Enabling single cell analysis of copy number variation in breast cancer

Jacqueline Marin[1], Saurabh Parikh[1], Arnaud Da Cruz Paula[2], Shirin Issa Bhaloo[2], Britta Weigelt[2], Alex Li[1], Khushali Patel[1], Ania Wronski[1], Anup Parikh[1], Jorge S Reis-Filho[2]

[1] Mission Bio, South San Francisco, CA, USA. All are employees and shareholders.
[2] Memorial Sloan Kettering Cancer Center, New York, NY

Introduction

- There is a lack of data on CNV in single cells in solid tumor samples
- We demonstrate single cell DNA sequencing using the Tapestri instrument for solid tumor samples (Figure 1)
- Informatics pipeline that can detect CNV and SNV in breast cancer tissue (Figure 2)
- Ability to uncover clonal architecture, resistance mechanisms and progression of disease at a single cell level.

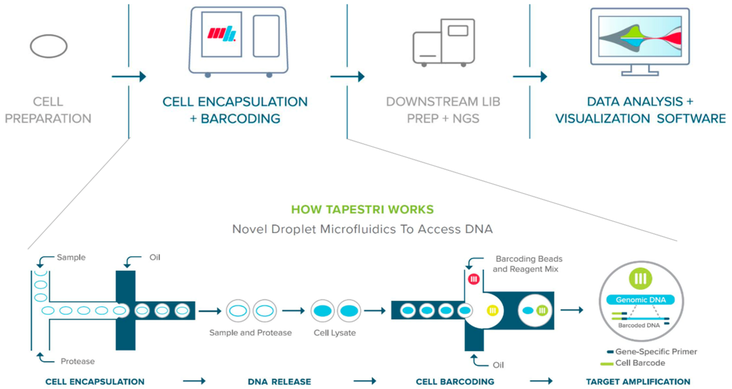


Figure 1: Tapestri workflow

Methods

- Panel designed in collaboration with Dr. Jorge Reis-Filho to capture SNV and CNV.
- Minimum of 5 amplicons designed per CNV region
- Nuclei Isolation protocol was optimized to eliminate nuclei aggregates
- 3 patient samples run on Tapestri instrument and analyzed via pipeline (Fig 1 & 2)

