PB3439: Multiomic Single Cell Analysis of Primary Pancreatic Ductal Adenocarcinoma Enhanced by Fixation

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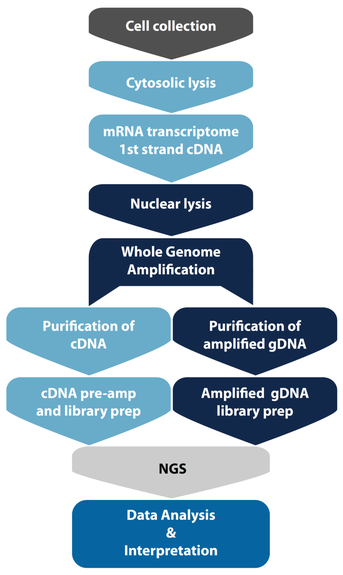
Introduction

We have developed a method for improved multi-omic retrieval of genomic DNA and mRNA from challenging cell types. Application of a non-formaldehyde fixation protocol enabled us to preserve single cells for genomic and whole transcriptomic amplification. We have married this process to ResolveDNA™ and ResolveOME™ technologies to recover 99% of the genome and full-length mRNA transcripts. We have demonstrated that the quality of data from fixed and live single cells is indistinguishable in a NA12878/HG001 cell line. Following proof of concept, we have applied this technique to enhance recovery of genomic data from a notably difficult sample, pancreatic ductal adenocarcinoma (PDAC) primary cells and cell lines. scRNAseq protocols have been challenging to apply in PDAC given high intrinsic nuclease activity and dense desmoplastic stroma.

Here, we demonstrate resolution of genetic and transcriptomic heterogeneity in PDAC patient derived cell lines and a primary tumor. Dual measurement of DNA and mRNA from single cells provides a unique platform to study therapeutic resistance, given the varied mechanisms of cell intrinsic resistance to treatment.



Methods

Samples: ~1 million cells from each of 4 cell lines, AsPC1, Panc0203, Panc1, MiaPaca2, were sorted live by FACS. An equivalent number of each were removed from culture and pelleted. This pellet was resuspended in 100uL of BioSkryb fixative. This suspension was incubated for 15 minutes on ice. After which the cells were again pelleted, and buffer exchanged for FACS sorting. Live and propidium iodide enriched fixed cells were sorted into a 96 well plate. Multiple plates of single cells were assayed using ResolveOME. Primary PDAC cells were fixed following the above method. Live and fixed primary patient cells were sorted by pan cytokeratin (CK) enrichment, and or propidium iodide presence or absence. ResolveDNA and ResolveOME assays were performed. Patient tissues were acquired on IRB protocol #: 18-469 through DF/HCC.

ResolveOME workflow: Cells were lysed and subject to mRNA amplification and 1st strand cDNA synthesis. Nuclei were subsequently lysed for whole genome amplification, and both cDNA and gDNA were purified for downstream amplification and library prep. These steps were performed by BioSkryb Genomics.

BaseJumper analysis: Single Cell data was generated using BaseJumper™ DNAQC and RNA Expression pipelines. Count matrices were exported and analyzed using Seurat to generate UMAP projections and perform differential expression analysis. CNV plots were generated using BaseJumper.

Results

Figure 1: Fixation protocol resolves cell types without disturbing mRNA expression
Single PDAC cells were sorted into two 96 well plates, Unfixed & Fixed. 8 Cells were sorted per cell line, per condition into each plate for a total of 64 cells. Amplification using ReolveOME was was performed on each cell to resolve genetic and transcriptomic heterogeneity.

