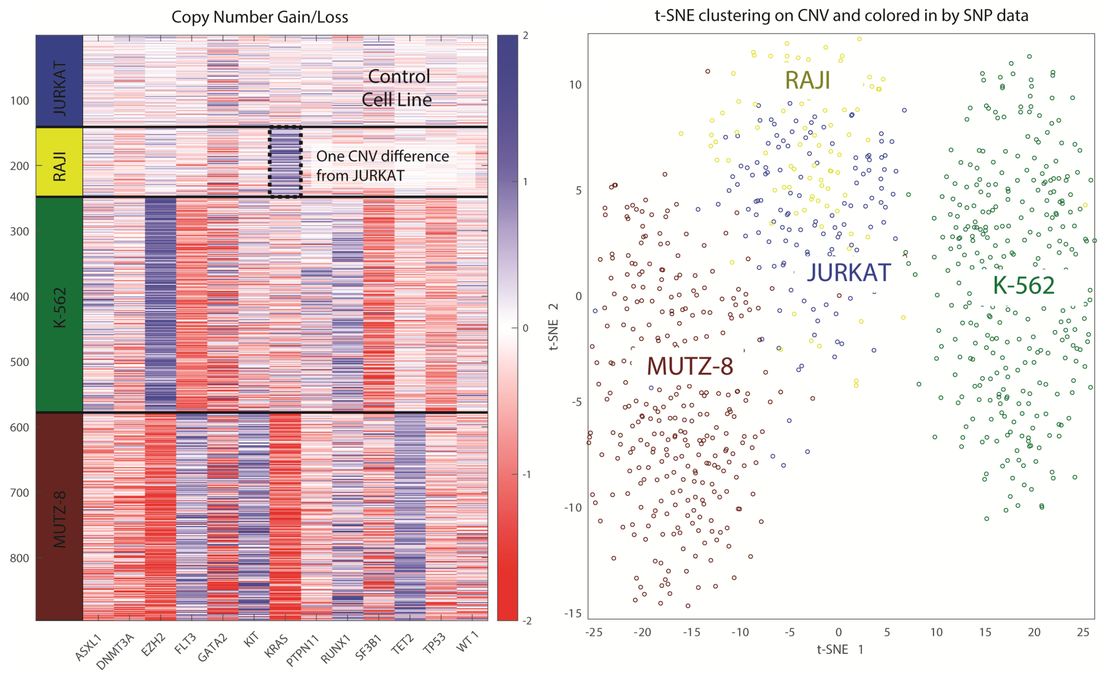
Co-detection of mutations and copy number variations in thousands of single-cells using an automated platform

Adam Sciambi [1], Nianzhen Li [1], Pedro Mendez [1], Aik Ooi [1], Nigel Beard [1]
[1]Mission Bio, Inc. South San Francisco, CA, USA

