Automated processing of solid tissues into single cells or nuclei for genomics and cell biology applications with the Singulator™ 100 and 200 systems.

S. Jovanovich*, J. Bashkin, K. Chear, N. Khan, K. Kumbagowdana, B. Leisz, R. McIntosh, D. Meyer, N. Pereira
S2 Genomics, Livermore, CA.

Single-cell sequencing is revealing the next level of complexity in biological systems. Single-cell sequencing workflows for multiple applications require reproducible generation of high-quality single-cell or nuclei suspensions.

S2 Genomics developed and commercialized the patented Singulator™ 100 System to automate the dissociation of solid tissues into single cell or nuclei suspensions in single-use cartridges using coupled enzymatic or chemical dissociation and mechanical disruption. S2 Genomics is now developing the Singulator 200 System™, with higher sample throughput, improved temperature and force control, and other advancements.

Validation data will be presented for processing <1 to 400 mg samples of fresh mouse and human solid tissues into single-cell suspensions with high viabilities and yields. In addition, the Singulator can process fresh or frozen mouse and human tissue samples into high-quality nuclei in ~5 min.

Singulator-prepared samples have been analyzed by single-cell RNA sequencing (scRNA-seq), single-nuclei RNA sequencing (snRNA-seq), and assay for transposase-accessible chromatin using sequencing (ATAC-seq).

The Singulator Systems can automatically process fresh tissue samples into single cell suspensions, while nuclei can be isolated from fresh, frozen, or OCT preserved tissue. **Figure 1** shows the Singulator 100 for processing a single sample. **Figure 2** shows the Singulator 200 capable of processing two samples with random access. The Singulator 200 has SingleSense™ real-time pressure and temperature control for precise, gentle dissociation.

The Singulator platforms are open with respect to reagents: use S2 reagents or your preferred formulations from any source. Enhanced software let's you create and share the right process for your tissue.

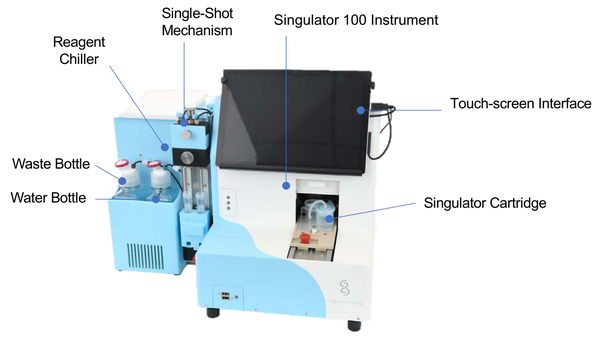

Figure 1. Singulator™ 100 System, including the Singulator instrument, single-use cartridge, chiller for nuclei reagents, and Single-Shot Mechanism for cell preparation reagents.

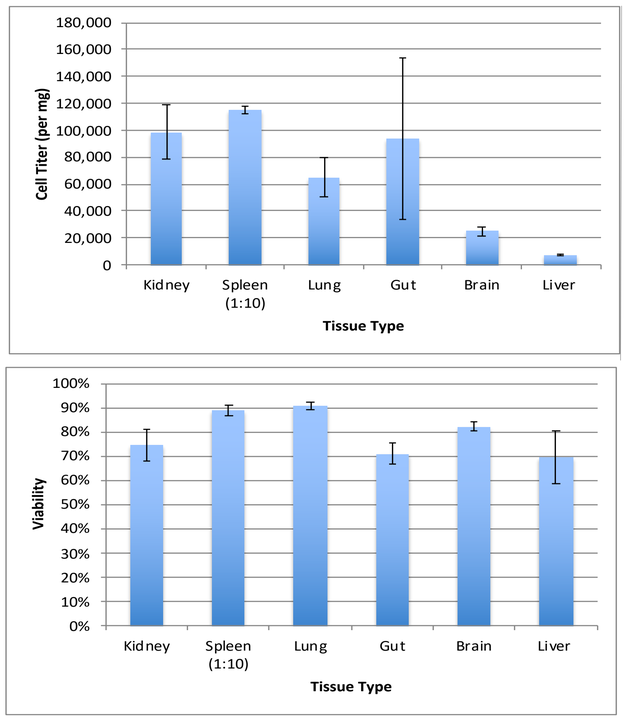

Figure 3. Automated dissociation of mouse tissues into single cells. Tissue-specific yields (top) are obtained with high viabilities (bottom).

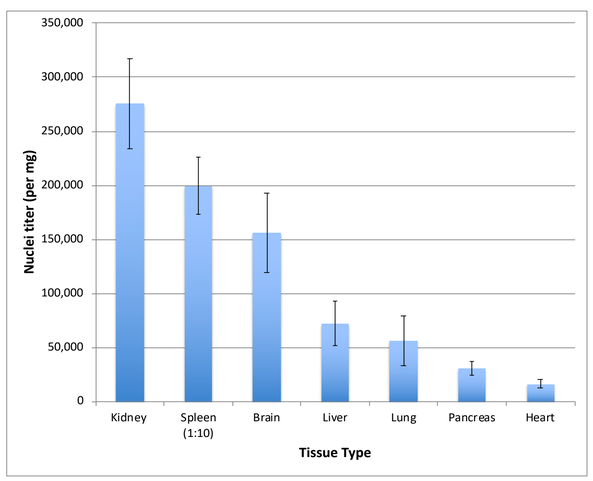

Figure 4. Automated dissociation of mouse tissues into nuclei. Tissue-specific yields are obtained in ~five minutes.

