Enrichment of viable cells from patient samples with LeviCell technology as input into the ResolveOME™ single cell multiomic workflow

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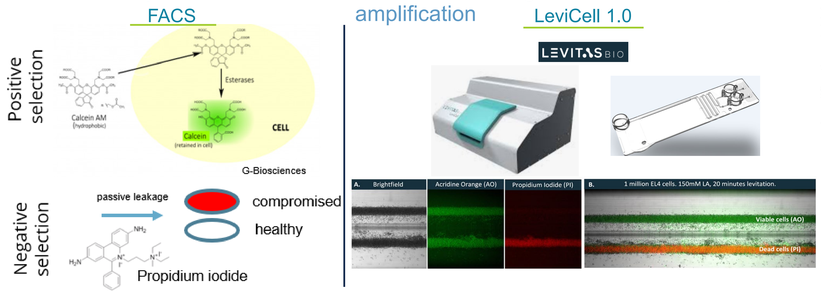
Introduction

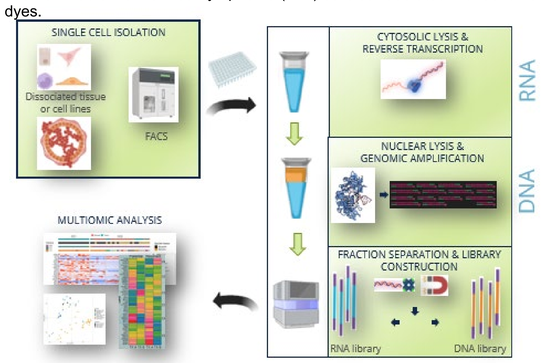
Single cell multiomic analysis is at the forefront of studies driving insight into tumor clonal evolution and somatic mosaicism in normal tissues, as well as into edited cell line surveillance. The ability to generate unified genomic and transcriptomic information with fidelity is wholly dependent on the input of viable cells into the amplification chemistry, otherwise concurrent genotype and phenotype are not accurately captured. We present the coupling of the Levitas LeviCell 1.0 instrument, where viable vs dying/dead cells differentially levitate in a microfluidic cartridge within a magnetic field, to single cell dispensing technologies providing input into ResolveOME multiomic amplification technology. Primary cryopreserved bronchoalveolar lavage cells were either directly inputted into FACS or HP D100 instruments for dispensing into 96 well PCR plates or inputted into the LeviCell 1.0 prior to dispensing. For FACS dispensing, even with the use of Calcein-AM and propidium iodide dual viability staining, levitation of primary cells (2 independent samples, 2 replicates each) prior to dispensing increased the percentage of cells yielding genomic amplification product from 51.25% to 88.75% and, for transcriptomic yield, from 52.50% to 97.50%. A similar trend of reduced reaction yield dropouts was obtained for HP D100 dispensing for both genomic amplification and cDNA yield, albeit with a more modest mean improvement of 17%--yet LeviCell viability enrichment importantly reduced the amount of sporadic clogging of the HP D100 microfluidic cartridge. Low-coverage genomic sequencing metrics assessing library complexity and amplification uniformity were overall comparable between pre- and post-levitation, as were the transcriptomic sequencing metrics of mitochondrial transcript percentage, exonic:intergenic read ratio, and number of expressed genes detected. The marked reduction of reaction failures or sub-optimal reaction yields while maintaining robust sequencing metrics with this LeviCell-dispensing-ResolveOME workflow is a facile solution for maximizing the utility of rare and valuable samples, as well as for maximizing the success rate of a given ResolveOME run with often challenging clinical samples.

Introduction

Cell viability enrichment is crucial for downstream genomic and transcriptomic amplification

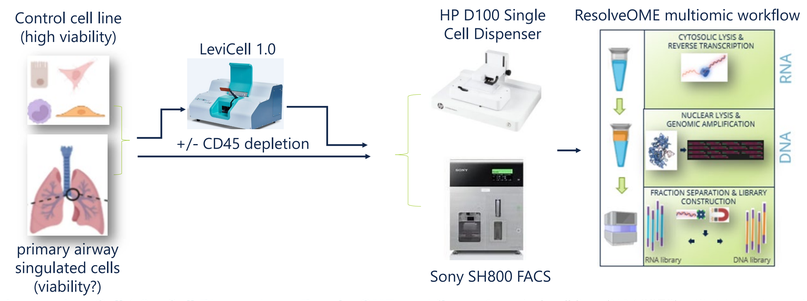


Strategy employed for viability assessment of single cells with FACS (left). A dual method of positive selection with Calcein AM and negative selection with propidium iodide maximizes viable cell output from FACS. **LevitasBio LeviCell 1.0 system (right).** Suspended cells flow throw microfluidic cartridge subjected to a magnetic field and a contrast agent, enriching viable cells through buoyancy properties. The bottom image shows separation of viable and non-viable murine T-cell lymphoma (EL4) cells corroborated with acridine orange and propidium iodide viability dyes.