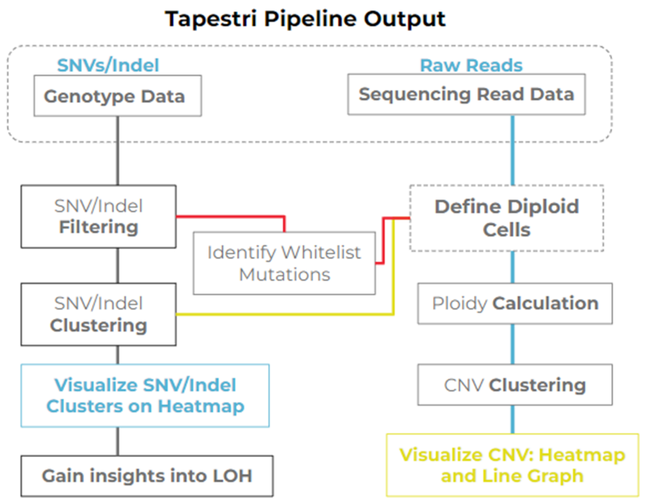


Figure 2: Bioinformatics Pipeline

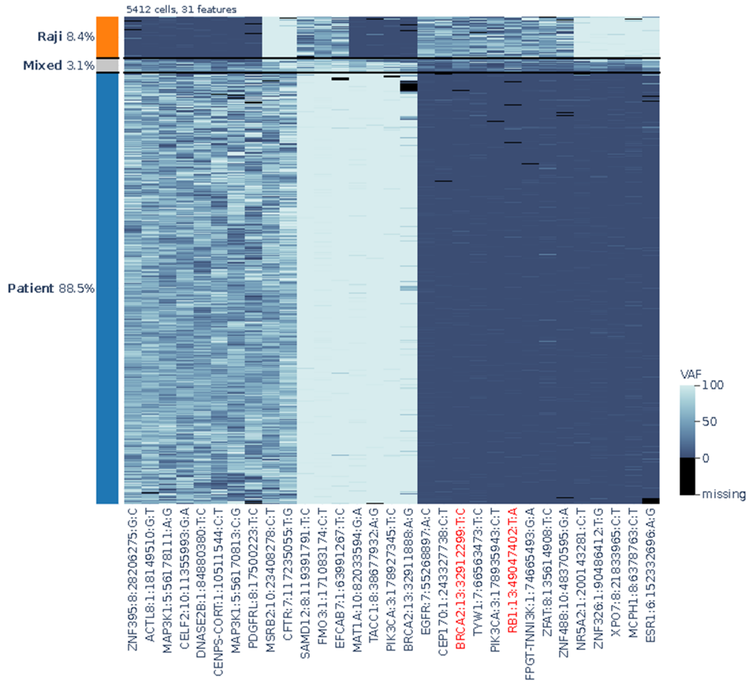
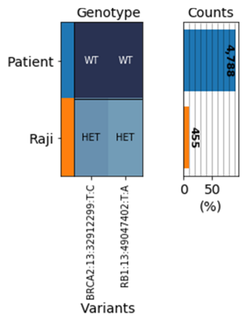


Figure 3: SNV calling
Clones are initially identified using the SNV signature. This patient had one clone with the spike-in Raji cells also identified.

Results

- We have developed the only end to end solution for simultaneous detection of SNV and CNV in solid tumors at a single cell level.
- Tapestri was able to identify SNV (**Figure 3**) and CNV (**Figure 4**) in clinically relevant samples.
- Copy gain was seen in *EGFR* (**Figure 5**) which is amplified in 20+% of all breast cancers and is linked to metastasis.
- Copy loss was seen in *BRCA2* and *RB1* (**Figure 5**). Both events are associated with poor clinical prognosis



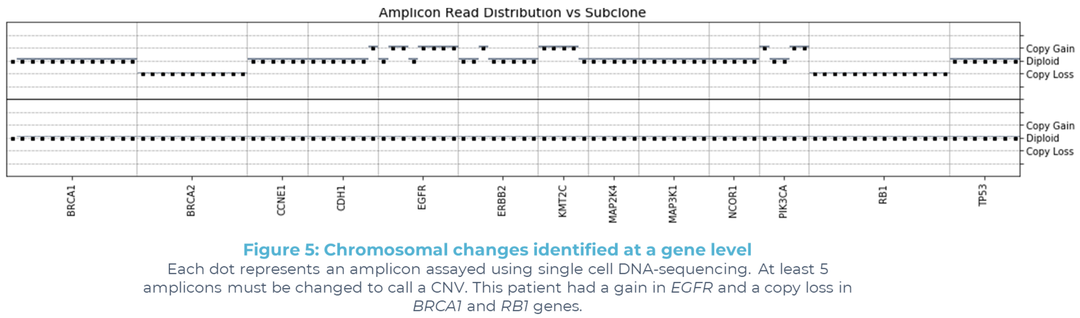


Figure 5: Chromosomal changes identified at a gene level
Each dot represents an amplicon assayed using single cell DNA-sequencing. At least 5 amplicons must be changed to call a CNV. This patient had a gain in *EGFR* and a copy loss in *BRCA1* and *RB1* genes.

The Tapestri Solution

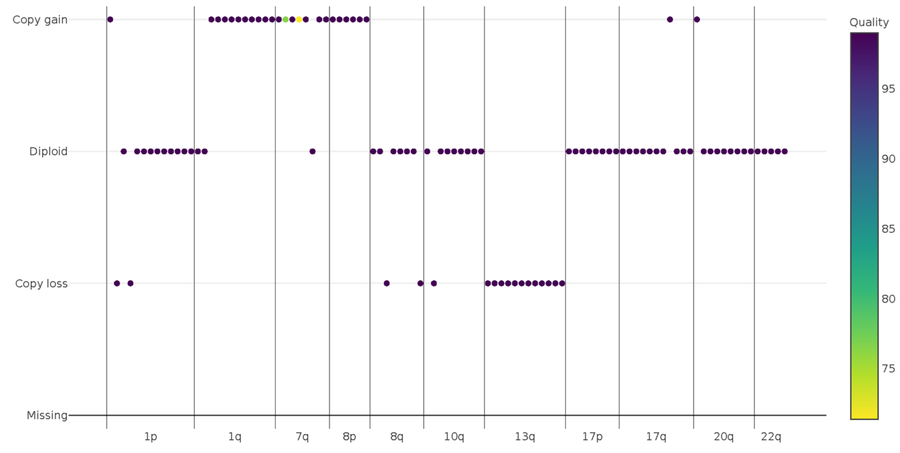


Figure 4: Chromosomal changes identified at an arm level
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Conclusion

