Molecular Biology Basic Lab Skills

On your bench you will find a number of labeled tubes containing solutions you will use in the following exercises, and some empty 1.7ml microfuge tubes that you will use. Before you start each part, make sure you collect the tubes you will need for that part. You also have a bottle of autoclaved Nanopure water. You have a 100-ml beaker to dispose of any solutions. You will need a 20ul micropipetter, a 100 or 200ul micropipetter, and 1000ul micropipetter and diposable tips that fit each, access to a vortexer and access to a low-speed benchtop microfuge and to a high-speed benchtop microfuge.

Part 1. Mixing Solutions with different viscosities and densities.

Introduction: Molecular biology uses a lot of solutions with dilute salts, optimized to make enzymatic reactions work well. Since only a small number of salts are used, we make concentrated stock solutions, sterilize them, and the make specific dilutions (mixing an exact ratio with water) for the process we are carrying out. You have to pipette the stock solutions carefully to get just the right volume, and any small droplets left behind have a big impact on the final results, so you need to rinse the tip with some of the water in the target container in order to get a good result.

1. Add 500ul of Solution A to Solution B – observe carefully both the pipette tips (do any droplets cling to the sides so they are left behind) and how the solutions 'layer' – which one is denser (on the bottom)?
    a. Can you get all of each solution out of the tip and into the tube?
    b. Does the speed that you pull up and push out the solution make a difference?
2. After capping the tube, try mixing the two solutions by several methods:
    a. Finger mixing (flick the bottom of the tube fairly hard)
    b. Vortexing
    c. Pipetting up and down
3. Are droplets left on the sides of the tube? If so, use the benchtop minifuge to briefly spin two balanced tubes. Did this help?
    a. Do the solutions eventually mix, or do they separate?
    b. If the layers separate, try to remove the top layer to a fresh tube, without leaving any behind or getting any of the bottom layer in the new tube.
        i. What is the volume in the new tube?
        ii. What is the volume remaining in the old tube?
4. Add 500 ul of Solution C to Solution D – answer the same questions as above.
5. Add 500 ul of Solution B to Solution C – answer the same questions as above.
6. If the layers separate, try to remove the top layer to a fresh tube, without leaving any behind or getting any of the bottom layer in the new tube.
    a. What is the volume in the new tube?
    b. What is the volume remaining in the old tube?
7. Now use the 5 ml serological pipette and the green pipette pump, and measure 2 ml of the solution combinations (A + B, C+D, B+C) each into a 15ml tube.
    a. How hard is it to measure the liquids and deliver them fully?
    b. How hard is it to mix the solutions completely
    c. To centrifuge these you will need to carefully BALANCE two tubes, then spin them in the larger format centrifuge for 1 minute.

Part 2 – Preparing a mixture of solutions, where the order of addition matters.

Introduction: in molecular biology reactions you are often combining 5-10 chemical solutions to get a final mixture that works for the reaction you want to do. Each chemical is available as a stock, but you use different amounts of the each one. Some chemicals will not stay in solution if they are in the presence of a concentrated solution of another chemical, so the order in which you add the solutions is very important. Another important factor is that some solutions are frozen – you thaw them right before you use them – then you must mix them (usually by vortexing), centrifuge briefly to collect everything at the bottom of the tube and then measure out the amount you need to use for your reaction. Carry out the following instructions as carefully as possible – make a note wherever you have a question about why the order is written in the way we have it, or if you notice anything odd about your solution – especially if you see anything that looks like particles or dust coming out the solution when you mix two solution.

1. Label a 1.5 ml microfuge tube that you are going to use as the final reaction mixture, into which you will measure the stock solutions.
2. Our goal is to make a total final volume of 200ul of a 'reaction mix' in two tubes.
    a. Add 60 ul of Molecular Biology grade water -use the 100ul micropipetter, set at 60
    b. Add 20ul of 3M sodium acetate (NaOAC) - use the 20ul pipetter, set at 20
    c. Add 40ul of Herring sperm DNA - use the 100ul pipetter, set at 40
    d. Add 70ul of isopropanol – use the 100ul pipetter, set at 70.
    e. Vortex the mixture, then spin for 15 minutes in the microfuge, set at 12,000 x g
        i. Do you see any particles? (this is called precipitate)?
    f. Check the volume using the 200ul micropitter set at 170 (if there a bubble of air at the bottom of the tip, your volume came up short, if there is a bit of solution left in the tube your volume is too big).
        i. Discard the solution
    g. Add 40 ul of water with the 100ul micropipetter to the tube, vortex and centrifuge for 10 seconds with the benchtop minifuge to collect all of the liquid.
    h. If you did have precipitate, did it disappear?
    i. What is your final volume?– What is the total volume supposed to be?