Making media and basic microbiology techniques in the high school laboratory

Sources for ingredients: Companies such Carolina Biological (www3.Carolina.com) of Fisher (www3.fishersci.com) can provide you with all of the ingredients that are mentioned below as well as inoculating loops.

Expenses: For approximately $240 or less (prices vary depending on the quantities you order) it is possible to purchase sufficient materials to make somewhere in excess of 500 SWC agar plates. This number of plates would be sufficient to perform this exercise with about 125 students (figuring 4 plates per student).

Making an inoculating loop

(inoculating loops can be purchased for about $2.00 each or made as below)

materials: pencil, about 8 cm of thin wire, tape, needle nose pliers

1) Tape the end of the wire to the pencil so that about 3 cm of the wire stick out beyond the pencil.
2) Use the needle nose pliers to curl the end of the wire into a small loop (the smaller the loop the better, the optimum size is about 1-2mm diameter).

Making a glass spreading rod

materials: glass rod about 15 cm long (can use glass pasteur pipettes as a substitute), bunsen burner (a propane torch can substitute for a Bunsen burner)

1) Hold the glass rod over the bunsen burner so that about 5 cm stick out beyond the flame, make sure the glass rod is perpendicular to the flame.
2) After a few seconds the glass will soften and the end of the rod will swing down, remove the glass from the flame and hold still for a few seconds so that the glass hardens into an "L". Use caution: The glass will be hot for several minutes.

Making Seawater Complete (SWC) media

Materials: natural or artificial seawater (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:

750 ml seawater
250 ml water
5 g peptone
3 g yeast extract
3 ml glycerol
15g agar

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 degrees C.

If you don't have an autoclave:
1) Mix the media ingredients in a container sufficient for boiling 1 liter of media.
2) Cover and boil media for at least 30 minutes. Be careful not to boil over.
3) Allow media to cool 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 degrees C.

Sterilze glassware. Glassware can be sterilized in a steam autoclave for 20 minutes at 15 psi or by baking empty glassware in an oven at 350° F for 2 hrs. Tops of tubes and bottles should be sealed with aluminum foil to prevent contamination. Glass pipettes should be in glass or metal containers covered with aluminum foil or bundled in foil packets.

Prepare liquid media. Add the appropriate amount of nutrient broth powder (typically 8g per L) to 250 ml water then add 1.25 g maltose (5 g per L). Sterile in a steam autoclave for 20 minutes at 15 psi or by boiling for 30 minutes. Note boiling will not result in complete sterility but should be sufficient for most teaching purposes; to minimize potential contamination place media in a pre-sterilized glass container, seal with foil, and then place in water and bring to a boil. Begin timing after the water has started to boil. Dispense 10 ml by sterile pipette into 25 sterile glass tubes with closures. When cool add 10 ul (about one small drop from a dropper) of 25 mg/ml ampicillin to 1 tube for each group. *Serratia marcesens* D1 is naturally resistant to streptomycin and so to reduce the chance of contamination you can optionally also add 10 ul of 25 mg/ml streptomycin to all tubes.

Prepare PBS dilution blanks. Prepare 1.5 L of 1XPBS. Dispense 9ml into 140 test tubes and sterilize by autoclaving. If boiling then boil first and when sterile dispense aseptically into pre-sterilized test tubes.

Prepare agar plates. Prepare 500 ml and 1,250 ml of nutrient broth + 0.5% maltose and add 15 g/L of agar to each. Sterilize as described above. Allow to cool until you can just hold your hand

against the bottle for about 3-5 seconds (~60º C). If streptomycin is desired add 1 ml per L of 25 mg/ml stock at this time and mix well. Pour ~25 plates from the 500ml portion and label -Ap. Add 1 ml per L of the 25 mg/ml ampicillin stock to the 1,250 ml portion and mix well. Pour ~60 plates from the 1,250 ml portion and label +Ap.