Introduction

Lung cancer represents approximately 20% of all cancer deaths in the United States and is one of the leading causes of cancer-related deaths worldwide. The majority of lung cancer cases are diagnosed as nonsmall cell lung cancer (NSCLC). NSCLC is the most common subtype, accounting for approximately 85% of lung cancer cases, and it is characterized by relatively slow-growing tumors. FFPE tissue is often used for molecular analysis because it is stable and easy to store and handle. However, the quality of DNA extracted from FFPE tissue is often lower than that obtained from fresh tissue.

Methods

Sample Preparation

DNA was extracted from FFPE tissue using FFPE lysis buffer. The DNA was then quantified using Qubit and a Qubit fluorescence spectrophotometer.

Library Construction

Library construction and sequencing of the DNA were performed using NextSeq 500 and NextSeq 550 instruments. Sequencing was performed in a 300-cycle run, generating 75 bp paired-end reads.

Analysis

The sequencing data was analyzed using the BWA algorithm. The alignment results were then further analyzed using the Genome Analysis Toolkit (GATK) to identify single-nucleotide variants (SNVs) and small insertions and deletions (indels).

Majority of Variants are identified in both sample types

We identified 111 single-nucleotide variants (SNVs) and 25 insertion and deletion (indel) events in the sequencing results from both FFPE and plasma DNA samples. The majority of these variants were found in both sample types, indicating that the sequencing methods were effective in detecting both types of mutations.

Variant location

To determine if mutations were evenly distributed throughout the genome, we created a histogram showing the frequency of mutations across the genome. The data was normalized to the average number of mutations per Mb, and the y-axis shows the number of mutations per Mb.

Variants Found at ClinVar Pathogenic Locations

Here we show:

- 135 variants found in only FFPE DNA
- 34 variants found in only cfDNA
- 22 variants found in both FFPE and cfDNA

Below is a table of the sequencing coverage for all the samples. Each sample had sequencing coverage of 60X or greater of the target region.

Conclusions

In summary, we were able to identify 135 variants in the FFPE DNA samples and 35 in the cfDNA samples. The majority of these variants were found in both sample types, indicating that the sequencing methods were effective in detecting both types of mutations. The data suggests that FFPE DNA is a reliable source of DNA for molecular analysis, and it can be used in conjunction with cfDNA to identify additional variants.