

Investigation of Novel Fixation Method for Single-Cell DNA and RNA Sequencing

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Cancer has been and continues to be one of the most difficult diseases to detect, treat, and cure. Due to mutations in our genome resulting in abnormal masses of cell growth, it is vital to explore the differences that makeup these abnormal cells as compared to normal cells. RNA sequencing (RNA-seq) has recently become a method of choice that allows for identification of global phenotypic changes between tumors and normal tissues. With the popular bulk RNA-seq technique, many cells in a tissue are used for transcriptome analysis. From the average of these cells, bulk RNA-seq tells us major transcriptional differences between normal tissue and cancerous tissue and goes even further to distinguish between cancer types. However, in single cell RNA sequencing, the different gene expression and the amount of gene expression can be analyzed across every different cell within a cancerous/normal tissue. Single cell RNA sequencing requires cells to be individually isolated. The method relies on central dogma of molecular biology and a clear link between RNA and protein abundance. Both single and bulk RNA seq require RNA to be extracted, converted to cDNA, amplified via PCR, and sequenced and matched with a reference genome. The amount of matches can then be quality controlled and normalized, leaving a quantifiable number for a particular gene and the amount expressed.

While the single cell RNA-seq process is greatly developed, there are many challenges in the initial isolation step. There have been many isolation protocols created like limiting dilution but they continue to be inefficient with lower quality of data. Many protocols also fail to store the nuclei for long periods of time, creating a stressful time constraint when working with samples. In this experiment, we plan to utilize RNA extracted from nuclei fixed with a novel fixating protocol in hopes of storing nuclei for longer periods of time. With this added protection, we hope to match the quality of data from fresh RNA extractions. We will utilize sequencing libraries and the R program to determine the quality of matches of this new isolation procedure as compared to RNA from untreated cells and RNA freshly extracted from the nuclei. Through this data analysis, we examine how long nuclei can be preserved and the quality of the RNA extracted from it. This will greatly expand our accessibility and time with single cell analysis in the future which aids vital cancer immunotherapy and detection development.