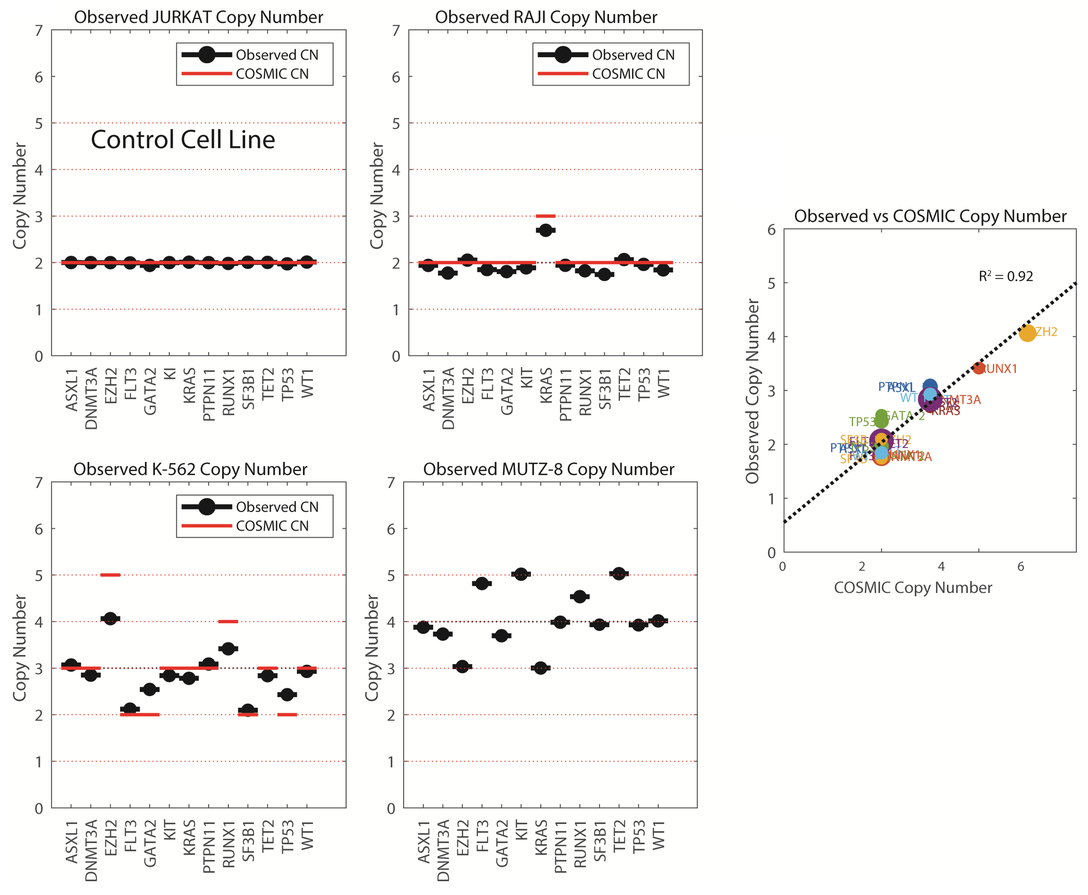
Abstract

Understanding tumor heterogeneity and clonal evolution has major implications in oncology research. Both chromosomal structural change/copy number alteration/variation (CNA/CNV) and driver gene mutation events are critical in cancer initiation and progression. Single-cell sequencing elucidates unique insights on cancer heterogeneity at unparalleled sensitivity, however current methods cannot detect CNV and point mutations in the same cells or at scalable costs. Previously we have developed a high-throughput single-cell DNA analysis platform that leverages droplet microfluidics and a multiplex-PCR based targeted DNA sequencing approach and demonstrates high sensitivity detection of SNVs and indels in the same cells and generation of high-resolution maps of clonal architecture based on mutational profiling. Here we present that using the same approach, by a combination of optimized amplicon panel design, improved biochemistry, and novel data analysis algorithms, we simultaneously characterize point mutations, small indels, and gene-level CNVs in the same single-cells of thousands of cells. Amplification or loss of function in oncogenes and/or tumor suppressors is detected reliably. Single cells were first clustered into subclones based on CNV then further dissected by their mutational profiles. Using known mixed samples, CNV alone confidently detects subclones of < 5% frequency, with subclonal identities confirmed with mutational genotyping. When CNV is combined with mutational analysis, rare subclones of <0.1% prevalence were detected. This facilitates the generation of more accurate clonal evolution maps and deeper understanding of cancer progression mechanisms. Additionally, the system provides capabilities for quality control of gene edited cells, further advancing cancer research and therapy.

