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Efforts to transform Heterosigma akashiwo using an Agrobacterium-mediated approach V.3

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ABSTRACT

This protocol was developed for transformation of Heterosigma akashiwo by Agrobacterium tumefaciens. H. akashiwo does not grow on solid medium, so transformants are grown "in bulk" and should not be considered clonal. We have had limited success with this protocol and may require further optimization.

Preparing an Agrobacterium starter culture

1 Plant transformation binary vectors (here pCambia1300 and its variants) were introduced into *Agrobacterium tumefaciens* (GV3101(pMP90)) via electroporation.

Cells were grown at 29 C for 2 days on agar-solidified LB (Luria-Bertani) medium with antibiotics (Kanamycin 50 µg/ml; Rifampicin 25 µg/ml; Gentamycin 25 µg/ml). Positive colonies carrying the plasmid were identified using PCR.

Preparing an Agrobacterium transformation culture

2 Agrobacterium starter cultures (5 ml LB broth) with appropriate antibiotics were grown overnight at 29 C at 200 rpms.

Approximately 16-18 h prior to the algal transformation a larger culture (25 ml) was inoculated with 25 µl of the starter culture

The following day, Agrobacterium cultures which had reached an OD_{600} of 1.5 were pelleted at 9,000 rpm for 5 min.

The bacterial pellet was resuspended in transformation medium consisting of half-strength Guillard's f Medium (f/2) with 200 µM acetosyringone, to achieve a final OD_{600} between 2 and 2.5. The cells were then left in the dark for 2 h at room temperature to activate the *vir* genes of Agrobacterium that are induced by the acetosyringone treatment.

Preparing H.akashiwo for co-cultivation

3 Algae were grown in f/2 medium at 22 C with 16h light:8h dark.

The starting number of cells was between 5 and 10 million in multiple trials

Algal cells were collected in a round bottom tube (Oakridge) and centrifuged at 2,000 rpm for 2 min.

Cells were gently rinsed in f/2 and centrifuged at 2,000 rpm for 2 min.

Co-cultivation

4 Algal cells were gently mixed with the Agrobacterium culture and layered on top of a 0.25% (w/v) agarose gel matrix. There were 2 to 4 million algal cells mixed with 18 to 25 million Agrobacterium per construct during co-cultivation in a volume ranging from 6 to 12 ml.

Our initial pilot assays with liquid (f/2) co-cultivation resulted in a high percentage of algal cell death. We observed that a semi-solid agarose gel matrix worked better with greater recovery of live algal cells.

After a 24 h incubation at room temperature, the co-cultivation mix was centrifuged at 2,000 rpm for 2 min.

Algal cells were rinsed with f/2 twice and resuspended in 20 to 40 ml of f/2 supplemented with Cefotaxime (150µg/ml; bacteriostatic agent).

Algal cells were grown in 10 ml aliquots in sterile multi-well plates under standard conditions and allowed to recover for 5 d.

Selection to identify putative algal transformants

5 Cells were placed in selection medium (f/2 supplemented with Hygromycin 10 and 20 µg/ml and Cefataxime 150µg/ml) at room temperature under standard growth conditions for 7d

Test results and Outcomes

6 We have had limited success with this protocol. There were six independent transformation trials carried out. In contrast to the co-cultivation recovery step, in general, there was little or no viability of the putative transformants after antibiotic selection as indicated by the presence of cellular debris and absence of intact living cells when visualized under a microscope. Also, H. akashiwo does not grow on solid medium, so transformants are grown 'in bulk' and cannot be considered clonal. The viability of transformants when treated with Hygromycin (between 10 µg/ml and 20 µg/ml) needs be reevaluated and the selection procedure may need further optimization as well.

Zendo Link

7 http://doi.org/10.5281/zenodo.439653