Optimization of RNA Isolation from Organoids for RNA Sequencing

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1. Abstract

RNA sequencing (RNA-Seq) is increasingly employed by researchers in clinical trials and drug development [1]. Patient-derived organoids (PDO) have emerged as a promising model for Pancreatic ductal adenocarcinoma (PDAC) research and personalized medicine. As a part of the TOR lab's commitment to study chemotherapeutical drug's mechanism of action through transcriptional profiling using the PDO model, this study aims to establish foundational pipeline for RNA isolation from organoids post-treatment. The drug screening from 384-well was adapted to a larger setting to ensure enough RNA yield for sequencing. RNA was isolated post-treatment and purified using TRI-reagent combined with a column based extraction kit. The study found that organoids with the initial count of 2 to 3 x 10^5 cells, treated with 5-Fluorouracil (5-FU) at the inhibitory concentration 20, provided sufficient RNA for sequencing. The removal of Matrigel and addition of chloroform phase separation step further improved RNA quality and integrity, as degraded samples were obtained with the column alone. For further improvements, it is recommended to test alternative sample lines, extraction kits, and smaller well plates.

2. Introduction

RNA-Seq is a molecular technique for identifying and quantifying gene expression levels. In a therapeutic context, transcriptional profiling provides insights into the complex mechanisms of pharmaceuticals, potentially leading to the development of more targeted treatments [1][2].

Organoids have emerged as a promising three-dimensional in-vitro model. These structures are made up of cells that replicates the characteristics of a specific organ [3]. PDO has become a powerful preclinical tool for PDAC research and personalized medicine, demonstrating their ability to retain the genetic and phenotypic characteristics of the original tumor, thereby facilitating the development of tailored therapeutic approaches for individual patients [4][5]. The integration of PDOs and transcriptional profiling holds great promise in advancing precision oncology for PDAC, allowing researchers and clinicians to develop more effective and personalized treatment approaches [2][3][4].

3. Aims and Leading Questions

The first objective of this thesis is to assess the feasibility of adapting the 384-well plate drug screening setup to a larger well-plate format, to yield adequate RNA quantities for bulk RNA sequencing.

- Does utilizing a 12 well plate for the drug screening assay facilitate the recovery of RNA in optimal quantity and quality?
- Is the RNA quality and integrity affected by the presence of Matrigel?

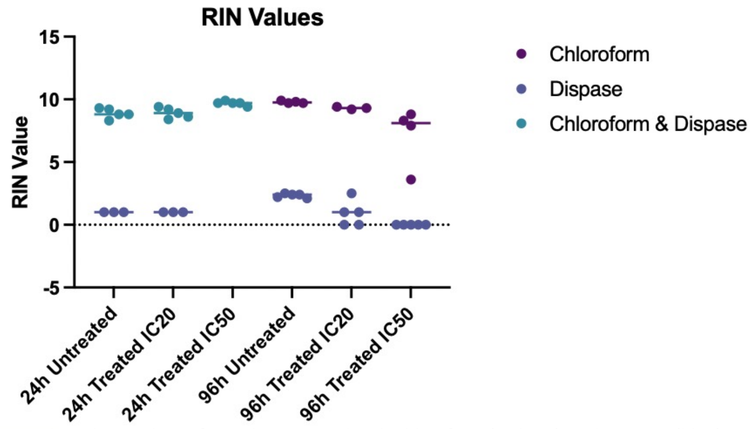
4. Materials and Methods

The drug screening assay of patient-derived xenograft organoids was adapted from a 384-well plate to a 12-well plate setting. Various variables were tested for seeding, including different starting cell amounts and different Matrigel coating volumes. After two days, the organoids were treated for 24 and 96 hours with 5-Fluorouracil at inhibitory concentrations of 20 and 50. RNA extraction was performed post-treatment. Two distinct protocols, TRI-reagent and column-based kits, were tested and combined for RNA purification. The quantification was primarily done using a spectrophotometer. Quality and integrity assessments were mainly conducted with:

- RNA 260/230 and 260/280 purity ratios obtained from the spectrophotometer
- RNA Integrity Number (RIN) value measured by the Bioanalyzer

5. Results

Trials on the 12-WP, with an initial cell count of 2 to 3 x 10^5 and treatment using the IC20 concentration of 5-FU, enabled the recovery of sufficient RNA. However, Matrigel was found to interfere with RNA extraction, as its removal using dispase led to a statistically significant increase in RNA concentration from treated organoids. There was also a noticeable trend of increasing purity ratios (260/230 and 260/280), although the average RIN value remained at 1. To enhance RNA quality, a phase separation step with chloroform was introduced, resulting in higher RIN values.



Fiigure 1: Plotted RIN values of samples prepared with Chloroform (violette), with Dispase (blue) and with both Chloroform and Dispase (turquoise) (Villapando, 2024)

6. Discussion & Conclusion

The drug screening protocol was effectively adapted to a larger 12-well plate format, though additional purification steps were necessary to obtain high-quality RNA. Despite encountering unexpected challenges, a protocol was successfully optimized to isolate RNA within a concentration range suitable for RNA sequencing. To further improve the process, it is recommended to test alternative cell lines, extraction kits, and smaller well plates.

References

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[5] Faried et al., 2022, pp. 1-10

Figures

Figure 1 Villapando, A. (2024). *Plotted RIN Values of samples prepared with Chloroform, Dispase and both Dispase and Chloroform*. medi.