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Biolistic transformation of *Isochrysis galbana*

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We use this protocol and it's working

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Troubleshooting

Preparing tungsten beads

- 1 Weigh out 60 mg tungsten into a microfuge tube
- 2 Wash in 1 mL 100% ethanol. Vortex. Centrifugation at 13000rpm for 1 minute then remove ethanol
- 3 Wash four times in 1 mL molecular grade water. Centrifugation at 13000rpm for 1 minute. Remove the water.
- 4 Add 1mL of water to beads and transfer 50 μ L aliquots (whilst vortexing frequently to avoid bead sedimentation) into microfuge tubes and freeze for later use.
- 5 Add 4 μ g plgNAT plasmid DNA (previously prepared using Qiagen miniprep) to a 50 μ L aliquot of tungsten beads. The DNA volume added should not be more than 10 μ L.
- 6 Add 50 μ L of 2.5M CaCl_2 and 20 μ L of 0.1M spermidine to the tungsten and DNA and vortex gently
- 7 Leave the tube for 10 minutes to sediment the beads. Centrifuge briefly for 3 seconds at 2000rpm to sediment beads. Remove supernatant.
- 8 Wash beads in 250 μ L of 100% molecular grade ethanol. Vortex and briefly centrifuge for 3 seconds at 2000rpm. Remove the supernatant.
- 9 Add 50 μ L of 100% molecular grade ethanol. Whilst frequently vortexing to prevent sedimentation, add 10 μ L aliquots to individual macrocarrier discs and leave to dry.
- 10 Store macrocarrier discs coated in DNA-tungsten beads inside a closed Petri dish lined with Whatmann filter paper at 4°C until needed.

Biolistic transformation

- 11 Grow cultures of *Isochrysis galbana* for 4-7 days prior to transformation. Target cell density should be about 5×10^5 - 1×10^6 cells/mL
- 12 Clean biolistic PDS-1000/He particle delivery system (BIORAD, CA, USA). Wipe outside and inside of the biolistic chamber with ethanol. Immerse rupture discs, macrocarrier

- holders and stopping screens briefly in ethanol and leave to dry
- 13 Connect PDS-1000 apparatus to a helium supply and a vacuum pump
 - 14 Set up biolistic apparatus according to manufacturers instructions.
 - 14.1 Take out rupture disc retaining cap and place rupture disc inside and place assembly back inside
 - 14.2 Assemble macrocarrier launch assembly. Place stopping screen onto shelf. Place macrocarrier disc in macrocarrier holder and position over stopping screen (with beads now facing downwards). Screw assembly together.
 - 14.3 Place macrocarrier launch assembly onto the uppermost shelf of the biolistic chamber.
 - 15 Prepare Isochrysis cells immediately prior to loading to avoid drying out. Filter 150 mL of *I. galbana* (NCMA 1323 from NCMA, USA) culture onto a 3 µm cellulose nitrate membrane filter (diameter 47 mm, Whatmann from GE Healthcare Life Sciences, UK)
 - 16 Wash cells with 3-5 mL of biolistic loading buffer to remove excess salt. Leave filter damp so as not to dry the cells completely.
 - 17 Transfer the filter containing cells to a small Petri dish (47 mm diameter).
 - 18 Place the Petri dish onto the target plate shelf in the biolistic chamber 6cm below the macrocarrier launch assembly.
 - 19 Perform biolistic transformation process
 - 19.1 Turn on vacuum pump and biolistic chamber.
 - 19.2 Remove the air in the biolistic chamber by setting the vacuum button to VAC until the pressure was 27 in.Hg.
 - 19.3 Switch the vacuum button to hold.



- 19.4 Press and hold the FIRE button until the rupture disc bursts and beads have bombarded the cells.
- 19.5 Release the FIRE button and set the vacuum button to VENT to allow air into the chamber
- 20 Remove the Petri dish and replace its lid. Tungsten beads should be visible on the centre of the filter.
- 21 Wash the cells off the filter using 3 mL 50% salinity F/2 and place into a sterile cell culture dish.
- 22 Leave cells to recover overnight (16 h) in light chamber incubator
- 23 Count cells using a haemocytometer to determine the number of surviving cells post transformation (ideally should be similar to starting concentration). Subdivide cells into a 24 well plate, 10 μ L of cells per well, and make the volume in each well up to 2 mL with 50% salinity F/2 media containing 80 μ g/mL nourseothricin. Include control wells containing non-transformed cells in media with and without nourseothricin.
- 24 Observe cells each week to look at cell health (motility, cell integrity). Perform cell counts in each well. If growth is observed in wells containing 80 μ g/mL nourseothricin, take these cells and maintain on nourseothricin at 120 μ g/mL in 24 well plates (2 mL volume) for 2 weeks and then at 150 μ g/mL in 10mL culture tubes for two weeks.