Abstract #1663

# Analytical Methods to Identify Tumor Heterogeneity and Rare Subclones in Single Cell DNA Sequencing Data from Targeted Panels

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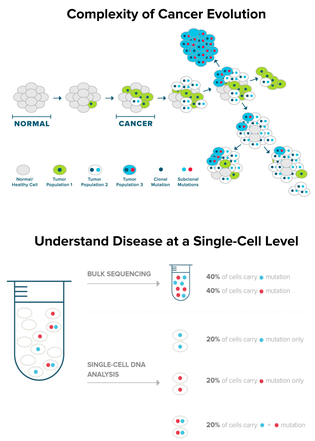
**Conflicts of interest:** S.S., M.M., S.W., D.K., N.V., K.G., A.S., N.L., A.P., R.D., H.V., K.J. are employees and shareholders of Mission Bio, Inc.

## Abstract

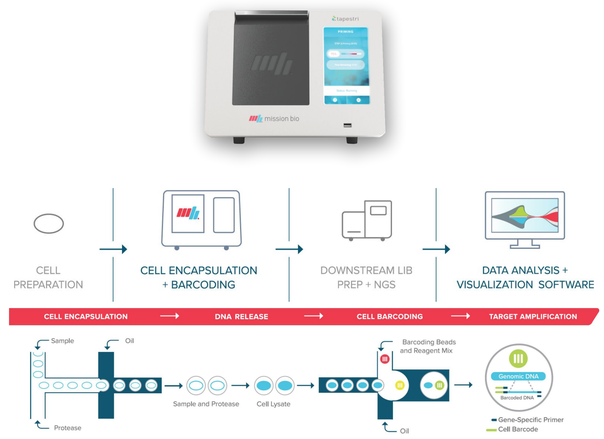
**Background:** With the advancements of single cell sequencing technologies it is now possible to interrogate thousands of cells in a single experiment. Single-cell RNA-Seq has been available for several years but high-throughput single-cell DNA analysis is in its infancy. Therefore, it is essential to develop new capabilities for assessing genetic variation present in rare cells and to better understand the role that these cells play in the evolution of tumor progression. To address these challenges and enable the characterization of genetic diversity in cancer cell populations, we developed a novel approach to identify mutation signatures which define subclones present in a tumor population.

**Methods:** Here we present subclone identification method using data generated on the Tapestri single-cell DNA platform and analyzed by Tapestri analytical workflow. The pipeline steps involve obtaining raw reads from the sequencer, removing adapters, aligning and mapping the reads, calling individual cells, and identifying genetic variants within each cell. After filtering for high quality variants, we then filter for data completeness to ensure only high quality data is used in downstream processing. The variant-cell matrix is then subjected to identification of subclones. Top variants defined the signature of each subclone are also identified. To validate our methodology, we used two different targeted sequencing panels on model systems with known truth mutations. Our pipeline shows the distinct clusters correlating with titration and cell line ratios. Cluster associated signature mutations were also identified. The pipeline can be used for multi sample analysis with time-series data from diagnosis to relapse or from primary site to metastasis to understand clonal diversity. These data demonstrate the utility of the Tapestri platform, the analytical pipeline, and associated data visualization capability. Our approach addresses key issues of identifying rare subpopulations of cells down to 0.1%, and transforms the ability to accurately characterize clonal heterogeneity in tumor samples. This high throughput method advances research efforts to improve patient stratification and therapy selection for various cancer indications.

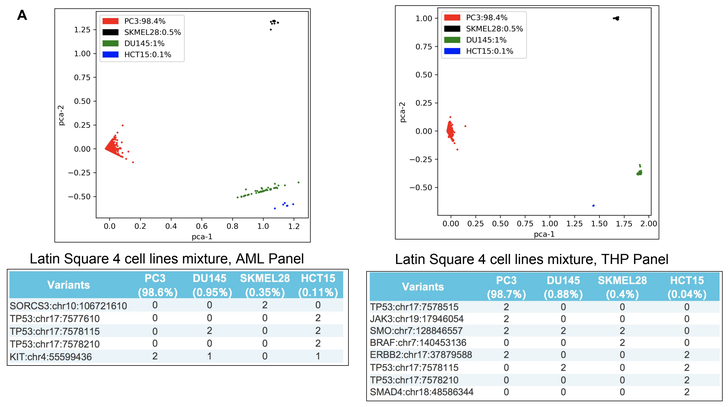
## Why Single-Cell ?




