Scaling Single-Cell Sequencing: Multiplexing Innovations for Cost-Effective High-Throughput Profiling

Sanika Khare, Dominic Skinner, Nick Pervolarakis, Aimee Beck, Amy Hamilton, Jerushah Thomas, Dmitry Pokholok, Ashley Woodfin, Frank Steemers, Maggie Nakamoto, Felix Schlesinger

Scale Biosciences, 3210 Merryfield Row San Diego CA 92121

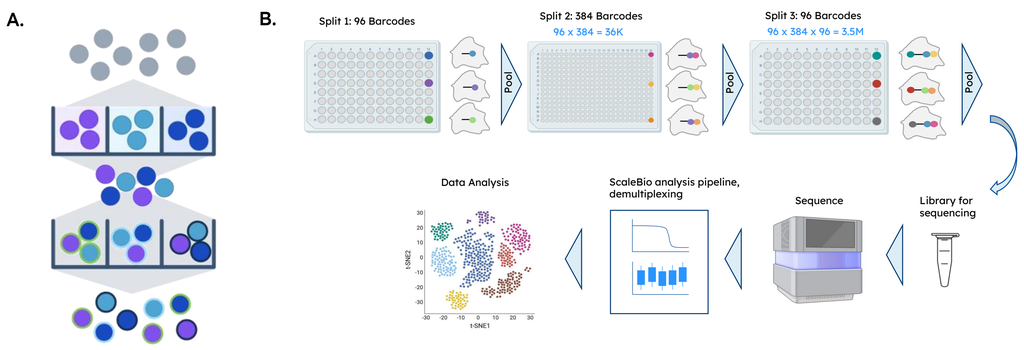
Background

Problem
Although plate-based fixation-compatible workflows have increased accessibility of single-cell profiling these workflows can still be challenging to use with complex samples, such as tissue, due to cell loss during preparation of single cell suspensions, variable recovery throughout the workflow, and arduous sample preparation leading to prohibitively long workflows.

Solution
Here we increase usability and flexibility of the ScaleBio™ Single Cell RNA Sequencing Kit by adding cell hashing to the workflow. Addition of these hashes enables pooling of cells from dissociated tissue samples before any centrifugation steps, drastically increasing cell recovery in upstream steps and preserving more of the sample for downstream recovery. Additionally, these hashes can increase sample throughput beyond the current 96 samples enabling larger screens.

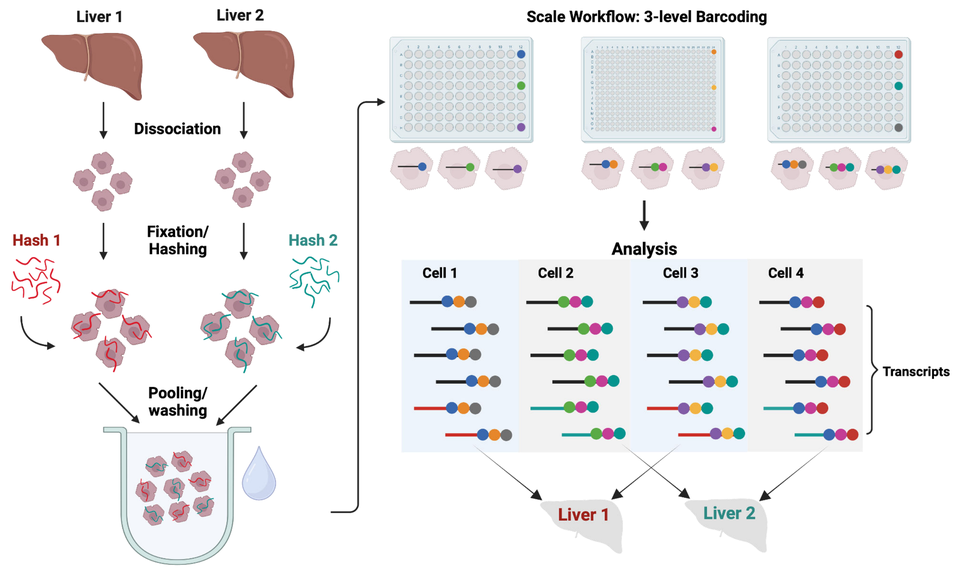
Workflow
Cells or nuclei were hashed and taken through the ScaleBio workflow. Signal and background of the hashes were examined in different workflows (pooling of the samples before and after washing) as well as in different sample types (cell lines, PBMCs, and nuclei isolated from dissociated mouse liver and kidney).

