Improving Single Cell Workflows Using the Singulator 100

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Introduction

Manual methods for the isolation of cells or nuclei can be lengthy, laborious, and prone to variability. The Singulator 100 by S2 Genomics is a cartridge-based, benchtop system that automates the dissociation of solid tissue into single cell or single nuclei suspensions. At the Stowers Institute, the Sequencing and Discovery Genomics Department worked with researchers to convert their manual isolation methods for a variety of organisms and tissue types into automated protocols on the Singulator.

Existing manual isolation methods generally consisted of adapted techniques from publications that were optimized based on organism, tissue type, and downstream application. On the Singulator, cell and nuclei protocols were run with the standard, pre-set protocols already installed on the instrument, using pre-formulated reagents from S2 Genomics. The customizable Singulator protocols were also utilized, allowing the use of buffers, enzymes, and runtime parameters modified to resemble the manual methods as closely as possible.

Singulator nuclei isolation protocols required 7-12 minutes to complete and generated single nuclei suspensions with well-maintained nuclear morphology. Singulator cell isolation protocols took 30-70 minutes to complete. When researchers had well-established manual protocols, the Singulator generated single cell suspensions with expected cell counts and viability percentages >85%. Automating less-optimized manual dissociation protocols generated results with lower quality, but the automated Singulator workflow allows for precise control over run metrics, which will expedite troubleshooting difficult methods. This poster describes how researchers at the Stowers Institute are generating high-quality, reproducible results and improving downstream applications by automating single cell and single nuclei workflows using the Singulator 100.

The Singulator 100

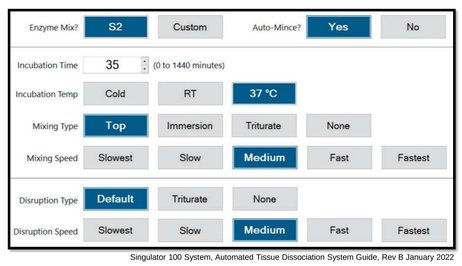
The Singulator 100 utilizes single-sample, single-use cartridges to dissociate solid tissues into single cell or single nuclei suspensions. The Single-Shot mechanism allows for users to provide their own reagents for dissociations, while the attached reagent chiller houses S2-formulated nuclei isolation/storage reagents.

Samples are added to the dissociation chamber, and the cartridge is placed inside the instrument. Once the run is initiated, reagents are sent to the cartridge, and the cartridge cap mechanically disrupts tissues following the protocol outlined in the setup screen (seen in the following section).

The dissociation is visible in real-time by a video feed from within the instrument. Once the run is completed, the sample is removed from the cartridge and can be taken through downstream workflows.

Automating Manual Methods

The Singulator comes pre-programmed with several cell and nuclei isolation protocols. These protocols can be modified, or custom protocols can be created and saved. By adjusting run parameters such as incubation time and temperature, mixing speed, and disruption type, members at the Institute were able to create custom protocols that closely resembled existing manual workflows.



Singulator 100 System, Automated Tissue Dissociation System Guide, Rev B January 2022

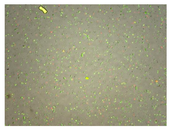
Mouse Brain Cell Dissociation

The S2 Mouse Brain protocol pre-installed on the instrument was used with the pre-formulated S2 Mouse Brain enzyme. The Mouse Brain protocol ran at 37°C and included a 40 min incubation, with the mixing and disruption speeds at the slowest settings.

The Mouse Brain protocol was then repeated, with edits to the incubation time (25 minutes), as well as the mixing speed (fast) and disruption speed (slow). For the enzyme, a papain solution was used rather than the S2 Mouse Brain enzyme.

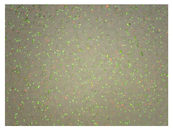
Singulator protocol with S2 reagents

Total cell concentration: 4.29 x 10^5 cells/mL
Live cell concentration: 3.96 x 10^5 cells/mL
Dead cell concentration: 3.24 x 10^5 cells/mL
Viability: 92.4 %
Average cell size: 6.4 µm
Total cell number: 1930
Live cell number: 1784
Dead cell number: 146



Modified Singulator protocol with lab-made reagents

Total cell concentration: 3.15 x 10^5 cells/mL
Live cell concentration: 2.59 x 10^5 cells/mL
Dead cell concentration: 5.53 x 10^5 cells/mL
Viability: 82.4 %
Average cell size: 12.1 µm
Total cell number: 1417
Live cell number: 1168
Dead cell number: 249



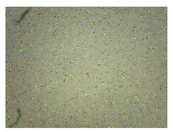
Planaria Manual Dissociation Comparison

Planaria were minced by hand before addition to the Singulator cartridge. The instrument was run with the Mouse Tumor protocol, CMFB was utilized in the buffer and enzyme locations (non-enzymatic digestion), and incubation took place at RT for 30 min.

The manual protocol was performed at the same time as the Singulator run, and the results were compared. Subsequent test runs with whole planaria resulted in the organisms retreating from the light used in the camera system, which prevented dissociation.