Ability to call biologically relevant genomic changes in clinical tissue

Gain	Loss
chr1q	chr13q
chr7q	
chr8p	

Single Cell DNA Sequencing Applications for Breast Cancer

- Clonal Mosaicism and Neoplastic Transition
- Clonal evolution in primary tumors
- Metastatic dissemination & Epithelial to Mesenchymal transition (EMT)
- Drug Resistance Mechanisms

Mission Bio Tapestri Solution

- Simultaneously measures **SNVs, indels**, and arm-level **copy number variations (CNVs)** including **loss of heterozygosity (LOH)** at the single-cell level across up to 10,000 cells
- Unambiguously identifies **variant zygosity** and **mutational co-occurrence**
- Detects **rare cell populations**

AACR #6635

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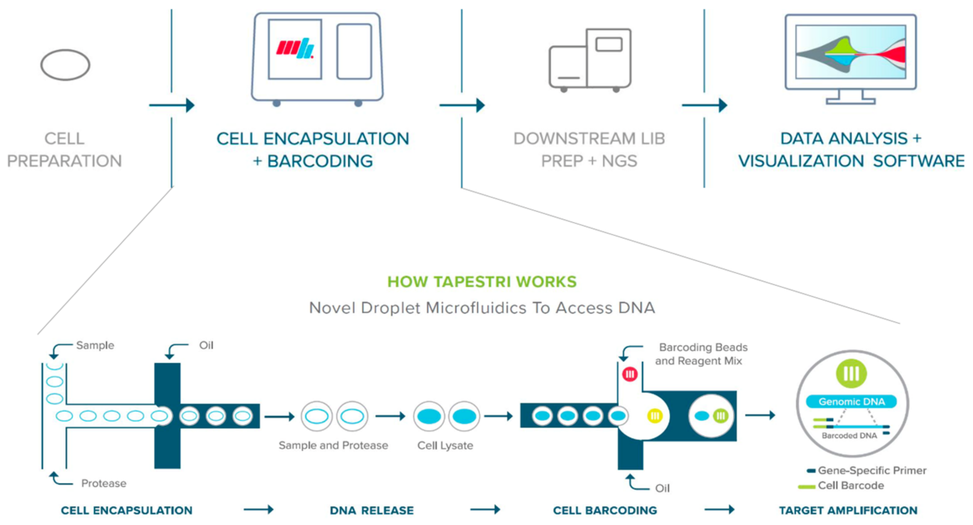

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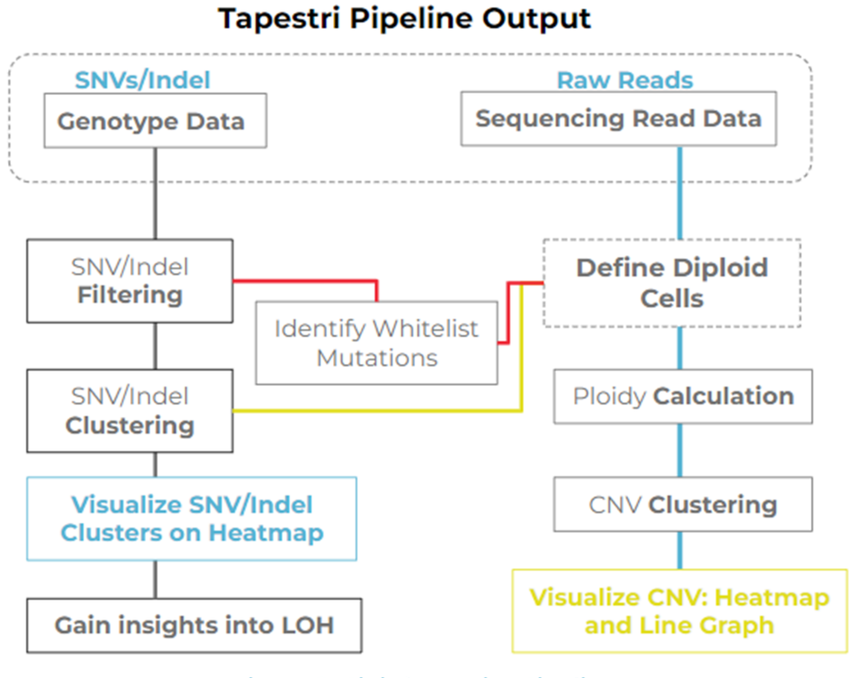

