A multimodal single-cell workflow to interrogate cellular responses to cancer therapy

#2261

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Abstract

Understanding the genomic landscape of cancer in single cells can be valuable for the characterization of molecular events that drive evolution of tumorigenesis and fostering progress in identifying druggable regimens for patient treatment scenarios. We report a new approach to measure multiple modalities simultaneously from up to 10,000 individual cells using microfluidics paired with next generation sequencing. Our procedure determines targeted protein levels, mRNA transcript levels and somatic gDNA sequence variations including copy number variants. This approach can resolve over 20 proteins, 100s of targeted transcripts and DNA amplicons. We employ oligo-conjugated antibody panels to probe cell surface markers and targeted RNA and DNA amplification to resolve gene expression levels and genomic variants. Cell suspensions are first stained with the antibody panel then loaded onto the Mission Bio Tapestri for generation of a droplet. This droplet biochemistry allows for concurrent cell lysis and release of gDNA/mRNA. A second droplet formation event brings together a barcoded bead and multiplex PCR amplification reagents. After amplification, the combined libraries are sequenced yielding a multimodal readout. We applied this technique to several dynamic immunology and oncology in vitro models. We explored the relationship between genotype-to-phenotype in breast carcinoma cell lines, PBMC cell population responses to mitogenic perturbations and imatinib impact on retinoblastoma and BCR-ABL driven leukemic cells. Our pilot studies demonstrate the utility of multimodal resolution in further illuminating cellular states and biological responses in cancer cells.

Methods

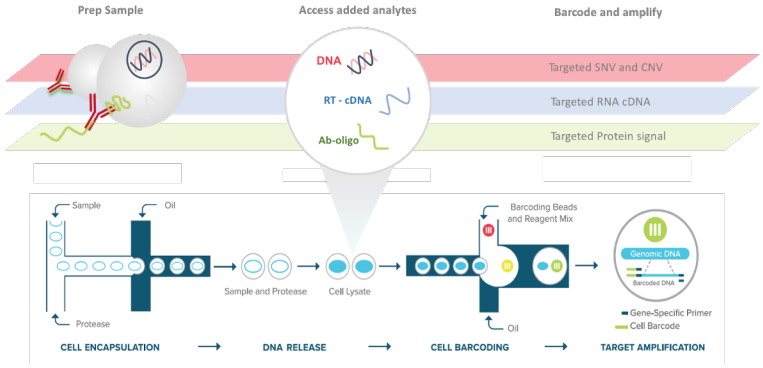
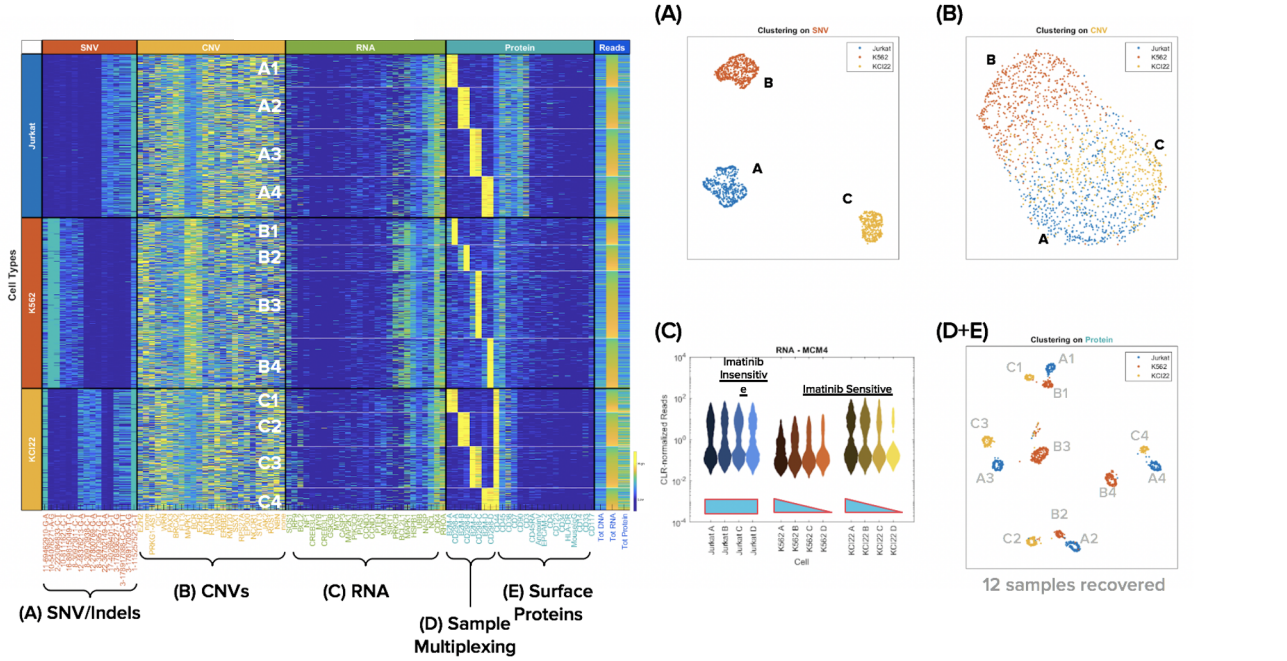