Figure 1: Combinatorial indexing technology uses a plate-based workflow to barcode cells, easily increasing throughput without the need for any instrumentation.



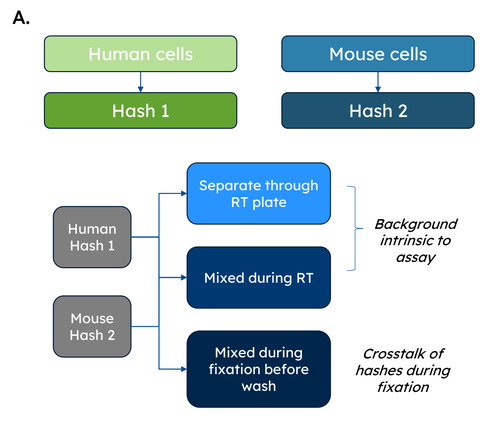
A. In combinatorial indexing, cells or nuclei pass through a unique combination of barcoded wells using pooling and splitting, resulting in a unique combination of barcodes being attached to target molecules for each cell.
B. Schematic shows how 3 rounds of indexing produce 3.5 million unique index combinations. After the plate-based workflow libraries are then sequenced and indices demultiplexed to assign reads to individual cells or nuclei

Figure 2: Cell hashing can be used to label and pool samples upstream of the ScaleBio scRNA workflow.



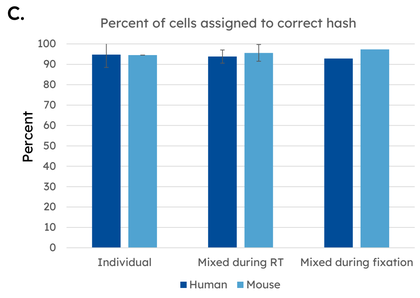
Cell hashing is performed on single cell or nucleus suspension. After labeling cells/nuclei with hash oligos fixative is added. Cells/nuclei are then pooled before washing, vastly increasing cell recovery for low-cell-number samples. After washing cells/nuclei are taken through the ScaleBio scRNA workflow. After analysis with the ScaleBio analysis pipeline each cell/nucleus can be assigned to its sample of origin based on the hash oligo detection.

Figure 3: Barnyard experiments show low background and good ability to identify cells with the correct hash regardless of whether hashed samples are mixed before or after washing.



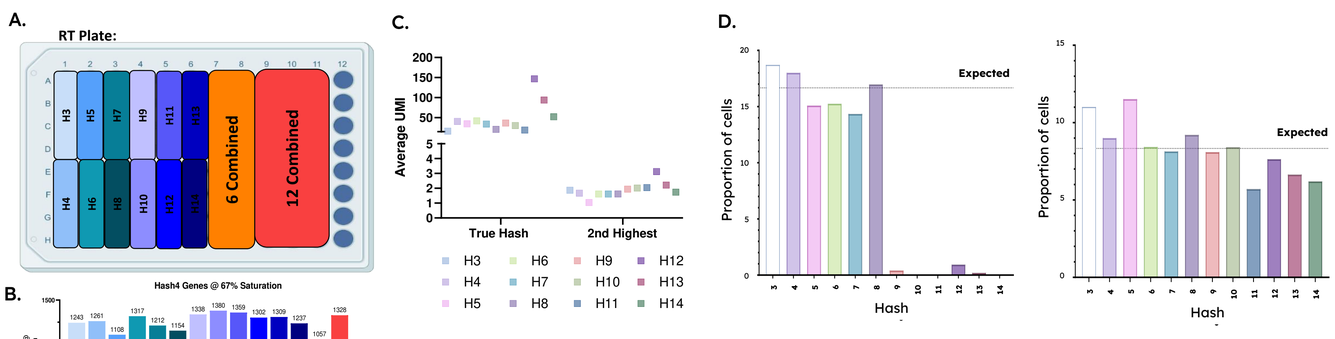
B.

