June 20th, 2011 – Skills in Four parts

You have been given 5 tubes, each containing ~5 ml of a solution (A,B,C,D,E).Most are aqueous but concentrated salt, sugar, detergent, and one is oil-based. You also have a tube of water, for making dilutions or rinsing tips. Finally, you have one small tube labeled 'Sample A' on the green lid that is blackish – this is for Part 4.

Part 1. Mixing Solutions with different viscosities and densities. A lot of stock solutions are very concentrated compared to the desired working strength. You have to pipette carefully and rinse the tip in order to pick up and deliver the correct volumes.

- Add 500ul of Solution A to Solution B – observe carefully both the pipette tips and how the solutions 'layer' – which one is denser?
  - Can you get all of each solution out of the tip and into the tube?
  - Try mixing the two solutions
    - Finger mixing
    - Shaking
    - Pipetting up and down
  - Are droplets left on the sides of the tube?
- Add 500 ul of Solution C to Solution D – answer the same questions as above.
- Add 500 ul of Solution C to Solution E – answer the same questions as above.

Part 2 – Serial Dilutions are used to make a less concentrated stock solutions if you will need several working stocks. The idea is not to change the measurement setting, and to use the first dilution as the starting place for the next one in the series. It can take a while to get the concept, but it is easy to keep track of what you are doing when you always add the same volume and the difference in volume in adjacent tubes makes it easy to tell if you have done the next step.

Using Solution A as the thing you are diluting with water, make 2-fold serial dilutions:

- Label five tubes: 1,2, 3, 4, 5
- To each tube add the same volume of water (here I use 500ul = 0.5ml as an example but you can use whichever of the pipetters you have)
- Add 0.5 ml of Solution A (500ul) to the water in tube 1 and mix thoroughly – make sure no droplets are on the sides (centrifuge if necessary to collect all the solution in the bottom).
- Remove 500ul from Tube 1 and add it to the water in Tube 2.
- Mix thoroughly and collect as before

- Remove 500 ul from Tube 2 and add to the water in Tube 3, mix and collect as before.
- Remove 500 ul from Tube 3 and add to the water in Tube 4, mix and collect as before.
- Remove 500 ul from Tube 4 and add to the water in Tube 5, mix and collect as before.

Now you should check that you did each dilution correctly. You can line up the tubes and see if the volumes look the same (tube 5 should have twice as much as the others).

Other ways to verify that you have the expected volume in each tube

- Re-measure each volume with the pipette (ask to be shown how this is done)
- Weigh the sample on an appropriate balance (you will need to verify that solution A does not have a detectably different mass-per-volume than water – weight 1 ml of solution A to test this).

Part 3 – Preparing a mixture of solutions. In molecular biology most solutions can be purchased as concentrated stocks – you then add a bit of this and dilute the whole thing in order to the desired final concentrations of each component. Although some come as molar stocks, often they are shipped as 10X or 5X stock solutions. Often they are frozen so they need to be thawed before you can use them. In that case you would thaw them,mix thoroughly, spin briefly in a centrifuge to collect all of the solution in the bottom of the tube and then use the to make working solutions. Often the order of addition is important too, so be careful to work straight down the list, unless otherwise directed.

- Label the tube you are going to measure the stock solutions into (one of the green-capped microfuge tubes).
- Our goal is to make a total final volume of 200ul of a 'reaction mix'.
  - Add 20 ul of Solution A (which is a 10X stock), using the 100ul pipetter
  - Add 40ul of Solution B (which is a 5X stock), using the 100ul pipetter
  - Add 2ul of Solution E (which is a 10% stock), using the 10ul pipetter
  - Mix these together, spin down in the centrifuge to collect all of the volume
  - Use the 100ul pipetter to make sure you have the correct volume (62 ul).
  - If the volume is correct add 133 ul of water with 1000ul pipetter, mix carefully and centrifuge to collect all of the liquid.
  - What is the total volume supposed to be?
    - The difference from 200ul is the amount held out so you can add your sample.

When you measured it, what was the actual volume?

What is the final concentration of each reagent when you have brought the volume to 200ul with your sample?

Part 4 - Recovering Pellets and Separating Layers of Liquids. In many molecular biology applications you make a material come out of solution so that it is no longer soluble, and then you spin it in a centrifuge to collect it at the bottom of the tube – you remove the solution, leaving this pellet of collected material behind. The goal is to remove as much of the liquid as possible without touching or disturbing the pellet. The other common task it to combine two liquids that do not mix, so that one type of material will dissolve in one liquid and something you are trying to remove will dissolve in the other liquid. You then spin the sample to separate the two types of liquids. Then you remove the top layer to another tube. The goal is to remove as much as possible of the top layer, but not so much that you get any of the bottom layer – this always means that you will lose a little bit of the top layer

- Pelleting
  - To Sample A (the green-capped microfuge tube) add 200ul of water.
  - Mix
  - Centrifuge for 5 minutes.
  - Remove the liquid from the pellet.
  - If your pipette tip touches the pellet, resuspend the pellet with 100ul of water and try again.
- Separating Liquid Layers
  - Mix 300 ul of the solution in tube A with 300ul of the solution in tube C.
  - Centrifuge for 2 minutes
  - Remove the top layer to a new tube
    - Did you get all of the top layer off?
    - Was this solution A or C? Why do you think so?
    - Measure the volume of each layer and record it

Repeat a few times to gain practice, these are very common operations.