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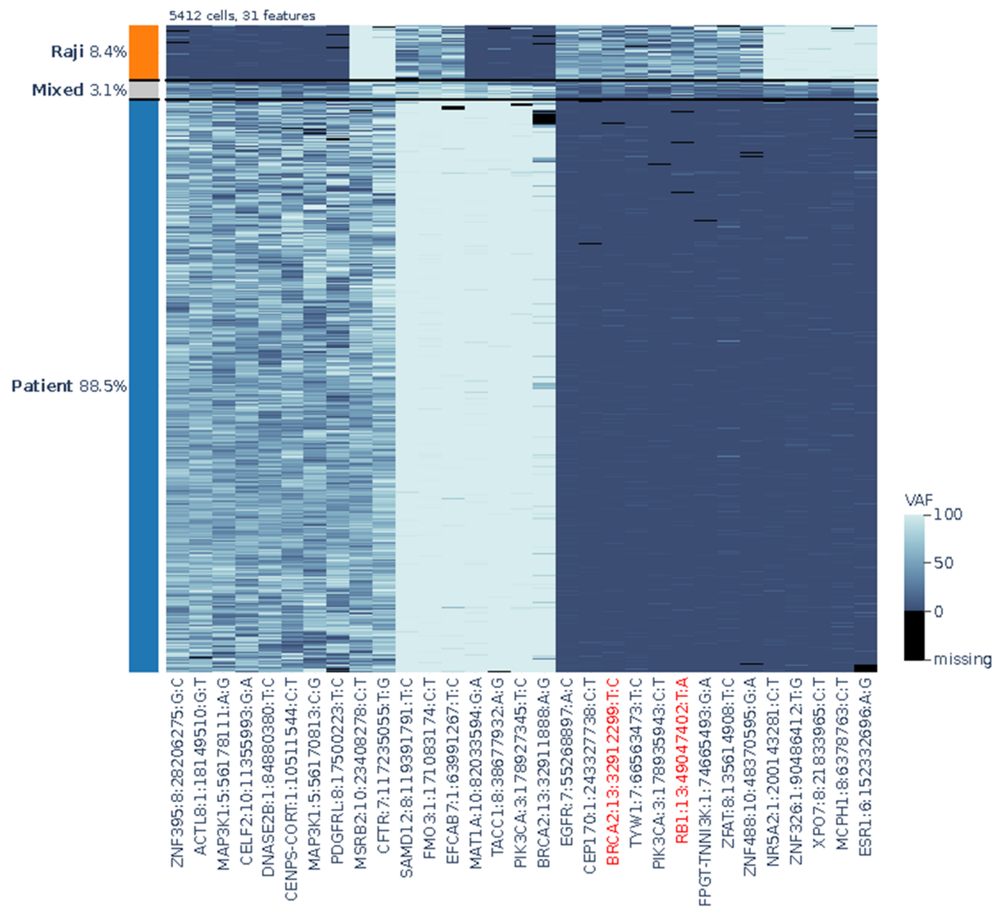

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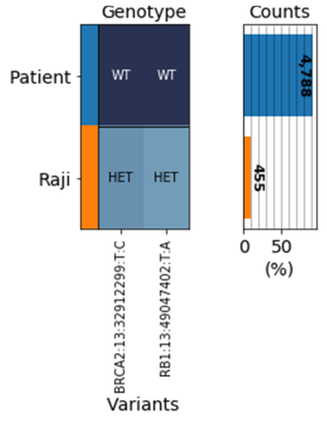

