

## Freshwater Media Protocol (FW\_70)

*Last Edited: November 3rd, 2022*

### 1. Obtain desired masses for ingredients

- ☐ Obtain “X” volume of DI Water
- ☐ Obtain mass such that **Sodium Pyruvate** and **Casamino Acids** have a concentration of 1g/L of Media
- ☐ Obtain mass such that concentration of **Agar** = 15g/L of Media
  - ☐ Desired volume:
  - ☐ Mass of Sodium Pyruvate:
  - ☐ Mass of Gellan Gum:
  - ☐ Mass of Casamino Acids:

### 2. Preparing the media

- ☐ Add “X mL” of DI water to a clean glass bottle, place on a stir plate and add a sterilized stir bar.
- ☐ Add “0.001X g” of **Sodium Pyruvate** and **Casamino Acids**
- ☐ **Add “0.015X g” of Gellan Gum**
- ☐ Stir until a homogeneous-looking mixture is formed.
- ☐ Close the bottle halfway through and autoclave for 20 minutes.

### 3. Plating

- ☐ After autoclaving, pour approximately 20mL of media onto each petri dish.

\*If the media starts solidifying before finishing the plating, proceed to place the bottle into a heat plate, then draw 20mL using a serological pipette into each petri dish manually.\*