Practice Making Dilutions

You have been given two samples that contain DNA oligomers (Oligo-1 and Oligo-2)that have a length of 50 bases. Each is at approximate 100uM concentration. One of the oligomers has been labeled with a fluorescent dye (Cy3, which looks pink when it is concentrated) while the other is just DNA (UL for unlabeled). You are going to use these solutions and make a series of dilutions 10-fold dilutions whose concentrations you will check both spectrophotometrically and using gel electrophoresis.

Note: Concentrated DNA (at 100uM or higher) is fairly stable at room temperature in our storage buffer so long as you don't contaminate it. Wear gloves. Make sure you add a buffer solution that is between pH 7-8.0 and contains EDTA (why are these important points?). Make sure the tip of your pipettor has not touched anything but the stock solution and target solution – don't reuse the tip!

Serial dilution:

Make a 10-fold serial dilution series, with 200ul (starting) volume in each sample, in a 6-member series:

1. Make 10ml of 3X SSC working stock from your 20X SSC stock.
    a. ___ ml 20X SSC
    b. ____ml of ddH_2O
2. Label the tubes Oligo 1: 1-1,1-2,1-3,1-4,1-5,1-6 and Oligo 2: 2-1,2-2,2-3,2-4,2-5,2-6
    a. In each tube pipette 180 ul of 3X SSC
    b. To the tube labeled Oligo1-1 add 20ul of the 100uM stock. Vortex (DNA this short is not sheared by vortexing). Use a quick-spin to collect the solution in the bottom of the tube.
    c. To Tube labeled Oligo1-2 add 20ul of the Oligo1-1 solution, vortex, spin
    d. To Tube labeled Oligo1-3, add 20ul of the Oligo1-2 solution, vortex, spin
    e. To Oligo 1-4 , add 20ul of the Oligo1-3 solution, vortex, spin.
    f. To Oligo 1-5 add 20ul of the Oligo 1-4 solution, vortex, spin.
    g. To Oligo 1-6 add 20 ul of the Oligo 1-5 solution, vortex, spin.
    h. Repeat the series, so you have a *replicate dilution series* for Oligo1-1. How might you check that your process was consistent and accurate?
3. Repeat the process for Oligo2, again repeat it so you have a replicate.
4. Store the sample tubes at 4C for spectrophotometry measurements to be done next lab.

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5. Straight dilution: make 1 ml of a 1uM solution (in 3X SSC) of Oligo-1 and of Oligo-2, repeat (so you have 4 tubes of 1ml solutions). Store at 4C with your other tubes.
6. Make 1 ml of a 0.01uM solution (in 3X SSC) of Oligo-1 and Oligo-2, repeat (so you have 4 tubes of these 1ml solutions). Store at 4C with your other tubes.
    a. What did you have to do to make this solution (what micropipettes or intermediate steps did you have to use)?

Note: be sure to label every tube with your initials and some type of identifier for the series. Label the rack as well.

Technique notes:

Did you rinse the tip into the solution as you made each dilution? For the concentrated Cy3 labeled stock you can often see a think film, or a few droplets, of the stock clinging to the sides. If you rinse the tip several times (3-5 is usual) you will wash this material into the solution – your delivered volume will be more accurate.

Did you use the same tip for the serial dilutions? If you did, but did not rinse it, then you may have carried over a small volume of the concentrated solution.

Why should you never go back into an original stock reagent bottle with a tip that has been used to deliver reagent into a solution mixture, or even water?

One steps 5 and 6 did you realize that you were re-creating solutions that you had already made? Which of the dilution series matched 5? Which matched 6?