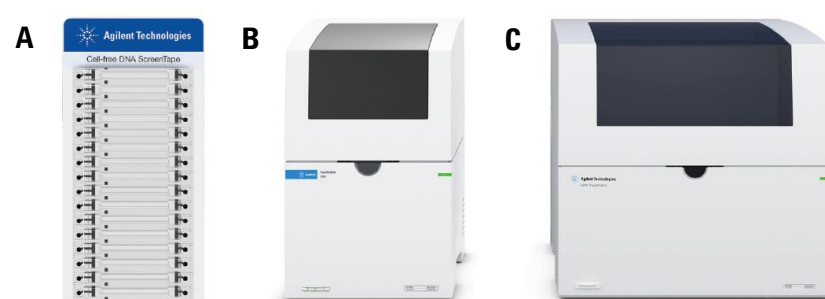


## Introduction

Cell-free DNA (cfDNA) is a valuable source for potential new biomarkers and low input library protocols for NGS workflows allow access to this dynamic genetic information. Accurate quantitation of cfDNA samples is essential to determine suitable input amounts for cfDNA library preparation. CfDNA samples may contain high molecular weight (HMW) material which can negatively influence library preparation and sequencing depth. Therefore, reliable quantitation of cfDNA requires a method that separates DNA fragments by size, such as automated electrophoresis. In addition, quality assessment with regards to HMW DNA contamination is an essential parameter for cfDNA samples.

## Agilent TapeStation systems



Components of the Agilent TapeStation system. A) Cell-free DNA ScreenTape device with 16 individual gel lanes. B) 4150 TapeStation instrument for automated electrophoresis of 1-16 samples per run. C) 4200 TapeStation instrument for automated high through-put electrophoresis of up to 96 samples per run.

## Conclusions

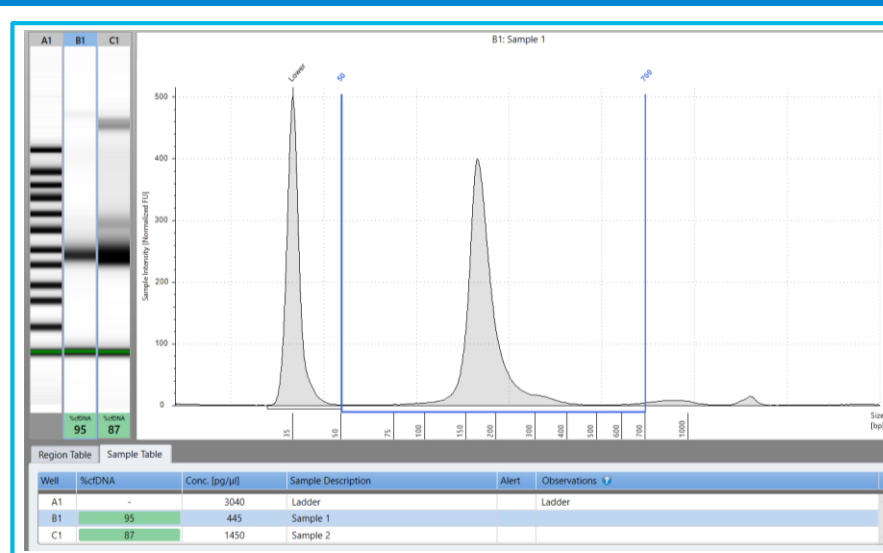
The Cell-free DNA ScreenTape assay is suitable to compare yields and quality of cfDNA samples down to a low concentration range.

Cell-free DNA samples show various characteristic patterns with a prominent peak at ~170 bp when separated by the Cell-free DNA ScreenTape assay.

