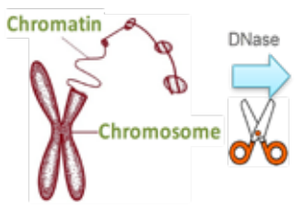
Correlation between mutations found in FFPE tumor tissue and paired cfDNA samples

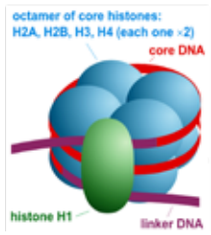
Brittany Niccum PhD, Cindy Heath, Lauren Saunders PhD, Antonia Hur, and Asmita Patel
Beckman Coulter Life Sciences, Indianapolis, IN

Introduction

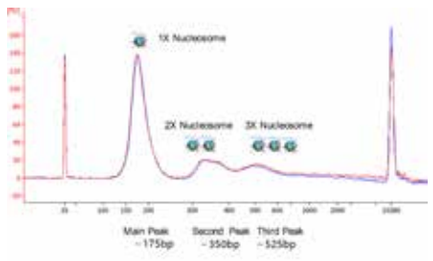
Liquid biopsies represent a promising area of facilitating cancer research as taking blood is less invasive than tumor biopsies. Cell-free DNA (cfDNA) consists of small (150 – 500 bp) DNA fragments that circulate in the blood. Levels of cfDNA tend to be low in healthy, non-pregnant patients and increased in patients with cancer, pregnancy, or extensive tissue damage. cfDNA is believed to be derived mostly from apoptotic cells and a source for biomarkers for a variety of diseases.



Apoptotic or necrotic cell death results in near-complete digestion of native chromatin from normal cell, tumor or fetus.



Each 160-175 bp DNA is wrapped ~1.67 times around one nucleosome. These protein-bound DNA fragments preferentially survive digestion and are released into the circulation, and can be recovered from peripheral blood plasma as cfDNA.



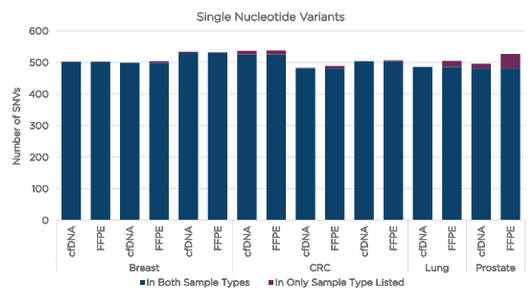
Typical cfDNA peaks characterized by Agilent 2100 Bioanalyzer, with a main peak at 175 bp, second and third peaks at 350 and 525 bp.

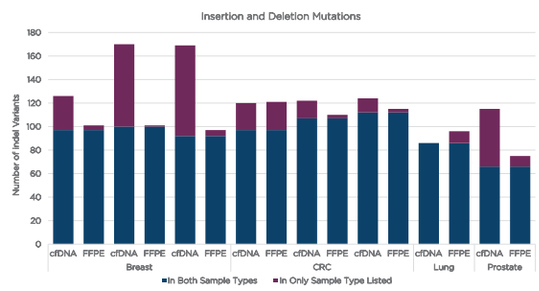
As a non-invasive way to detect disease cfDNA is extracted from blood; however, there is some concern that cfDNA does not contain the same biomarkers as tumor tissue. Tumor tissue is typically removed and stored as formalin-fixed, paraffin-embedded tissue, a process that preserves the morphological structures well but chemically modifies and degrades the nucleic acids.

Despite the difficulties, FFPE tissue (shown on the left) is often used to look for cancer-associated mutations; however it does not always correlate with the mutations seen in cfDNA. In this poster we present a comparison of matched FFPE and plasma samples to determine how many mutation are seen in both tissues. We also look at where the mutational mismatches appear in the chromosome. We found chromosomal regions have different mismatch rates, and we use this to draw conclusions about the best chromosomal locations for biomarkers. We also look at the different results that can come from using multiple different panels.

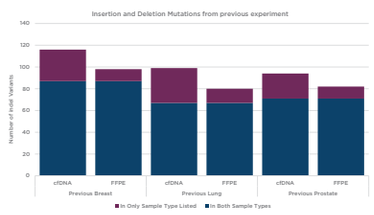
Insertion and Deletion are more often found in cfDNA than DNA extracted from FFPE

Following sequencing the reads were mapped to the genes used in the hybridization panel. Any variants were analyzed further. We separated out the insertion and deletion mutations from single nucleotide variants (SNVs). Mutations found in only FFPE DNA or only cfDNA were compared to each other. The majority of the mutations found were SNVs. There were very few SNVs not found both in the cfDNA and FFPE DNA; neither of the sample types found significantly more SNVs. In contrast, when looking at insertion and deletions (indels), significantly more indels were identified in the cfDNA sequencing (student t-test P=0.03). This is especially true for the cfDNA paired Breast tumors and the prostate tumor. The lung sample showed the opposite result, and one of the CRC tumors showed similar numbers of indels found in both sample types.





