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Protocol status: Working
We use this protocol and it's working

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Day 1

- 1 Streak out marine microbes onto Marine Agar plates and incubate overnight at 25-28°C.

Note

Some strains take longer to grow to single colonies. Incubation times longer than 24 hours may be required for some slower growing strains. Monitor growth in future steps accordingly.

Day 2

- 2 Inoculate a single colony into 5mL Marine Broth (2216) media in the late afternoon/ early evening. Incubate overnight at 25°C, shaking at 200 rpm.

Day 3

- 3 Measure optical density (OD_{600}) of overnight culture growth and document
- 4 Pipette 100 μ L of overnight culture from each strain onto Marine Agar plates containing either Strep (for background resistance), Kan or Gent (MIC) at each concentration (25 μ g/mL, 50 μ g/mL, 100 μ g/mL and 200 μ g/mL).
- 5 Use beads or plate spreader to spread the culture evenly on the plate. Remove the beads and incubate overnight at 25°C overnight.

Day 4

- 6 Observe and document growth on all concentration plates.
For the **MICs** (Kan/Gent) identify the lowest concentration of media in which **no colonies are observed**.

Note

If there are no colonies at any concentration, screen lower concentrations of Kanamycin/Gentamicin in the media (i.e. 5, 10, 15, 20 μ g/mL)

- 7 For the **Streptomycin Resistant strain**, select a single colony from the highest dose of **Streptomycin** that has growth and **streak it out** onto a plate containing the same concentration AND a plate containing the next highest concentration of antibiotics. Incubate overnight at 25C.

Day 5

- 8 Observe and document growth on both the MIC plates (2 days old) and the Streptomycin resistance plates. If Streptomycin resistance is sustained at less than 200 μ g/mL, then continue step 7, passaging the strains onto higher concentrations of antibiotics. Once robust growth occurs at 200 μ g/mL, inoculate a single colony into 5mL Marine Broth containing Strep 200 μ g/mL and incubate 25°C overnight, shaking at 200rpm.

Day 6 or later

- 9 Store culture in cryovials for long term storage. Add 500 μ L overnight culture to 500 μ L 50% glycerol, pipette mix and store in the -80C for future use.