ResolveOME[1] multiomic workflow. To achieve near-complete and uniform single-cell genomic amplification and full-transcript RNAseq from the same individual cell, single cells are first dispensed into 96 or 384 PCR plates followed by cytosolic lysis and reverse transcription. Primary template-directed amplification (PTA)[2] of the genomic DNA then is performed, with subsequent affinity separation of the amplified cDNA. Separate DNA and RNA libraries are sequenced, and the multiomic data is analyzed by BaseJumper software from BioSkryb Genomics.

Methods



Levitas LeviCell 1.0 – Cell Dispensing – ResolveOME workflow. A control cell line (NA12878) with high inherent viability or primary human cryopreserved bronchoalveolar lavage cells (~5E5 cells each) were subjected to the LeviCell 1.0 workflow (w/LeviSelect CD45 depletion) according to the manufacturer's instructions and single cells subsequently dispensed into PCR plates by Sony SH800 FACS or HP D100 cell dispensing, or directly dispensed in the absence of levitation. ResolveOME multiomic chemistry was performed downstream of dispensing, where genomic sequencing was performed with 2M 2X50 reads per cell and RNA sequencing performed with 150,000 reads per cell.

Conclusions

- For patient samples, which often are of poor integrity, levitation prior to FACS or cell dispensing significantly improved the fraction of successful single-cell genomic and transcriptomic amplification reactions from ResolveOME multiomic chemistry
- DNA and RNA performance metrics were overall concordant between pre- and post-levitation samples
- For precious clinical samples, first enriching for viability by levitation, even in the presence viability gating in FACS, maximizes ResolveOME reaction output

Results

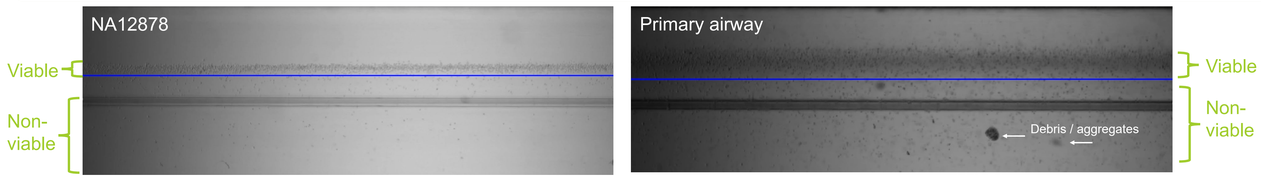


Figure 1: Separation of viable cells by levitation. Images of LeviCell 1.0 microfluidic cartridge enriching viable cells. The blue line indicates the viability threshold set based on image of tight band of viable, levitating cells, relative to sedimenting non-viable cells or debris/aggregates. A healthy control cell line NA12878 (left) has minimal debris and dead cells, while a primary airway cell sample has significantly more non-viable cells and debris clumps. ~500,000 cells were used as input.

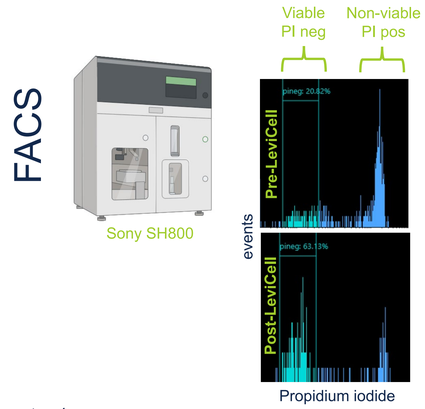
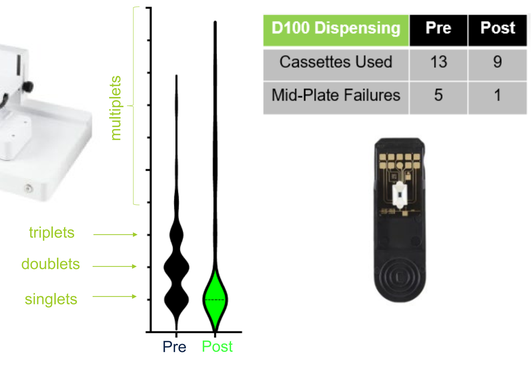


