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

Lauren Saunders and Asmita Patel - Beckman Coulter Life Sciences

Introduction

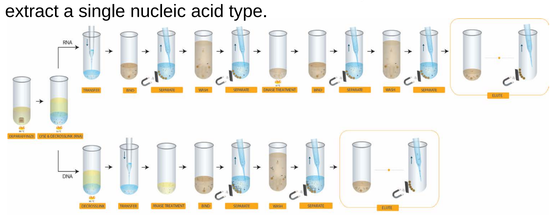
Liquid biopsies represent a promising area of facilitating cancer research as taking blood is less invasive than tumor biopsies. The cell free DNA (cfDNA) present in the blood includes DNA derived from cancer cells and cancer biomarkers can be detected in the extracted cfDNA. However, cfDNA is a less direct view of what is happening in the tumor, and can have a different genetic profile than the tumor tissue itself.

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. This tissue is often used to look for cancer-associated mutations despite these difficulties; however, it does not always correlate with the mutations seen in cfDNA.

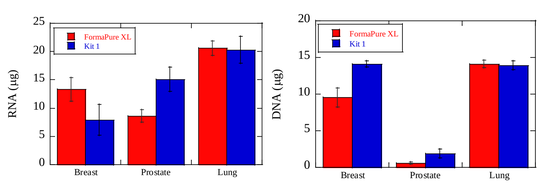
In this poster we present a comparison of matched FFPE and plasma samples to determine how many mutations are seen in both tissues. We also look at where the mutational mismatches appear in the chromosome. Different chromosomal regions can have different mismatch rates, and we use this to draw conclusions about the best chromosomal locations for biomarkers.

Extraction of DNA and RNA from FFPE with FormaPure XL

FormaPure XL (under development) is a new kit from Beckman Coulter Life Sciences that improves the lysis capabilities for larger sample volumes or very difficult to digest tissues. FormaPure XL can extract both RNA and DNA or the entire lysate can be used to extract a single nucleic acid type.

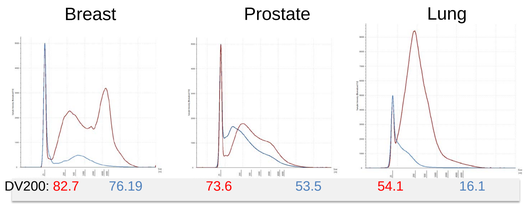


DNA and RNA Yield. Seven 10 µm FFPE curls were extracted in triplicate with both FormaPure XL and a competitor's kit. Yields for both RNA and DNA are shown below. Yields were measured using Quant-It Ribogreen and Picogreen, respectively. Yields varied with tissue block, but high yields were seen with both kits.

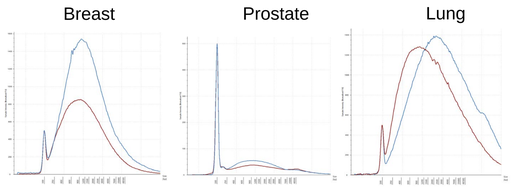


Extraction of DNA and RNA from FFPE with FormaPure XL

Quality of RNA from FFPE. Comparison of FormaPure XL RNA (red) with Kit 1 RNA (blue). FormaPure XL RNA is significantly larger than Kit 1 RNA, as seen by both the traces and the DV200 values.

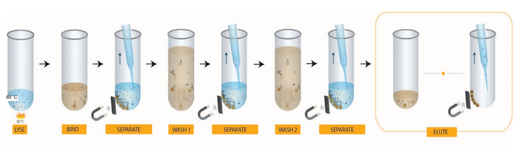


Quality of DNA from FFPE. Comparison of FormaPure XL DNA (red) with Kit 1 DNA (blue).

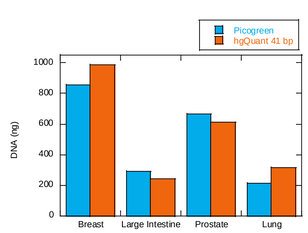


Extraction of cfDNA from plasma

DNA was extracted from 1.5 mL plasma using the Apostle MiniMax kit. This kit uses a bead-based chemistry and is automatable. The workflow is shown below.

