Abstract 5563: Single Day Workflow for High-Quality Whole Genome Analysis of Thousands of Single Cells with ResolveDNA

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Introduction

In acute myeloid leukemia (AML), 30% of samples carry mutations in FMS-like tyrosine kinase (*FLT3*)[1]. FLT3 inhibition has clinical benefit, but resistance to FLT3 inhibitors like quizartinib arises through multiple mechanisms. Identifying rare clones with on and off-target resistance mutations requires large-scale, single-cell screening.

What mechanisms of acquired resistance can we reveal using whole genome single-cell sequencing?

To further define the clonal heterogeneity of a tumor we have enabled the processing of thousands of single cells with enhancement of the ResolveDNA® workflow. This automated chemistry maintains high allelic balance with uniform coverage of >95% of each single cell's genome. We paired this with our semiautomated library preparation and IDT xGen™ Exome enrichment to elucidate a mechanism of quizartinib resistance in AML MOLM-13 cells. This automated, scalable chemistry, provides a one-day workflow we leveraged to identify rare clones from a heterogenous cell population.

Methods

Thousands of Cells in One Day – *Proof of Concept*

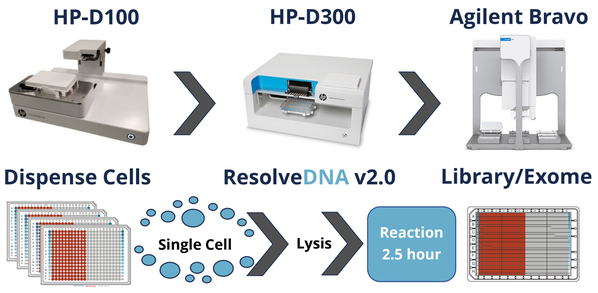
A 1:1 mixture of NA12878 cells with MOLM-13 cells were sorted into a 1536 well plate. Individual cells were amplified with ResolveDNA and libraries prepped for lowpass (n=68) and deep sequencing (n=6). Bioinformatics analysis was performed using BaseJumper™ (BioSkryb Genomics) to determine cell line identity.

In Vitro Model of Quizartinib Resistance

MOLM-13 cells were maintained in 2nM quizartinib. After 2 months of continual 2nM quizartinib dosage (replenished every 3 days with fresh media) a population of resistant cells was isolated.

Discovering Mechanisms of Quizartinib Resistance *In Vitro*

2,208 single cells were sorted. 184 resistant and 184 parental cells were sequenced by lowpass. 1,104 were processed at 272plex for whole exome sequencing. Bioinformatics analysis was performed using BaseJumper.



ResolveDNA Automated WGA	–	**BioSkryb**
Semiautomated Library Prep	–	**BioSkryb**
Low Pass DNA Sequencing	–	**Illumina NextSeq2000**
Exome Preparation	–	**IDT xGen Exome Hyb Panel v2**
Deep Sequencing	–	**Illumina NovaSeq**
BaseJumper Analysis	–	**BioSkryb**

Results

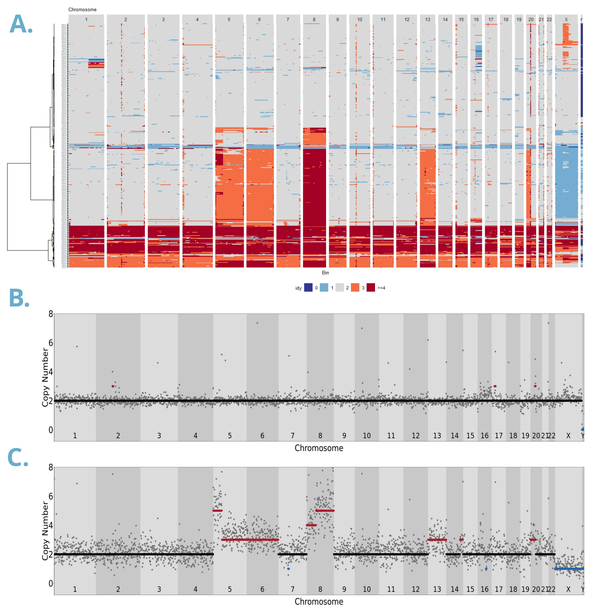


Figure 1: ResolveDNA can be used to determine cell identity based on genomic alterations. **A.** From the mixture of single cells, we were able to identify the lineage of each by copy number variation. **B.** Copy number variation observed in a cell identified as NA12878 cell. **C.** Copy number variation observed in a cell identified as MOLM-13.

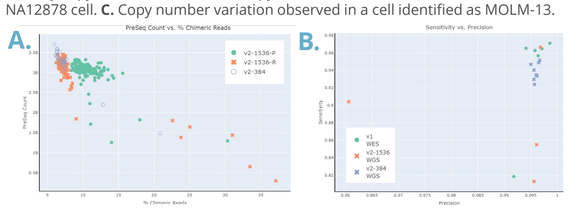


Figure 2: The ResolveDNA automated workflow maintains performance metrics versus manual, low-input cell ResolveDNA workflow. **A.** Low pass sequencing quality. **B.** Precision and sensitivity are comparable between 384 and 1536 workflows.

