Evaluation of pre-analytical variability in circulating cell-free DNA using digital PCR and molecularly-tagged sequencing

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Abstract

Circulating cell-free DNA (cfDNA) analysis holds promise for novel diagnostics in cancer medicine. However, little is understood about the effect of pre-analytical factors on DNA quality and on downstream applications. We compared 3 different blood collection protocols using paired plasma samples from 22 healthy volunteers (EDTA tubes processed within 1 hour, Cell-free DNA BCT tubes at ambient temperature processed within 24 hours and 72 hours) and evaluated differences in cfDNA yield, quality and integrity. Using an in-house multiplexed droplet digital PCR assay, we found no significant difference in cfDNA yield or integrity across blood collection protocols. To assess whether cell-stabilizing preservative in specialty tubes may induce low-abundance noise in cfDNA, we performed molecularly-tagged targeted deep sequencing and developed an informatics approach for enumeration and variant calling from uniquely tagged DNA fragments. Sequencing results showed no significant evidence of preservative-induced cfDNA damage across tested blood collection protocols. Our results suggest that plasma DNA obtained up to 3 days following collection in Cell-free DNA BCT tubes may be used for downstream sequencing in patients with cancer.

Introduction

Cell-Free DNA (cfDNA)

- Released from normal or tumor cells during apoptosis and necrosis
- Present at variable concentration in plasma and highly fragmented (150-200 bp)
- Circulating Tumor DNA comprises 0.01% to 90% of total cfDNA in cancer patients

Applications of cfDNA

- Non-invasive prenatal diagnostics
- “Liquid biopsy” in cancer patients
- Monitoring organ transplant patients

Knowledge Gap

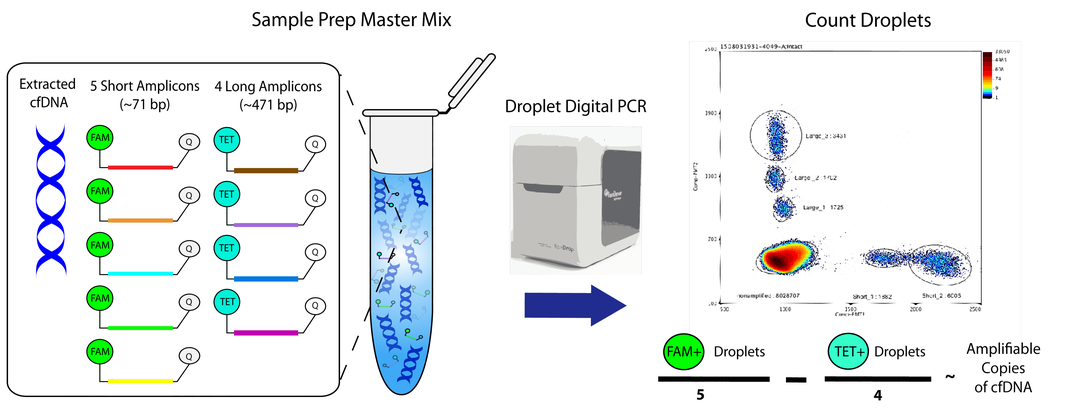
- Lack of uniformity and appropriate controls in pre-analytical phase
  - Sample collection methods
  - Sample processing methods
  - Methods for extraction and quantification

Purpose of the Study

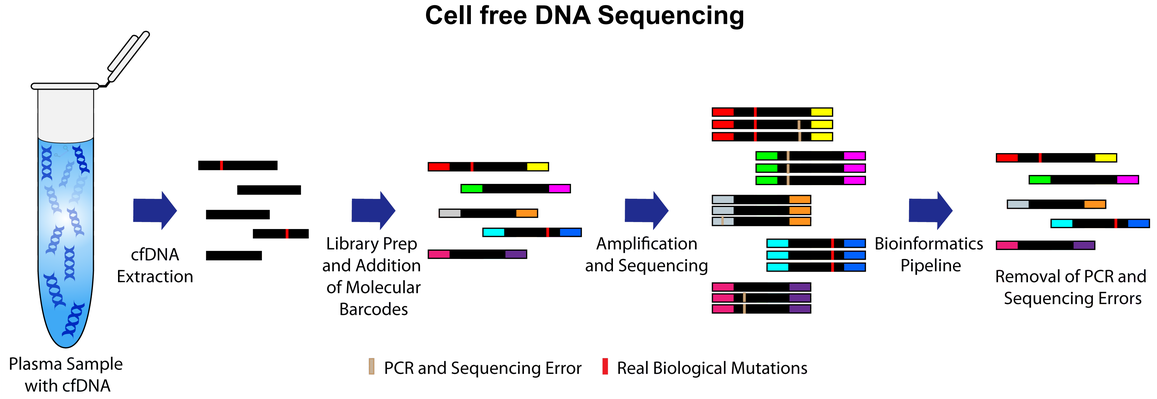
- Compare 3 different blood collection protocols using paired plasma samples from 22 healthy volunteers
  - EDTA processed within 1 hour
  - STRECK Cell-free DNA BCT tubes at ambient temperature processed within 24 and 72 hours
- Evaluate cfDNA yield, integrity, and quality