	Run alone	Mixed before RT	Mixed before washing
Correct hash UMIs	12,511	12,525	1,212
Incorrect hash UMIs	35	70	25



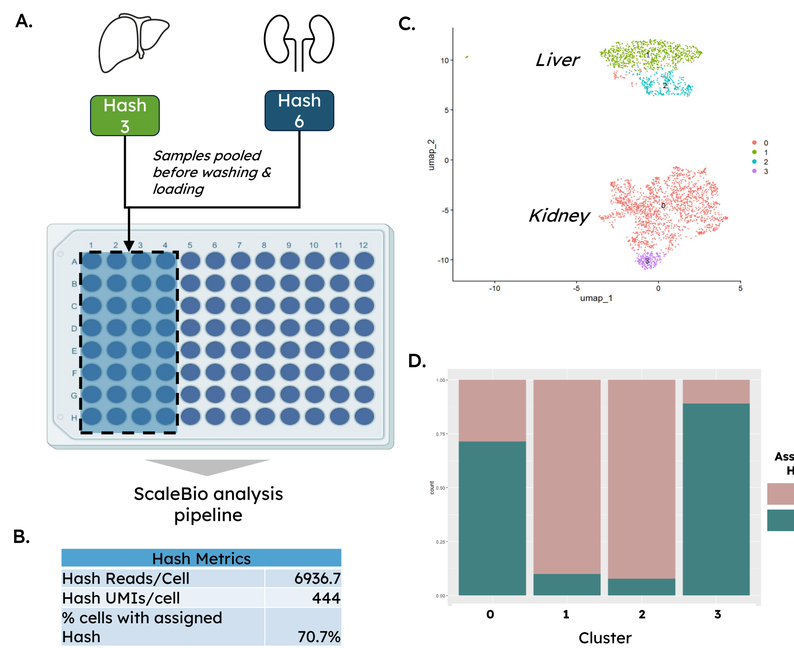
A. Experimental model system used to determine signal and background of hashes.
B. Table showing the number of UMIs recovered from the correct and incorrect hashes in the experimental workflow shown in Figure A. The number of UMIs from the incorrect hash does not increase when mixing cells prior to washing, suggesting that background introduced from incorrect binding of hashes is not significant.
C. Percent of cells with the correct hash identified when mixing hashed cells at different steps of the workflow. Data shows that mixing of hashed cells before any washes does not interfere with the ability to call the correct hash.

Figure 4: Multiplexing with individual and pooled hashes recovers expected proportions of PBMCs and does not impact RNA assay performance.



A. Schematic of RT plate. PBMCs were labeled with one of 12 hashes and run individually or combined (6 or 12 samples) using the ScaleBio scRNA workflow.
B. Median genes recovered from RNA library were similar across all 12 individual hashes as well as in pooled hashed samples.
C. Average UMI per hash. Detection shows uniform high distribution in true hash and consistently low background for all 12 hashes.
D. Expected proportions of cells are recovered from each of the 6 (left) or 12 (right) samples run in the pooled conditions.

Figure 5: Cell hashing of tissue-derived nuclei yields quality data with good sample identification.



B.

Hash Metrics	
Hash Reads/Cell	6936.7
Hash UMIs/cell	444
% cells with assigned Hash	70.7%

A. Schematic of experiment. Nuclei isolated from mouse liver and kidney were incubated with hash oligos, pooled during fixation, and taken through the ScaleBio analysis pipeline.
B. Metrics showing reads/UMIs recovered per cell as well as % of cells with a hash identified.
C. UMAP comprised of cells that were assigned to either Hash 3 (liver) or Hash 6 (kidney), colored by cluster
D. Proportion of cells in each cluster identified in Figure C that were assigned to each hash. Data shows that majority of nuclei from clusters 0 and 3 (kidney) were assigned to hash 6, while majority of nuclei from clusters 1 and 2 (liver) were assigned to hash 3, as expected.
E. Split UMAP of cells colored by Hash3 and Hash6 detection, scaled by relative UMI counts. Yellow cells in the third column would indicate high counts of both Hash3 and Hash6. This result shows specific hashing of cells without overlap of hash UMI's within a cell.

Conclusions

- ScalePlex hash oligos are robustly captured in the ScaleBio scRNA workflow, enabling higher sample throughput, introducing new stopping points, and simplifying workflows.
- Hashing shows high signal and low background when tested on barnyard samples, PBMCs, and tissue-derived nuclei despite pooling of hashed samples before any wash steps.
- Hash oligos can be used to efficiently trace cells back to their original sample in barnyard cells, PBMCs, and tissue-derived nuclei.