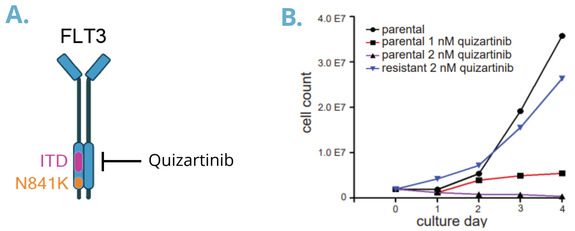


Figure 3: Generation of quizartinib-resistant cell lines. **A.** FLT3 activation promotes proliferation through PI3K, AKT, MAPK, STAT5 and RAS pathways. An internal tandem duplication (ITD) or activating point mutation typically drives this phenotype[1, 3]. Quizartinib binds proximal to the ATP binding site near the ITD, locking the inactive conformation of FLT3, inhibiting downstream phosphorylation. **B.** Quizartinib-resistant cells (R) demonstrated growth rates approximating the parental (P) in DMSO dose-controlled experiments. However, in the same time period parental MOLM-13 cell growth is inhibited by 1 and 2nM quizartinib.

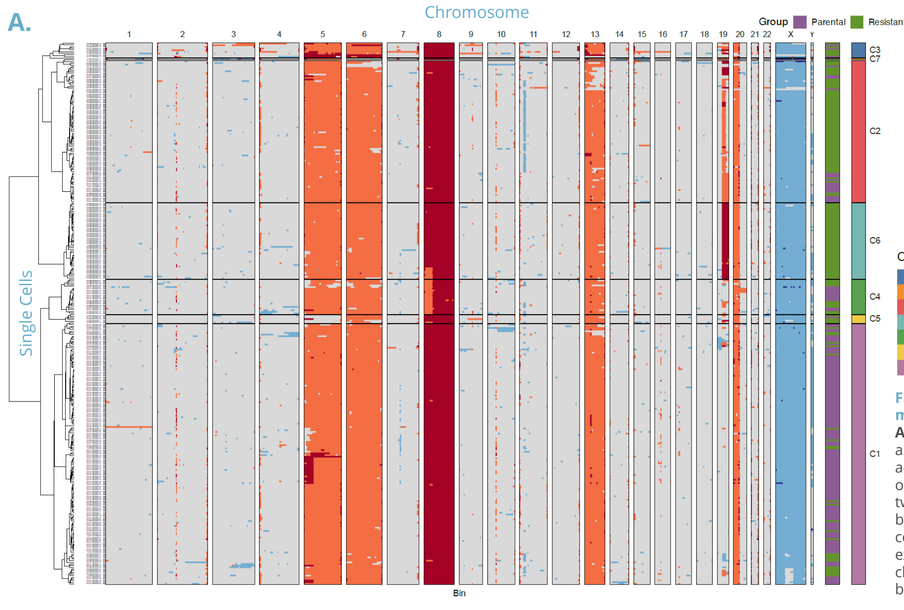


Figure 4: Multiple potential resistance mechanisms exist at the single-cell resolution. **A.** Relative to the P cells, R cells exhibit diverse aneuploidy on 19q seen in Clusters 2 and 6. R cells additionally lose the addition on 5p seen in some of the P cells. Within the Resistant population, two dominant clusters emerge, Cluster 2 marked by loss of a region in one copy of Chr 11p and concomitant gain in a section of 19q. Cluster 6 exhibits a full gain of 19q with no significant change in Chr 11. **B.** MDA plot showcasing biological variance due to drug resistance

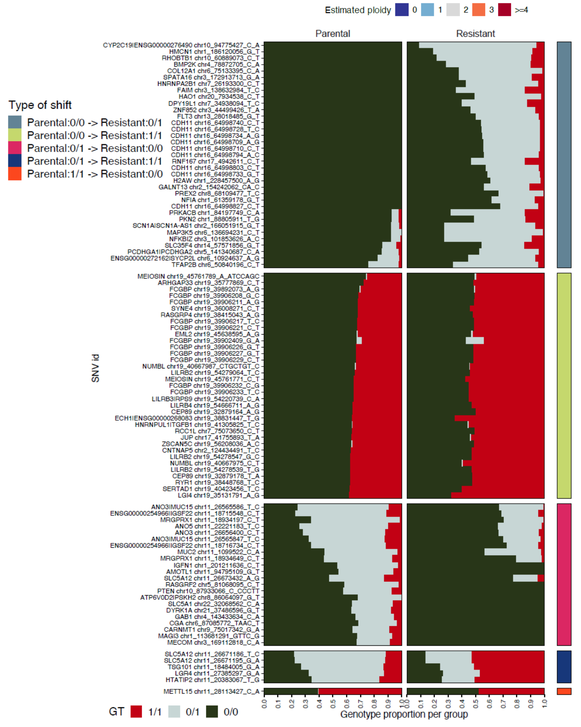


Figure 5: Single nucleotide variants identified by WES showcase significant genotype differences between the Parental and Resistant cells. Colored groups show five state changes across the cells. The evolution of genotypes across cells is visible as allelic states migrate. Of particular note is the FLT3 N841K mutation (Group 1) which is a key driver to resistance of quizartinib.

Conclusions

- We have demonstrated a high throughput workflow to detect rare single cell clones in a heterogenous sample by WES of thousands of genomes at a cost of < $7/cell
- CNV clustering reveals dynamic chromosomal changes resulting directly from treatment, which would not have been detectable by low cell throughput or bulk sequencing
- Dramatic genomic plasticity demonstrated by CNV and SNV analysis is orders of magnitude higher than expected from differential gene expression by end counting methods
- This method demonstrates the ability to detect rare mutants, potentially providing clinicians with novel insights to improve therapeutic treatment aims

References

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2. Zawistowski et al. biorXiv (2022). https://doi.org/10.1101/2022.04.29.489440
3. Jiang et al. Blood. (2004) https://doi.org/10.1182/blood-2004-02-0712