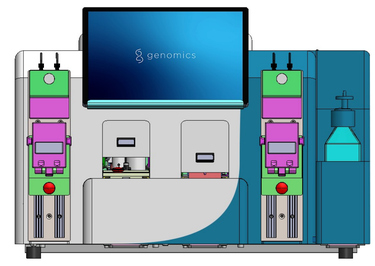

Figure 2. Singulator™ 200 System for processing two samples in single-use cartridges, with integrated chiller for nuclei reagents, and dual Single-Shot Mechanisms for delivering cell preparation reagents.

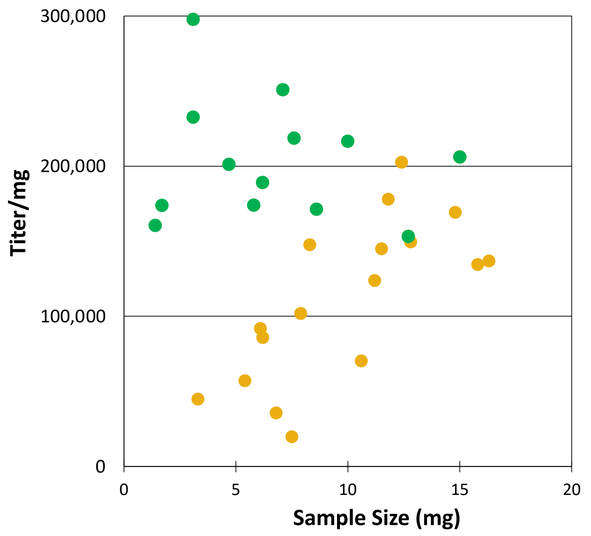

Figure 5. Dissociation of small samples with the NIC+ cartridges. Yield of mouse kidney nuclei per mg vs. sample size is shown for standard (yellow) and NIC+ (green) cartridges.

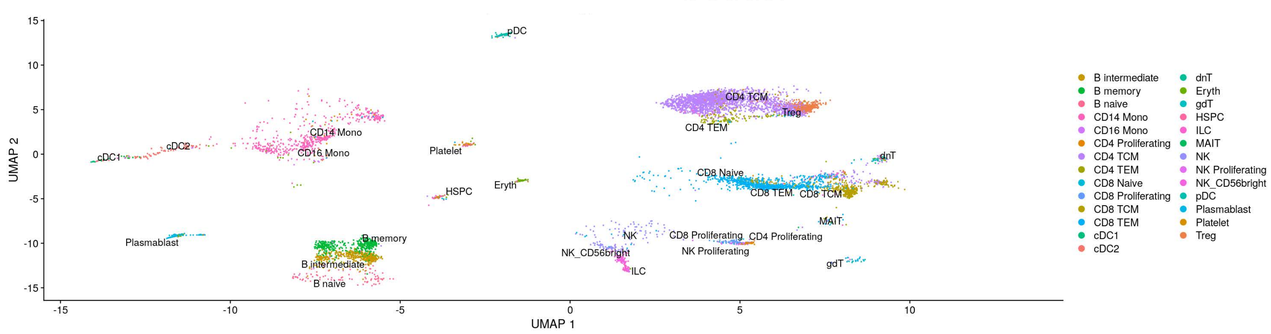

Figure 6. scRNA-Seq of TILS. Human lung samples were dissociated with the Singulator 100 with the cell isolation cartridges, and TILs purified by magnetic separation (StemCells) before scRNA-Seq library construction with a Chromium. 7,676 TIL cells were sequenced with analysis by Azimuth using the PBMC data set.

Figure 3 shows data from processing fresh mouse samples into cell suspensions using the Singulator 100 System with standard cartridges.

Samples that are difficult to process into single cells, such as frozen samples, or human prostate, heart, or muscle, can be quickly and routinely processed into nuclei suspensions for ATAC-Seq or snRNA-Seq analysis (**Figure 4**).

The recently introduced NIC+ cartridges produce nuclei efficiently with samples *as small as 1 mg,* including biopsy samples. **Figure 5** illustrates the improved efficiency of and yields of the NIC+ cartridges for mouse kidney nuclei isolations. Similar results are obtained from frozen samples and other tissues.

The Singulator Systems are being applied for a wide range of studies, from basic biology to translational research. **Figure 6** shows scRNA-Seq results from tumor infiltrating leukocytes (TILs) purified from a human lung tumor with magnetic beads.

Summary: The Singulator 100 System automates processing fresh or frozen tissue into filtered suspensions of single cells or nuclei. Samples down to 1 mg can now be routinely processed for nuclei analysis.

*stevanj@s2genomics.com

Supported in part by National Institutes of Health award number R41 HG010129-01 to Dr. Jovanovich.