# Assessing the Tumor Mutational Burden of Patients with Pancreatic Cancer using Cell Free DNA (cfDNA)

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## Background

Pancreatic cancer has little to no effective indicators for early detection prompting late diagnosis and poor prognosis. Its position in the body also presents an obstacle for tests and treatment. Analyzing cfDNA allows us to detect gene mutations and serves as a biomarker for PDAC. Liquid biopsies are far less invasive and give a more in-depth analysis than tissue biopsies.

## Significance & Aim

- Implement a minimally invasive approach for early detection and monitoring tumor progression and treatment
- Overcome limitations of tissue biopsy including tumor heterogeneity, invasive nature, and inaccessibility

## Methods

- Blood was collected from 19 patients at 3 timepoints
  - Before treatment
  - During treatment
  - After treatment
- Whole blood was spun down to create visible layers
  - Plasma
  - Buffy Coat
  - Erythrocytes
- cfDNA was isolated from plasma using the QIAmp Circulating Nucleic Acid Kit to use as an experimental group
- Germline DNA was isolated from PBMC's using Qiagen DNeasy Blood & Tissue Kit to use as a control group
- DNA was quantified using Qubit to measure DNA concentration
- DNA was prepared for sequencing using ligation-based library prep.
- DNA quality was analyzed using TapeStationD1000 measuring base pair size
- DNA will be sequenced using custom targeted panel and high-throughput NexSeq 500/550 V2.5 kit

## DNA Processing Pipeline

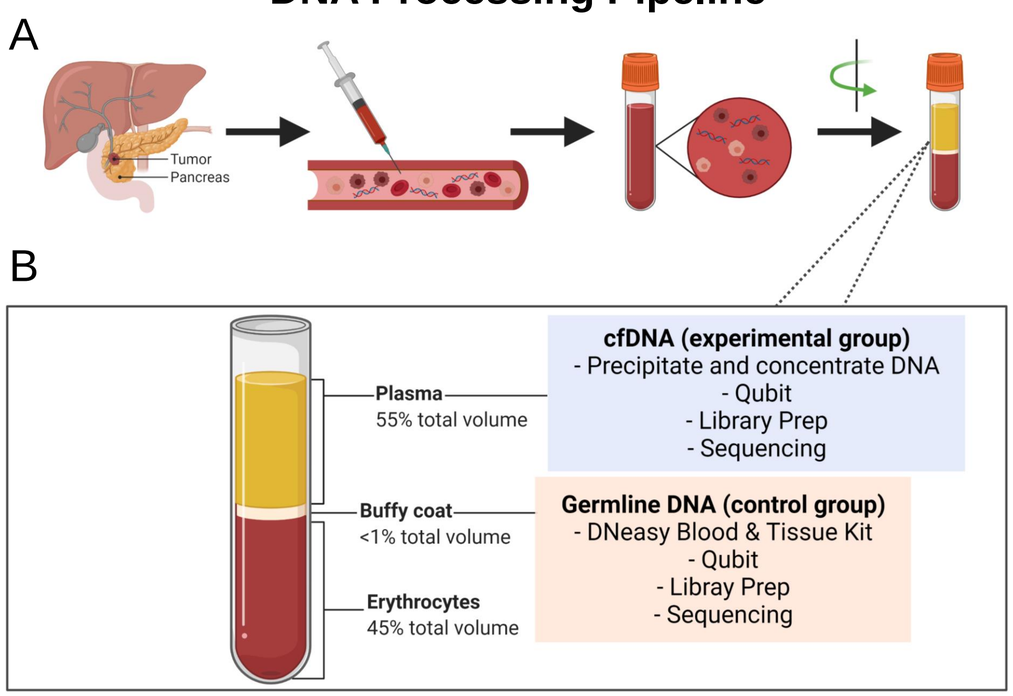


**Figure. 1(A)** Tumor releases cfDNA into the bloodstream. Blood is extracted from patient bloodstream and spun down. **(B)** Blood is separated into 3 layers then taken down a sample processing pipeline isolate, purify, amplify, and sequence DNA.

