Bacteria

*Vibrio coralliilyticus* (ATCC BAA-45)

*Vibrio shiloi* AK1 (ATCC BAA-91)

Culturing from agar stabs sent from Max (stored at 4C):

Day 1:

Bring a marine agar plate to room temperature

Using a sterile pipette tip/toothpick, touch the bacteria growing in the punctured spot of the stab culture

Streak to singles

Incubate the plate overnight @ 30C

Day 2:

Repeat the following day if (a) no single colonies can be isolated (overgrown) or (b) no colonies are present.

If you can isolate a single colony, pick it up with a sterile toothpick and inoculate a flask containing 50 mL of marine broth

Incubate the flask overnight @ 30C with shaking

Day 3:

Prepare glycerol stock: Pipet 500 uL of the bacterial culture into a labeled cryovial containing 500 uL of 30% glycerol and store @ -80C

Culturing from glycerol stock

Streak out strains on marine agar plates (include clean plate)

Incubate O/N @ 30C

Pick single colonies from each plate

Inoculate in 10 mL sterile marine broth

Incubate O/N @ 30C with shaking

Washing

Centrifuge cultures @ 3000 rpm for 5 minutes

Decant supernatant

Resuspend pellet in 10 mL filtered seawater

Centrifuge cultures @ 3000 rpm for 5 minutes

Decant supernatant

Resuspend pellet in 10 mL filtered seawater

\*\*always include media blank\*\*

Quantify CFU @ OD 0.100

Select the “Cell Culture Module” in the NanoDrop software

Blank the instrument to the double-washed media blank

Measure OD of double-washed cultures

Record initial OD

Dilute the cultures until OD = 0.1

Measure OD of diluted culture

Record final OD

Clean the NanoDrop with 10% bleach

Prepare 10-5, 10-6, and 10-7 dilutions of each culture and media blank

\*\*These dilutions are meant to provide plates that are neither too sparse nor overgrown. \*\*Adjust dilution factors if necessary to create countable plates (~100 colonies)

Plate 100 uL of each dilution of each culture/media blank onto marine agar plates

Incubate O/N @ 30C

Count colonies

Calculate CFU/mL = colonies/(volume plated \* dilution factor)

Example:



Record CFU @ OD 0.1