Zooxanthella Isolation Protocol

From Coffroth Lab – August 2005

Protocol:

1. Preserve a piece of the original tissue.

2. Chop a piece of tissue (1-2 cm2 depending on species and thickness) into small pieces on a glass slide and then transfer into a homogenizer

3. Grind in about 2 ml of f/2 and pour through a 125 um mesh into a 15 ml tube. Rinse mesh with 1 ml f/2 for a final volume of about 3 ml

4. Transfer chunks from the mesh to a sterile 50 ml tube or flask containing 25 ml f/2

5. Transfer 0.5 ml of homogenate to a sterile 50 ml tube or flask containing 25 ml f/2. (Note: aliquots of the homogenate can also be transferred to antibiotic media – see notes on media)

6. Spin the remainder for 5 min at 5000 rpm.

7. Transfer 0.5 ml of the supernatant to a sterile 50 ml tube or flask containing 25 ml f/2.

8. Pour off supernatant and top with about 10 ml f/2. Spin for 5 min at 5000 rpm.

9. Transfer 0.5 ml of the supernatant to a sterile 50 ml tube or flask containing 25 ml f/2.

10. Pour off supernatant and resuspend in about 5 ml f/2

11. Mix and then transfer 0.5 ml to a sterile 50 ml tube or flask containing 25 ml f/2.

12. Transfer 1 drop of the mixed pellet to a sterile 50 ml tube or flask containing 25 ml f/2.

13. Transfer 1 drop of the mixed pellet to each of 5 drams.

14. Place the rest of the suspension in a sterile 50 ml tube or flask containing 25 ml f/2.
15. Keep your fingers crossed ?

Follow-up:
To assure relatively clean zoox it is important to initially do frequent transfers into antibiotic media and f/2 as soon as growth is observed (every 4-7 days).