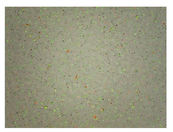
Singulator Protocol

Total cell concentration: 4.90 x 10^4 cells/mL
Live cell concentration: 4.19 x 10^4 cells/mL
Dead cell concentration: 7.08 x 10^3 cells/mL
Viability: 85.5 %
Average cell size: 9.9 µm
Total cell number: 2206
Live cell number: 1887
Dead cell number: 319



Manual Protocol

Total cell concentration: 5.04 x 10^4 cells/mL
Live cell concentration: 4.03 x 10^4 cells/mL
Dead cell concentration: 1.01 x 10^4 cells/mL
Viability: 80.0 %
Average cell size: 7.1 µm
Total cell number: 2270
Live cell number: 1817
Dead cell number: 453

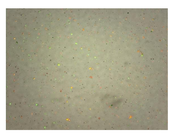


Snail Eye Protocol Adjustments

The PI lab's manual protocol for snail eyes would dissociate cells with only 40-60% viability. Based on that manual protocol, automation on the Singulator was tested using the cold setting (6-10°C), with a 15 min incubation, slowest immersion mixing, with the slowest disruption. The enzyme and buffer concentrations were the same as those used in the manual protocol.
After seeing the results, the Singulator run was repeated with a reduced incubation time.

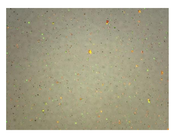
Singulator protocol 15 min Incubation

Total cell concentration: 1.24 x 10^5 cells/mL
Live cell concentration: 5.31 x 10^4 cells/mL
Dead cell concentration: 7.06 x 10^3 cells/mL
Viability: 42.9 %
Average cell size: 7.4 µm
Total cell number: 557
Live cell number: 239
Dead cell number: 318



Singulator protocol 10 min Incubation

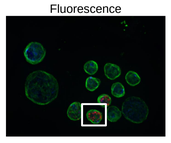
Total cell concentration: 1.58 x 10^5 cells/mL
Live cell concentration: 8.46 x 10^4 cells/mL
Dead cell concentration: 7.30 x 10^3 cells/mL
Viability: 53.7 %
Average cell size: 6.1 µm
Total cell number: 710
Live cell number: 381
Dead cell number: 329

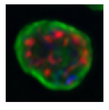


Fly Ovary Nuclei Isolation

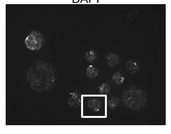
Manual isolation methods required mincing with forceps by hand, and the collection of ovaries and nuclei isolation had to be done at the same time, without the ability to freeze tissue. Singulator testing of frozen tissue generated nuclei with intact nuclear envelopes and synaptonemal complexes, and the GFP-tagged proteins of interest were not quenched. Many genotypes could be isolated in a short amount of time, allowing for the comparison of post-processing events like spreading and immunofluorescence.

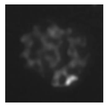
Fluorescence



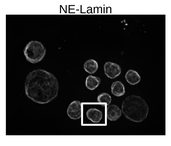


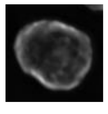
DAPI





NE-Lamin

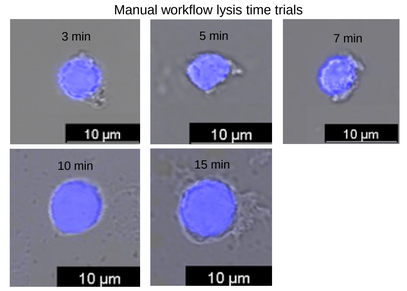




Mouse Intestine Nuclei Isolation

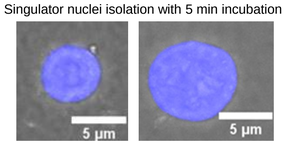
A manual intestinal epithelial cell isolation protocol was used to create a single-cell suspension, then the lab performed a manual nuclei isolation protocol. The nuclei lysis time was tested to try to achieve a lysis that worked well for all types of intestinal epithelial cells.

Manual workflow lysis time trials



The pre-programmed Low Volume nuclei protocol was run on the Singulator, modified to include a 5-minute incubation step (the default setting for that protocol is for no incubation). S2 reagents were utilized for the nuclei isolation and storage buffers.

Singulator nuclei isolation with 5 min incubation



Conclusion

The mouse brain samples worked well on the Singulator, reducing the dissociation time required by the manual method by greater than an hour, also reducing the cell death caused by hypoxia and mechanical trauma and eliminating the need for FACS sorting. Planaria samples processed on the Singulator did not require such fine mincing as the manual protocol, and the planarian microbiota that previously contaminated single cell suspensions in tissue culture media using the manual method were not present two days after the Singulator run. Similar cell counts and viabilities to the manual planaria protocol will allow the lab to save time and increase reproducibility through automation. Viability scores for the snail samples were overall lower, but in-line with results achievable by the lab's manual protocol. A 10% improvement after adjustments to the Singulator protocol suggest the Singulator will be beneficial when troubleshooting and optimizing the protocol further, as the lab now has automated control over run variables.

The manual fly nuclei isolation involved mincing tissue with forceps, which produced inconsistent results, and the lysis step of the manual mouse nuclei isolation was not ideal for all types of intestinal epithelial cells. In both instances, the Singulator reduced the time necessary for isolation, and the isolated nuclei displayed well-maintained nuclear morphology.

Summary

The Singulator 100's automated isolation workflows allow researchers to generate reproducible results by maintaining control over protocol variables that might otherwise be difficult to regulate when performing manual methods. While the protocols require testing to maximize output and quality for each organism or tissue, the ability to edit the protocols allows for precise adjustments of run parameters, which expedites troubleshooting and optimization. Manual protocol steps cannot always be directly translated to the Singulator, and the inability (at the time of this poster's creation, Singulator v2.0 software) to edit the duration of the disruption step can result in fragile tissues having decreased viability scores. As the software is updated to allow greater control over the run parameters, fragile tissues may better tolerate the automated workflow.

The consistent results from the Singulator 100 have allowed labs at the Stowers Institute to reduce isolation time and increase sample size, leading to the design of additional experiments that were not possible with the manual methods.

Acknowledgements

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