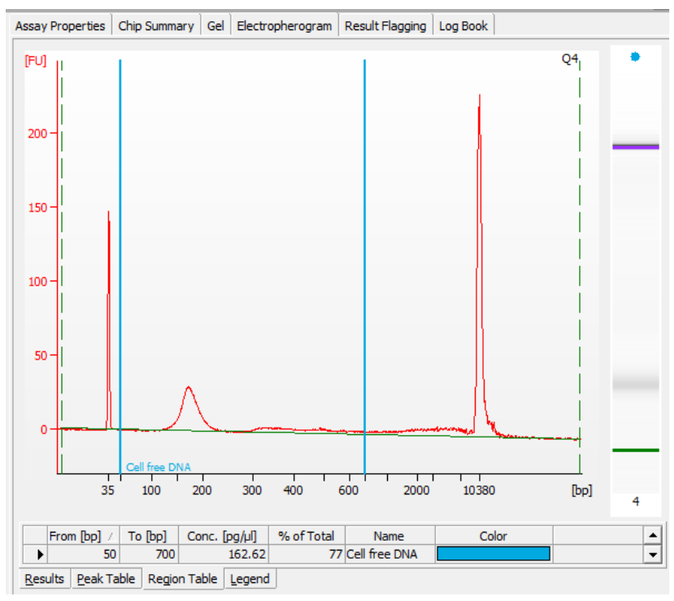
Experimental

Blood was collected in K_3EDTA collection tubes (Sarstedt) and centrifuged within 60 minutes (10 min, 2000 x g, 20°C). The plasma was carefully removed and centrifuged once more (10 min, 6000 x g, 20°C). The samples were aliquoted and stored at -80°C. cfDNA was extracted from 1 ml plasma using the QIAamp MinElute ccfDNA Mini Kit (Qiagen) and eluted in 40 µl TE buffer. The cfDNA samples were analyzed with the Agilent 2100 Bioanalyzer system using the Agilent High Sensitivity DNA Kit (p/n 5067-4626). Unless stated, manufacturers' protocols and guidelines were followed.

Results and Discussion

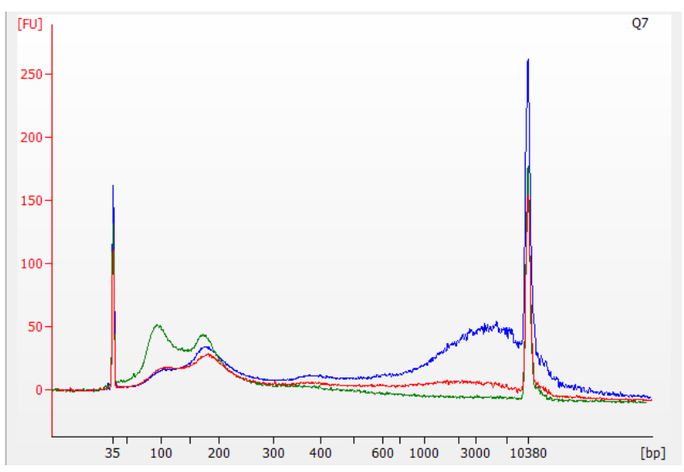
Quality control of cell-free DNA samples

- Electrophoresis of cfDNA samples typically exhibit a broad peak at ~170 bp, reflecting the association of DNA with histone proteins.



A typical profile of cfDNA analyzed with the High Sensitivity DNA assay on the 2100 Bioanalyzer system. The sample is evaluated with smear analysis with a region from 50-700 bp to include all cfDNA specimen. The region table displays the cfDNA concentration and % of cfDNA in the total sample.

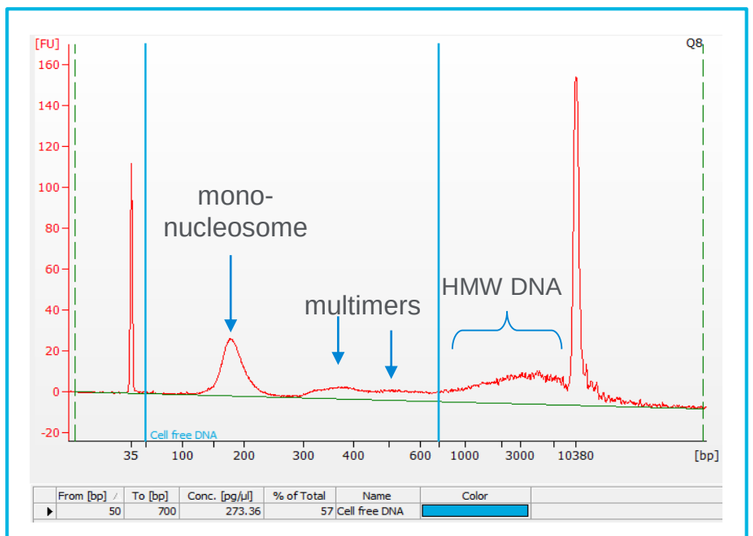
- Cell-free DNA samples may contain larger DNA fragments dependent on multiple variables, such as preanalytical sample treatment or extraction method.
- During NGS library preparation, high molecular weight DNA can bind to adapters leading to low library yield.
- Purity and concentration assessment of the cfDNA samples is essential prior to NGS library preparation.



Electropherogram overlay of cfDNA samples containing various levels of high molecular weight DNA. The % of total of cfDNA allows for detailed comparison of sample purity. The sample with the highest purity (green, 93 % cfDNA) shows no significant high molecular weight material in contrast to a slightly contaminated sample (red, 78 % cfDNA) and highly contaminated sample (blue, 55 % cfDNA).