## DNA Qualification

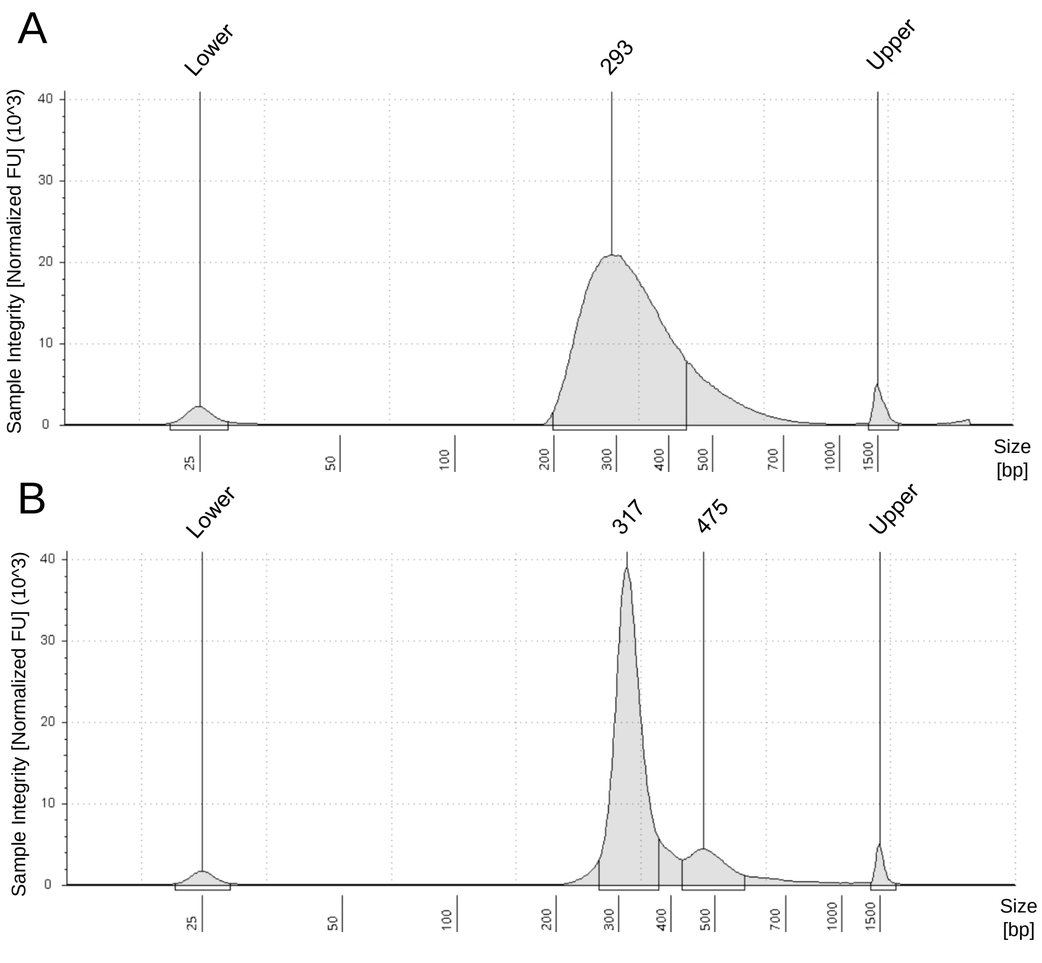


**Figure. 2(A)** Final library from high-quality sample analyzed using TapeStation D1000 assessing average base pair size. **(B)** Final library from low-quality sample analyzed using TapeStation D1000 assessing average base pair size.

## DNA Quantification & Qualification across various timepoints throughout treatment

A

| Timepoint | Average Input Concentration (ng/μL) | Average Amplification Concentration (ng/μL) | Average bp Size |
|---|---|---|---|
| Germline | 48.2 ± 26.2 | 152.8 ± 64.5 | 339 ± 15 |
| Baseline | 9.0 ± 7.3 | 90.9 ± 88.1 | 365 ± 35 |
| Treatment | 6.3 ± 3.6 | 132.1 ± 66.5 | 362 ± 13 |
| Post-treatment | 4.3 ± 2.4 | 157.0 ± 83.8 | 358 ± 17 |

B

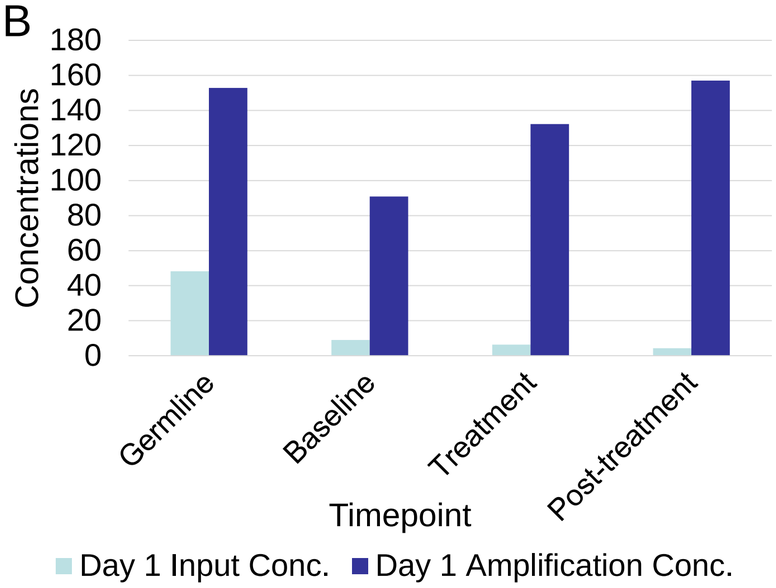


**Figure. 3(A)** Average base pairs and DNA concentrations indicate adequate quality and quantity to proceed with library preparation. **(B)** Initial DNA concentrations after library amplification increased by 3, 10, 21, and 37-fold for germline, baseline, treatment, and post-treatment, respectively.

## Results

Average base pair size fell between 300-400 base pairs for each timepoint, as can be seen in figure 3A, indicating high quality DNA and successful molecular-barcode insertion during library preparation. Increase in initial DNA concentration, as seen in figure 3B, indicates successful amplification during Day 1 library preparation and sufficient DNA quantity to proceed with sample processing pipeline.

## Conclusion

This sample processing pipeline is capable of producing high quality DNA in quantities sufficient for targeted next generation DNA sequencing. Sequencing cfDNA using a custom targeted panel meant to detect key mutational sequences associated with pancreatic cancer can help personalize cancer treatments while possibly predicting and improving patient outcomes.