# Addressing Mitochondrial Heteroplasmy Using Next Generation Sequencing

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## Introduction

The importance of the mitochondria to cell life and proliferation is well known. Adenosine triphosphate (ATP) is vital to the transport of macromolecules and is the main source of energy for cellular function. Further, mitochondria have their own genome (size ranging from 14-16 kb) and undergo replication separately from the rest of the cell. They acquire their own mutations and have their own pattern of inheritance.

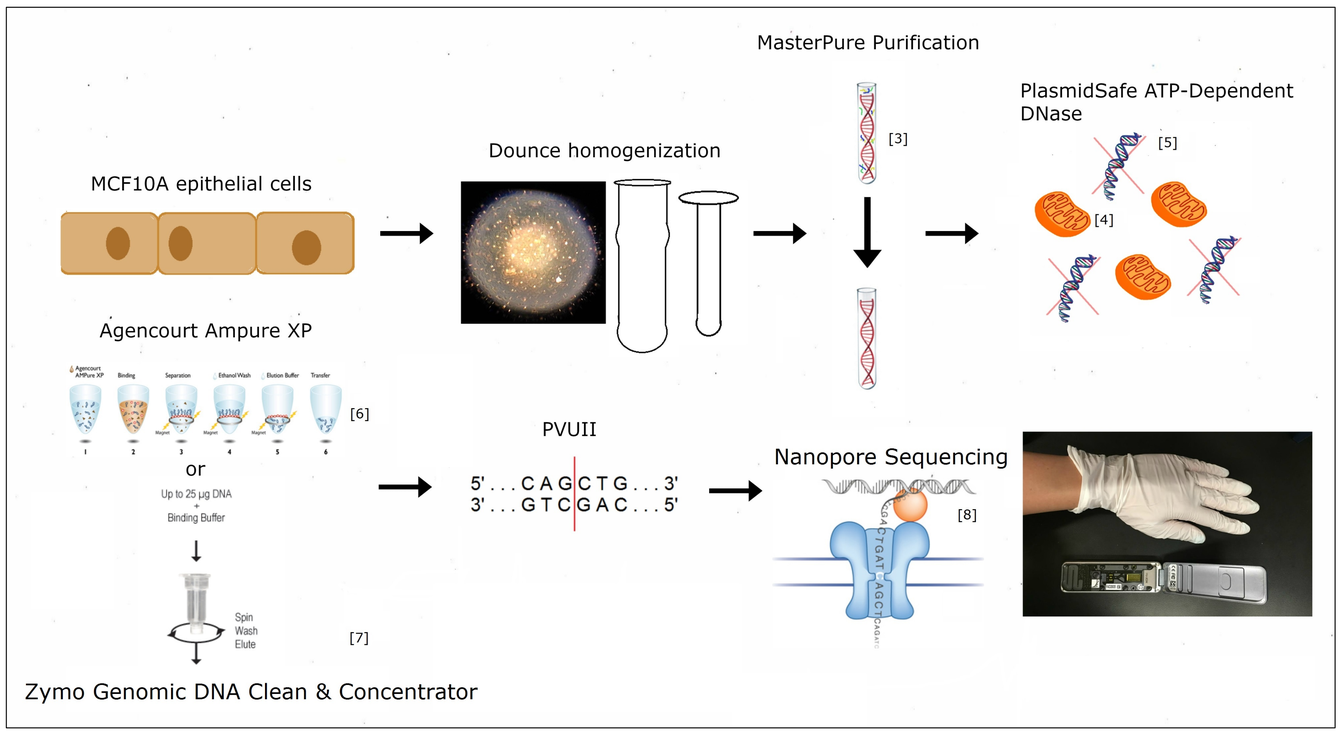
Mitochondrial heteroplasmy is the phenomenon in which a cell contains more than one mitochondrial DNA (mtDNA) genotype.[1] Altered presence of heteroplasmy has been associated with a number of human diseases.[2] Better understanding heteroplasmy would offer new insight into these diseases and potentially be useful for developing treatments.

Despite its importance and relatively short length, the mitochondrial genome has only been sequenced using non-full length amplicons. Short sequences are not ideal for studying heteroplasmy, or for assessing native modifications such as methylation. Fragmented amplicons and PCR bias render data not ideal for studying heteroplasmy. Therefore, sequencing the mitochondrial genome in its entirety would greatly advance our understanding of complexity and heteroplasmy in mtDNA, and is the focus of this project.

## Materials and Methods

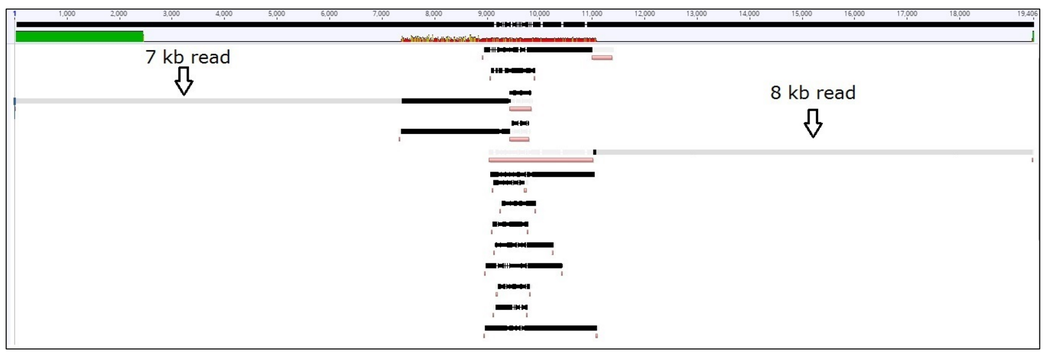
First, cells were swollen using a hypotonic buffer and broken apart using a Dounce homogenizer. The mitochondria were then separated out using differential centrifugation. Next the sample underwent purification with MasterPure to remove contaminants and further isolate the mtDNA. PlasmidSafe Purification was used to hydrolyze all linear DNA, leaving circular mtDNA intact. The remaining contaminants were removed using either Agencourt AMPure XP (uses magnetic beads to separate DNA from the rest of the sample) or Zymo Genomic DNA Clean and Concentrator (unique spin column for low volume elution). Lastly, the sample was cut with restriction enzyme PVUII to linearize the mtDNA,and sequenced using Oxford Nanopore's MinION. (see fig below)

