Automated processing of solid tissues into single cells or nuclei with the Singulator™ 100 system: scRNA-Seq, and ATAC-Seq data on Human and Mouse Tissues.

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Single-cell sequencing is revealing new levels of complexity of biological systems, but experimental workflows require reproducible generation of high-quality single-cell or -nuclei suspensions. S2 Genomics developed and commercialized the Singulator™ 100 System to automate the dissociation of solid tissues into single cell or nuclei suspensions in single-use cartridges using enzymatic or chemical dissociation with mechanical disruption.

Here we present the utility of the Singulator 100 for preparation of cells and nuclei from human and mouse tissues for subsequent analysis by single-cell RNA sequencing (scRNA-Seq) and assay for transposase-accessible chromatin (ATAC-Seq). We also demonstrate the use of RNase inhibitors directly in the Singulator sample cartridges for preservation of RNA quality during nuclei isolations.

The Singulator 100 System can automatically process fresh tissue samples into suspensions of single cells, while nuclei can be isolated from fresh, frozen, or OCT preserved tissue. **Figure 1** shows the Singulator 100, which can use S2 reagents or alternative formulations.

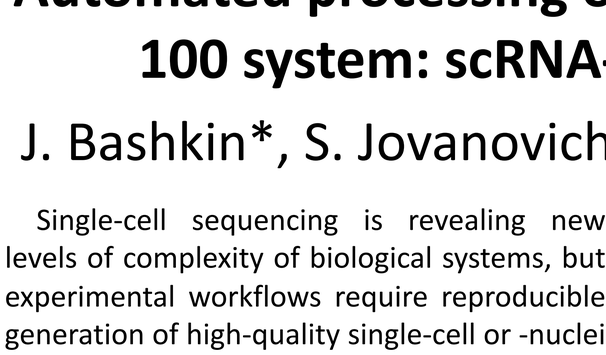

Figure 1. Singulator 100 System, including the single-use cartridge, chiller for nuclei reagents, and Single-Shot Mechanism for delivering cell reagents.

TILS From Tumors: Cells can be isolated from tissue in high yield and viability and used for a variety of downstream analyses. We explored the feasibility of isolating tumor infiltrating lymphocytes (TILS) from solid tumors processed on the Singulator, using lung cancer as a model system. Three matched sets of normal and tumor (two squamous cell carcinomas and one adenocarcinoma) patient tissue samples were dissociated into cells on the Singulator. TILS were then isolated from the tumor-derived cell suspensions using magnetic beads. Cells from normal, tumor, and TILS were then analyzed by scRNA-Seq; the data highlights differences in cell-type representation and gene expression. **Table 1** shows the yield and viabilities of the cells isolated from the normal and tumor tissue samples. **Figure 2 and Table 2** show the scRNA-Seq data from the nine samples. **Figure 3** and **Table 3** show results from UMAP and AZIMUTH analyses.

Sample	Sample Size (mg)	Average Titer (x10^6)	Viability
Patient 1: Normal	500	0.28	93%
Patient 1: Tumor	240	5.88	97%
Patient 1: TILS	N/A	1.43	97%
Patient 2: Normal	300	0.67	94%
Patient 2: Tumor	260	6.73	94%
Patient 2: TILS	N/A	1.31	97%
Patient 3: Normal	490	2.64	96%
Patient 3: Tumor	250	5.07	100%
Patient 3: TILS	N/A	1.21	98%

