Chloroplast transformation of the liverwort Marchantia polymorpha

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Abstract

This protocol was adapted from Chiyoda et al., 2007 and Boehm et al., 2015

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Guidelines

-use desiccated ethanol for the steps 10.-14. of microprojectile preparation

-the chloroplast transformation vector used here is pCS Cl0*b. Linearising the vector using NotI increases transformation efficiency (Boehm et al., 2015). The linearised vector is ethanol precipitated and resuspended in TE buffer to obtain the concentration of approximately 1 ug/ul (quantification using NanoDrop is sufficient)

Before start

-filter sterilise the $CaCl_2$ solution and store aliquots in -20 °C

-store spermidine aliquots at -80 °C

-wash stopping screens with dH_2O and sterilise by autoclaving

-sterilise the cellophane discs by autoclaving

Protocol

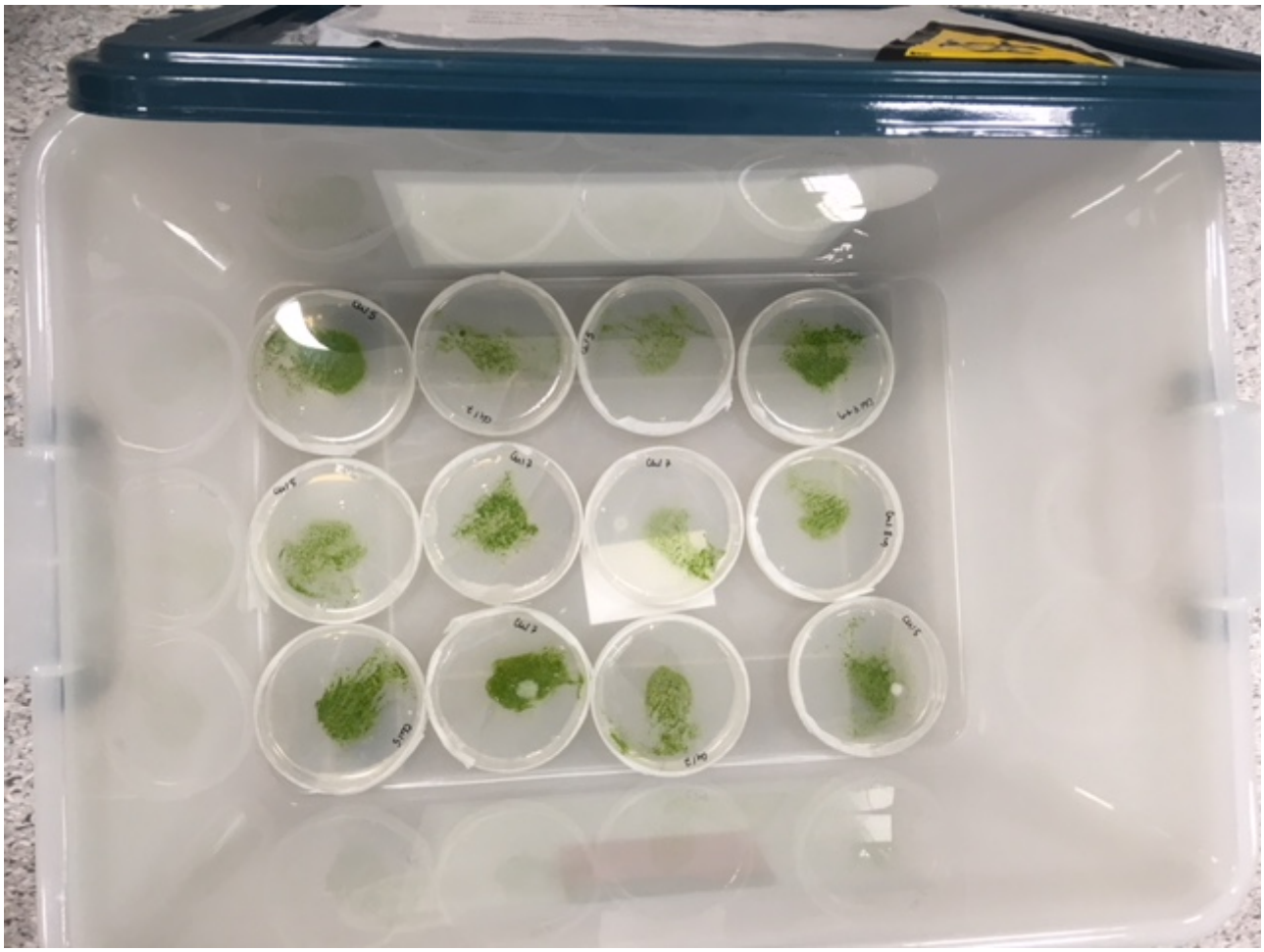
Spore preparation

Step 1.

1. Collect intact sporangia in sterile dH_2O in an eppendorf tube. Surface sterilise the sporangia using 1 % sodium dichloroisocyanurate (NaDCC), rinse three times with 1 ml sterile dH_2O, remove water. Add 100 ul sterile dH_2O per sporangium. Break the the sporangia open, resuspend spores by pipetting.

2. Place the 100 ul of spore suspension on the center of a sterile cellophane disc on a plate containing modified Johnson's agar medium (Honkanen et al., 2016 https://doi.org/10.1016/j.cub.2016.09.062).

3. Germinate spores on plates for 7 days under continuous light (50-60 umol photons m^{-2} s^{-1}) at 21 °C.