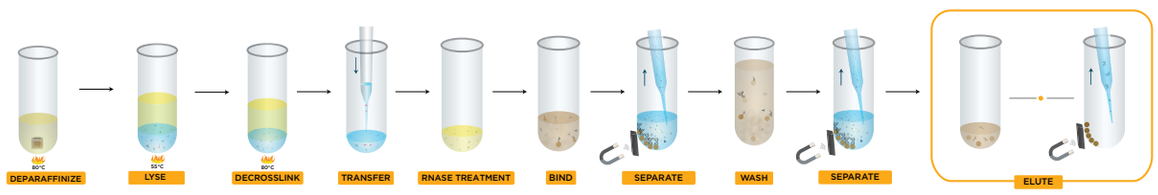
A previous study conducted using three samples, showed results similar to this study. All tumor types showed more indels found in only the cfDNA samples. These results suggest that more indels can be found using only cfDNA samples than only FFPE DNA samples. This result however does seem to be tumor specific and more work should be done to elucidate which tumors types this could be true. Lung tumor samples in the previous data set were different from the data from this study, which suggests that we need more data sets to conclude if cfDNA has a different mutational profile than FFPE DNA.



Methods

Sample Preparation

DNA was extracted from the FFPE tissues using FormaPure XL Total automated on a Biomek i7 hybrid. DNA concentrations of 4 10 uM curls were estimated using the Quant-iT Picogreen; yields varied between blocks. Some blocks had very low yields, most likely due to tissue distribution in the block. The extraction was repeated with 7 10 uM curls. DNA from FFPE was sheared on a Covaris S220 following the 200bp shear protocol. cfDNA was extracted from 1 mL of plasma using the Apostle MiniMax™ High Efficiency cfDNA kit. cfDNA yield varied between the samples as expected. Concentration was estimated using the Kapa hg-Quant kit.



FormaPure XL DNA workflow

Apostle MiniMax™ High Efficiency cfDNA kit workflow

Library Construction

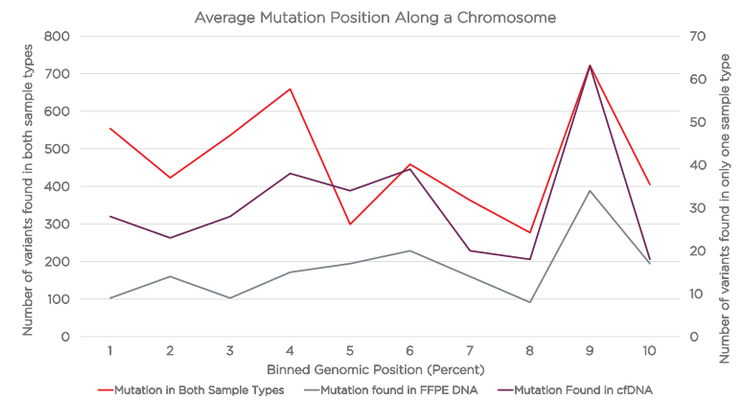
Sheared FFPE DNA and cfDNA was processed with Swift Accel-NGS 2S Hyb DNA Library Kit. DNA input was 100ng. Following the library construction, the Swift Pan-Cancer Hyb Panel was used to gene enrichment. The hybridized libraries were sequenced on the Illumina NextSeq 550.

Analysis

The sequencing data was analyzed using the BWA-enrichment tool on Illumina BaseSpace mapped to the genes in the panel. Below shows the coverage of reads for each of the samples.

