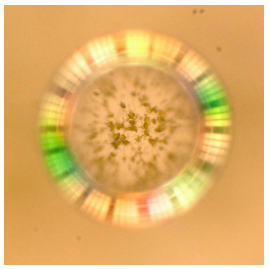
Sep 24, 2019

Marchantia chloroplast isolation



DOI
dx.doi.org/10.17504/protocols.io.4×6gxre

Eftychis Frangedakis[1]

[1]University of Cambridge

OpenPlant Project

Eftychis Frangedakis
University of Cambridge, Plant Sciences

DOI: https://dx.doi.org/10.17504/protocols.io.4x6gxre

Protocol Citation: Eftychis Frangedakis 2019. Marchantia chloroplast isolation. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.4x6gxre

Protocol status: Working
We use this protocol and it's working

Created: June 29, 2019

Last Modified: September 24, 2019

Protocol Integer ID: 25310

Keywords: marchantia chloroplast isolation, maize chloroplasts for protein import study, maize chloroplast, protocl from mark settle, marchantia, protein import study, modifiction of the isolation

Abstract

This protocol is a modifiction of the **ISOLATION OF MAIZE CHLOROPLASTS FOR PROTEIN IMPORT STUDIES** protocl from Mark Settles (modified from Ken Cline).

Guidelines

*Pre-cool o/n Buffer GR and pestle and mortar.

Materials

MATERIALS

Percoll™ Plus **GE Healthcare Catalog #**17-5445-01

Miracloth **Merck Millipore (EMD Millipore) Catalog #**475855

GR Buffer (1L)

50 mM Hepes-KOH pH7.5	50 ml 1 M Hepes (23.8g/100ml) [238.3 - Melford]
0.33 M Sorbitol	100ml 3.3 M Sorbitol (300g/500ml) [182.2 - Melford]
1 mM MgCl2	1ml 1M MgCl2
1 mM MnCl2	1ml 1M MnCl2 (19.7g/100ml) [197.91 - Sigma]
2 mM EDTA	4ml 0.5M EDTA pH8

add fresh

5 mM Na-ascorbate	1 g Na-ascorbate [Acros Organic]
1% BSA	10g BSA

Troubleshooting

1 Prepare 20 ml 30% Percoll solution by mixing 6ml Percoll, 14ml Buffer GR.

2 Prepare 10 ml 70% Percoll solution by mixing 7ml Percoll and 3ml Buffer GR.

3 Add 15ml of 30% Percoil soution in a 50ml Falcon tube. Very carefully underlay 6ml of 70% (denser) Percoll solution using a 5ml Gilson pipette (A in Figure).

4 Keep the gradient on ice until further use.

5 Weigh 10g of thallus (grown in 12h light : 12h dark cycle – harvest the tissue 2-3 hours at the start of the light cycle - to reduce starch accumulation) (B in Figure).

6 Homogenize the tissue using a mortar and pestle into 100ml buffer GR (C and D in Figure).

7 Filter homogenate through two layers of Miracloth into two 50ml falcon tubes (E and F in Figure)

8 Centrifuge at 1200 g for 7 min (G in Figure)

9 Remove supernatant and resuspend the pellet carefully in 2ml of buffer GR.

10 Transfer the resuspended pellet to the top of the Percoll Gradient using a cut-off pipette tip (H in Figure).

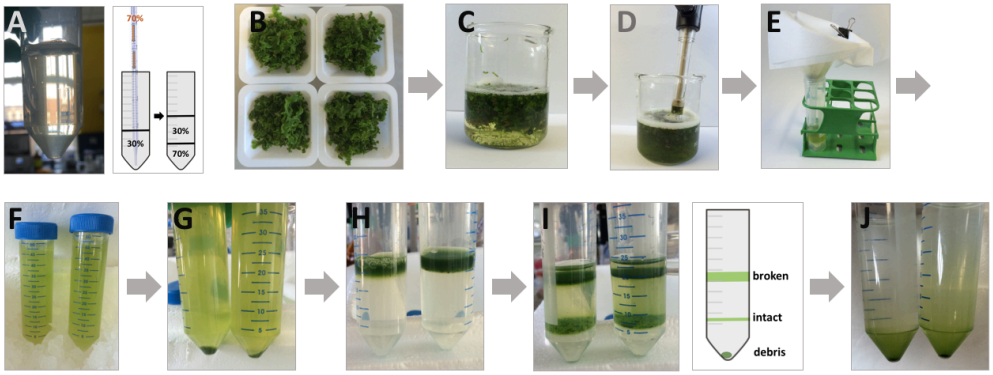
11 Spin at 7000g for 17 min at 4ºC. USE A SLOW ACCELERATION AND TURN DECELERATION OFF. Broken chloroplasts will reside in the top fraction, intact chloroplasts will reside in the interface of the two Percoll layers (I in Figure).

12 Carefully transfer intact chloroplasts from the interface, to a new 50 mL Falcon tube.

13 Wash intact chloroplasts with 25ml of Buffer GR (without BSA). This step removes residual Percoll. Centrifuge at 1500g for 5min at 4^{o}C (J in Figure).

14 Flash freeze pellet if not used immediatly and store at -80^{o}C

15



16