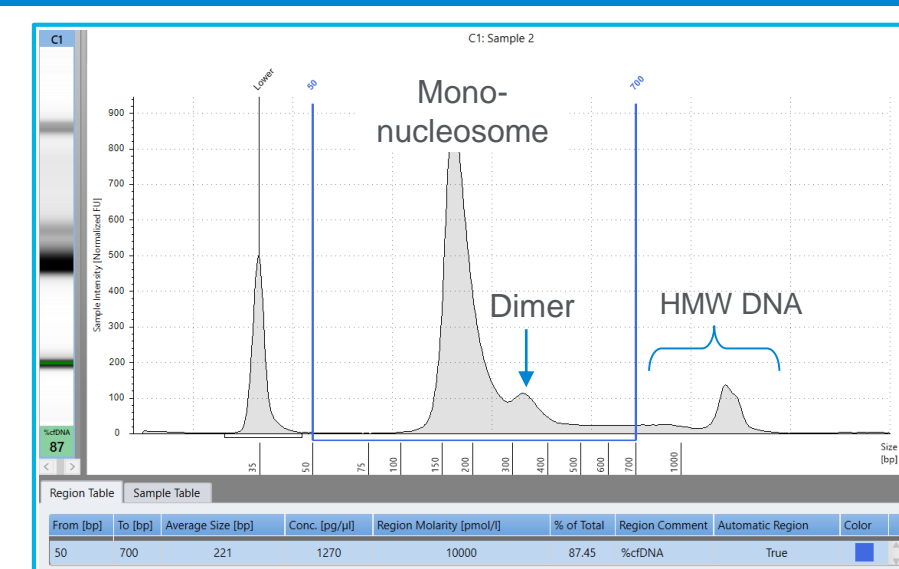
Automated region analysis allows for separation and quantitation of cfDNA subcomponents apart from high molecular weight material. The quality of the cfDNA sample is determined by the value %cfDNA.

## Cell-free DNA electropherogram profiles

Electrophoresis of cfDNA samples with the Cell-free DNA ScreenTape assay typically exhibit a broad peak at ~170 bp, reflecting the association of DNA with one histone protein. The predominant peak is sometimes followed by less abundant DNA fragments representing nucleosome multimers. Some samples may also display high molecular weight (HMW) DNA larger than 700 bp. Cell-free DNA is evaluated with a pre-set region from 50-700 bp to include all cfDNA specimen apart from HMW DNA. The sample table displays the total DNA concentration and the percentage of cfDNA in the total DNA sample (%cfDNA). The region table displays the concentration of the cfDNA specimen separately from the high molecular weight (HMW) DNA. Changes to these example electropherograms are typical for cfDNA samples, with additional peaks or variations in peak intensities.



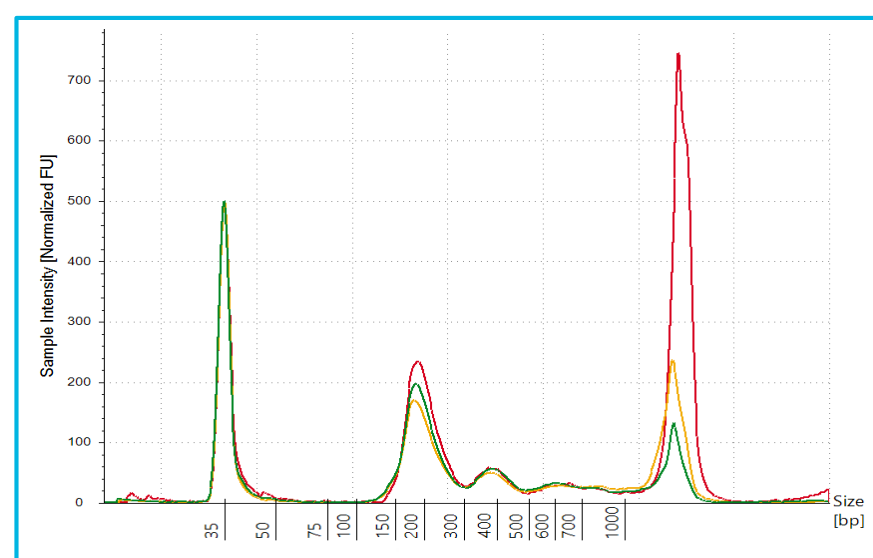
Cell-free DNA sample analyzed with the Cell-free DNA ScreenTape assay. The broad peak at 170 bp represents the mononucleosomal fragment. Cell-free DNA is evaluated with a pre-set region from 50-700 bp. The sample table displays the total DNA concentration and the percentage of cfDNA (%cfDNA).



