High-throughput single-cell targeted DNA sequencing from frozen, fixed and preserved solid tumor samples reveals complex genomic variation and clonal propagation

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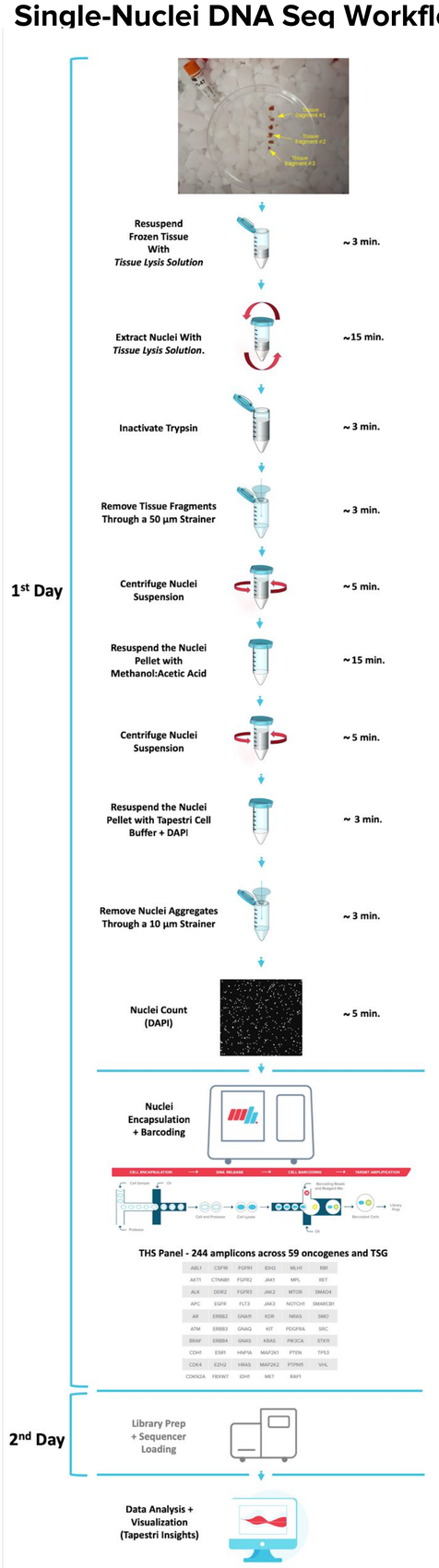
Abstract

Biologically annotated specimens such as frozen, fixed and preserved tissues are key sources of cells for genomic analysis. Bulk NGS using archived solid tumor samples is inadequate to fully characterize somatic variation buried in the landscape of cellular populations. Single-cell targeted DNA sequencing provides an essential solution to elucidate and map genomic variation in such materials. Although the study of frozen, fixed and preserved tissues at the single-cell level is compromised by preservation processes, the isolation of nuclei allows the recovery of suitable gDNA templates. Common tissue disaggregation processes can be complicated by persistence of conglomerated cellular components, ruptured nuclei, and other insoluble extracellular matrices. For challenging samples, we developed a nuclei isolation protocol that demonstrates optimal performance for high-quality targeted DNA sequencing from archived human solid tumor samples.

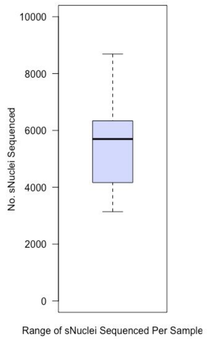
This process begins with physical maceration of ~10-100 mg preserved tissue or 20-100 uM sections, suspension followed by enzymatic treatment, filtration and centrifugal collection. After cell straining, nuclei are ready for counting, staining and sequencing. Fluorescent microscopy using membrane, cytoplasmic and nuclear stains reveal highly purified intact nuclei, recovering at least 500K nuclei from 30-50 mg of tissue containing >70% nucleated cells by H&E-staining. Many human samples provide outstanding quality nuclei suspensions, including cryopreserved cell lines, PBMCs, bone marrow, liver, brain, breast, colon, lung, prostate and melanomas. Fixed and preserved specimens also yield nuclei readily interrogated by targeted DNA sequencing panels. Nuclei suspensions are readily processed with the Tapestri Platform for single-cell DNA analysis. Leveraging droplet microfluidics and barcoding, the workflow enables high uniformity of ~90%, low ADO of ~10%, and typical nuclei recovery rates exceed 10%. Up to 20,000 nuclei can be interrogated in each run with catalog or custom panels for any tumor type. Here, a 59-gene tumor hotspot panel was used to study five melanoma metastases. The single-cell data enabled the unique reconstruction of tumor sample clonal phylogeny unresolved by bulk analysis. Also, low prevalence metastatic subclones masked in bulk NGS data were detected in normal liver samples. In summary, we show that diverse types of archived tumor tissues are readily dissociated with this universal nuclei protocol. Researchers now have a highly sensitive, targeted, customizable solution for revealing genomic variation and clonal propagation in complex archived solid tumor samples.