Tumor Tissue	Sample Name	Mean Region Coverage Depth	Uniformity of Coverage (Pct > 0.2*mean)	Target Coverage at 1X	Target Coverage at 10X	Target Coverage at 20X	Target Coverage at 50X
Breast 1	cfDNA	1946.5	99.50%	100.00%	100.00%	100.00%	100.00%
Breast 1	FFPE	1322.4	98.10%	100.00%	100.00%	100.00%	99.90%
Breast 2	cfDNA	1677.2	89.50%	100.00%	100.00%	100.00%	99.50%
Breast 2	FFPE	1306.5	99.10%	100.00%	100.00%	100.00%	100.00%
Breast 3	cfDNA	1051.4	90.50%	100.00%	99.90%	99.90%	99.80%
Breast 3	FFPE	1931	98.50%	100.00%	100.00%	100.00%	100.00%
CRC 4	cfDNA	1973.2	99.30%	100.00%	100.00%	100.00%	100.00%
CRC 4	FFPE	1239.8	89.50%	100.00%	100.00%	100.00%	99.50%
CRC 5	cfDNA	2108.1	99.40%	100.00%	100.00%	100.00%	100.00%
CRC 5	FFPE	1480.1	98.70%	100.00%	100.00%	99.90%	100.00%
CRC 6	cfDNA	1772.4	99.00%	100.00%	100.00%	100.00%	100.00%
CRC 6	FFPE	2656	99.60%	100.00%	100.00%	100.00%	100.00%
Lung 7	cfDNA	1775.8	99.50%	100.00%	100.00%	100.00%	100.00%
Lung 7	FFPE	336.9	87.90%	100.00%	99.60%	99.60%	92.30%
Prostate 8	cfDNA	1436.4	99.10%	100.00%	100.00%	100.00%	99.90%
Prostate 8	FFPE	598.6	91.70%	99.90%	99.80%	99.80%	98.60%

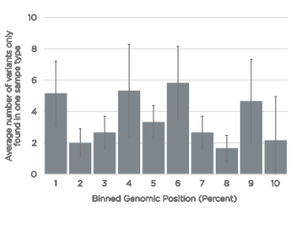
Variants at the beginning of a chromosome are less likely to be found in DNA extracted from FFPE tissues than in cfDNA



To determine if mutations were evenly distributed throughout chromosomes, each chromosome was divided into 10 bins; the first 10% of each of the 23 chromosomes were treated as one bin. The mutations were pooled into the 10 bins for each chromosome. This was done separately for mutations found in only DNA from FFPE, only cfDNA, and found in both FFPE DNA and cfDNA.

The graph to the left shows how the mutations mapped across the 10 bins. A Pearson's correlation was done to test for how differently the mutations mapped across the 10 bins. Mutations found only in FFPE tissue were found to not correlate (P=0.5) as well as mutations found only in cfDNA (P=0.8) to the position of mutations found in both FFPE DNA and cfDNA. A similar result was found in a previous study that was conducted for AACR 2019 (reference below).

When looking at the graph at the top, mutations found in both sample types have a peak that does not occur in the mutations found only in FFPE DNA. This could suggest that there are mutations that are not found in the FFPE tissue that are found in cfDNA at the start of the chromosomes.



Level					Mean
Bin 60	A				5.8
Bin 40	A	B			5.3
Bin 10	A	B			5.2
Bin 90	A	B	C		4.7
Bin 50		B	C	D	3.3
Bin 30			C	D	2.7
Bin 70			C	D	2.7
Bin 100				D	2.2
Bin 20				D	2
Bin 80				D	1.7

Levels not connected by the same letter are significantly different

The figure above from AACR 2019, shows the average number of variants found in each bin of the chromosome for three sample types; the error bars are the standard deviation of the three sample types.

The chart above from AACR 2019, represents the average number of variants found in each bin of the chromosomes. A student T-test was used to determine how different the bins were to each other. Each level that is not connected by the same letter are significantly different from each other. The levels with letter A are significantly different from levels in with letter D

Conclusions

Here we show that sequencing of cfDNA captures the majority of variants that are found when sequencing FFPE DNA. One result shown here is that more indels are identified using cfDNA than with FFPE DNA. This is especially shown here with breast tissue, while this is inconclusive with lung tissue. More samples should be tested before any conclusions can be derived.

Another interesting finding is the distribution of variants across the genome. There is some indication that variants sequenced using cfDNA is correlated better to the variants found with either cfDNA or FFPE. This result could be used to better understand if there is bias that occurs when sequencing FFPE DNA and where this bias could be from such as cross-linking could be more apparent in parts of chromosomes.

Reference: Saunders, L., Hur, A., Niccum, BA., Patel, A. (2019) "Correlation between mutations found in FFPE tumor tissue and paired cfDNA samples" AACR Annual Meeting 2019. Atlanta, Georgia March 29-April 3,2019. DOI:10.1158/1538-7445.AM2019-2233 (AAG-5153PST03.19)

AAG-6085PST10.19

BECKMAN COULTER Life Sciences