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

Dalia Dhingra, Aik Ooi, Adam Sciambi, Shu Wang, Saurabh Gulati, David Ruff
Mission Bio, Inc., South San Francisco, California, USA

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 imatinib impact on 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.

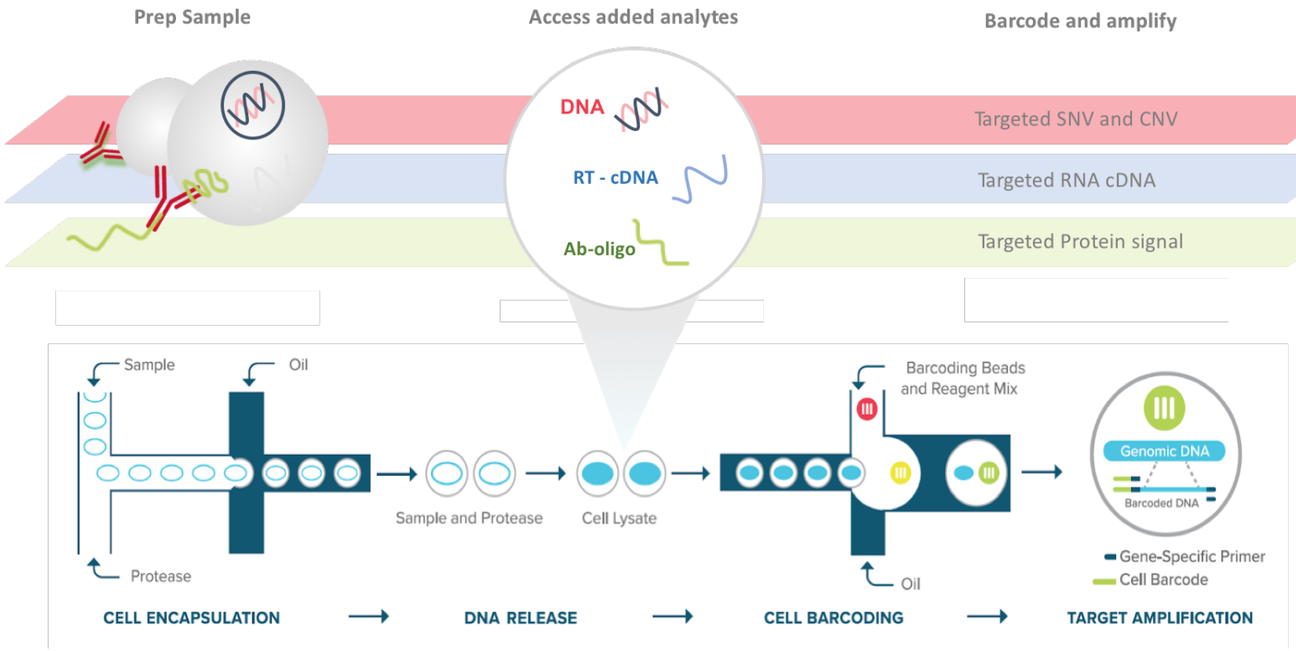


Figure 1: Single cell workflow for paired genotype and phenotype on the Tapestri platform

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Contact: dhingra@missionbio.com

# SNVs, CNVs, gene expression, and protein expression from the same single cells

This system utilized K562, KCL-22 and Jurkat cell lines. The 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 received no dose. The cells were cultivated and harvested for the Tapestri multi-omic workflow. The cells were be stained with a 17-plex Antibody-oligo conjugate panel, washed and loaded onto Tapestri. The cells were then interrogated with a 56-plex RNA target panel consisting of immune response and pathway response genes. An 88-plex gDNA panel was also be applied for the detection SNVs and CNVs.

