An Amplification-based Assay for Better Characterization of Circulating Cell-Free DNA Purified from Plasma

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1. Abstract

Introduction:
Quantity and quality of ccfDNA from plasma is highly variable, with frequent contamination of larger, genomic DNA as a consequence of hemolysis during plasma processing. ccfDNA yields are typically low, with tumor or fetal DNA at significantly lower frequencies compared with normal or maternal. Due to the inherent variability of ccfDNA, knowing the quantity of DNA is not in itself reliably predictive of the amount of true ccfDNA. In this poster, we describe novel methods for measuring ccfDNA quality and quantity utilizing a multiplexed qPCR assay.

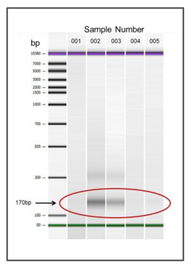
Methods:
DNA was purified from ccfDNA from plasma and serum samples using multiple methods. A multiplexed qPCR assay was designed that included three different amplicon sizes (75, 150, and 300bp). The quantitative differences between the increasingly larger amplicon sizes were calculated as a ratio to determine the level of degradation of ccfDNA samples. The size of ccfDNA fragments cluster around 170bp, with smaller populations at 340bp and 510bp. Since gDNA is expected to be much larger in size, the ratio of 75bp to 300bp targets can help predict the ratio of ccfDNA to gDNA. ccfDNA samples with low 75/300bp ratios are indicative of gDNA contamination.

Results:
Testing of ccfDNA showed high degradation ratios when the 170bp population was prevalent. In experiments with samples that contained more gDNA, a lower ratio was observed. Additionally, we could reduce the degradation ratio of "clean" ccfDNA samples by adding genomic DNA into the eluate.

Conclusions:
Data derived from a multi-size target qPCR assay can be very effective in determining the characteristics of a ccfDNA sample eluate. Using such a method can drive researchers to triage samples and make informed decisions about what downstream methods to use. Concentrating on less complex panels vs. highly multiplexed panels or whole exome sequencing for degraded samples can ensure getting the most useful information out of an individual sample, thus saving time, cost, and loss of information about precious samples.

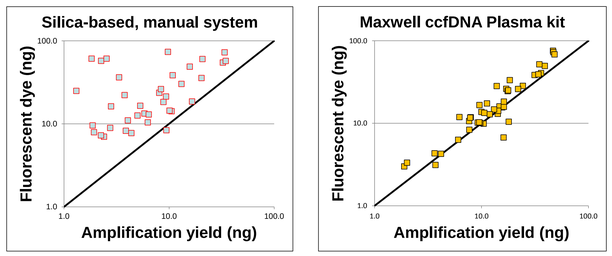
2. Characteristics of circulating, cell-free DNA (ccfDNA)

- Present in plasma
- Highly fragmented
  - Majority of ccfDNA ≈170 bp
- Low concentration
  - 10-30ng/ml of normal plasma or less
- Rapid turnover
  - Half life of 30 minutes
- Compatible with downstream assays



3. Why not use fluorescent quantitation?

- Eluate fluorescent dye quantitation compared with digital PCR quantitation



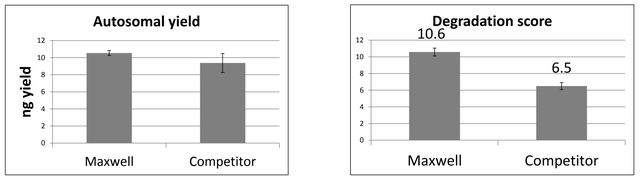
- Competitor is heavily skewed towards quant overestimation by dye due to carrier RNA

4. Amplification-based kit in development

- Single-well, multiplexed assay
- 3 targets for measurement of degradation or genomic DNA contamination: 75 bp, 150 bp, and 300 bp
- Built-in internal positive control to measure inhibition
- Sensitivity to 3.2 pg/µl input
- Master mix with simple set up
- As little as 1 µl per amplification of sample

5. Concept of degradation score

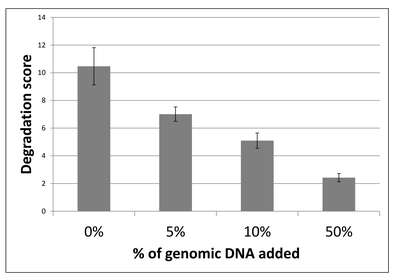
Using matched plasma, tested the Maxwell ccfDNA chemistry vs a manual, competitor system



- Inhibition not observed in any samples
- With seemingly equivalent yields, the degradation factor (short target/longer target) is higher for the Promega chemistry meaning that there is more 170bp as a fraction of total in the Promega samples

6. Genomic spike into ccfDNA reduces degradation score

- A representative ccfDNA sample was spiked with gDNA
- Promega amplification assay was run to determine a degradation score



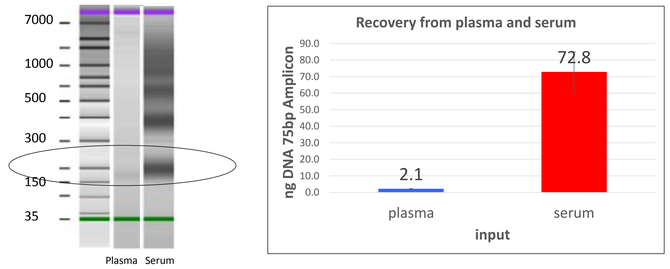
- Increased gDNA contamination results in a lower degradation score
- A low degradation score indicates purification of larger genomic DNA
- Additional wt DNA from hemolysis of white blood cells can cause problems in mutation detection of ccfDNA

The Maxwell® RSC, Maxwell® RSC Chemistries, and Agilent TapeStation are **For Research Use Only. Not for Use in Diagnostic Procedures**.

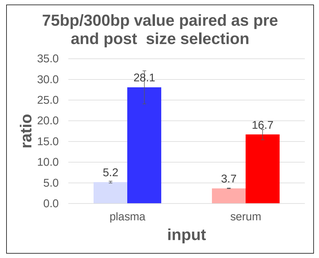
The Promega amplification assay and size selection resin are in development **For Research Use Only. Not for Use in Diagnostic Procedures**.

7. Plasma vs serum ccfDNA characteristics

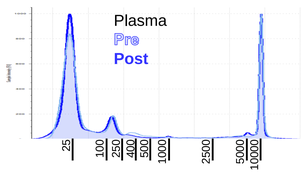
- Plasma yields a small amount of 170bp ccfDNA, whereas serum yields a larger amount of amplifiable DNA appearing as an apoptotic ladder

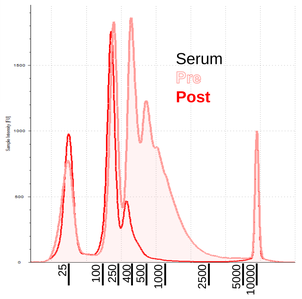


8. Increased degradation scores by size selection



- Use of size selection resin to eliminate larger material decreases background and increases ratio of the 170bp fragment
- Promega resin in development used to exclude materials greater than 400bp





9. Conclusions

- Data derived from a multi-size target qPCR assay can be very effective in determining the characteristics of a ccfDNA sample eluate.
- Using such a method can drive researchers to triage samples and make informed decisions about what downstream methods to use.
- Concentrating on less complex panels vs. highly multiplexed panels or whole exome sequencing for degraded samples can ensure getting the most useful information out of an individual sample, thus saving time, cost, and loss of information about precious samples.

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