Objective Qualification of Cell-free DNA by Applying the %cfDNA Quality Metric

Annika Dorn and Whitney Pike
Agilent Technologies

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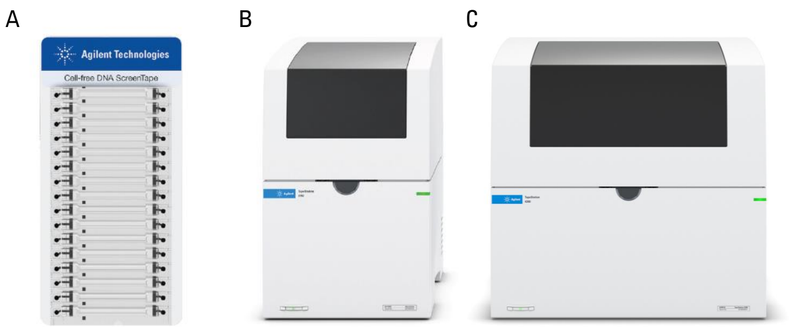
Introduction

Next Generation Sequencing (NGS) of cell-free DNA (cfDNA) has the potential to reveal new biomarkers for various conditions. However, library preparation of cfDNA samples remains a challenge due to restricted amount of starting material and variable size distribution of cfDNA fragments. The yield and quality of cfDNA can vary and a lot of care is required to obtain appropriate cfDNA starting material for downstream applications. Further, cfDNA samples may contain high molecular weight (HMW) DNA that can negatively affect library yield and sequencing quality. Therefore, quality control of cfDNA starting material is essential to ensure the success of downstream experiments. The Agilent TapeStation system with the Cell-free DNA ScreenTape assay is based on automated electrophoresis and thereby offers an objective and reliable qualification of cfDNA samples by applying the %cfDNA quality score.

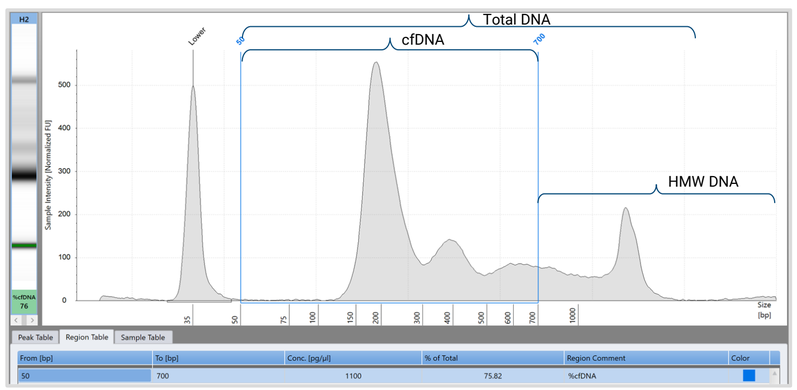
Methods

Six independent plasma samples were processed with two different extraction kits. CfDNA was extracted from human blood plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen) and Mag-Bind cfDNA kit (Omega Bio-tek), respectively. The extracted cfDNA samples were analyzed with the Cell-free DNA ScreenTape assay in combination with the 4150 and 4200 TapeStation systems (Agilent Technologies).

Agilent TapeStation systems



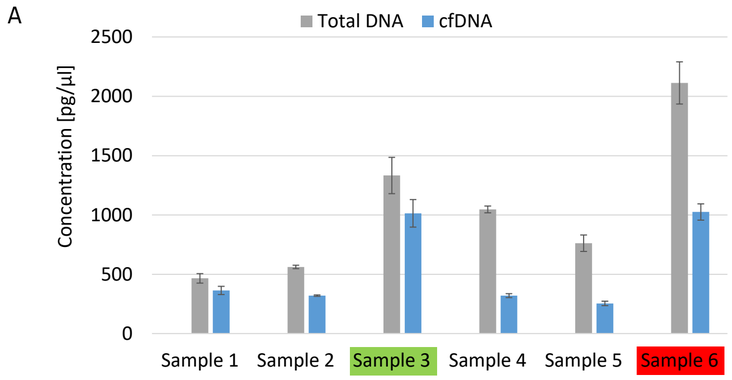
Components of the Agilent TapeStation system.
A) Cell-free DNA ScreenTape device with 16 individual gel lanes. B) 4150 TapeStation instrument for automated electrophoresis of 1-16 samples per run. C) 4200 TapeStation instrument for automated high-throughput electrophoresis of up to 96 samples per run.

