Teacher Media prep recipes for *P. flu* protocol

Phosphate Buffered Saline (PBS) (for dilution process)
7.65 g NaCl
0.72g Na_2HPO_4 (anhydrous)
0.21g KH_2PO_4
1L deionized H_2O

1) weigh and measure dry ingredients first , then add to DI water to reach final volume.

½ Strength Tsoy Agar + Xgal Plates
15 g Tryptic soy w/ dextrose
***Note: If your Tryptic Broth does not have a carbon source (such as glucose) you will need to add 2.5 grams of dextrose to your solution. The bacteria require sugar to thrive.
15g Agar (biological)
1L deionized H_2O

1. Autoclave for 30 minutes
2. Add 1 mL 20mg/mL X-Gal Solution when media has cooled, ensuring that the media is still warm enough for the agar not to harden. X-Gal must be dissolved in 1ml Dimethylsulfoxide (DMSO) by vortexing. *addition of X-gal is what gives the colonies the Blue and White coloring on the plates. see teacher resources for the science behind this.*

Pour plates before media has cooled too much, after adding the Xgal. Makes about 40 plates.

X-GAL prep: To add to 1L of media
Weigh out 20mg of X-GAL
Dissolve in 1ml DMSO (Dimethylsulfoxide)
Vortex to mix
Ensure that X-Gal is kept in the dark or wrapped with aluminum foil as it is light sensitive. This product does not have to be sterilized.
Freeze both the powder and your surplus stock solution.

King's B Media (for culture tubes)
10 g Protease Peptone No. 3
0.75 g K_2HPO_4
7.5 mL Glycerol
489.5 mL Water

1. Add 3 mL of 1M $MgSO_4$ x $7H_2O$ (see below)
2. Autoclave solution for 30 minutes

***For increased glycerol concentrations, add 2.5 mL of additional glycerol, and subtract 2.5 mL of H_2O. Ex: 2% = 10 mL Glycerol, 487.5 mL Water. (for increased evolution rates ie: faster)

1M MgSO4 Stock
30 g $MgSO_4$ (anhydrous) or 61.6 g $MgSO_4$ (heptahydrate)
250 ml deionized H_2O

1) combine salts and water
2) autoclave after addition to King's B solution, and any remaining stock solution

Other notes: (from taylor @ Vaughn Cooper's lab)

1) use the 2.5% King's B Media when running the evolution tubes.
2) the 1 M $MgSO_4$ should be prepared and autoclaved, along with the King's B Media, which should contain the proper amount of Protease Peptone Number Three, KH_2PO_4, Glycerol and Water, which when all combined together should reach a final volume of 497 mL. *For example, for the 2.5%, we add 484.5 mL of water to the dry ingredients, followed by the addition of 12.5 mL glycerol. Once both the Queen's B and 1M $MgSO_4$ have been autoclaved, we then add the 3 mL of $MgSO_4$ to the media.*
3) The T-Soy Broth media is used only on Day -1. The teacher may need to use it to set up tubes prior to class use if they are preparing their own cultures to plate ie: Day -3. This is simply the day when we choose a single isolated colony from an isolation streak and put it into 5 mL of T-Soy to allow the cultures to grow, then plate the cultures 2 days before the students use the plates for day -1