A second method we employed included whole DNA extraction from cells, digestion with single cutter PVUII and Blue Pippin automated size selection for fragments the size of the mitochondrial genome.

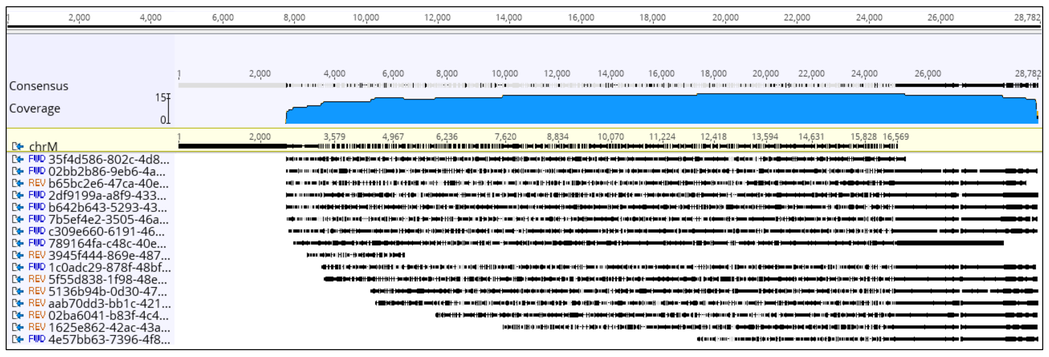



## Results

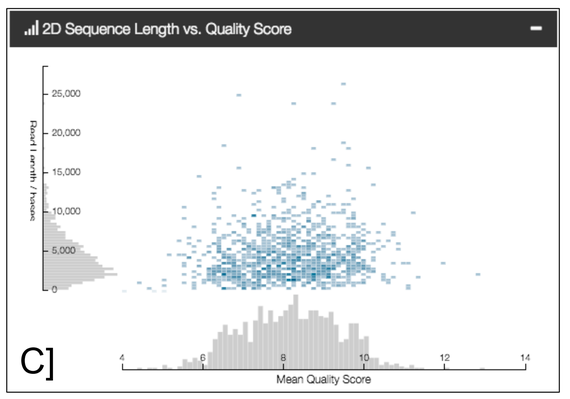
A]

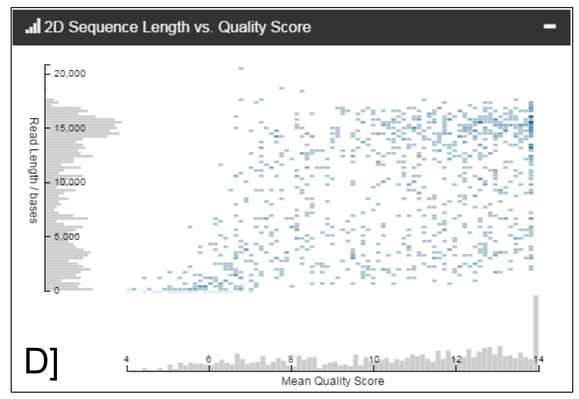



B]




- Successful assembly of a mitochondrial genome using native (non-amplified) mtDNA fragments using Geneious
- Fragments aligned to their respective sections of the mitochondrial genome (see figs ab)
- 1 mtDNA read out of every 100 reads
- Figure A: Sequence alignment of sample derived using the Dounce homogenization protocol described in methods, reads 7-8 kb long
- Figure B: Sequence alignment of sample using the alternative Blue Pippin size selection method, reads 16 kb long




- Quality score represents the probability that a base was read incorrectly. The higher the quality (Q) score, the smaller the probability of error. For example, a score of 10 would indicate that the probability of an incorrect base call was 1 in 10 (90% accuracy), and a score of 20 would indicate the probability as 1 in 100 (99% accuracy).
- Figure C: Quality score report for the reads from the described methods
- Figure D:Quality score report for the reads from alternative Blue Pippin size selection method

## Works Cited

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## Conclusions/ Discussion

- Using two separate methodologies, we were able to sequence full length mtDNA from the cell line MCF10A (breast epithelial cells)
- Although this method is able to yield long, native mtDNA sequences, improvements in throughput and read length are necessary. We were able to successfully concentrate genomic DNA while eliminating contaminants, but a significant amount of the desired material was lost at each step- in order to gather enough mtDNA to sequence, the initial mitochondrial isolation required a total of 1-5 billion cells. Despite this, there was significant enrichment of the sample so that 1 in every 100 reads were mitochondrial, as opposed to 1 in every 400 in unenriched samples.
- The longest length read from the Dounce homogenization protocol described in methods was 8kb long, but using the second Blue Pippin size selection protocol read length increased to 16 kb
- Future developments will include optimization of an alternative protocol which involves total DNA isolation, then PVUII digestion and automated size selection for fragments 12-18kb to preferentially enhance for mtDNA. We also hope to investigate methylation patterns and how they vary between cell lines and disease/normal samples.