Making Seawater Complete (SWC) media

SWC media is the media that you will use to grow the luminescent bacteria that we will work with later in this exercise. It can also be used to grow *Vibrio fischeri* which is a luminescent strain of bacteria that can be purchased from Carolina Biological.

Materials: sea salt (you can get instant ocean salt mix from any pet store that sells marine fish), tap water, agar, yeast extract, peptone, glycerol, 20 sterile petri plates, container suitable to boil 1 liter of liquid, heat source to boil water

SWC agar recipe:

500 mlL water, 12 g sea salt, 2.5 g peptone, 1.5 g yeast extract, 1.5 ml glycerol (aka: glycerin) 7.5 g agar (if making solid media)

If you don't have an autoclave:

1) Mix the media ingredients in a container sufficient for boiling 1 liter of media.

2) Cover media with foil, bring to a boil, and then simmer media for at least 30 minutes. Be careful not to boil over and mix frequently so you do not burn the agar at the bottom.

3) Pour media into sterile petri plates while it is still hot. About 15-20 ml per plate. Be aware that the agar will solidify once the temperature falls below 45 degrees C so work fast.

Tip: Keep the plates closed as much as possible. The longer that the lids are off the plates the greater the chance of contamination.

If you have an autoclave:

Mix all of the above ingredients together and autoclave for 20-40 minutes. After media has cooled to the point where you can just stand to touch the container (about 50-60 degrees C) and pour into sterile petri plates. Be aware that the agar will solidify once the temperature falls below 40 C.

Making a Freezer Stock

With proper preparation most bacteria can be stored alive indefinitely in the freezer. To accomplish this 0.2 ml of glycerol (aka:glycerin) are added to 0.8 ml of freshly grown culture and the tubes are mixed well then the culture is frozen and stored in the freezer. For very long term storage you want to freeze the culture as quickly as possible and store in freezer that has a temp below -20C (ie: a deep freeze freezer). However, as long as you do not use a 'frost free freezer' (frost free freezers stay frost free by cycling between 'warmer' and 'colder' temperatures – which is bad for keeping bacteria alive in the freezer) most cultures will keep for at least a few years in any freezer that holds its temperature moderately well. To freeze your culture you can use a dry ice-ethanol bath. Get some rubbing alcohol and some dry ice (many ice cream stores, and some sporting good stores or fishing supply stores) use a hammer to break up the ice in the bottom of a rugged container and cover with the alcohol. After a few minutes put in your tube using tongs. It will freeze in a minute or two and be ready for the freezer (be aware that the alcohol will remove sharpie labels and you will to wipe off the alcohol and use tape to label your tubes after they are frozen). If you can not get dry ice then you could just try to freeze your culture in the freezer and wait a few days and plate it out to see if it survived the process.