Agrobacterium tumefaciens Mediated Transformation of pCB302-gfp-MBD into S. microadriaticum via Bead Beating

1. Placed 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.

a. Mini-bead beater [S. Micro]= 3.0 x10^5 total cells from flask: S. Micro ASP-8A 8/2/19 100 mL; 2

b. Lambert Bead Beater [S. Micro]= 9.6 x 10^5 total cells from flask: S.M. 75 mL + 1mL ASP-8A 8/21/19

2. Then, added 350 µL of 20% polyethylene glycol (PEG) to the suspension.

3. Shook the tube in a bead beater at 4200 rpm for 90 seconds.
    a. Mini-bead beater [S. Micro]= 1.6 x10^4 cells/mL
    b. Lambert Bead Beater [S. Micro]= 6.0 x10^4 cells/mL

4. After shaking, transferred the cells to a new sterile 2 mL cryotube and washed to remove the PEG by pelleting at 3,000 g for 3 minutes then resuspending in 1 mL ASP-8A.

a. Mini-bead beater [S. Micro]= 0 visible cells

b. Lambert Bead Beater [S. Micro]= 4.4 x10^4 cells/mL (some seen swimming)

5. Added 150 µL of Agrobacterium culture (OD600= 1.5) harboring the pCB302 plasmid.
6. Incubated in fresh ASP-8A medium without antibiotics in the dark for 2 days before selection.
7. To select, added 50 µg/mL Kanamycin and 50 µg/mL Ampicillin. In addition, 50 µg/mL Kanamycin was always present to prevent any bacterial growth during the selection process.
    1. 50 µL of 1000x kanamycin
    2. 25 µL of 50 µg/mL Ampicillin