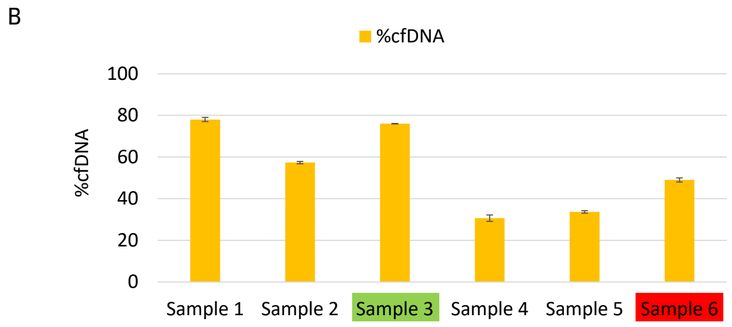


The Cell-free DNA ScreenTape assay offers total DNA quantification, separate cfDNA quantification, and a %cfDNA quality score. A pre-set cfDNA region of 50 to 700 bp is assigned to separate cfDNA from high molecular weight (HMW) DNA. Using region analysis, the concentration of cfDNA apart from HMW DNA is automatically evaluated by the software. The %cfDNA quality score is provided as an additional quality parameter, determining the percent cfDNA subcomponents in the total DNA sample. The %cfDNA quality score provides a reference allowing for quick decisions of the cfDNA sample quality.

Results

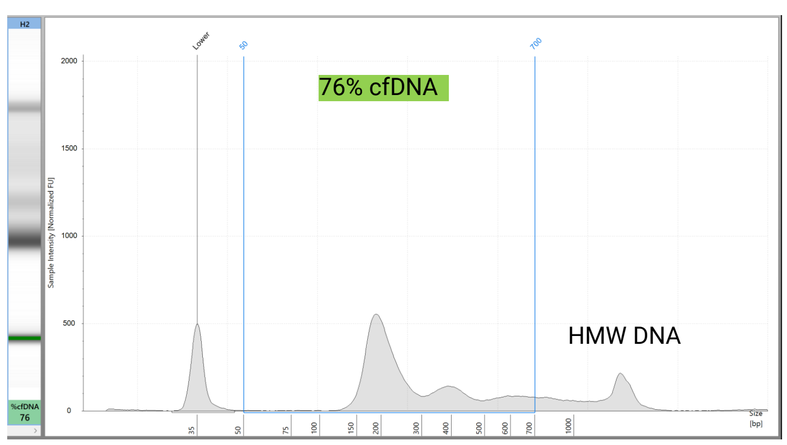
The Cell-free DNA ScreenTape assay in combination with the 4150 and 4200 TapeStation systems was used to analyze cfDNA extracted from six different samples.
(A) Comparison of total DNA and cfDNA concentration. Each of the six samples contains varying amounts of total DNA, with sample 6 showing the highest total DNA concentration. Although much lower in total DNA, cfDNA concentration in sample 3 is comparable to sample 6.
(B) The %cfDNA score allows for an objective qualification of cfDNA samples. Samples 1-3 display a high %cfDNA score from 57-78%, indicating a higher purity of cfDNA samples. Samples 4-6 are much lower in %cfDNA with scores from 31-49%, indicating large amounts of HMW DNA contamination.





The Cell-free DNA ScreenTape assay does not utilize an upper marker, thus allows for the simultaneous detection of cfDNA (region from 50 bp to 700 bp) and HMW DNA (peak to the right). For a fast and automated qualification of the sample, the %cfDNA value is depicted below the gel view on the left. Sample 3 displays a %cfDNA score of 76%, indicating a low amount of HMW DNA. Although the total DNA concentration of the sample is relatively low with 1333 pg/µl, the yield of cfDNA is high (1014 pg/µl).