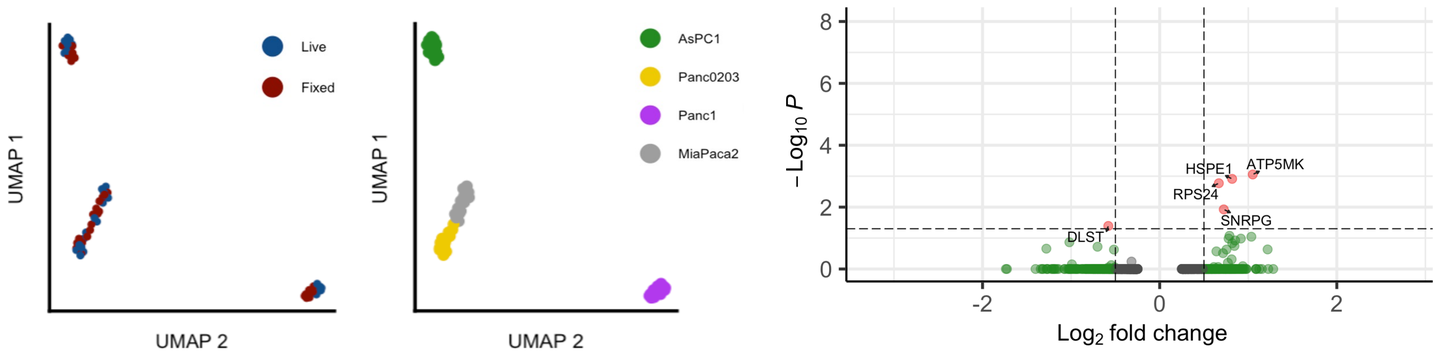


Figure 2: Genetic heterogeneity within cell lines. Copy number variation profiles were plotted for clones in each cell line. Aberrant copy number profiles were observed in all sequenced cells. At least two distinct clones were present in each cell line sequenced (n=16 cells per line).

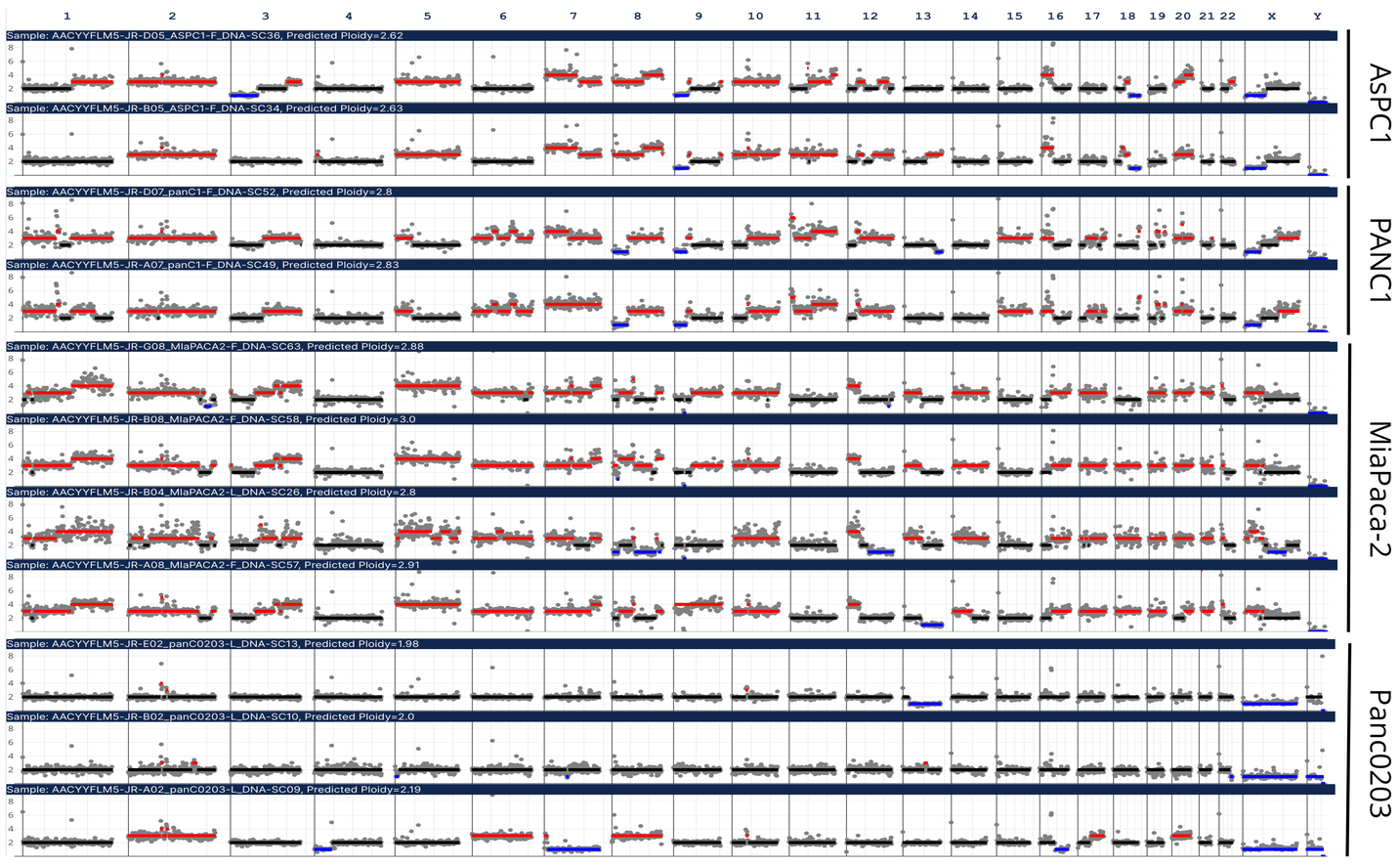


Figure 3: Matched gene representation for live and fixed cell lines.
Proof of concept data demonstrating the utility of the fixative for multiomic single cell applications. Here we focus specifically on transcriptome recovery with ResolveOME. The proportion of exonic reads exceeds 70% with negligible intergenic contamination for both live and fixed single cells. The average protein coding gene count for each cell exceeded 4,000 for both live and fixed cells.