Figure 2: Cell levitation upstream of cell dispensing: effect on viability, singlets, and clumping. Left: Cryopreserved, singulated human airway lavage cells were subjected to propidium iodide staining and sorting by Sony SH800 FACS with or without prior LeviSelect CD45 depletion and LeviCell 1.0 levitation. The LeviCell workflow markedly decreased the relative proportion of PI-positive, non-viable cells. Right: The same primary airway sample was subjected to HP D100 cell dispensing with or without prior LeviCell 1.0 treatment. Levitation reduced the fraction of triplet and doublet well dispenses with concomitant increase in singlet wells as reported by the HP D100 software. In addition, LeviCell 1.0 treatment prevented clogging of the HP D100 cartridge by the patient sample, resulting in a savings of consumable cassettes.



D100 Dispensing	Pre	Post
Cassettes Used	13	9
Mid-Plate Failures	5	1

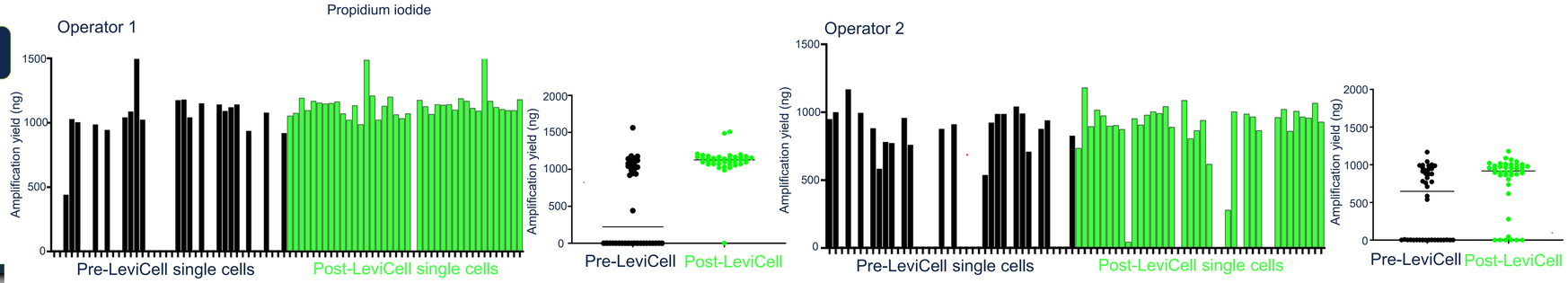


Figure 3: ResolveOME genomic amplification of single cells in the presence or absence of LeviCell 1.0 viability enrichment. FACS-sorted single airway cells were subjected to the ResolveOME multiomic workflow following LeviCell 1.0 treatment (Post-LeviCell) or directly dispensed in the absence of levitation (Pre-LeviCell). Amplification yield of the genomic arm of the ResolveOME multiomic chemistry is shown for both conditions, across two operators. Levitation significantly reduced the number of zero- or low-yielding ResolveOME reactions for both operators.

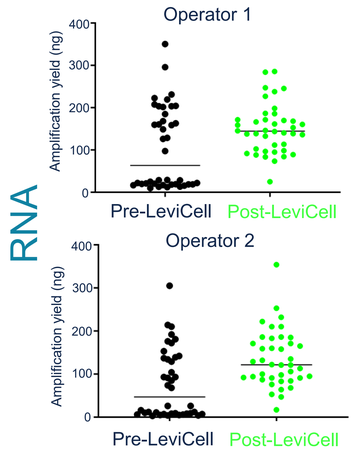
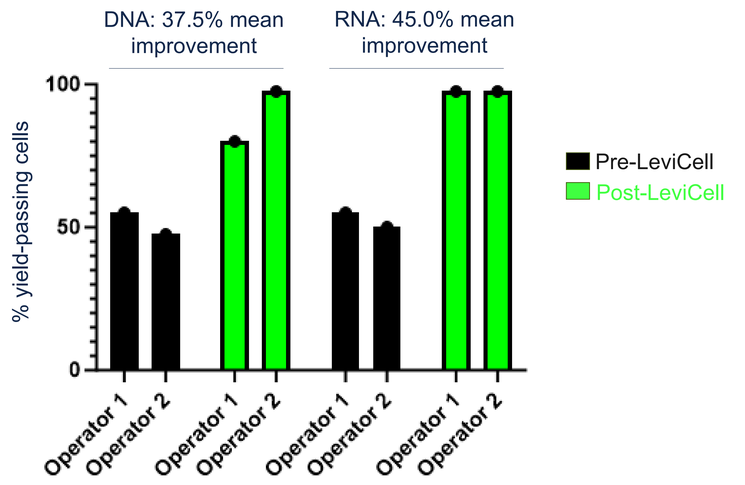


Figure 4: Cell levitation upstream of cell dispensing increases the frequency of yielding ResolveOME reactions at both the DNA and RNA levels. Left: Transcriptome amplification yields and reaction dropout frequency Pre- and Post-levitation. The RNA improvement with levitation qualitatively mimics the improvement observed for the DNA arm of ResolveOME (Figure 3). Right: Quantitative summary of the DNA and RNA yield improvements observed with LeviCell 1.0 treatment of primary airway cells. A passing cell was defined as >500 ng of genomic amplification yield or >20 ng of RNA amplification yield.