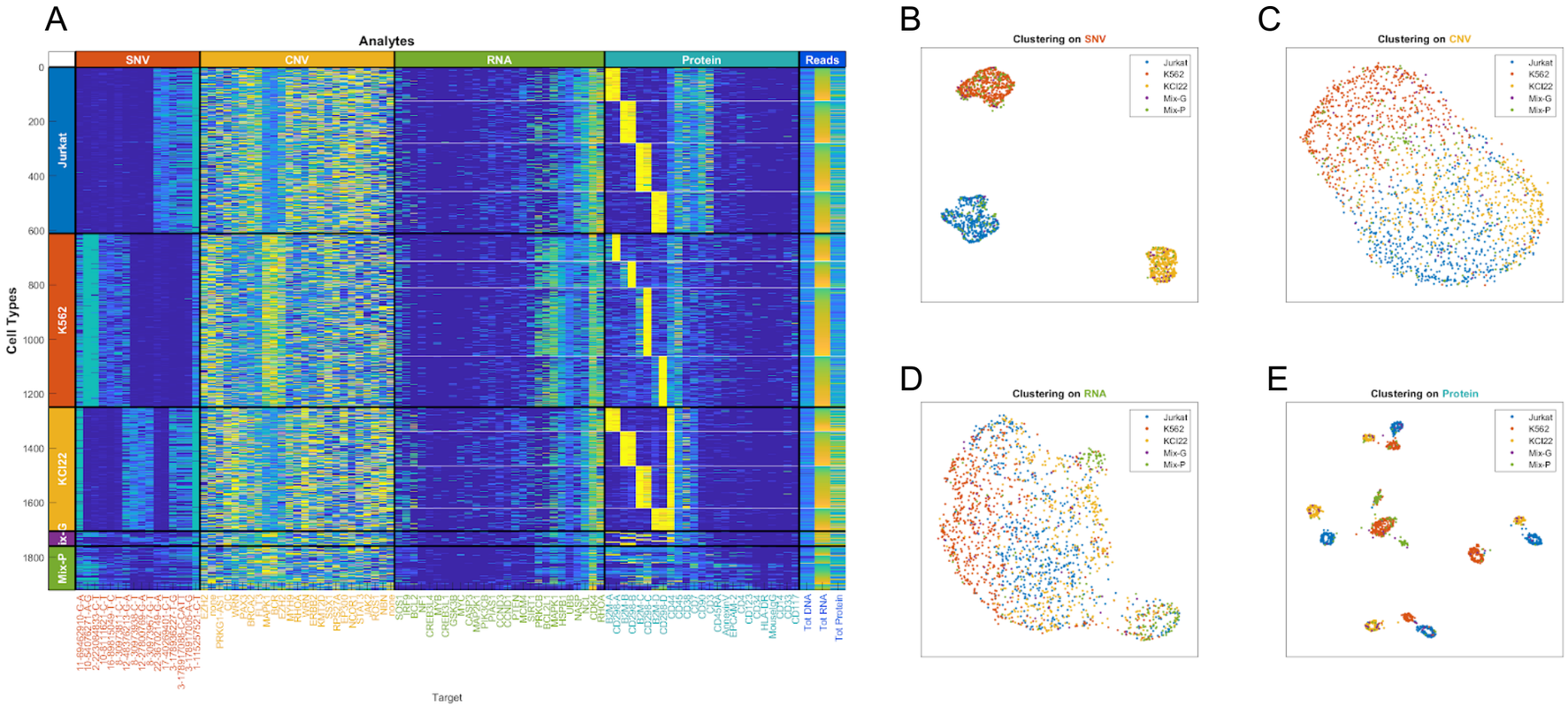