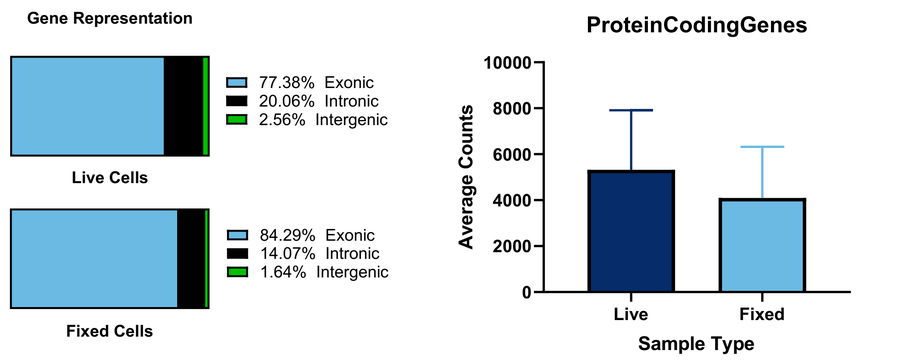


Figure 4: Uniform transcript coverage for live and fixed cell lines.
Coverage over the length of each gene was plotted from the 5′ to 3′ body. Sequenced cells have uniform gene coverage in both live and fixed conditions.

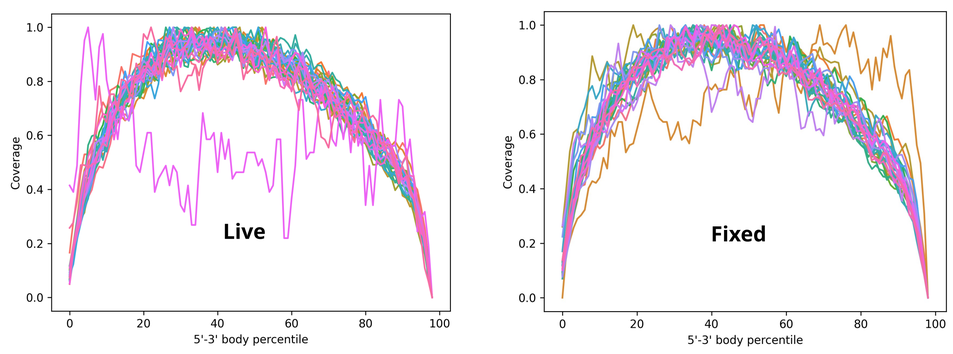
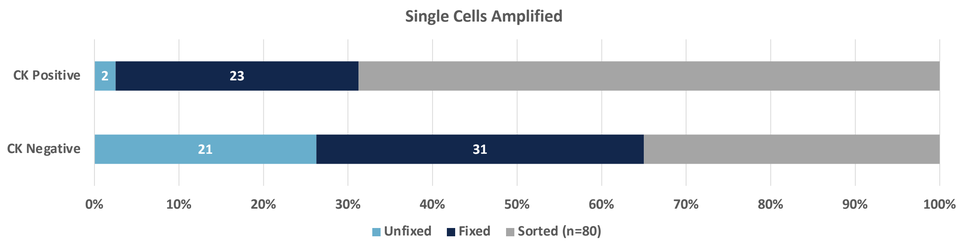


Figure 5: Fixation enabled recovery of live primary cells
Single PDAC cells were sorted into two 96 well plates, Unfixed & Fixed. 80 Cells were sorted into each plate. 40 cells on each plate were FACS sorted by CK positive and 40 by CK negative. Amplification of primary PDAC cells using ResolveOME was determined. For both CK positive and CK negative cells, fixation enhanced recovery of genetic material from FACS sorted cells.



References

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2. Zawistowski et al. biorXiv (2022). https://doi.org/10.1101/2022.04.29.489440

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Conclusions

- Genetic heterogeneity was observed in PDAC cell lines from unbiased sequencing of just 16 cells
- Fixation of cells prior to ResolveOME genome and transcriptome sequencing does not impact gene expression and can be used for samples with difficult handling requirements

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