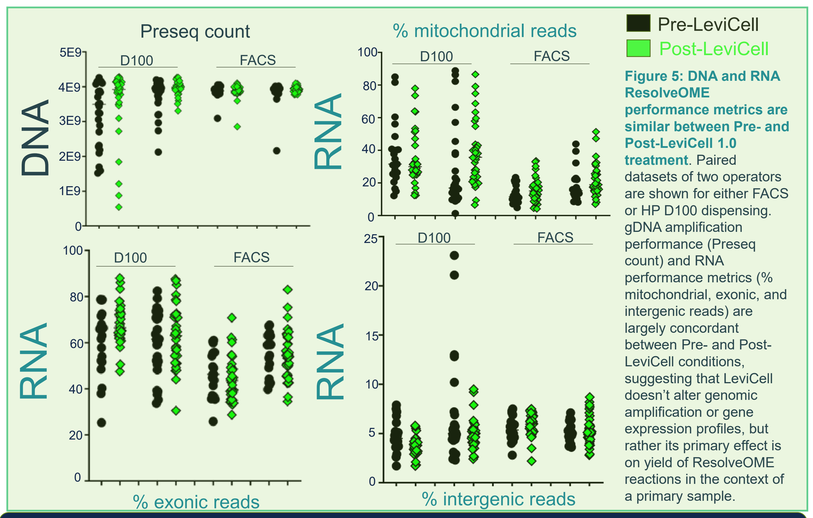


Figure 5: DNA and RNA ResolveOME performance metrics are similar between Pre- and Post-LeviCell 1.0 treatment. Paired datasets of two operators are shown for either FACS or HP D100 dispensing. gDNA amplification performance (Preseq count) and RNA performance metrics (% mitochondrial, exonic, and intergenic reads) are largely concordant between Pre- and Post-LeviCell conditions, suggesting that LeviCell doesn't alter genomic amplification or gene expression profiles, but rather its primary effect is on yield of ResolveOME reactions in the context of a primary sample.

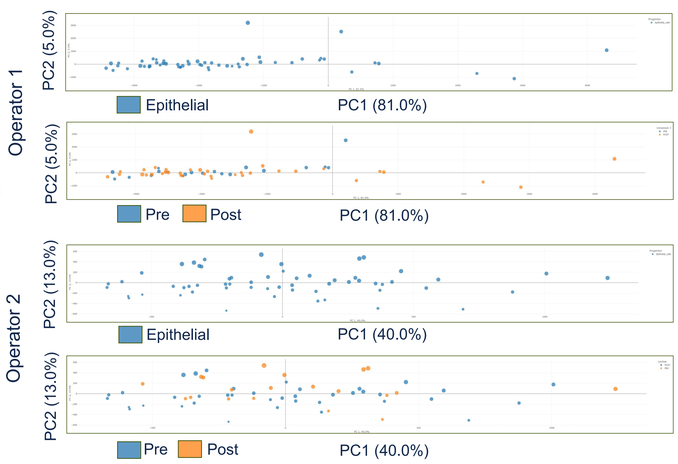


Figure 6: PCA analysis of ResolveOME gene expression of Pre- and Post-LeviCell airway cells. BaseJumper software PCA plots of gene counts showing concordance between Pre- and Post-LeviCell 1.0 for cell ID typing (top plots) and gene expression profile (bottom plots) between ResolveOME operators. This suggests that there is not pronounced alteration of gene expression as a function of LeviCell treatment paired with downstream Sony SH800 FACS sorting.

References

1. Marks JR et al. Unifying comprehensive genomics and transcriptomics in individual cells to illuminate oncogenic and drug resistance mechanisms. bioRxiv preprint: **doi:** https://doi.org/10.1101/2022.04.29.4894402.
2. Gonzalez-Pena, V. *et al.* Accurate genomic variant detection in single cells with primary template-directed amplification. *Proc Natl Acad Sci U S A* 118, e2024176118 (2021).

Acknowledgements

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