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Glass bead transformation of Heterosigma akashiwo V.3

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol was developed for the glass bead-mediated transformation of *Heterosigma akashiwo*. This species does not grow on solid medium, so transformants are grown "in bulk" and should not be considered clonal. We have repeated this protocol with success and have been able to maintain transformants on selection medium for several months.

Troubleshooting

1 Harvest exponential growth *Heterosigma akashiwo* culture (1-2 × 10^6 cells mL^{-1}) by centrifugation at 1500 rpm for 90 s in a 50 mL sterile screw-cap tube. Remove the supernatant by pipetting.

2 Resuspend the cell pellet in a small volume of MAX Efficiency® Transformation Reagent for Algae (Invitrogen, Thermo Fischer Scientific, USA) or in 384mM Sorbitol.

3 Transfer 300µl of cells to a 1.5 ml microcentrifuge tube and add up to 50 µl of linearized plasmid DNA (1 – 10µg). Mix by tapping the mixture and incubate for 10 minutes at room temperature. Plasmid sequences can be found at http://doi.org/10.5281/zenodo.439653.

4 Weigh approximately 300 mg of glass beads (425-600µm, Sigma-Aldrich, USA) into a 2 ml microcentrifuge tube and add the cells-DNA mixture to the glass beads. Vortex at full speed for 10 secs and let it sit on bench for 15 mins.

5 Transfer the mixture in equal amounts to 4 wells of 12 well plate with 2 ml of fresh f/2 medium with cefotaxime (100µg/ml, to control bacterial growth).

6 Incubate in dim light for 1 day and then transfer to normal light conditions for another 2 days. After 3-4 days, add the appropriate antibiotic and incubate under normal conditions.

Expected Results

7 Transformants should be visible after 20-30 days. Continue to transfer the live cells to fresh medium every 10-12 days with antibiotic and analyse transformants by PCR once sufficient culture is obtained.