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Working

Biolistic transformation of polar diatom Fragilariopsis cylindrus

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ABSTRACT

Transformation system for the polar diatom *Fragilariopsis cylindrus* using microparticle bombardment.

EXTERNAL LINK

https://ueaeprints.uea.ac.uk/66542/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Hopes A (2017). Expanding the molecular toolbox in diatoms: developing a transformation system, CRISPR-Cas and Inverse Yeast-1-hybrid. PhD Thesis, University of East Anglia.

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GUIDELINES

Keep cells and plates at 4^{o}C where possible throughout the protocol. Where brief room temperature steps are required, such as shooting cells, keep plates on ice before and transfer back to ice/4^{o}C as quickly as possible.

1 Culturing Fragilariopsis cylindrus. Grow Fragilariopsis cylindrus in Aquil media + Si (Price, 1989; https://ncma.bigelow.org/algal-recipes) at 4oC in 24 hour light. When culturing pre-chill the media and keep F. cylindrus on ice. Innoculate cells at a starting concentration of 50,000 cells/ml.

2 Making plates for shooting and selection. Make 1.5% Aquil agar plates for shooting and 0.8% Aquil agar plates supplemented with 100ug/ml zeocin (final concentration) for selection. To make plates make 2x concentrated SOW and 2x concentrated agar in separate bottles. Autoclave, cool to 50oC and mix. Add in Aquil nutrients once the SOW and agar have been mixed. For selective plates add in zeocin (stock concentration 100mg/ml). Pour plates and store at 4oC once set. Make sure plates are chilled to 4oC before using.

3 Plasmid. A plasmid containing a FCP:shble cassette and a FCP:egfp cassette have been used to transform F. cylindrus. Shble confers resistance to the antibiotic zeocin and the egfp has a human codon bias. An endogenous FCP promoter and terminator have been used. The plasmid can be found at Addgene: https://www.addgene.org/85987/

4 Preparation for shooting. Preparation of particles for shooting is based on the method by Kroth (2007). Weigh 60mg of M10 (0.7µm diameter) tungsten particles (BioRad, Hercules, CA) into a sterile 1.5 mL reaction tube. Add 1ml 100% ethanol and vortex for 3-5 minutes. Centrifµge at 12000 rpm for 1 minute and discard supernatant. Add 1ml of sterile water, resuspend, centrifµge for 1 minute at 12000 rpm and discard supernatant. Repeat two more times. Resuspend in 1ml of water and pipette 50ul aliquots into fresh Eppendorf tubes. To avoid sedimentation of particles during this step vortex frequently. Store aliquots at -20°C for up to two weeks. Make a 2.5M CaCl2 solution (store at -20oC) and a 0.1M Spermidine solution (store at -20oC and use within one month).

5 Harvest cells. Harvest cells during exponential growth (5x105 – 1x106 cells/ml). Use 5 x 107 cells for each shot. Filter cells at 4oC onto a 47

mm diameter 1.2 µm isopore filter (Merck, catalog number: RTTP04700) using vacuum filtration. Place the filter into the centre of a pre-chilled 1.5% agar Aquil plate. Keep plates at 4oC until needed for shooting, at which point transfer to ice.

6 Coat particles for shooting with the plasmid. Particles are coated according to Kroth (2007). Thaw aliquots of CaCl2, spermidine and tungsten particles. Resuspend tungsten particles by briefly vortexing. For 5 shots, pipette 50µl of tungsten particles, 5µg of plasmid (in 5-10µl of water), 50µl of 2.5M CaCl2, and 20µl of 0.1M spermidine into a 1.5ml Eppendorf tube. Vortex for 1 minute. Centrifuge briefly and remove supernatant. Add 250ul of 100% ethanol and vortex until homogenous. Centrifuge briefly and remove supernatant. Add 50µl of 100% ethanol and vortex. Store on ice and use particles within 1 hour. Sterilise macrocarriers in 70% ethanol and leave to dry. Place into a sterilised macrocarrier holder (sterilised by 70% ethanol or autoclave), vortex prepared particles to resuspend and pipette 10ul into the centre of the macrocarrier. Leave to dry (make sure the ventilation hood is switched off to prevent particle migration) – this only takes a few minutes and proceed to shooting.

7 Microparticle bombardmdent. A PDS-1000/He biolistic microparticle delivery system (Bio-Rad Laboratories, catalog number: 1652257) was used to transform F. cylindrus. Prior to shooting autoclave the rupture disc retaining cap and microcarrier launch assembly. Wipe the particle gun inside and out with 70% ethanol. Set-up the device according to manufacturer's instructions. Dip a rupture disc (1550 psi) into 100% isopropanol, leave to dry and assemble into the rupture disc retaining cap. Clean helium lines by firing a shot without cells and particles. Perform particle bombardment as follows: Load a rupture disc into the retaining cap and install into the assembly. Load a stopping screen and the prepared macrocarrier holder into the microcarrier launch assembly. Place the plate with the chilled cells into the chamber (at room temperature) at a flight distance of 6cm. Create a vacuum of 25 Hg in the biolistic chamber, shoot and immediately return the cells to ice. Turn the filter paper upside down to bring the cells into contact with the Aquil plate surface and incubate at 4oC for 24 hours under standard conditions to allow cells to recover before selection. Shots are carried out in triplicate.

8 Selection. Keeping the plate on ice, rinse cells from the plate/filter with 500µl – 1ml of chilled Aquil media. Divide the cells between 5 selective plates on ice and spread with a sterilised drigalski spatula. Incubate cells at 4oC under standard conditions. Colonies typically take 3-7 weeks to appear at which point they can be screened by colony PCR or transferred to liquid media for further growth.

9 References. Price, N.M., Harrison, G.I., Hering, J.G., Hudson, R.J., Nirel, P.M., Palenik, B. and Morel, F.M., 1989. Preparation and chemistry of the artificial algal culture medium Aquil. Biological oceanography, 6(5-6), pp.443-461. Kroth, P.G., 2007. Genetic transformation; a tool to study protein targeting in diatoms, chap. 17. Methods in molecular biology, 2nd edn., Totowa, NJ, USA: Humana Press.