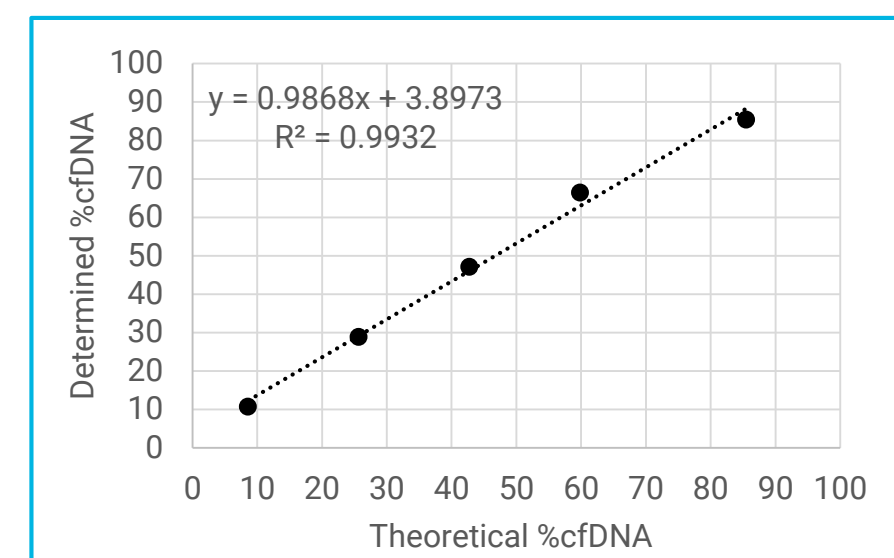
Typical profile of cfDNA analyzed with the Cell-free DNA ScreenTape assay. The electropherogram shows the mononucleosome, dimers, and HMW DNA. Multimers are included in the %cfDNA region (50-700 bp) and are separated from HMW DNA. The region table displays the concentration of cfDNA specimen and percentage relative to the total DNA sample (%cfDNA).

## Cell-free DNA qualification

Cell-free DNA samples may contain larger DNA fragments dependent on multiple variables, such as preanalytical sample treatment or extraction method. During NGS workflows, high molecular weight DNA can negatively affect library yield and sequencing. The Cell-free DNA ScreenTape assay allows to define the quality of the cfDNA sample with the value %cfDNA. This quality metric evaluates the percentage of cfDNA subcomponents in the total sample DNA. The value %cfDNA allows to define quality thresholds to decide whether a sample contains sufficient cfDNA relatively to the total DNA content to continue the downstream process. The accuracy of the %cfDNA metric was evaluated by adding sheared genomic DNA (gDNA) to a cfDNA reference sample. The pure reference sample had a quality of 85 %cfDNA. The sheared gDNA and reference sample were pooled at a variety of ratios to mimic samples covering a quality range from 10 %cfDNA to 85 %cfDNA. The results showed excellent correlation with an R<sup>2</sup> value of 0.993.



Electropherogram overlay of cfDNA samples with various sample qualities. Sample A (green, 72 %cfDNA) shows low abundance of HMW material > 700 bp reflecting the highest quality of the sample set. Sample B (orange, 57 %cfDNA) contains a higher amount of HMW material reflecting a lower sample quality. Sample C (red) with the lowest quality value of 41 %cfDNA shows a significant amount of HMW material.