Table 1. Yields and viabilities for cells isolated on the Singulator 100 for the different patient samples. Patient 1: adenocarcinoma; Patients 2, 3: Squamous cell carcinoma. Fresh, anonymized samples obtained under an approved IRB protocol. Tissues were manually minced and processed on the Singulator using S2 Genomics' Lung Cell Isolation Reagent reconstituted in DMEM with the Lung Cell Protocol. The resulting cell suspensions were transferred to 15 ml conical tubes and spun at 300 g for 5 minutes at 4ºC.. The supernatant was removed and the samples were resuspended in 1 mL of RBC lysis buffer; lung and tumor samples incubated for 3 minutes and 2 minutes, respectively. The samples were then topped off to 8 mL with DMEM and spun at 300 g for 5 minutes at 4ºC and supernatant removed. Tumor cells were purified with a 30% Percoll gradient centrifugation at 300 g for 8 min. The supernatant was removed and the sample was and resuspended in PBS with 1% BSA. For all samples, the lung cells were resuspended in 1 mL of DMEM with 10% FBS, then strained sequentially through 70 µm, 40 µm, and 30 µm Flowmi® cell strainers. Cells were stained with AO/PI and quantified for yield and viability using Nexcelom K2 Cellometer. The lung and tumor cells were loaded onto the 10x Chromium™ following the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) User Guide protocol (CG000315 Rev B) with a 10,000 cell target. The remaining tumor cells were spun down, resuspended in the recommended media (PBS with 2% FBS and 1 mM EDTA) and TILs isolated using StemCell Technologies' EasySep™ Release Human CD45 Positive Selection Kit (Cat# 100-0105). After purification, the TILs were spun at 300 g for 5 min with a refrigerated (4ºC) swinging-bucket centrifuge. The supernatant was removed and the sample resuspended in 1 mL of PBS with 1% BSA and loaded onto the 10x Chromium.

Sample	Estimated # of Cells	Mean Reads/Cell	Median Genes/Cell
Patient 1 Normal	7,713	16,274	1,584
Patient 1 Tumor	8,085	24,751	669
Patient 1 TILs	7,676	20,545	1,069
Patient 2 Normal	6,831	15,811	1,408
Patient 2 Tumor	8,500	28,345	1,459
Patient 2 TILs	8,668	22,748	1,302
Patient 3 Normal	6,716	31,019	1,949
Patient 3 Tumor	6,458	26,865	1,936
Patient 3 TILs	6,192	25,962	1,302

Table 2. Summary of sequencing output from Cell Ranger™.

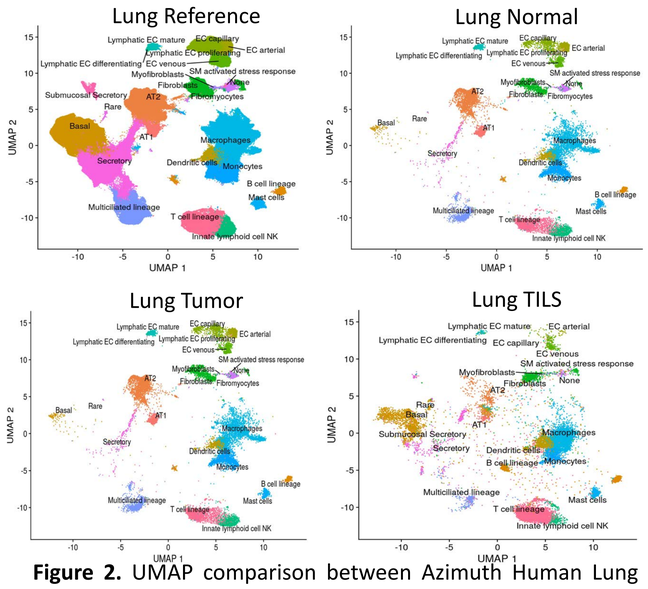

Figure 2. UMAP comparison between Azimuth Human Lung Reference Dataset and Patient Normal, Tumor, and TILS samples pooled bioinformatically. The tumor samples shows a slight increase in basal and T cells, with a decrease in EC cells. The TILS show an increase in lymphocytes relative to the tumor tissue.

Cell Type	Normal	Tumor	TILs
AT1	3.4	0.8	0
AT2	25.2	5.6	0
B cell lineage	4.6	18.4	18.8
Dendritic cells	1.6	3	4
EC arterial	2.9	0.2	0
EC capillary	2.3	0.3	0
EC venous	3	1.5	0
Fibroblasts	7.3	16.9	0
Lymphatic EC mature	2.7	0.1	0
Macrophages	20.1	26.3	5.2
T cell lineage	11.5	15.7	66.9
T cell lineage (Patient 2)	6.1	33.3	67.1
T cell lineage (Patient 3)	11.7	19.7	59.8

Table 3. Comparison of cell type representation as determined by Azimuth analysis of the scRNA-Seq data for Patient 1 sample, across Normal, Tumor, and isolated TILS lung samples. Enrichment of T cell lineage cells and depletion of lung epithelial cells illustrates the effectiveness of the TILs isolation. Data for Patient Samples 2 and 3 were generally comparable; the T cell lineage enrichment specifically for Patient 2 and 3 samples are also listed. Only cell types that exhibited differences in representation are listed.

