Acoustic cell separation platform for efficient preparation of single cell/nuclei suspension from complex samples for genomics analysis

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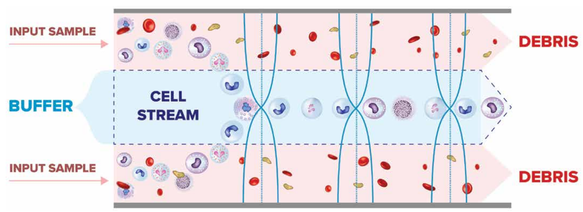
INTRODUCTION

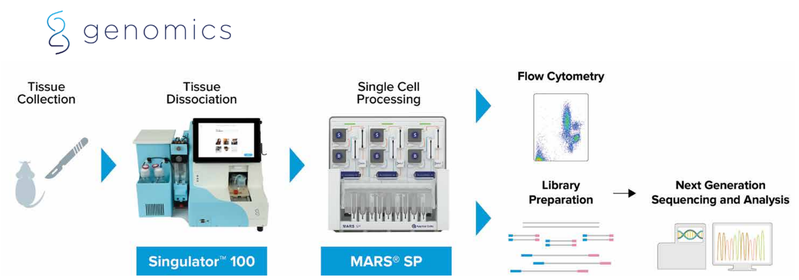
The scale and capabilities of single-cell RNA-sequencing methods have expanded rapidly in recent years, enabling major discoveries and large-scale cell mapping efforts. Sample types go beyond simple cultured cell line cells and blood cells to all types of tissues: fresh or frozen. In certain cell types, single nuclei isolation is much easier than single cells and they are more resistant to physical stress, thus single nuclei have become another source for accessing genomic information. The purity and integrity of single-cell/nuclei isolated from tissues are critical to the success of generating sequencing data at the single-cell resolution on commercial platforms. Conventionally developed isolation and clean-up protocols involve the use of many centrifugation steps, MACS or even FACS. The process is very time-consuming and costly. We have developed a high-speed Acoustic cell processing platform as part of our multi-physics approach to enable robust separation of single cells/nuclei from cellular debris and reproducible results from complex tissue samples.



Schematic explanation of MARS Acoustic Cell Separation/Washing process: cell suspension that contains live cells, dead cells and cellular debris passes through a microfluidic channel in a laminar flow with the "wash buffer". When the acoustic field is turned on live cells will move to the "wash buffer" from the original suspension but dead cells and debris will exit the channel before they move to the "wash buffer". The flow rate of the sample and "wash buffer" are both 0.8mL/min.

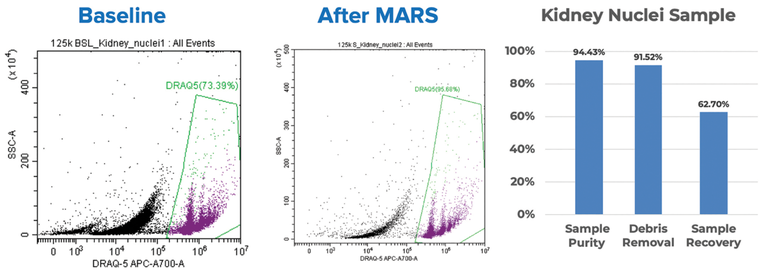
The MARS SP system contains three acoustic cell washing modules.

