Agrobacterium Tumefaciens Bead Beating Protocol Pcb302 into S. Microadriaticum

1. Place 1.3 x10^7 Symbiodinium cells in 1 mL culture medium (ASP-8A) in a 2mL cryotube containing a dry volume of 200 µL (about 500 mg) acid-washed, sterile glass beads.
2. Then, add 350 µL of 20% polyethylene glycol (PEG) to the suspension.
3. Shake the tube in a bead beater at 4200 rpm for 90 seconds.
4. After shaking, transfer the cells to a new sterile 2 mL cryotube and wash to remove the PEG.
5. Add 150 µL of Agrobacterium culture (OD600= 1.5) harboring the pcb302 plasmid.
6. Incubate in fresh ASP-8A medium without antibiotics in the dark for 1-2 days before selection.
7. To select, add 1 mg/mL BASTA and 50 µg/mL Kanamycin and 50 µg/mL Ampicillin. In addition, 50 µg/mL Kanamycin should always be present to prevent any bacterial growth during the selection process.
8. The cultures should then be maintained in the same solution (Final volume 25 mL) in 50 mL sterile tubes under the standard photoperiod conditions and monitored for the appearance of bright-green fluorescence detected by microscopy (under phase contrast and epifluorescence 40x and 63x).

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