4-cell line mix using the Tapestri Single-Cell DNA AML Panel

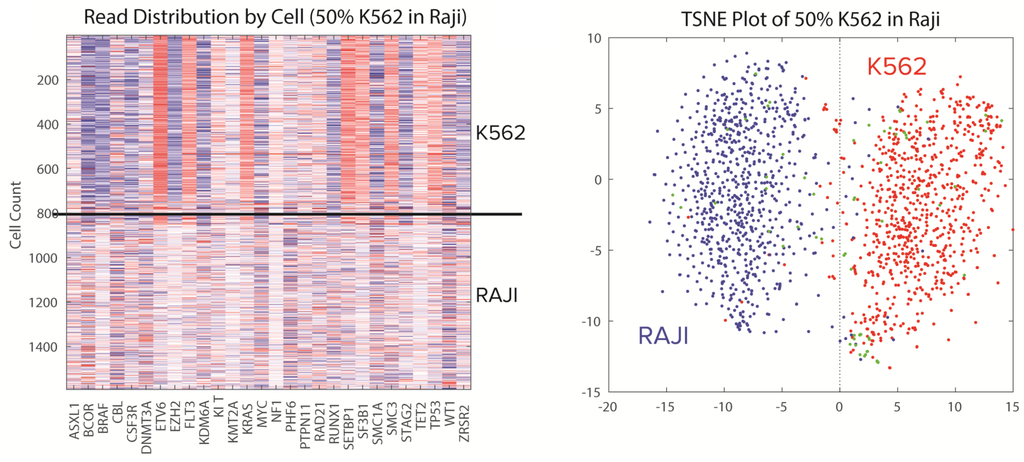


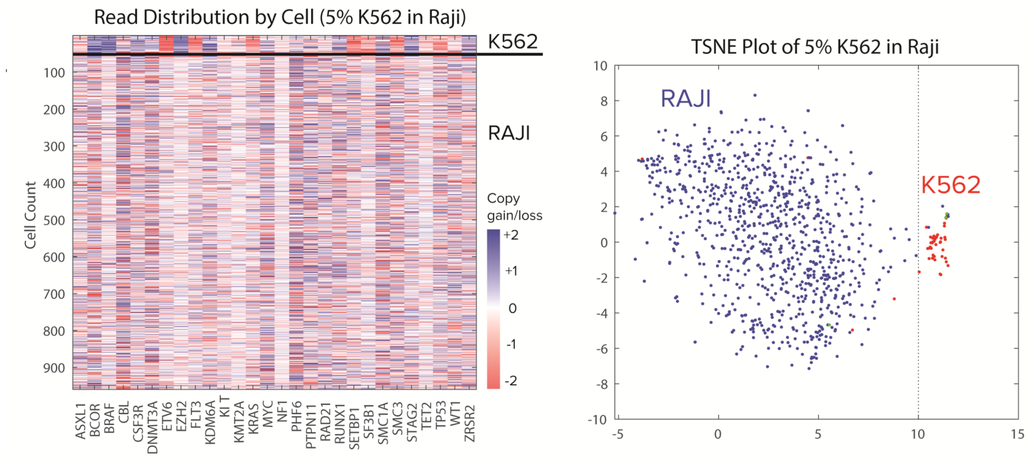
Mutz-8, Raji, K562 and Jurkat cells were mixed together at 43%, 26%, 20% and 11% respectively and analyzed using the Tapestri Platform and Single-Cell DNA AML Panel. Normalized percentage of sequencing reads using the amplicons in the AML panel were used to calculate CNVs for each gene tested. Jurkat cells were used as a control cell line with a known diploid status for all genes tested. The identities of the 4 cell lines were resolved using unsupervised clustering of the CNV data and visualized with a heat map. In addition, CNV data were grouped on a t-SNE plot and data were color-coded based on SNV genotypes previously established from pure cell lines. The data showed clear correlations between CNV-based clustering and cell-line specific SNV signatures, and showed for the first time, CNV and SNV measurements from the same cells.



Plotting the calculated CNV value across all genes shows averaged integer gene-specific CNV calls in the Mutz-8, Raji and K562 cell lines. Observed CNVs across 13 genes in the Raji and K562 cells compared to known CNVs obtained from the COSMIC database showed high correlation with $R^2 = 0.92$.

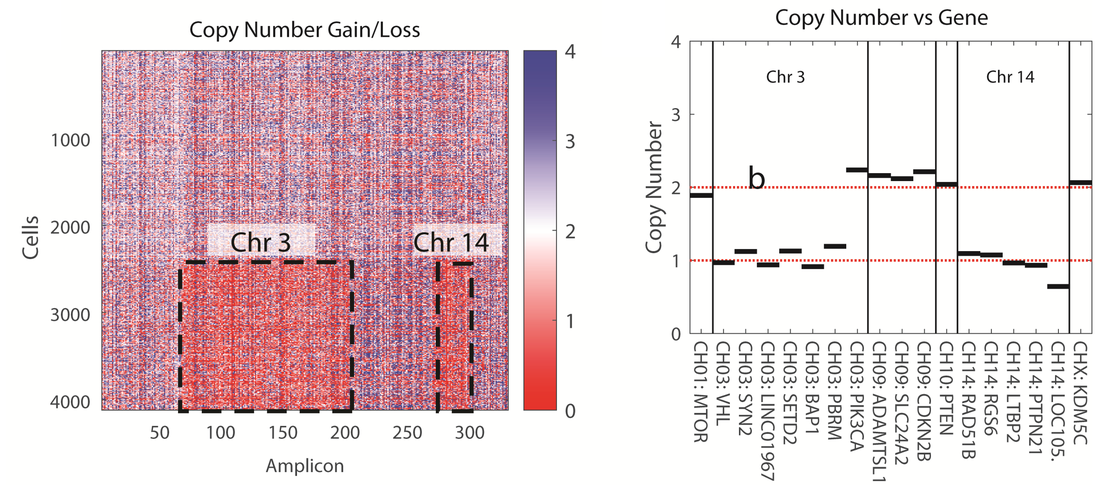
Spike-in experiment using the Tapestri Single-Cell DNA Myeloid Panel