## Mission Bio Tapestri Workflow Overview




## Up to 0.1% High Sensitivity in Subclone Identification and Clonal Signature

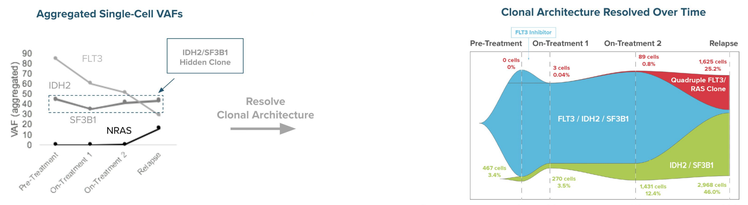



| Variants | PC3 (98.6%) | DU145 (0.95%) | SKMEL28 (0.35%) | HCT15 (0.11%) |
|---|---|---|---|---|
| SORCS3:chr10:106721610 | 0 | 0 | 2 | 0 |
| TP53:chr17:7577610 | 0 | 0 | 0 | 2 |
| TP53:chr17:7578115 | 0 | 2 | 0 | 2 |
| TP53:chr17:7578210 | 0 | 0 | 0 | 2 |
| KIT:chr4:55599436 | 2 | 1 | 0 | 1 |

| Variants | PC3 (98.7%) | DU145 (0.88%) | SKMEL28 (0.4%) | HCT15 (0.04%) |
|---|---|---|---|---|
| TP53:chr17:7578515 | 2 | 0 | 0 | 0 |
| JAK3:chr19:17946054 | 2 | 0 | 0 | 0 |
| SMO:chr7:128846557 | 2 | 2 | 2 | 0 |
| BRAF:chr7:140453136 | 0 | 0 | 2 | 0 |
| ERBB2:chr17:37879588 | 2 | 0 | 0 | 2 |
| TP53:chr17:7578115 | 0 | 2 | 0 | 2 |
| TP53:chr17:7578210 | 0 | 0 | 0 | 2 |
| SMAD4:chr18:48586344 | 0 | 0 | 0 | 2 |

To validate the analytical methods, Latin square 4 cell lines assay was performed using two targeted panels AML and THP. **A).** Distinct clusters assigned by truth variants correlates well with titration and cell line ratios in both AML and THP assays. Rare subclone and clonal signatures were detected at ~0.1%. **B).** Successful cluster and distinguish spiking-in cell line in PBMC sample at 1%.

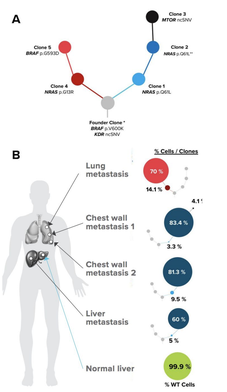
## Longitudinal Analysis of AML Samples and Detection of Remission Clones




- Expansion of **double-mutant relapse clone** never detected w/bulk NGS
- Acquisition of **RAS clone** detected too late w/bulk NGS

To understand which mutations are real drivers of AML versus ones that are just passengers or contributors, longitudinal analysis using AML panel was performed to reveal the therapy resistant clones. Pre-treatment leukemia sample, on-treatment and relapse BM samples were analyzed by single-cell sequencing. The analysis resolves evolution of 3 subclones basing on combination of 4 mutations, helps to understand clonal composition of cancer for making dynamic changes in treatment.

## Clonal Distribution and Phylogeny Analysis to detect Rare Subclones and Tumor Purity




| Clones | Wild-type Clone | Founder Clone | Clone 1 | Clone 2 | Clone 3 | Clone 4 | Clone 5 |
|---|---|---|---|---|---|---|---|
| Tissues containing clone | All | N/A | Liver Chest wall 1 Chest wall 2 | Liver Chest wall 1 Chest wall 2 | Chest wall 1 | Lung | Lung |
| MTOR ncSNV | WT | WT | WT | WT | HET | WT | WT |
| NRAS p.Q61L | WT | WT | HET | HOM | HOM | WT | WT |
| KDR ncSNV | WT | HET | HET | HET | HET | HET | HET |
| BRAF p.V600K | WT | HET | HET | HET | HET | HET | HET |
| NRAS p.G13R | WT | WT | WT | WT | WT | HOM | HOM |
| BRAF p.G593D | WT | WT | WT | WT | WT | WT | HET |

**A).** A phylogenetic tree illustrates the evolutionary path of each clone. **B).** Clonal distribution in all tissue samples. *founder clone. **Unlike in clone 1, the wild-type allele was not detected in clone 2. **C).** All clones identified across all tissue samples using the Tapestri Platform. The inferred putative founder clone is based on the presence of the mutational pattern in all clones. WT is wild-type for that allele, HET is heterozygous for that mutant allele, and HOM is homozygous for that mutant allele. **D).** Triple-mutant clone distribution in all tissue samples. Rare subclones detected in normal liver at 0.15% (5 cells). **E).** The distribution of mutant cells in all samples (shown in gray) indicate varying levels of tumor purity. Using single-cell genotype data the wild-type cells can be directly detected and removed from downstream analysis.