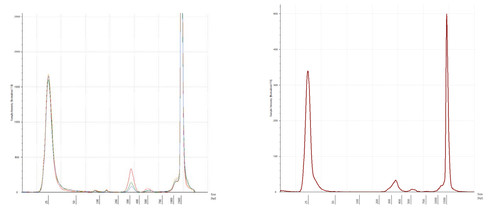


DNA Yield. Yield was calculated by both Quant-it Picogreen and KAPA hgQuant using 41 bp primers. The quantification agreed well with both methods, which is expected for high quality DNA fragments.



Library Preparation

Libraries were created from DNA (25 ng) extracted with FormaPure and Apostle MiniMax using the Accel-NGS 2S Hyb DNA Library Kit. Those libraries (500 ng) were hybridized to the IDT xGen Pan-cancer Panel and further amplified as described in the protocol.



Library size. Representative traces of Accel-NGS 2S Hyb Libraries (left) and trace of pooled samples after IDT hybridization and amplification. Libraries are in the 200 – 400 bp range as expected.

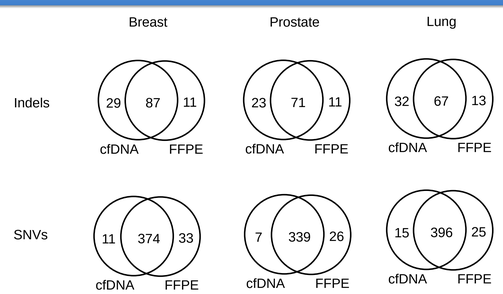
Sequence Quality

Sequencing was done on a NextSeq and Illumina BWA Amplification was used to create scaffold and identify mutations.

Sample Tissue	Sample Type	Percent Aligned Reads	Read Enrichment	Uniformity of Coverage (Pct > 0.2*mean)	Target Coverage at 1X	Target Coverage at 20X
Breast	cfDNA	99.70%	65.00%	95.40%	99.30%	98.80%
Prostate	cfDNA	99.60%	64.60%	95.20%	99.40%	98.90%
Lung	cfDNA	99.70%	65.20%	96.10%	99.80%	99.40%
Breast	FFPE	99.70%	58.00%	97.90%	99.90%	99.60%
Prostate	FFPE	99.70%	70.20%	96.80%	99.80%	99.60%
Lung	FFPE	99.70%	56.50%	97.60%	99.60%	99.00%

Sequencing metrics. All samples ran well and coverage and aligned reads agreed well across samples, as shown by the quality control metrics listed above.

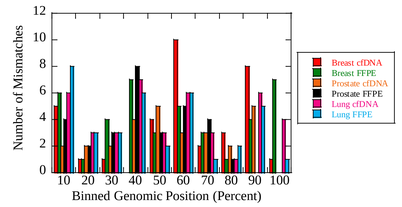
Comparison of cfDNA and FFPE Mutations



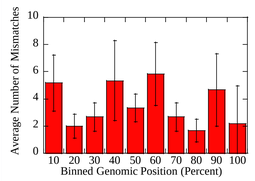
Mutation matches. Mutations seen in matched samples of cfDNA and FFPE were compared. Depending on the cancer type and mutation type (indel or SNV), up to one third of mutations were only seen in one sample type. More indels were seen in cfDNA, whereas more SNVs were found in FFPE.

Genomic Location of Mismatches

Frequency of Mismatches along chromosomes. All samples had similar areas of high mismatch and low mismatch, so samples were combined for analysis. Chromosome location was calculated as a percentage to account for different chromosome lengths. Mismatches were binned in groups of 10% of the chromosome.



Combined Mismatches. Average mismatches across all samples are shown below. Samples were analyzed using a Student's T test and grouped into different levels. The chromosomal regions analyzed have four different levels of sample mismatch shown below. Biomarkers in region D (the first 10 – 30%, 40 – 50%, 60-80%, or 90 – 100% of the chromosome) have the lowest number of mismatches between cfDNA and FFPE samples and would be most likely to be found by both sampling methods.



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 same letter are significantly different.

Conclusions

Both FFPE and cfDNA detect at least two thirds of the observed indels and at least 90% of the observed SNVs. As SNVs are more likely to be found in both tissue types, they are more suitable for biomarker use if looking across different tissues. This was true for all three cancers tested.

Different regions of the chromosome have different rates of mismatches in mutation detection between plasma and FFPE tissue. The first 10-30%, 40-50%, 60-80%, and 90-100% of the chromosome have the lowest rates of mismatch and provide the best locations for biomarkers detectible in both tissues. Future work will focus in increasing the sample size to further narrow down the areas of the chromosome with the highest likelihood of good mutation detection in both plasma and FFPE.