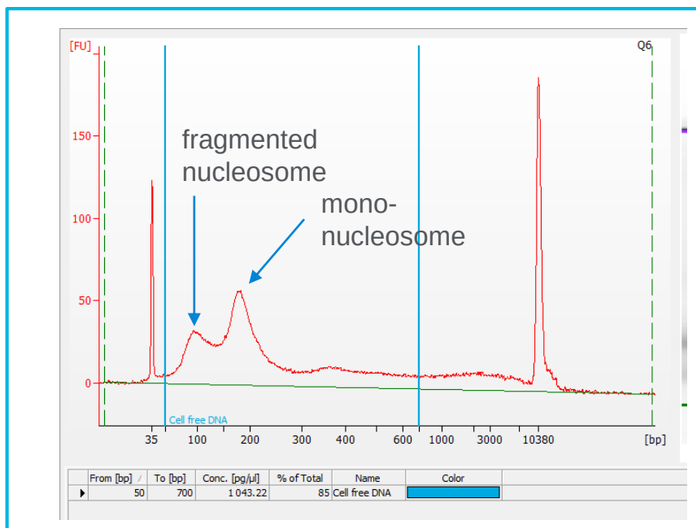
Cell-free DNA electropherogram profiles

- cfDNA samples commonly exhibit a main peak representing the mononucleosome
- The predominant peak is sometimes followed by less abundant DNA fragments representing nucleosome multimers.
- Some samples may display high molecular weight (HMW) DNA above 700 bp.



Cell-free DNA sample analyzed with the High Sensitivity DNA assay on the 2100 Bioanalyzer system. The electropherogram shows the cfDNA main peak (mononucleosome) and multimers thereof, which are included in the cell-free DNA region for quantification and separated from the high molecular weight DNA (HMW DNA).

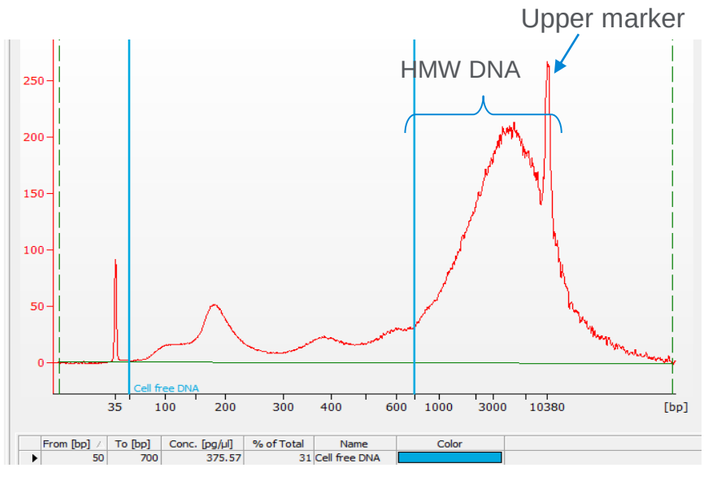
- cfDNA samples may exhibit an additional peak representing fragmented nucleosomes, with molecular weight about half of the mononucleosome.
- Changes in electrophoresis profiles are related to extraction methods and preanalytical treatment of the sample.



Electropherogram of a cell-free DNA sample analyzed with the High Sensitivity DNA assay. The electropherogram shows the cfDNA main peak (mononucleosome) and multimers. The smaller peak reflects fragmented nucleosomal DNA.

- cfDNA samples may exhibit significant high molecular weight DNA merging with the upper marker of the High Sensitivity DNA assay.

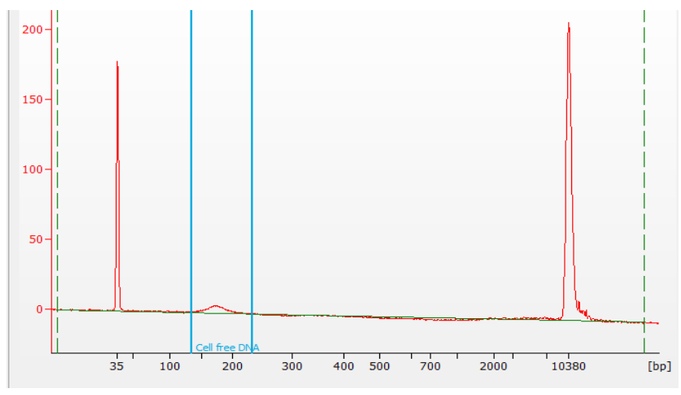
- NOTE: Sample concentration by the software is dependent on accurate area calculation of the upper marker. When peaks overlap with the upper marker, quantitation determination is compromised. The DNA concentration provided is only an approximation and not accurate in these types of profiles.



Electropherogram of a cell-free DNA sample analyzed with the High Sensitivity DNA assay. The electropherogram shows the cfDNA main peak (mononucleosome), multimers, fragmented nucleosomal DNA, and a large HMW DNA smear.

Sensitivity

- For accurate quantification of DNA smears, the High Sensitivity DNA assay recommends sample concentrations between 100 pg/µL and 10 ng/µL.
- cfDNA samples of limited abundance can be clearly detected at or above 25 pg/µL.



Electropherogram of a cfDNA sample with minimal concentration analyzed with the High Sensitivity DNA assay. The cfDNA smear has a concentration of 25 pg/µL, and can be clearly detected.

Conclusions

- Cell-free DNA samples show various characteristic patterns when electrophoretically separated.
- The High Sensitivity DNA assay is suitable to compare yields and purity of cfDNA samples down to a low concentration range.
- Region analysis allows for separation and quantitation of cfDNA subcomponents apart from high molecular weight material.
- The quantitation may be affected by presence of high molecular weight DNA within the sample, if it overlaps with the upper marker of the assay.