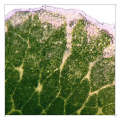

Sep 24, 2019

Marchantia chloroplast transformation

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1 Works for me dx.doi.org/10.17504/protocols.io.4v9gw96

OpenPlant Project

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1 Before tranformation place in a glass petri dish (Fig. 1-1) the macro-carrier holders (Fig. 1-2) , macro-carriers (Fig. 1-3) , stopping screens (Fig. 1-4) and ruptures discs (Fig. 1-5) and autoclave (Fig. 1-6 and 7)

2 DNAdel^{TM} nanoparticles are supplied as a 50 mg/mL suspension in binding buffer. To dissociate any aggregates prior to use, agitate the suspension, sonicate briefly for 30 s using an ultrasonic water-bath sonicator, vortex for 5 s and repeat.

3 Use 0.5 mg of nanoparticles per transformation.

4 Dilute DNAdel^{TM} nanoparticles into binding buffer to final concentration 30 mg/mL. For example, for 3 shots, mix 30 µL of 50 mg/mL DNAdelTM nanoparticles with 20 µL Binding Buffer (Seashell Technologies) into a 1.5 mL centrifuge tube.

5 Add 1.5-2 µg of plasmid DNA per shot planned. For example for 3 shots add 4.5-6 µg of plasmid DNA.

6 Add an equal volume of Precipitation Buffer (Seashell Technologies) (total volume of DNAdel^{TM} nanoparticles plus volume of Binding Buffer plus volume of plasmid DNA), vortex and incubate at room temperature for 3 min.

7 Centrifuge at 8000 xg for 10 s, discard supernatant, and wash the DNA coated DNAdelTM nanoparticles with 500 µL ice cold 100% EtOH.

8 Centrifuge at 8000 xg for 10 s again, discard supernatant, and resuspend the nanoparticles in 7 µL of 100% EtOH per bombardment planned. To resuspend the nanoparticles briefly sonicate using an ultrasonic water-bath sonicator. Usually two rounds of 5 s sonication.

9 Pipette 7 µl of DNA coated nanoparticles in the center of each macro-carrier and leave to dry (Fig. 2A). Spread across the center of macro-carrier surface with the use of a sterile pipette tip.

10 Two plates with 7 day old sporelings should be used per contruct transformation (Fig. 2B)

11 Using sterile tweezers place the stopping screen into the macro-carrier launch assembly (Fig. 2C).

12 Using sterile tweezers place the DNA loaded macro-carrier) into the macro-carrier launch assembly (Fig. 2D).

13 Screw the macro-carrier holder (with the plasmid DNA loaded macro-carrier) on top of the macro-carrier launch assembly (Fig. 2E).

14 Load the rupture disk into the retaining cup using sterile tweezers (Fig. 2F and G).

15 Screw firmly the retaining cup with the rupture disk onto the gas acceleration tube at the top of the bombardment chamber.

16 Place the macro-carrier launch assembly into the bombardment chamber, second position from the top, and close the door (Fig. 2H).

17 Place the opened plate with the sporelings on the target shelf (Fig. 2H).

18 Press the vacuum button (second red button from the left) to “vac” position until vacuum reaches 27-28 inches Hg and immediately move button to “hold” position. Then keep the fire button pressed until rupture disk bursts (pressure reaches ~1000 PSI) and then release the fire button.

