genomics

Singulator[™] 100 Cell Protocol Submission

Please fill out form as thoroughly as possible. For additional questions and support, email <u>community@s2genomics.com</u>. For more information on the Singulator[™] 100 and single-cell processing, head over to <u>www.s2genomics.com</u>.

General Information | Study Identification

Protocol Name:	Mouse Heart Cell Isolation
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Investigator Name: S2 Genomics Field Applications Team

Secondary Investigator(s):

Tissue Species: Male Mouse

Tissue State: Fresh

Pre-Singulator[™] 100 Processing | Run Summary:

- Singulator was heated 15 minutes prior to run

- Mouse Heart Enzyme (pending approval) was reconstituted in 20mL of HBSS and vial was inverted 10x and incubated for 15 minutes to thoroughly mix

- 98 mg of mouse heart was extracted, rinsed with Ca/Mg free HBSS, blotted with a Kimwipe, and minced into roughly 1mm pieces and added to a custom cell isolation cartridge with a 145um filter

Singulator[™] 100 Cell Protocol Parameters

Enzyme Mix: Cutsom Protocol Type: Mouse Lung Cells Auto Mince: OYes ONo	0	Custom Formulation: - please contact S2 Genomics, Inc. for more information on the Mouse Heart Enzyme - storage buffer was HBSS and 10% FBS
Incubation Time: ^{25 minutes}		Incubation Temperature: 37 °C
Mixing Type: Top		Mixing Speed: Fastest
Disruption Type: Default		Disruption Speed: Slowest

Post-Singulator[™] 100 Processing

Centrifuge Time & Speed: 300g for 5 minutes at 4°C

Additional Cleanups/Notes:

- final volume was upped to 8mL and spun down at 300g for 5 minutes to help remove any debris
- supernatant was discarded and the pellet was resuspended 1mL RBC lysis buffer for 15 seconds
- added 9mL of HBSS to neutralize RBC lysis and spun sample at 300g for 5 minutes at 4°C
- once spun, discarded supernatant and resuspended pellet in 1mL HBSS for downstream processing

Results:

- cell viability of 79% was observed using the Nexcelom K2 AOPI based cell counter with rougly 33,750 cells/mg

Investigator Email: community@s2genomics.com Secondary Email(s): Tissue Type: Heart Mass (mg): ^{98 mg}