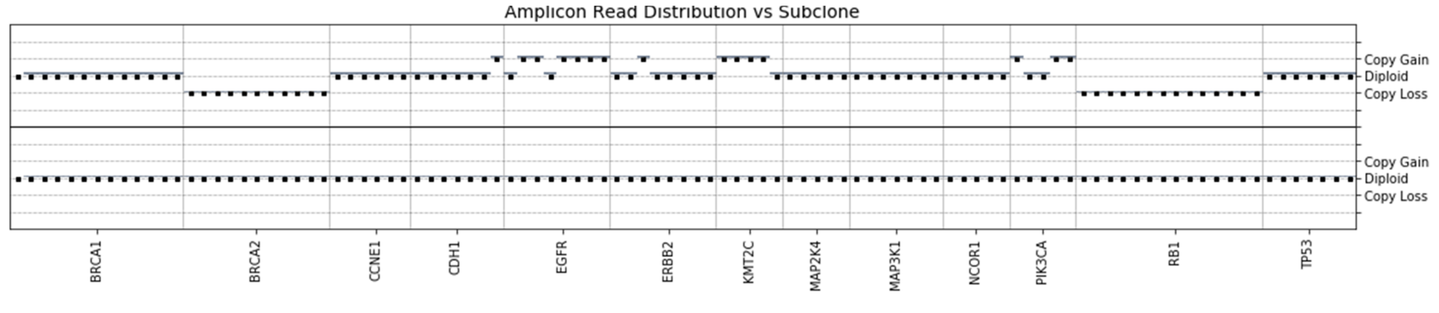

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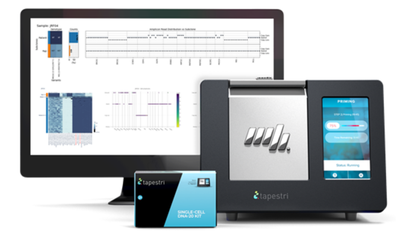

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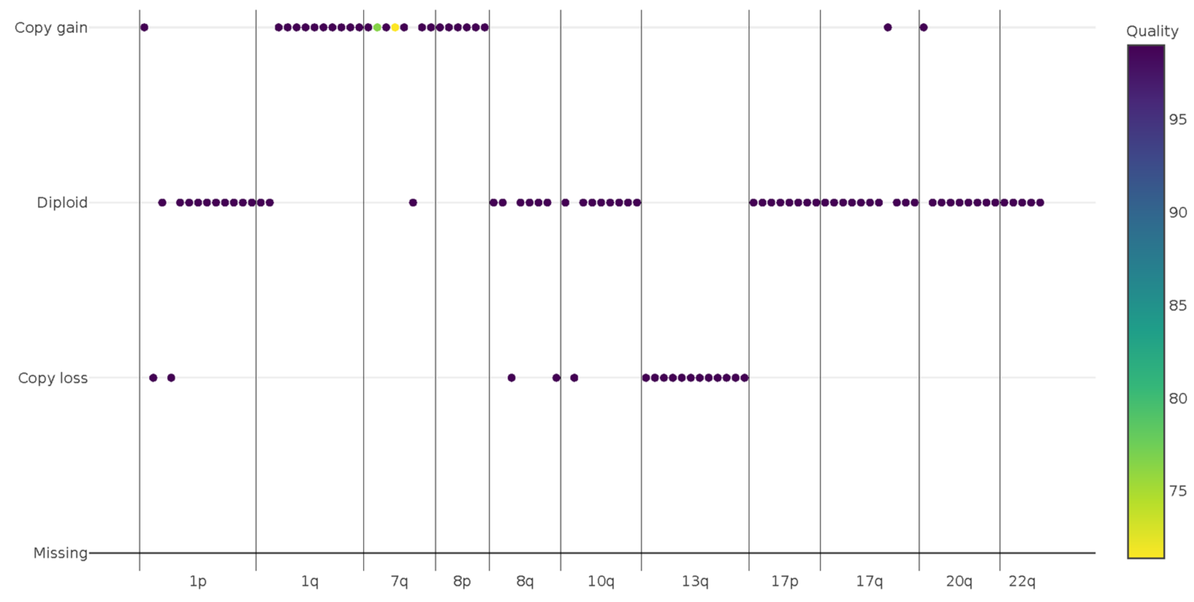

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