**Materials**

* Marine broth
* Agar
* RO water
* Ethanol
* Bleach
* Petri dishes
* Pipets
* Culture tubes
* Frozen cultures of V. coralliilyticus (virulent, non-quarantined), V. owensii (virulent, non-quarantined), and V. diazotrophicus (non-virulent)

**Instruments**

* Autoclave
* Incubator (standing and shaking)
* Spectrophotometer
* Microscope
* Hemocytometer

**Procedure for streaking out plates**

* Keep all surfaces sterilized with 70% ethanol.
* Prepare marine broth and agar according to instructions.
* Autoclave marine broth and agar.
* Allow media to cool without gelling.
* Pour media into petri dishes until 2/3 of the bottom surface is covered.
* Gently shake the plates to even the surface and cool until complete gelling at 4C.
* Remove each culture from -80C storage.
* With a sterile stick, remove a very small amount of cells from each frozen stock and gently streak to singles on marine agar plates.
* Allow each plate to incubate overnight (18 hours) at 27C.
* After incubation, this plate may be stored at 4C wrapped completely in parafilm for up to 10 days.

**Procedure for liquid culture**

* Keep all surfaces sterilized with 70% ethanol.
* Prepare marine broth according to instructions.
* Autoclave marine broth.
* Allow broth to cool completely before handling.
* Put 5 mL of marine broth in each of three sterile culture tubes.
* With sterile sticks, gently pick a single colony from each plate.
* Gently mix the stick in the media and throw the stick into biohazardous waste.
* Set each culture tube in a gently shaking incubator overnight (18 hours) at 27.
* Centrifuge each culture tube for 10 minutes at 3000 rpm.
* Pour off the media supernatant and resuspend in 0.04 uM filtered sea water.
* Prepare fresh liquid culture for each inoculation.

**Procedure for analyzing bacterial concentrations**

* Pipet 10 uL of each resuspened culture into a hemocytometer.
* Let it settle for 5 minutes.
* Count cells.
  + Dilute 1:10 in saline if cells are too concentrated to count. Dilute further if necessary.