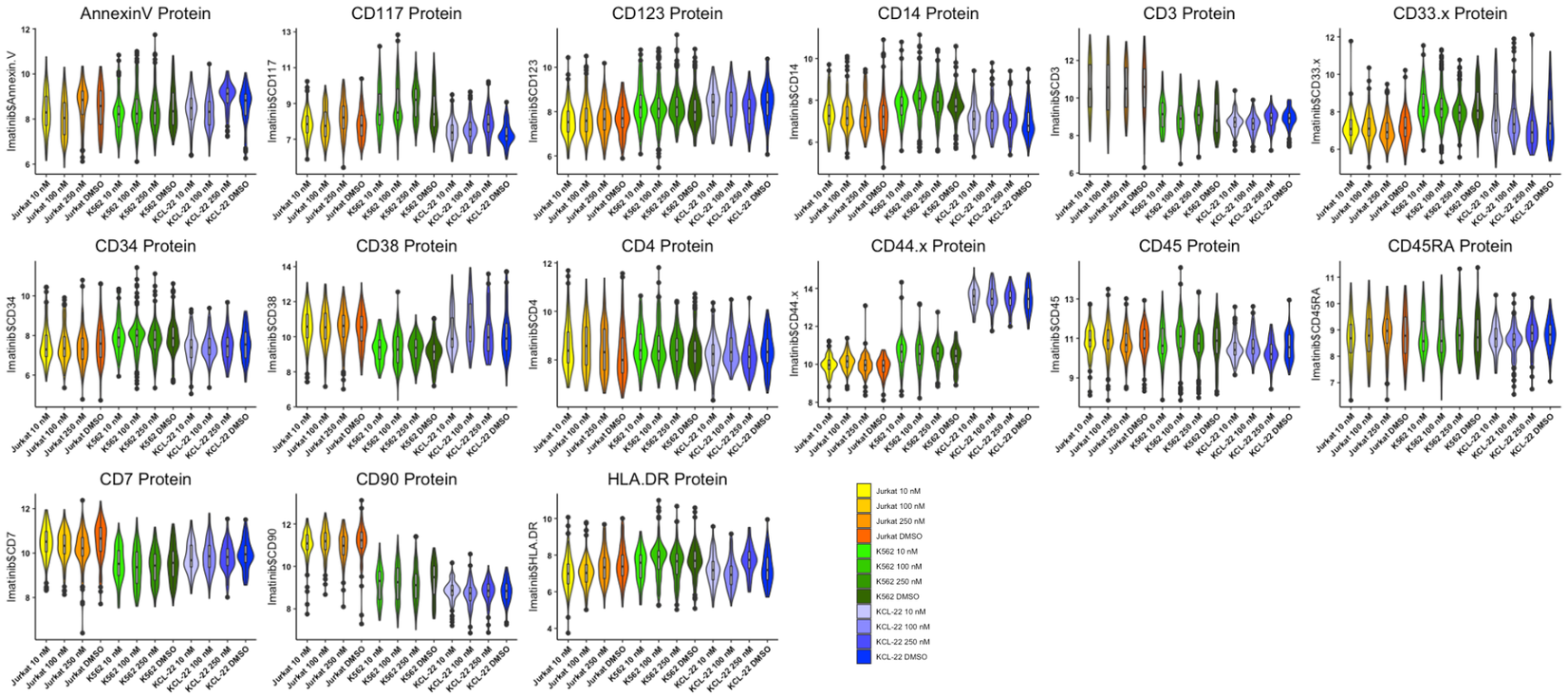