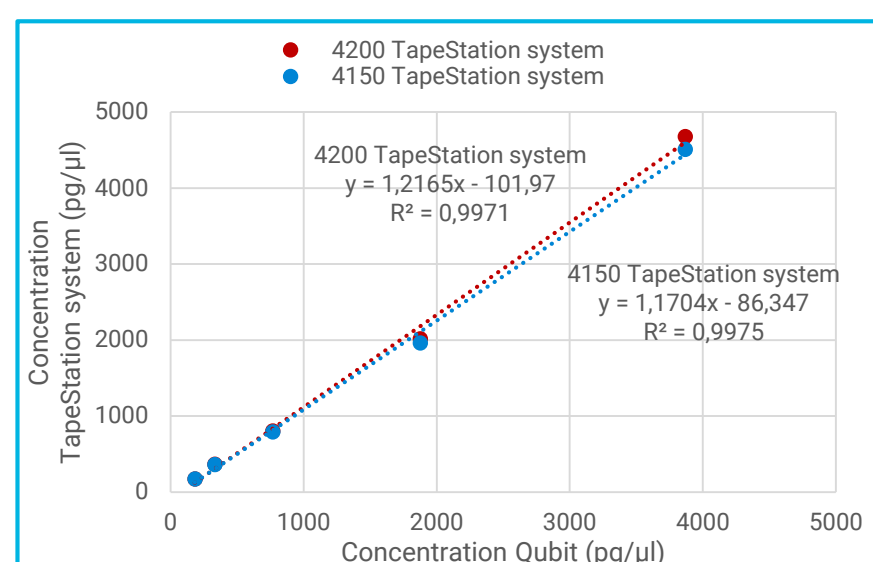


Accuracy of the %cfDNA metric evaluated using a reference sample with 85 %cfDNA and added high molecular weight DNA. The theoretical values are displayed on the x-axis, with a maximum of 85 %cfDNA represented by the pure reference sample. The prepared samples were analyzed with the Cell-free DNA ScreenTape assay and the results of the %cfDNA value are presented on the y-axis.

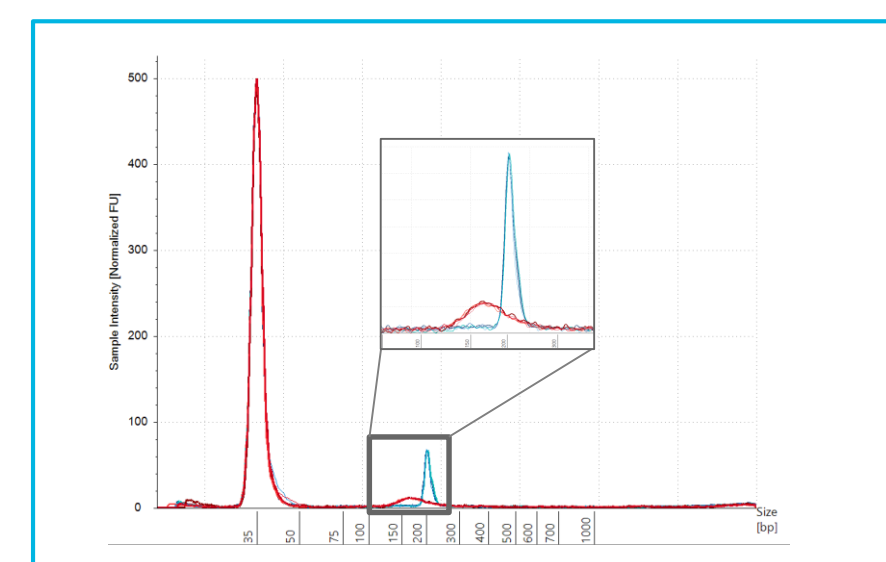
## Cell-free DNA quantification

The Cell-free DNA ScreenTape assay is capable of quantifying the cfDNA apart from HMW DNA, because they are size separated during electrophoresis. The quantification accuracy was evaluated from 200 to 4,000 pg/μl with a reference cfDNA sample and compared to the concentration determined by Qubit. The quantification accuracy of the cfDNA ScreenTape assay on both the 4150 and 4200 TapeStation systems displayed excellent correlation to the concentration measured by Qubit as seen by both R<sup>2</sup> values close to 1.

To demonstrate the high sensitivity of the Cell-free DNA ScreenTape assay, a cfDNA sample and a DNA fragment were diluted to 20 pg/μl each. The mononucleosomal peak is clearly detected above baseline with a signal-to-noise ratio greater than 3.



Quantification of a reference cfDNA sample in dilution series from 200 - 4000 pg/μL. Total DNA sample concentrations determined with the Cell-free DNA ScreenTape assay (n=24) on the 4150 and 4200 TapeStation systems were compared to DNA concentrations measured by Qubit (n=3).



Electropherogram overlay (n=5) of a 200 bp fragment (blue) and a cfDNA sample (red), each with a concentration of 20 pg/μl analyzed with the Cell-free DNA ScreenTape assay. The cfDNA peak is clearly visible above the background signal.