7 day old spores before shooting.

Microprojectile preparation

Step 2.

1. Suspend 0.6 um diameter gold microprojectiles (Bio-Rad) in 100 % ethanol to obtain concentration of 20 ug/ul (can make aliquots and store in -30 °C). I use 3.5 mg gold microprojectiles for each construct.

2. Centrifuge the gold particle suspension (20 ug/ul) at 6,000 g for 1 min. Discard supernatant.

3. Add 1 ml sterile water to the pellet and vortex thoroughly.

4. Centrifuge at 2,000 g for 3 min and discard supernatant. It may be hard to get the gold particles to pellet at this stage, but resist the temptation to spin fast/long to avoid clumping.

5. Add 320 ul sterile water to the pellet and vortex thoroughly.

6. While vortexing the tube add in following order: 350 ul of 2.5 M $CaCl_2$, 35-40 ul of 1 ug/ul vector DNA solution and 70 ul of 0.1 M spermidine.

7. Incubate on ice for 10 minutes with vigorous mixing once per minute for 10 s.

8. Quick spin at a small table top centrifuge for few seconds (just enough to pellet the gold particles)

9. Discard supernatant.

10. Add 500 ul ethanol to the pellet and vortex thoroughly.

11. Quick spin at a small table top centrifuge for few seconds.

12. Repeat steps 10.-12.

13. Resuspend gold particles into 80 ul ethanol.

14. Sterilise macrocarriers with 100 % ethanol and air-dry under flow hood prior to use.

15. Use a 7 ul aliquot of gold microprojectile suspension for each bombardment. Pipette in the middle of dried macrocarriers under flow hood, wait until completely dry.

