10x Genomics®

Sample Preparation Demonstrated Protocol

Moss Protoplast Suspension for Single Cell RNA Sequencing

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Notices

Manual Part Number

CG000090 Rev C

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Demonstrated Protocol

Moss Protoplast Suspension for Single Cell RNA Sequencing

1. Overview

This Demonstrated Protocol outlines how to obtain a single protoplast suspension from spreading earthmoss, *Physcomitrella patens* (Grandsen or Villesexel ecotypes), in preparation for use in 10x Genomics® Single Cell Protocols. Protoplasts are plant cells without a cell wall but with an intact plasma membrane.

2. Getting Started

2.1. Tips & Safety

Best practices for handling any protoplast or cell sample include using sterile technique, nuclease-free reagents and consumables, minimizing pipetting steps, and using wide-bore pipette tips where possible to minimize cell or protoplast damage.

To determine accurate protoplast or cell counts, best practices include sampling the protoplast or cell suspension at least twice and at least two counts on each sample (*i.e.* a minimum of four counts in total, based on two independent draws from the suspension). Consult Technical Note *Guidelines on Accurate Target Cell Counts* (Document CG000091) for more information. A hemacytometer was used for protoplast counting.

CRITICAL! *Follow tissue and material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.*

2.2. Materials

Supplier	Description	Part Number (US)
Rainin	Tips LTS 1ML Filter RT-L1000FLR Pipet-Lite LTS Pipette L-1000XLS+	17007954 17014382
Corning/Falcon	Cell Strainer 40 µm	352340
Thermo Fisher Scientific	INCYTO™ C-Chip™ Disposable Hemacytometer Trypan Blue Stain (0.4%) Cell Scrapers	22-600-101 15250061 08-100-241
Eppendorf	DNA LoBind Tubes 2.0 ml* Model 5430 Microcentrifuge Model 5430 Rotor 6X15/50ML with lid Model 5430 Rotor 30X1.5ML with lid	022431048 022620584 05-401-512 05-401-503
VWR	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 50 ml Sterile Polypropylene Centrifuge Tubes with Flat Caps, 15 ml Standard Orbital Shaker, Model 1000	82018-050 21008-103 89032-088
Sigma-Aldrich	D-Mannitol Driselase	M4125 D8037

*No substitutions are allowed. Items have been validated by 10x Genomics and are required for Single Cell workflow, training and system operations.

2.3. Literature

This Protocol was developed in accordance with Liu, Y.C., Vidali, L. *Efficient Polyethylene Glycol (PEG) Mediated Transformation of the Moss Physcomitrella patens*. J. Vis. Exp. (50), e2560, doi:10.3791/2560 (2011). Both Grandsen or Villesexel ecotypes were used and produced similar results.

2.4. Preparation – Wash Solution & Dissociation Solution

a) Prepare **50 ml** 8% Mannitol solution in distilled water (Wash Solution).

b) Dissolve 2% Driselase solution in **10 ml** 8% Mannitol solution (Dissociation Solution).

3. Preparation of Protoplast Suspension

NOTE

This Protocol was demonstrated using moss from a total of four 10 cm petri dishes, but can be scaled down based on sample availability.

a) Using a cell scraper, gently transfer the moss to a 50 ml centrifuge tube. Use Wash Solution to help transfer the moss into the tube.

b) Add Wash Solution up to **10 ml**.

c) Gently mix by inverting the tube 5 times.

d) Remove as much Wash Solution as possible by pouring; leaving moss solids in the tube.

e) Add **10 ml** Dissociation Solution to the 50 ml centrifuge tube containing the moss.

f) Incubate on the orbital shaker (speed setting 3) at **room temperature** for **1 h**.

g) Place a 40 µm cell strainer on top of a 50 ml centrifuge tube and filter the protoplast suspension into the tube.

h) Centrifuge the filtered suspension at **300 rcf** for **5 min**.

i) Remove the supernatant without disturbing the protoplast pellet. Leave only enough solution to cover the protoplast pellet.

j) Add **10 ml** Wash Solution into the tube. Using a 10 ml serological pipette, resuspend the protoplast pellet by gently pipetting 10 times or until protoplasts are completely suspended.

k) Centrifuge at **300 rcf** for **5 min**.