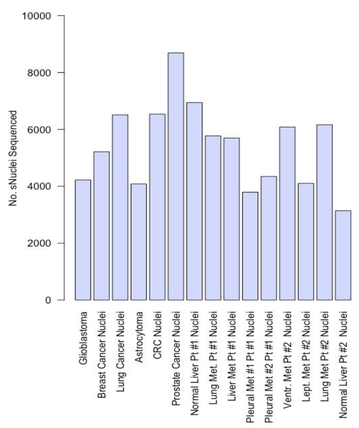
Single-Nuclei DNA Seq Workflow



Results

Nuclei Protocol Enables Any Tissue Type



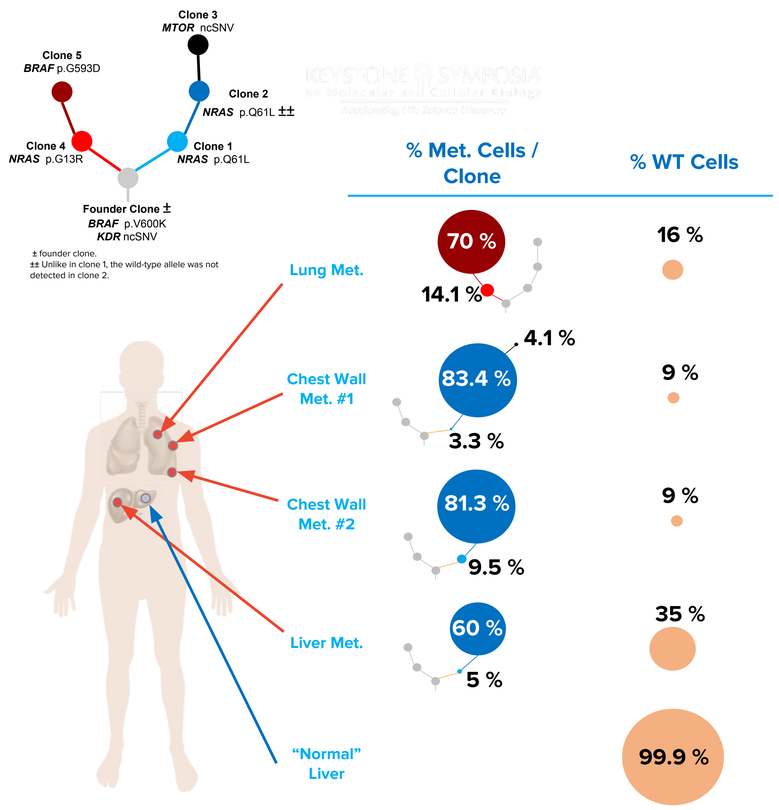


- Average of 5,419 single nuclei sequenced per sample

True Clonal Architecture of Met. Melanoma (Patient 1)

Clones	Founder Clone	Clone 1	Clone 2	Clone 3	Clone 4	Clone 5
MTOR ncSNV	-	-	-	45%	-	-
NRAS p.Q61L	-	58%	100%	100%	-	-
KDR ncSNV	MUT	38%	50%	51%	50%	50%
BRAF p.V600K	MUT	68%	78%	78%	70%	74%
NRAS p.G13R	-	-	-	-	100%	100%
BRAF p.G593D	-	-	-	-	-	29%

Phylogenetic Reconstruction (Patient 1)