Figure 2: (A) A heat map displays the DNA, RNA, and protein reads from each cell grouped by cell type. Protein tags were also used to multiplex samples on single Tapestri runs. UMAP was employed to visualize the cells clustered by (B) SNVs, (C) CNVs, (D) RNA, and (E) protein.

# Single cell protein expression

A




B

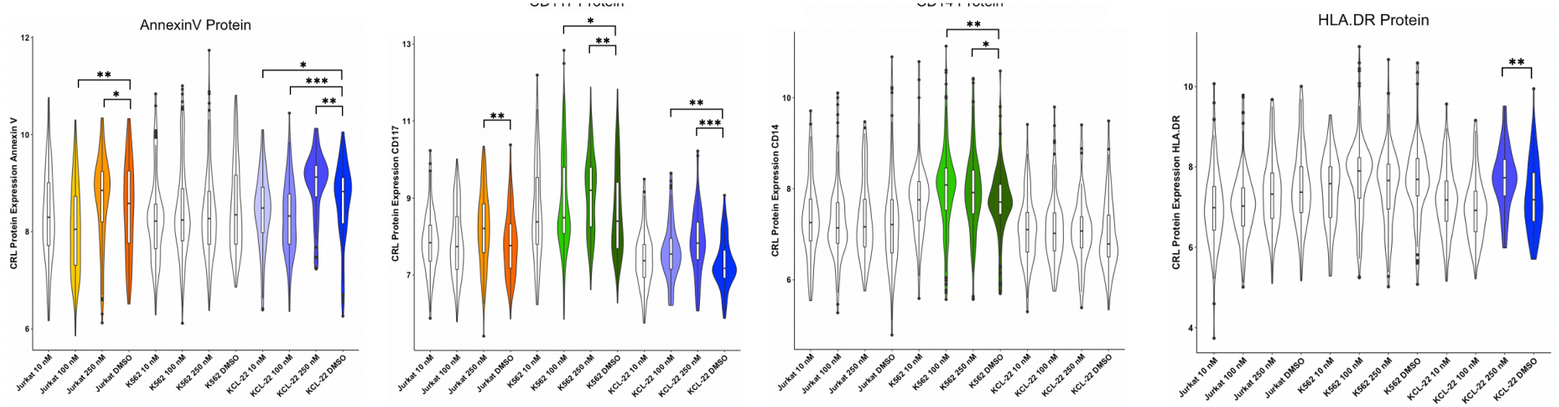


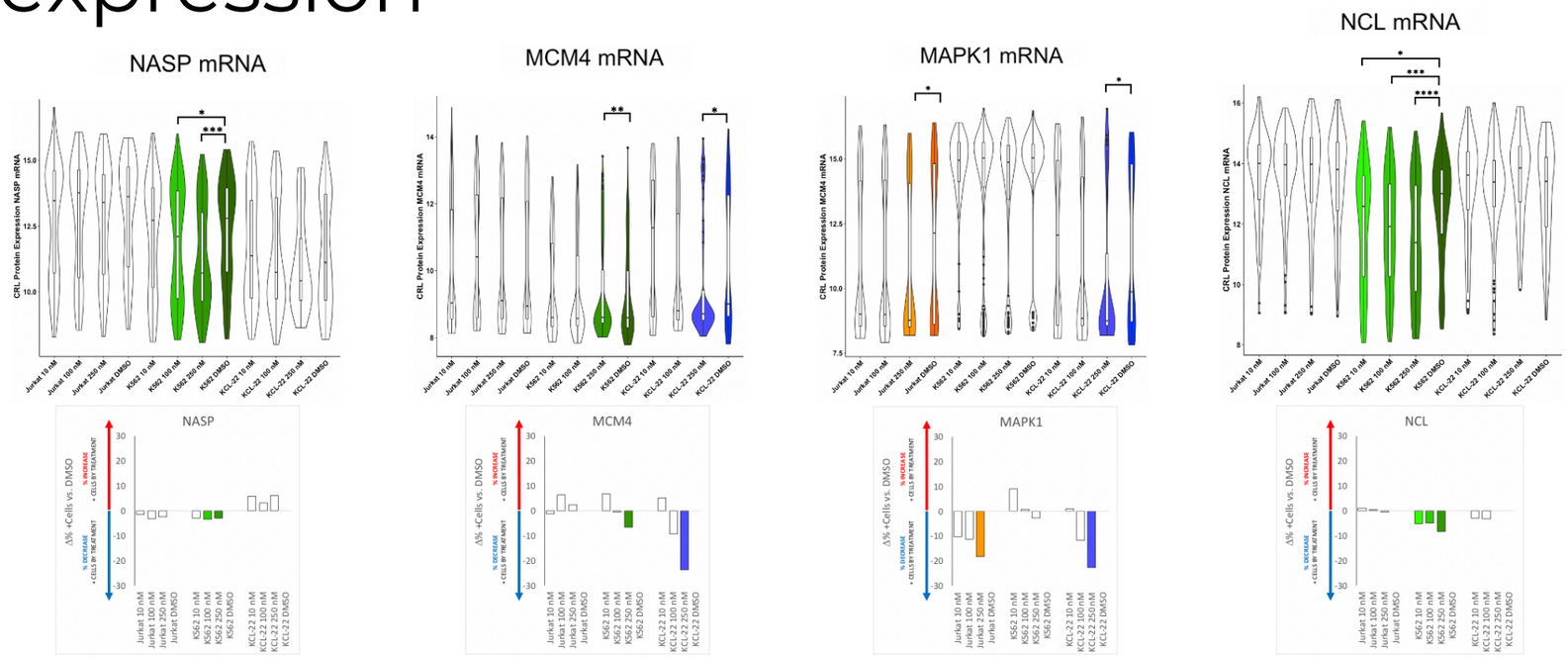

Figure 3: (A) Center Log-ration (CLR) transformed read counts for the four drug doses across 15 protein targets. (B) Four statistically significant protein targets determined by the Kolmogorov-Smirnov unpaired test or the unpaired t-test with Welch correction.
* P<0.05; ** P<0.01; *** P<0.001.