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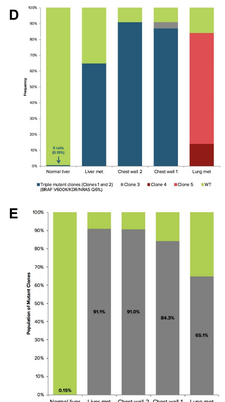
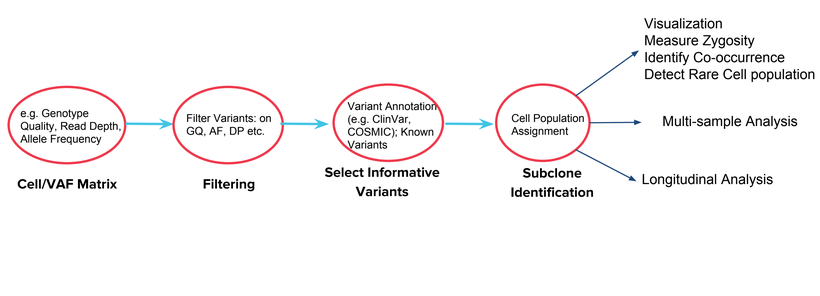
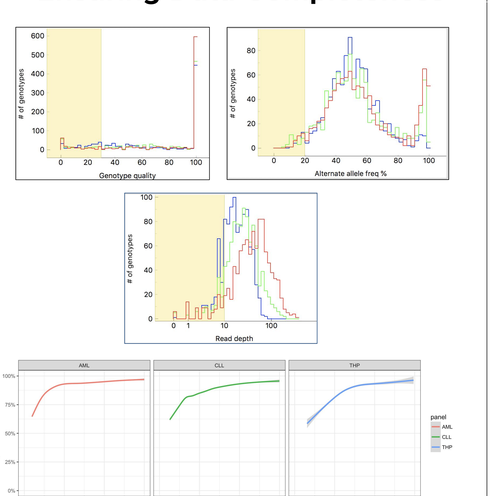



## Primary Analysis and Subclone Identification


## Ensuring Data Completeness




## Selecting Informative Variants

**Keeping Clinically Relevant Variants**

| Variant | Function | Protein | Coding impact | ClinVar | DANN | Whitelist |
|---|---|---|---|---|---|---|
| ASXL1:chr20:31022959:T/C | coding | ASXL1:p.L815P | missense | not provided | 0.31 | False |
| TP53:chr17:7578211:C/T | coding | TP53:p.R174Q | missense | Pathogenic | 1.00 | False |
| TP53:chr17:7577581:A/G | coding | TP53:p.Y196H | missense | Conflicting interpretations of pathogenicity | 1.00 | False |
| TP53:chr17:7578115:T/C | intronic | | | Benign | 0.65 | False |


| | Variant 1 | Variant 2 | Variant 3 | Variant 4 |
|---|---|---|---|---|
| Cell1 | 0 | 0 | 1 | 0 |
| Cell2 | 0 | 1 | 1 | 0 |
| Cell3 | 0 | 0 | 0 | 0 |
| Cell4 | 1 | 1 | 0 | 0 |

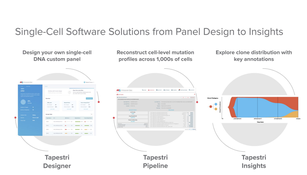
| Subclones | C1 | C2 |
|---|---|---|
| EZH2:chr7:148504854:A/AGACTT | Het | Hom |
| TP53:chr17:7577581:A/G | Het | WT |
| TP53:chr17:7578211:C/T | Het | WT |
| | | |
| Total | 10557 (77.79%) | 3015 (22.21%) |
| 1% K562 | 6467 (99.11%) | 58 (0.89%) |
| 50% K562 | 4090 (58.04%) | 2957 (41.96%) |

Tapestri INSIGHTS: RAJI-K562 Cell Line Mix

## Results and Conclusions

With the analytical method demonstrated above, we show the distinct clusters correlating with titration and cell line ratio. We were also able to identify the cluster associated signature mutations. These data demonstrate the utility of the Tapestri platform, and the analytical pipeline, and associated data visualization capability. Our approach has the potential to address the key issues of identifying rare subpopulations of cells and transforms our ability to accurately characterize clonal heterogeneity in tumor samples. This high throughput method of accurately characterizing clonal populations should lead to improved patient stratification and therapy selection for various cancer indications.

## Mission Bio Solution and Future Work




Catalog Panels for Hematology and Solid Tumor

**TAPESTRI SINGLE-CELL DNA PANELS**

| | # genes | # amplicons | Target regions coverage (Kbp) | Panel Uniformity |
|---|---|---|---|---|
| AML Panel | 19 | 50 | ~18.5 | >90% |
| CLL Panel | 34 | 286 | ~54.9 | >90% |
| Myeloid Panel | 47 | 330 | ~66.0 | >80% |
| Tumor Hotspot Panel | 59 | 244 | ~40.0 | >80% |