# *Biolistic delivery to* Ectocarpus *cells*

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**Bead preparation**

1. Weigh out 1µm gold beads in a tube and add water to 50 mg/ml final. The suspension can be kept for several months at 4°C

2. Vortex the beads for 1 min and then sonicate for several seconds so that they stay in suspension during the pipetting.

3. Whilst the beads are sonicating, pipette aliquotes of 20 µl 1.5 ml tubes. One aliquot is enough for two macrocarrier disks. These aliquots can be stored at 4°C in the same way as the stock solution.

4. Centrifuge an aliquot at 13,000 rpm for 10 secondes and discard the supernatant.

5. Add: 10 µl of 2.5 M $CaCl_2$, vortex for 1 minute and sonicate for 10 s

   4 µl 0.1 M Spermidine, vortex for 1 minute and sonicate for 10 s

   1 µl 1µg/µl plasmid DNA, vortex for 1 minute and sonicate for 10 s

   5 µl 8mM dye, vortex for1 minute and sonicate for 10 seconds

6. Incubate for 10 min at room temperature in the dark.

7. Centrifuge an aliquot at 13,000 rpm for 10 secondes and discard the supernatant.

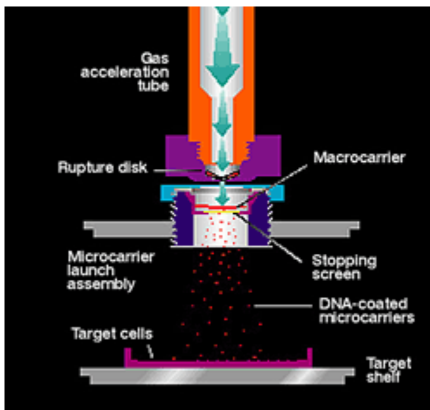
8. Add 20 µl of 100% ethanol, vortex for 1 minute and sonicate for a few seconds.

9. Whilst sonicating, pipette 9 µl each onto two macrocarriers. The disks should be placed flat on Whatman paper in a Petri dish.

10. Leave to dry at 4°C.

**Shooting**

11. Place the disk with the beads in the gene gun, together with the stop grill and the rupture disk, as indicated in the BioRad manual.




12. Cut the adult *Ectocarpus* filaments into small pieces and place them in the centre of a Petri dish containing seawater in 1.5 % Phytagel. Make sure that the filaments do not clump. They should cover a 1 to 2 cm circle in the middle of the dish. Remove as much liquid as possible.

13. Shoot the beads at a rupture pressure of 2000 psi with the sample 6 cm from the stop grill.

14. Take up the sample in seawater and put it in a culture room at 13°C.