16. Sterilise the rupture discs by dipping in isopropanol just before each shooting.

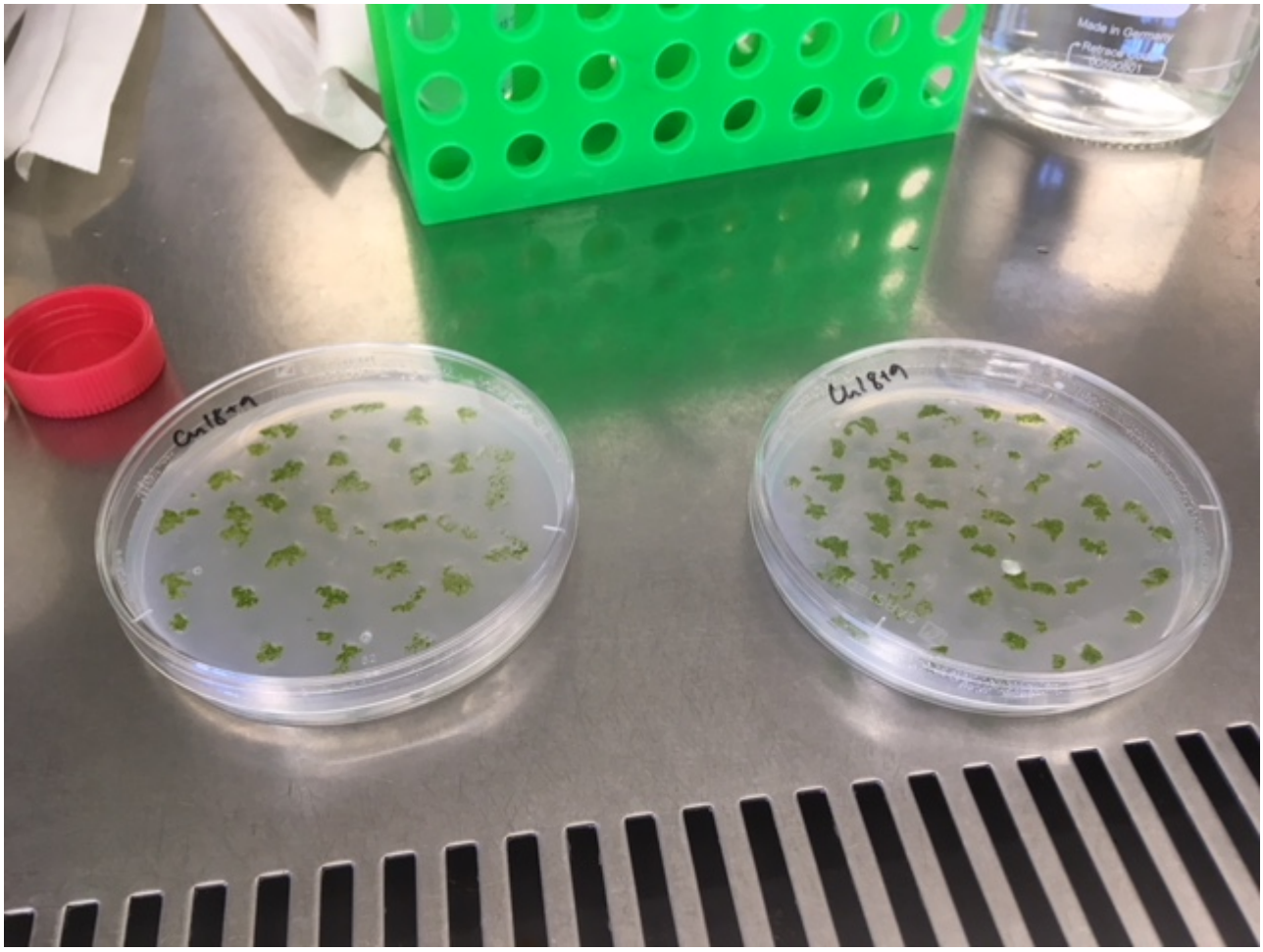
Particle bombardment and selection

Step 3.

1. Bombard sheets of 7 day old spores using a biolistic delivery system (PDS-1000/He, Bio-Rad) under a vacuum of 28 in Hg with a rupture disk of 900 psi (Bio-Rad) or 1,100 psi, the distance between the plate and the stopping screen 100-120 mm. Each plate can be shot twice to increase the chances of getting transformants. Note that if your biolistics delivery system has a hepta adaptor attached to it the rupture disc will only rupture at about 300 psi above the psi of the rupture disc (i.e. at 1,400 psi when using 1,100 psi rupture discs). This is ok. If using a hepta adaptor only shoot one macrocarrier from the middle channel.

2. Keep the plates overnight in continuous light (50-60 umol photons m^{-2} s^{-1}) at 21 °C.

3. Add few ml of sterile dH_2O onto each plate, use a sterile spreader to divide the spores from each plate onto two agar plates containing modified Johnson's media without sucrose and 500 mg/l spectinomycin. Try to separate the spores using sterile forceps. It will be difficult to separate the spores and spread them evenly. It is ok to leave them attached together.



Spores immediately after moving to selection plates.

4. Screen for green spectinomycin resistant plants after 3-4 weeks.

5. Successively subculture to obtain homoplasmic lines. Confirm by PCR after about three months.