19 Release the vacuum.

20 Bombard each plate twice (we observed increased efficiency with the second bombardment).

21 Remove the plate from the chamber.

22 Unload macro-carrier launch assembly and rupture disk retaining cup.

23 After finishing, tape the plate and palce back in growth chamber for two days.

24 Using a sterile scalpel transfer the sporelings on a selection plate conatining 500µg/mL spec

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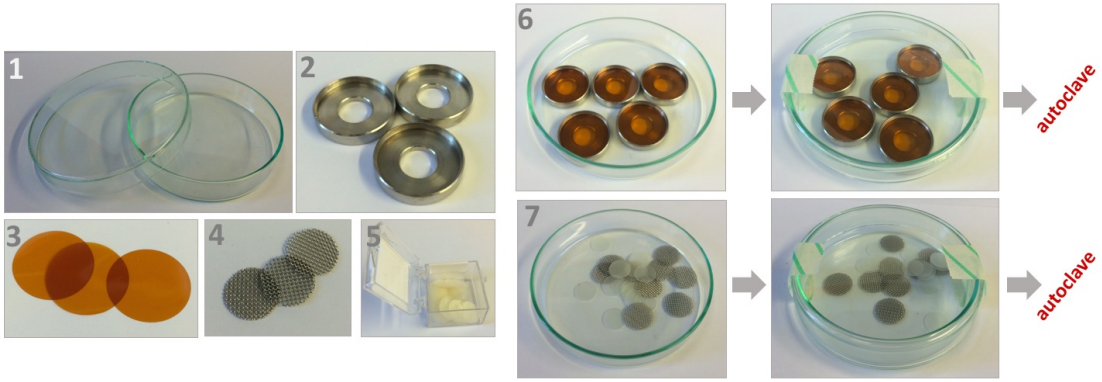

Figure 1

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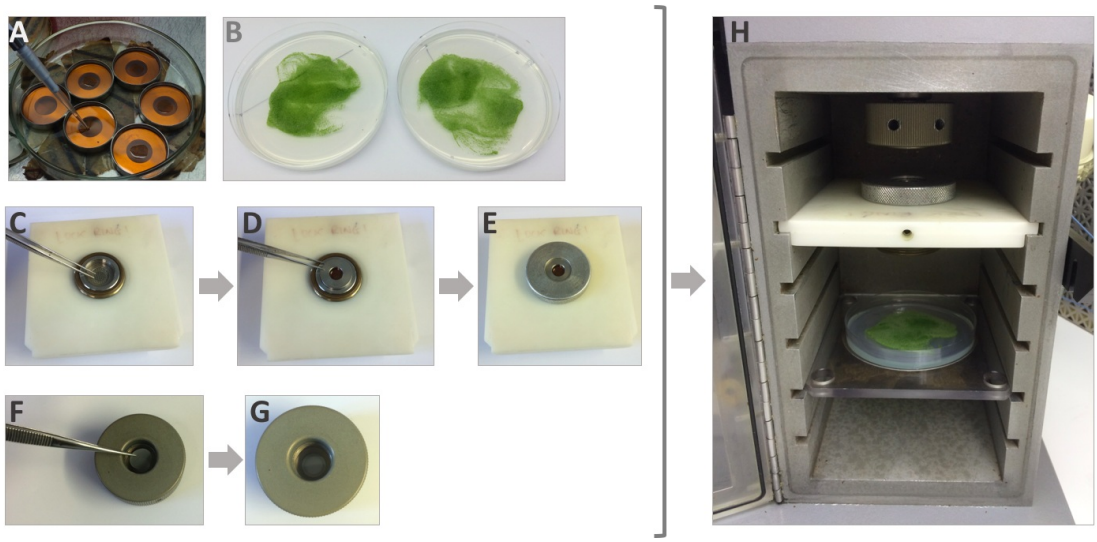

Figure 2

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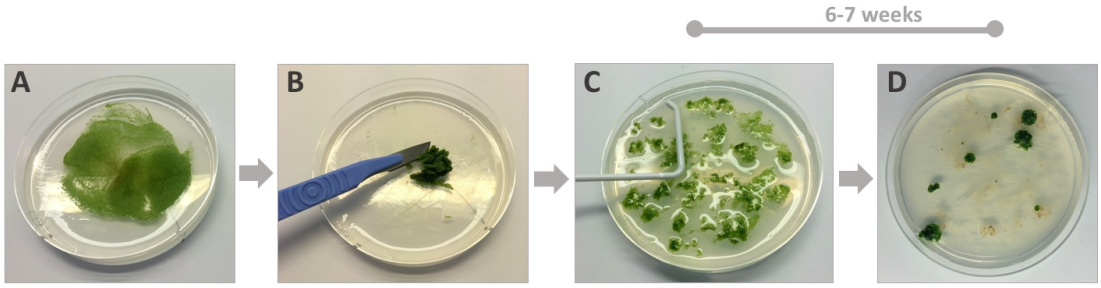

Figure 3