Single-Nuclei DNA Seq - High Quality Metrics

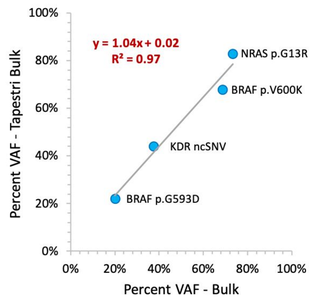
Sample ID	Seq No. Reads	Panel Uniformity*	ADO Rate**	Reads Amplicon/Nuclei	Single-Nuclei Throughput
Normal Liver	146 M	90.16 %	7.61 %	60x	6,943
Lung Met	108 M	91.39 %	5.14 %	58x	5,772
Liver Met.	131 M	89.75 %	6.06 %	65x	5,697
Chest Wall Met #2	113 M	87.30 %	3.57 %	85x	3,791
Chest Wall Met #1	118 M	86.07 %	8.04 %	78x	4,346

* Panel uniformity = % of panel whose coverage depth is ≥ 20% of the mean coverage depth
** ADO: % of cells called homozygous at a known heterozygous locus

Total = **26,549 nuclei**

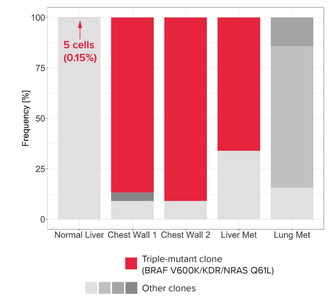
Bulk vs. Single-Nuclei Ensemble VAFs Correlation

High Correlation of Single-nuclei vs. Bulk

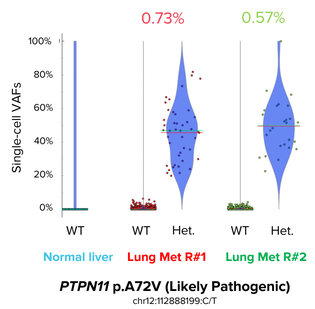


High Sensitivity & Consistency Detecting Very Low Frequency Cell Clones

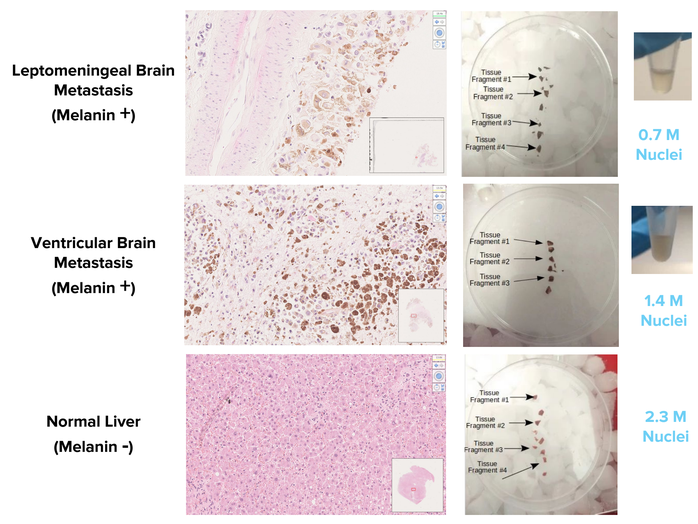
Oligo Metastatic Cells in “Normal” Tissue (Patient 1)



Reproducibility (Patient 2)



Single-Nuclei Library Prep is Resistant to Known Potent PCR Inhibitors



Sample ID	Seq No. Reads	Panel Uniformity*	ADO Rate**	Reads Amplicon/nuclei	Single-Nuclei Throughput
Lept. Brain Met.	148 M	92.21 %	8.23 %	89x	4,104
Vent. Brain Met.	117 M	90.57 %	6.07 %	50x	6,082
Normal Liver	146 M	90.16 %	7.61 %	60x	6,943

* Panel uniformity = % of panel whose coverage depth is ≥ 20% of the mean coverage depth
** ADO: % of cells called homozygous at a known heterozygous locus

Conclusions

- Extracted nuclei from fresh frozen tissue, generate high quality single-cell genomics data
- Single-cell data is highly correlated with matched bulk next generation sequencing (NGS) data for variant identification
- Single-cell data enables the unique reconstruction of tumor sample clonality and evolution unresolved by bulk sample analysis
- The Tapestri Platform is highly sensitive, detecting consistently, low-prevalence clones at a frequency as low as 0.15%, and which were unreported in bulk NGS data
- Known potent PCR inhibitors such as melanin, does not interfere with single-nuclei isolation, library preparation and sequencing

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