TECHNIQUES IN MOLECULAR BIOLOGY – DILUTIONS, SOLUTIONS, AND BASIC LAB MATH

Building on other Chemistry, Biochemistry and Biology courses we will learn to navigate the laboratory setting by calculating and preparing most of your own buffers. Yes you will see this again in biochemistry laboratory, BUT it is one of the critical skills for a graduating chemist/biochemist/biologist and repetition is important to master this skill set.

Using the lab math handout and notes from class you should be able to:

- Know which calculation to use when starting from a stock concentrate (mixture or single substance) vs a solid.
- Recognize the right glassware needed to make the appropriate solution
- Calculate percent w/vol to prepare agar plates or other compounds.
- Create your own buffers using a pH meter in class.

Practice problems: Using the online notebook, create an page in the *assignments folder* to answer all of the following questions. One page for all of the questions. Use a separate rich box entry for each question. For each problem describe what you are making show the calculations and enter in all equations in a neat and orderly fashion.

Starting Resources: 1 M Tris-Cl pH 7.0, 10% borate solution, 50X phosphate buffered solution (PBS), 5kg bottle of sodium chloride, 0.1 M EDTA, dehydrated powdered agar, yeast extract (powder), HEPES solid, 1 M HCl, 5 M NaOH, 1 M sodium acetate, glacial acetic acid (you look up the concentration!), 90% Ethanol in water, syber-safe dye (10,000X), dried agar (powder), 500 X kanamycin.

1) To finish preparing a buffer used to purify DNA the manufacturer indicates that you need to add enough ethanol to make a 10 ml solution 10% ethanol. Please calculate and describe how to create the final solution.

2) Calculate and describe how you would prepare a 100 ml solution of TEA buffer (look up the components) using the resources listed above.

3) Describe how you will prepare 150 ml of PBS. What is PBS? What are the concentrations of the components at final concentration?

4) Prepare 50 ml of agar gel containing the following: 10% agar, 10 mM Tris-Cl pH 7.5, 1X syber-safe dye, 0.5% borate and 20 mM NaCl. Show the calculations AND describe in a step-by-step manner HOW you would prepare this solution.

5) To grow cultures of bacteria you will need to prepare an LB media containing kanamycin. Calculate how to prepare first 500 ml of LB media and then after autoclaving and cooling the solution, how you would prepare 100 ml of LB media with kanamycin.