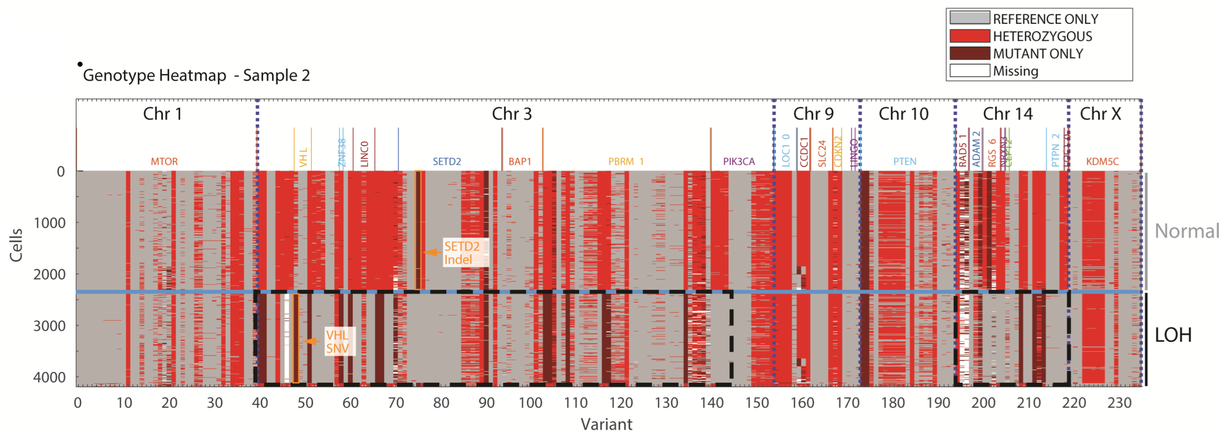


K562 cells were mixed with Raji cells at ratios of 50% and 5% and analyzed for SNVs and CNVs using the Tapestri Single-Cell DNA Myeloid Panel. Heat maps and t-SNE plots clearly showed clustering of 2 different cell types using CNV data and illustrate the ability of the platform to detect rare populations of cells based on CNVs.

Loss of Heterozygosity (LOH) in Renal Cell Carcinoma (RCC) Samples



RCCs have a high prevalence of LOH in several chromosomal regions, including chr. 3, 9 and 14. These chromosome deletions can result in the loss of critical tumor suppressor genes and enhance the progression of cancer. To test if the Tapestri Platform can infer LOH, an RCC tumor sample was analyzed using a custom panel targeting regions within chromosomes 1, 3, 9, 10, 14 and X. Plotting the calculated CNV values across amplicon position along the chromosomes showed potential areas of LOH in chromosomes 3 and 14. CNV status was calculated and plotted across chromosomes. Data showed that cells associated to LOH lost VHL, SETD2, BAP1 and PBRM1 among other genes on chr. 3 and RAD51B, PTPN21 and others on chr. 14.



Using the genotypes of WT, HET, and HOM called by Tapestri Pipeline software, clones with normal diploid copy numbers compared to clones with loss of copy number in each sample were distinguishable using hierarchical clustering and heatmap visualization. Again, the sample showed a population that had LOH in chr. 3 and chr. 14.

Conclusions

The Tapestri Platform co-detects CNVs and SNVs simultaneously in single cells from cell lines and tumor samples. Using catalog or custom Tapestri Single-Cell DNA Panels, loss or gain of gene copies is reliably determined. Since the same amplicons used to determine SNVs are used to calculate CNVs, researchers may apply the present CNV analysis strategy to previously generated Tapestri single-cell data. Here we present the first commercially available platform that detects CNVs, LOH, SNVs, and indels in the same cell.