Making Solutions and Plasmid Mini Preparations
2007-08 INS Spring Quarter – Lab 1

Goals: This lab will introduce you to techniques used applicable to research in molecular biology. You will learn how to make solutions for use in the laboratory. You will also learn how to isolate plasmid DNA from E. coli.

PART I. Making Solutions

A most important aspect of research in molecular biology involves the making of solutions. It is very important that a solution is made correctly, having the right pH, concentration, and sterility. Today each team will follow a plasmid mini-prep protocol to determine how to make each of the three solutions required for the procedure. After each team has been evaluated for their approach by the instructor, the bench will make the appropriate solutions for isolation of plasmid DNA from bacteria. The scanned version of the protocol is posted as PDF entitled: *INS Bio Lab 1 Miniprep*. For this lab you will need at least one copy of the protocol for each group of students.

Here are some tips for making your solutions:
A. Add items in a logical order. When adding solids and liquids together, it is necessary to determine how much of each item you will add BEFORE you begin. All solutions will be brought up to a final volume, so you can estimate what that is BEFORE you begin. Add some (about half) of the water first prior to adding the other reagents. Unless instructed otherwise, dissolve solids in the water and then add liquid reagents. You can then pH your solution and/or add water to bring up the final volume.

B. Calculations
Sometimes it may be more efficient to use molarity when calculating concentrations. A mole is defined as one gram molecular weight of an element or compound, and comprised of exactly 6.023 x 10^23 atoms or molecules (this is called Avagadro's number). The mass attributed to one mole of any element or compound is called its atomic weight (elements) or molecular weight, or formula weight for compounds. The number of moles of a given dry reagent can be calculated as: # of moles = weight (g)/ molecular weight (g)

Molarity is the unit used to describe the number of moles of a chemical or compounds in one liter (L) of solution. By this definition, a 1.0 Molar (1.0 M) solution is equivalent to one *formula weight* (FW = g/mole) of chemical dissolved in 1 liter (1.0 L) of solvent (usually water). Formula (or molecular) weight is always given on the label of a chemical bottle.

Molarity Calculation Examples:
Example 1: To prepare a liter of a simple molar solution from a dry reagent
Multiply the *formula weight* (or MW) by the desired molarity to determine how many grams of reagent to use:
Chemical FW = 194.3 g/mole; to make 0.15 M solution use
194.3 g/mole * 0.15 moles/L = 29.145 g/L

Example 2: To prepare a specific volume of a specific molar solution from a dry reagent
A chemical has a FW of 180 g/mole and you need 25 ml (0.025 L) of 0.15 M (M = moles/L) solution. How many grams of the chemical must be dissolved in 25 ml water to make this solution?
#grams/desired volume (L) = desired molarity (mole/L) * FW (g/mole)
by algrebraic rearrangement,
#grams = desired volume (L) * desired molarity (mole/L) * FW (g/mole)
#grams = 0.025 L * 0.15 mole/L * 180 g/mole
after cancelling the units,
#grams = 0.675 g

PART II. Plasmid Mini-Preparations

Follow the protocol provided to isolate plasmid DNA from the bacteria. We will inform you of any changes or details prior to the lab. Next week you will analyze your isolated plasmid DNA.