Reagent Flexibility/ATAC-seq: To demonstrate the flexibility of the Singulator with respect to reagents, and the use of isolated nuclei for ATAC-Seq, we isolated nuclei from mouse brain and lung tissues, using (a) S2 Genomics' Nuclei Isolation Reagent (NIR) and Nuclei Storage Reagent (NSR), or (b) a reagent formulation recommended for use with ATAC-Seq from Vendor A (**Figure 4**). Equivalent yields of ~60,000 nuclei/mg tissue were isolated, and bulk RNA quality (**Figure 5 top**), ATAC library quality (**Figure 5 bottom**), and cell-type representation (**Figure 6**) obtained using either reagent formulation were also similar.

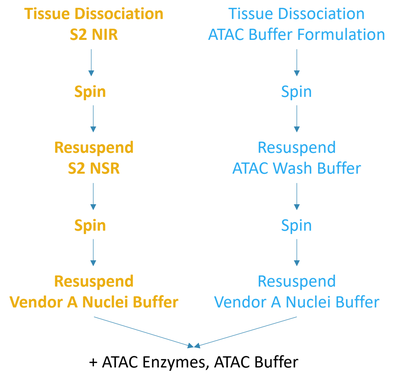

Figure 4. Comparative workflows, using the Singulator with either S2 or Vendor A reagents for nuclei isolation for ATAC-Seq.

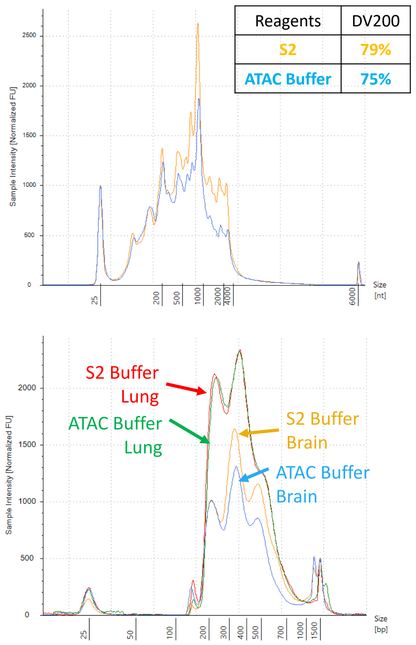

Figure 5. Comparison of bulk RNA (Top) and ATAC library (Bottom) quality for nuclei isolated from mouse brain or lung tissue, using the Singulator with S2 Genomics' or Vendor A reagent formulations (bulk RNA data for brain not shown).

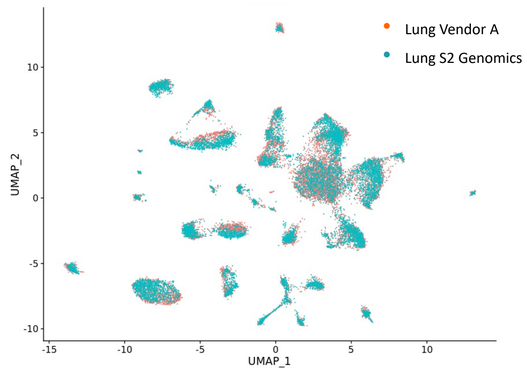

Figure 6. Comparison of clustering of scATAC-Seq for mouse lung nuclei isolated on the Singulator using either S2 Genomics' or Vendor A reagent formulations.

The Singulator 100 System automates processing fresh or frozen tissue into filtered suspensions of single cells or nuclei, which can be used for a variety of downstream applications, including isolation and analysis of TILS from tumor samples.

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