Figure 1
The Tapestri platform uses a two-step workflow. Cells are lysed in the first droplet. Amplification and barcoding of DNA, cDNA, and antibody tags are performed in the second droplet.

Results



Triomics with hashing (SNV/Indels, CNV, RNA, Protein, Sample Hashing). Three cell lines x 4 cancer treatment doses shows 12 different combinations of sample/treatment in one run.

K562, KCL-22 and Jurkat cultured cells were divided into four dosage groups: Three receive sublethal doses of the tyrosine kinase inhibitor Imatinib (Gleevec) at 10, 100, 250 nM and the fourth no dose. The cells were cultivated and harvested for the Tapestri triomic workflow. The cells were stained with a 17-plex Antibody-oligo conjugate panel, washed and loaded onto Tapestri. The cells were interrogated with a 56-plex RNA target panel consisting of immune response and pathway response genes, as well as an 88-plex gDNA panel. Separate libraries were made for each analyte with those from the same single cells sharing cell barcodes. Cells were identified based on SNVs detected from the DNA.

BCR-ABL fusion transcript	Cell line	Sensitivity	Specificity
p210 b2a2	KCL-22	93.6%	96.5%
p210 b3a2	K-562	96.8%	95.7%
p190	TOM-1	70.2%	99.7%

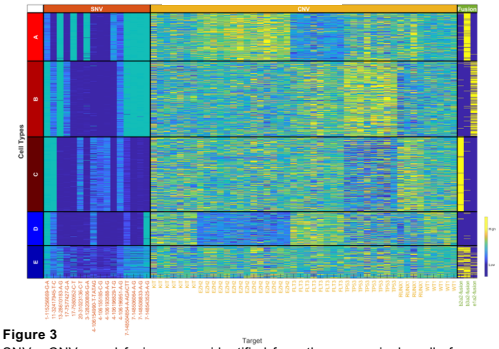


Figure 3
SNVs, CNVs, and fusions were identified from the same single cells from DNA reads and RNA reads.

A mix of K-562, TOM-1, KCL-22, and KG1 cells were mixed and run on Tapestri with an acute myeloid leukemia panel (128 amplicons) and primers to detect three BCR-ABL1 fusions transcripts.

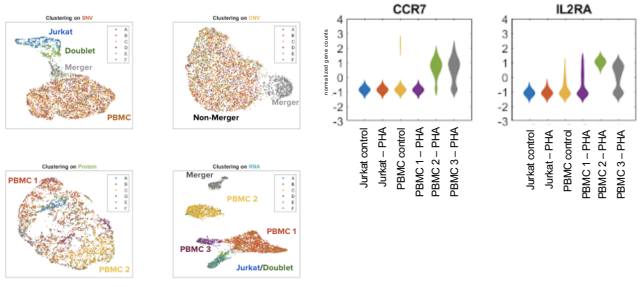


Figure 4
Triomics (SNV/Indels, CNV, RNA, Protein, Sample Hashing).

A mix of PBMCs with a spike in of Jurkat cells were split into two flasks, one stimulated with PHA and the second was the control. The cells were were stained with 17 tagged antibodies for protein expression and 4 tagged antibodies for cell hashing to differentiate between stimulated cells and the control cells. The gDNA and cDNA were amplified with an 88 plex DNA panel and 121 plex RNA panel, respectively. Separate libraries were made for protein expression, gene expression, and DNA but combined during analysis based on a shared cell barcode.

UMAP clustering based on SNVs (top left), CNVs (top right), protein (bottom left) and RNA (bottom right) can distinguish Jurkat cells from mergers and can identify three PBMC populations.

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