# Single cell RNA expression

Figure 4: (A) Four statistically significant protein targets determined by the Kolmogorov-Smirnov unpaired test or the unpaired t-test with Welch correction on CLR transformed read counts.
* P<0.05; ** P<0.01; *** P<0.001.
(B) Significantly affected mRNA targets in KCL-22 is shown here along with the targets highlighted in the PI3K-ALKT pathway.

We demonstrate the feasibility of conducting triomic measurements for a combination of targeted DNA amplicons (88-plex), targeted RNA amplicons (56-plex) and protein (17-plex) in individual single-cells using the Tapestri system. Using an Imatinib dose response scheme to perturb pathway responses in K562, KCL-22 and Jurkat cells, we identify statistically significant mRNA and protein expression signatures. These responses occur in a backdrop of SNV and CNV differences. We plan to move forward with further RNA-protein correlation studies employing diverse genotypic cell types.

A




B

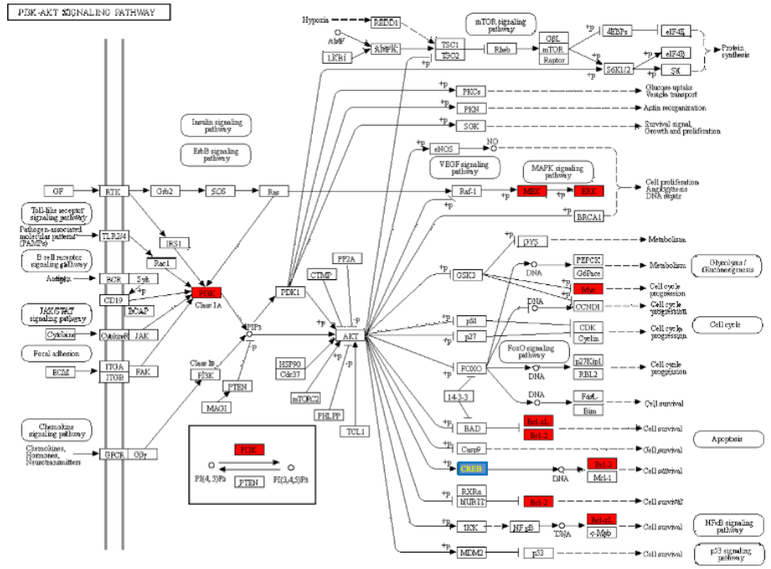
| % Cells affected by drug | Effect Score | Effect on Gene Expression |
|---|---|---|
| induction between 20-25% cells | 4 | Induction* |
| Induction between 15-20% cells | 3 | |
| Induction between 10-15% cells | 2 | |
| Induction between 5-10% cells | 1 | |
| Induction between 0-5% cells | 0 | |
| reduction between 0-5% cells | 0 | Reducction* |
| reduction between 5-10% cells | -1 | |
| reduction between 10-15% cells | -2 | |
| reduction between 15-20% cells | -3 | |
| reduction between 20-25% cells | -4 | |

* Compared against DMSO

A
A
A
A

| KCL-22 | | |
|---|---|---|
| Drug-effect Score | Gene_Symbol | Comments |
| -2 | BCL2 | |
| -1 | BCL2L1 | Dose-Dependent |
| 0 | CASP3 | Dose-Dependent |
| 3 | CREB3L4 | Dose-Dependent |
| -4 | HSPB1 | Dose-Dependent |
| 2 | IRF9 | Dose-Dependent |
| -2 | MAP2K1 | Dose-Dependent |
| -4 | MAPK1 | Dose-Dependent |
| -4 | MCM4 | Dose-Dependent |
| -3 | MYC | Dose-Dependent |
| -1 | NF1 | |
| -1 | PIAS1 | Dose-Dependent |
| -2 | PIK3CB | |
| -2 | SIRT1 | Dose-Dependent |
| -1 | SOCS2 | Dose-Dependent |
| -2 | STAT4 | Dose-Dependent |

**RED=down-regulation**, **BLUE=up-regulation**

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