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Biolistic Transformation of Amphidinium V.2

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We use this protocol and it's working

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Abstract

A method to transform the chloroplast of *Amphidinium carterae* using biolistics.
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Materials

pAmpPSBA

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To be completed in advance

1 Prepare artificial minicircle (pAmpPSBA) at 1mg ml^{-1}. pAmpPSBA contains a bacterial origin of replication and an ampicillin resistance marker, so transformation and growth using a suitable *E. coli* strain, followed by a miniprep, is the simplest method to achieve this. pAmpPSBA also contains a linearised minicircle containing the core region and a modified form of PSBA to confer resistance to the herbicide Atrazine.

2 Grow wild type *Amphidinium carterae* in *f/2* medium to early log-phase.

3 Prepare *f/2* plates (1.5% agarose in *f/2* medium) and leave to dry for 3-4 hours before storing at 4ºC. (This drying step is not strictly necessary, but allows the cells to be transferred to the plates much more quickly, which reduces the subsequent time between plating and shooting).

4 Autoclave stopping screens, macrocarriers and 1,550 PSI rupture disks (all supplied by BioRad).

Cell Preparation

5 Count the cells. For each plate to be transformed (including necessary controls), between 1-5×10^7 cells are needed. Harvest an appropriate volume for the number of plates to be shot.

6 Centrifuge the cells at low g (~1,500g) and resuspend the pellet in a very small volume (~500µl) of fresh *f/2* medium.

7 Spot the cells onto the centre of the *f/2* plate dropwise. Leave to dry. Cells should for a circle of no more than 2cm diameter to maximise the number of cells in the region bombarded by gold particles. Note that *Amphidinium* does not survive long term on solid plates, so aim to shoot as soon after drying as practical.

DNA Preparation

8 *DNA Precipitation is carried out using Seashell Technology's "DNAdel™ gold carrier" delivery system. 550nm gold particles are used throughout.*

9 Sonicate the DNAdel™ gold particles (supplied at 50mg ml^{-1}) to dissociate aggregates. 1-2 minutes should normally be sufficient, but the presence of aggregates can be

detected by eye.

10 Dilute the gold particles in the supplied 'binding buffer'* to a final concentration of 30mg ml^{-1}. Use 0.5mg gold per plate for transformation. *(e.g. 30ul gold particles and 20ul binding buffer for 3 plates)*.

* Supplied by Seashell Technology with the DNAdel™ gold carrier kit.

11 Add artificial minicircle to gold particles at a ratio of 2.5µg DNA per mg gold. Using very concentrated DNA stock keeps volume low and allows for high concentrations of DNA and gold to maximise DNA precipitation onto the gold particles.

DNA Preparation

12 Vortex the DNA-gold particle mixture briefly.

13 Add an equal volume of 'precipitation buffer'* to the DNA-gold particle mix and vortex briefly. Allow to stand for 3 minutes.

* Supplied by Seashell Technology with the DNAdel™ gold carrier kit.

14 Centrifuge at 10,000g for 10s to pellet the gold particles.

15 Remove the supernatant and add 500µl ice cold 100% ethanol. Vortex briefly.

16 Centrifuge at 10,000g for 10s to pellet the gold particles.

17 Remove the supernatant. Add 10µl ice cold 100% ethanol for each 0.5mg gold in the preparation. *(e.g. add 30µl for the 1.5mg gold prepared for shooting 3 plates).*

18 Briefly sonicate the solution to minimise aggregation and allow for reliable delivery. Again, 1-2 minutes will normally be sufficient, but sonication should continue untill even suspension of the gold particles is achieved.

Bombardment Preparation

19 Wash the macrocarriers in 70% ethanol and allow to dry.

20 Wash each rupture disk and macrocarrier in isopropanol and allow to dry.

21 Transfer a 10µl aliquot of the prepared gold particles (see above) to the centre of each macrocarrier. Allow to dry.

Bombardment

22 *Bombardment is carried out in a Biorad 'Biolistic PDS-1000/He' device using Biorad stopping screens, macrocarriers and rupture disks.*

23 Shooting is carried out using 1,550 PSI rupture disks. (These show higher rates of transformation that 1,100 or 1,350 PSI disks.)

24 Shooting is carried out in vacuum at ≥25 in. Hg.

25 Plates for transformation are placed in the middle slot in the Biolistics device *(i.e. the highest possible position for a plate. Higher slots are occupied by the rupture disk and macrocarrier).*

Post Shooting

26 *Cells should be resuspended as soon as possible after shooting to minimise cell death.*

27 Resuspend all cells from each plate in 5ml fresh *f/2* medium. As many cells as possible should be washed off the surface of the plate and collected in the desired vessel for growth.

28 Leave to recover overnight.

29 Dilute the cells as and apply selection criteria. *(e.g. each plate's cells are diluted to a final volume of 50ml with 2ug ml^{-1} Atrazine).*

30 Monitor growth by microscopy every 1-2 days. Untransformed *Amphidinium carterae* are typically dead at 10-12 days post-application of Atrazine.