Unraveling the Link Between Total cfDNA Quantified by Agilent TapeStation Cell-free DNA ScreenTape and Actionable cfDNA Molecules Measured on Downstream Assays

Isabell Priester[1], Nafiseh Jafari[2], Annika Dorn[1], Jason Saenz[2], Lauren Lee[2], Carlos Hernandez[2], Andrew Dunnigan[2], and Mayer Saidian[2]

[1] Agilent Technologies, Germany
[2] nRichDX Inc., USA

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Introduction

cfDNA holds immense potential as a non-invasive biomarker for various disease states, including cancer, prenatal testing, and transplantation monitoring. Researchers are exploring methods to obtain improved diagnostic sensitivity and specificity while maintaining consistency of results between different downstream applications.

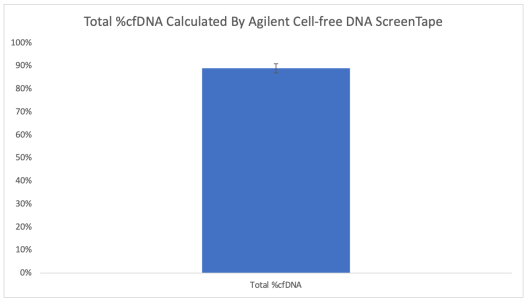
Among the downstream applications used for cfDNA analysis, the Agilent Cell-free DNA ScreenTape assay for TapeStation systems has emerged as a popular choice for total cfDNA quantification. The system employs automated electrophoresis to assess the fragment length distribution and concentration of cfDNA samples while offering a fast turnaround time and using minimal volume. Every sample assayed on the Cell-free DNA ScreenTape assay is given a %cfDNA quality score which automatically determines the percent cfDNA subcomponents in the total DNA sample.

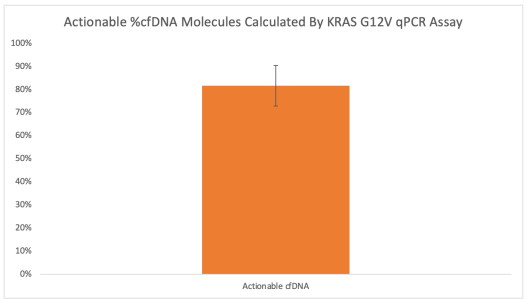
This study aims to show the correlation between total cfDNA quantified by Agilent Cell-free DNA ScreenTape assay and the detection of actionable cfDNA molecules through qPCR. Actionable cfDNA molecules refer to specific genetic alterations, such as mutations, copy number variations (CNVs), and epigenetic modifications, which have clinical relevance and potential therapeutic implications.

Materials & Methods

Thirty-eight replicates of a contrived sample at 5mL sample volume were spiked with a cfDNA reference standard containing the KRAS p.G12V mutation at a concentration of 10 ng/mL. All samples were extracted using the nRichDX Revolution Max20 cfDNA Isolation Kit. The cfDNA quality and quantity of the extracted samples were determined using Agilent Cell-free DNA ScreenTape assay. The percent recovery of actionable cfDNA molecules was determined by qPCR mutation detection assay on the QuantStudio 3 Real-Time PCR System.

Results





	Total %cfDNA	Actionable %cfDNA Molecules
Mean	89%	82%
Standard Deviation	0.02	0.09

Figure 1. Mean %cfDNA of 38 replicates analyzed by the Agilent Cell-free DNA ScreenTape assay (Left). The mean of actionable %cfDNA molecules of 38 replicates were analyzed by a qPCR assay for the KRAS G12V mutation. Error bars represent the standard deviation from each data set.

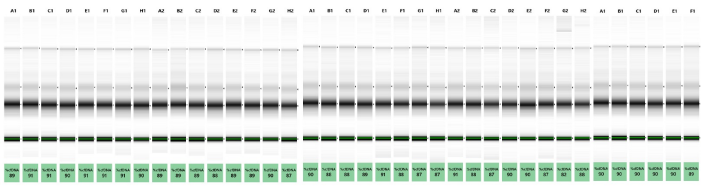


Figure 2. Gel electrophoresis of extracted nucleic acids size (bp) using the Agilent Cell-free DNA ScreenTape assay for TapeStation systems. All thirty-eight samples are represented showcasing the total %cfDNA of each samples.

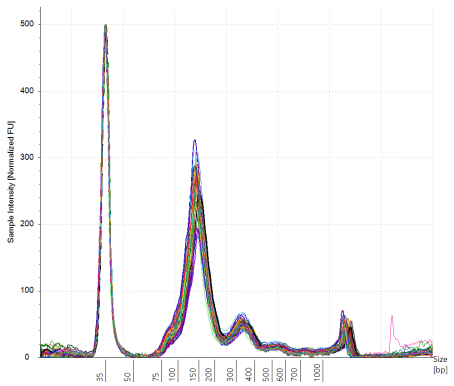


Figure 3. TapeStation electropherogram tracings of all thirty-eight samples showing extracted total cfDNA with a monomer, dimer, and trimer.

Conclusion

The nRichDX Revolution Max20 cfDNA Isolation Kit demonstrated high extraction efficiency, exceeding 89% of %cfDNA across a statistically powered experiment involving 38 samples. Validation through Agilent Cell-free DNA ScreenTape assay electropherogram analysis affirmed the high quality of the extracted cfDNA, consistently revealing monomers, dimers, and trimers in all samples. Further assessment via qPCR unveiled an actionable cfDNA mean recovery rate of 82%. These findings establish a meaningful correlation between cfDNA quantification using Agilent Cell-free DNA ScreenTape assay and the detection of actionable cfDNA molecules through qPCR.

The Agilent Cell-free DNA ScreenTape assay is a valuable tool capable of swiftly and accurately determining %cfDNA in samples. This metric directly corresponds to the quantity of recoverable actionable cfDNA in downstream applications. Notably, the Cell-free DNA ScreenTape assay offers the advantage of minimal sample volume requirements, making it an efficient initial quality control method for assessing the quantity and quality of extracted cfDNA samples before committing to more resource-intensive downstream procedures.

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*Agilent TapeStation systems are for Research Use Only (RUO). Not for use in diagnostic procedures.