METHOD

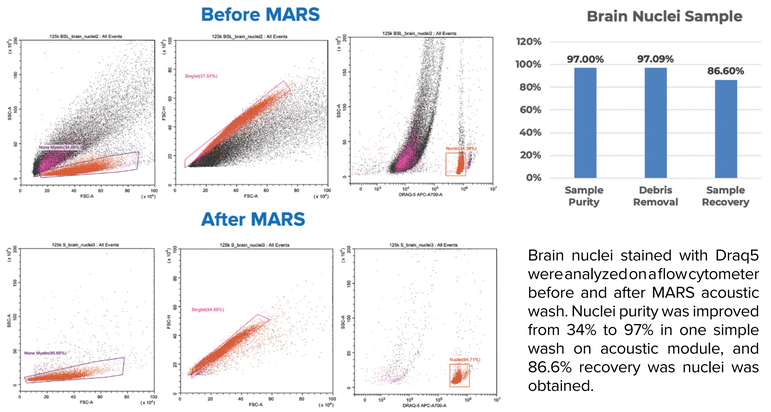


Schematic illustration of the workflow: solid tissues from various organs of the murine sample were processed on Singulator 100, and single-cell/single nuclei suspension was generated with pre-set programs. The cell/nuclei suspension was then processed on MARS acoustic module with a "Wash" program to separate cells/nuclei from debris. "washed" cells/nuclei will be ready for flow cytometer analysis or downstream single-cell genomics analysis.

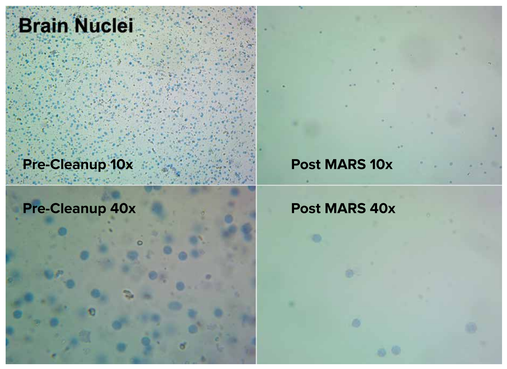
RESULTS – Nuclei Isolation from Debris



Kidney nuclei stained with Draq5 was analyzed on a flow cytometer before and after MARS acoustic wash. Nuclei purity changed from 73% to 96% and 63% of nuclei were recovered in the collection tube.



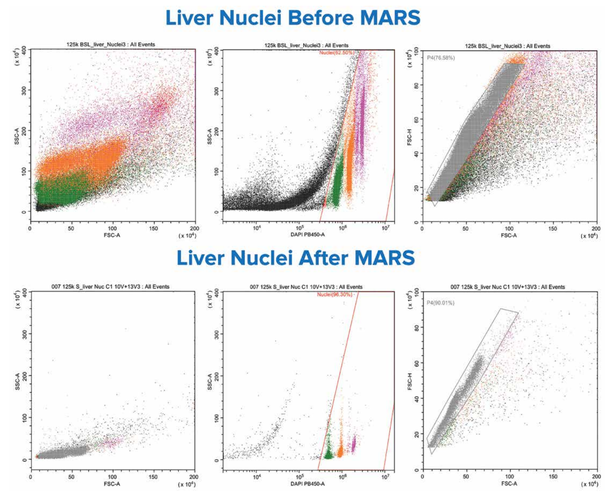
Brain nuclei stained with Draq5 were analyzed on a flow cytometer before and after MARS acoustic wash. Nuclei purity was improved from 34% to 97% in one simple wash on acoustic module, and 86.6% recovery was nuclei was obtained.



Brain nuclei purity was assessed by imaging generated on a cell counter with trypan blue staining. A two-step washing process was performed on MARS acoustic module where larger debris and smaller debris were removed separately from single nuclei suspension in two separate washing steps. Higher purity was achieved with a visual examination, but recovery was sacrificed with the two-step process.

Image data provided courtesy of S2 Genomics.

RESULTS – Single Nuclei Isolation from Debris



Liver nuclei stained with DAPI were analyzed on a flow cytometer before and after MARS acoustic wash. Liver nuclei purity changed from 62% to 96% from a two-step wash, and over 90% of nuclei were singlets after the MARS acoustic process.

RESULTS – Single Cell Isolation from Debris

Mouse brain tissue dissociation was processed on MARS Acoustic wash and Percoll based centrifugation process, the cell recovery and purity was accessed by flow cytometry. Percoll based method had lower recovery.

CONCLUSION

- MARS Acoustic cell separation platform provides an automatic solution for single-cell nuclei and single-cell preparation with high purity and high recovery for downstream analysis
- MARS, together with a Singulator, the automatic tissue dissociation system, provides the streamlined workflow for single-cell and single nuclei preparation from solid tissues.

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