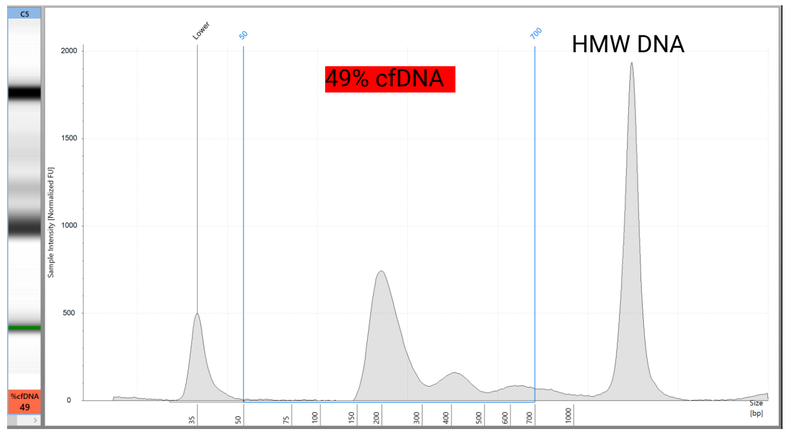
Sample 3



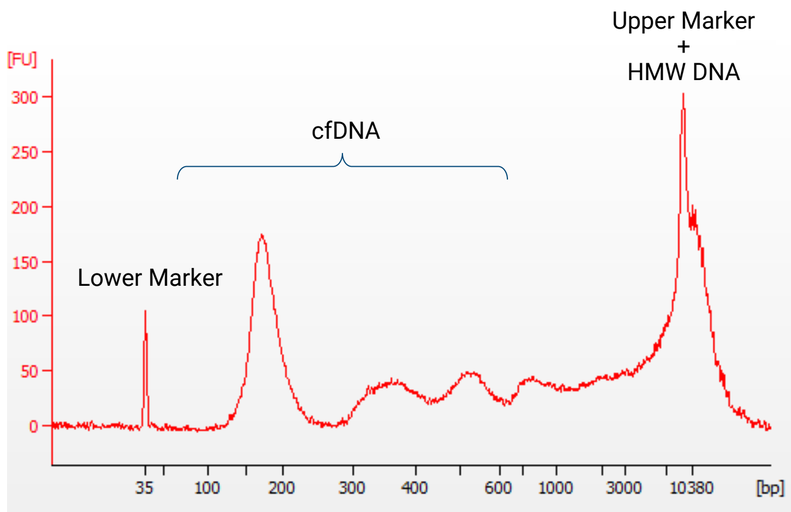
Results Continued

In contrast, in the electropherogram of sample 6 a high amount of HMW DNA can be detected. The low %cfDNA value demonstrates that although of high total yield (2113 pg/µl), this sample is comprised of only 49% cfDNA, resulting in a cfDNA concentration of 1026 pg/µl. Dependent on the amount of cfDNA, subsequent steps for NGS library preparation need to be adjusted. Therefore, accurate quantification of the cfDNA subcomponents in a sample is essential to obtain high quality NGS results.

Sample 6



When analyzing cfDNA samples with other QC methods, utilizing an upper marker, several problems occur. The HMW DNA cannot be detected clearly, as it co-migrates with the upper marker. Moreover, microfluidic channels can be clogged by HMW DNA. This compromises both quantification and qualification of the sample. Consequently, precious information about HMW DNA contamination is lost. This can compromise following steps for NGS library preparation and ultimately threatens sequencing quality.



Conclusions

- The Agilent TapeStation system with the Cell-free DNA ScreenTape assay offers objective and reliable qualification of cfDNA samples by applying the %cfDNA quality score.
- Detailed quantitative information on both total DNA yield and HMW DNA contamination can be obtained.
- Accurate quantification of cfDNA starting material, including any HMW DNA contamination is essential for NGS library preparation and generating reliable NGS results.
- Other QC methods that utilize an upper marker might miss critical information on HMW DNA contamination, thereby threatening the successful outcome of downstream experiments.

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