Repeat

l) Repeat steps i – k for a total of two washes.

m) Resuspend the protoplasts in approximately **1 ml** Wash Solution.

n) Determine the protoplast concentration using a hemocytometer. The target concentration is **2000 protoplasts/µl** (2 x 10^6 protoplasts/ml).

NOTE

The protoplasts are concentrated further than in cell-based 10x Genomics® Demonstrated Protocols to minimize the volume of protoplast suspension added to the Single Cell Master Mix, thereby minimizing the final concentration of Mannitol in the Single Cell Master Mix.

o) Determine the protoplast viability using a trypan blue staining method, for example.

p) If necessary, pass the protoplast suspension through a 40 µm cell strainer to remove large debris particles and cell clumps.

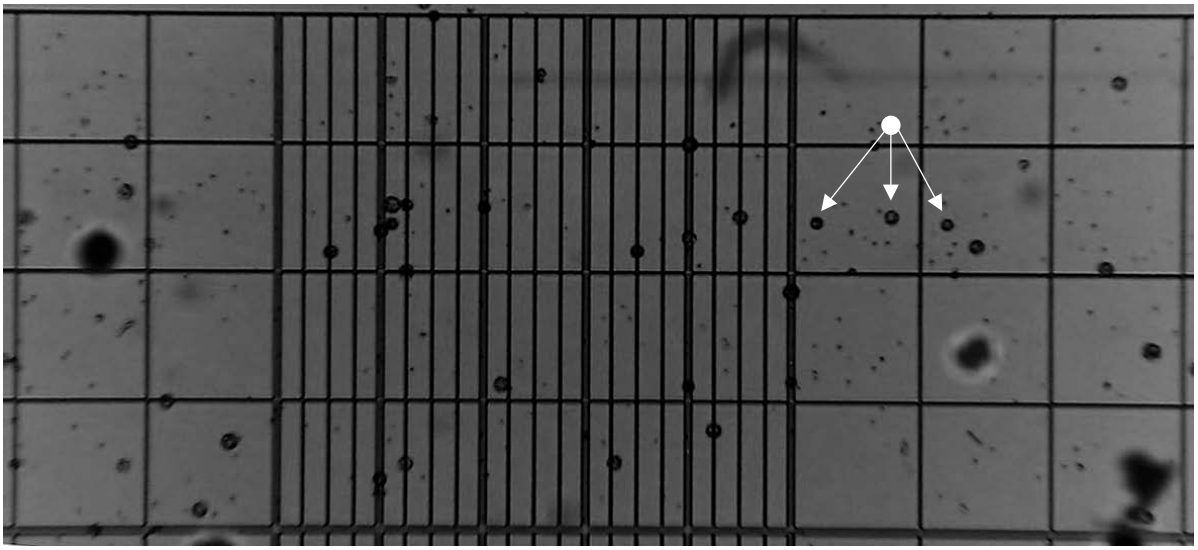
q) If necessary, dilute the protoplasts with additional Wash Solution to achieve the target protoplast concentration.

r) Once the target protoplast concentration is obtained, place the protoplasts on ice.

s) Proceed immediately with the 10x Genomics® Single Cell Protocol to avoid prolonged (>30 min) incubation of the prepared protoplasts on ice.

4. Results

Ideally, the protoplasts should be well singulated with little or no large debris particles. Very small particles of debris may be seen on the hemacytometer but do not appear to adversely affect the performance of 10x Genomics Single Cell Protocols. The percent viability of protoplasts is expected to be ~90% based on trypan blue staining.



Protoplasts are well singulated (white arrows). A few large and small debris particles can be seen in the sample but do not appear to adversely affect the performance of the 10x Genomics Single Cell Protocols.