Methods

Cell free DNA Integrity

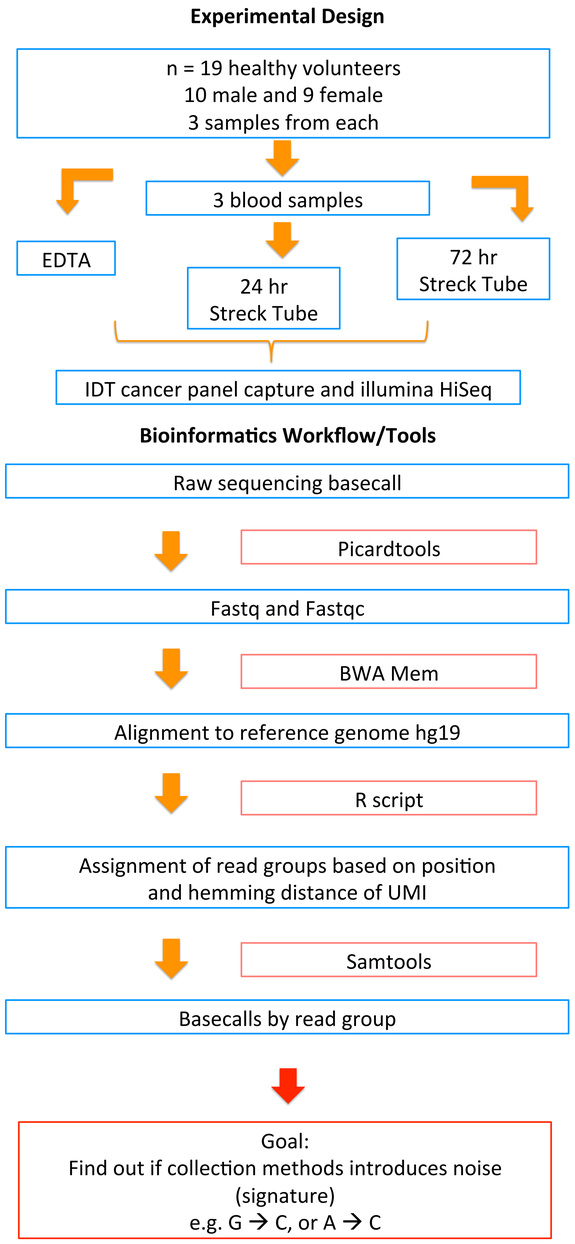


Cell free DNA Sequencing



Results

Experimental Design



Bioinformatics Workflow/Tools

Goal:
Find out if collection methods introduces noise (signature)
e.g. G → C, or A → C

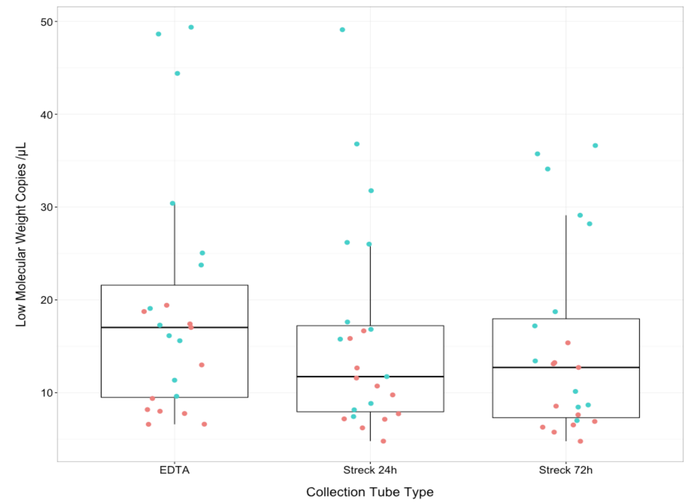


Figure 1: The figure above shows the low molecular weight copies per micro liter of plasma. The male subjects are colors in blue, while the female subjects are in red. Male subjects seems to have more variability in cfDNA levels.

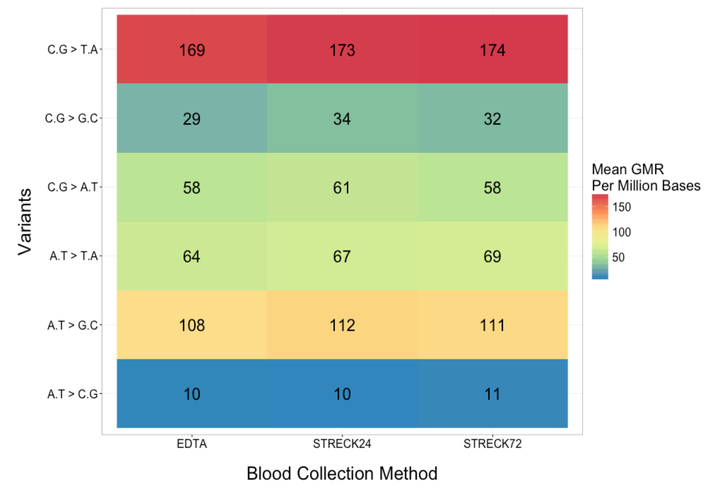


Figure 2: The figure above shows noise profile through mean global mismatch rate per million bases of six different variants in the three blood collection methods. The was no significant difference between in the noise profile of all the six variants in the three blood collection methods.

Discussion and Conclusion

Preliminary Analysis

- The cfDNA quantity and size distribution for the three blood collection methods were not significantly different
  - However there is a noticible decrease in low molecular weight cfDNA in both Streck methods
- The global mismatch rates (GMR) for each variant of the three blood collection methods were also not significantly different
  - However, some variants are slightly higher in Streck 72 than Streck 24 and EDTA
- Thus, plasma cfDNA obtained from Cell-Free BCT tubes upto 3 days shows not significant different from EDTA in regards to integrity and quality

Translational Significance

- Selection of a reliable, efficient blood collection method for cfDNA is required to evaluate utility of circulating tumor DNA as a biomarker.