Practice calculating Dilutions

A great deal of the time you will be using concentrated stock solutions to make working stocks. Describe how to make the following volumes of working solutions with the provided stock solutions.

Given these Concentrated Stock solutions:

1M NaCl
5M KCl
3M NaOAc (sodium acetate), pH 5.2
7.5M NH_4OAc (Ammonium acetate)
10% SDS
8M Urea
TEMED (pure, very long chemical name – used to polymerize acrylamide gels)
Solid agarose powder (100g)
3% agarose in 1X TBE buffer, 50 ml
1% Agarose in 1X TBE buffer , 50 ml
1% agarose in water for sealing
30% Acrylamide-0.8% bis-acrylamide in water
10X TBE (Tris-Borate-EDTA) buffer
10X TE (Tris-EDTA) buffer
10 % APS (ammonium persulfate)
ddH_2O (deionized and distilled, or 18MOhm)
70% EtOH (Ethanol in water)
95% EtOH (ethanol in water)
1M Tris-HCl, pH 8.3
1M Tris-HCl, pH 8.0
1M Tris-HCl, pH 7.5
100% isopropyl alcohol
20X SSC (sodium citrate buffer)
100% glycerol
1 mg/ml BSA (bovine serum albumin, a globular protein with a slight positive charge that is used to stabilize many reactions, like DNA ligation, restriction digestion and PCR amplifications)
10mg/ml salmon sperm DNA, sheared, in water
Mol. Biology grade H_2O – note that this is different from 10MOhm water, it has been specifically treated to remove nucleases and heavy metals.

Making working Solutions: Describe the pipetting steps you would use to make the following working stocks:

1. 1ml of 1X TE buffer
2. 1L of 0.5X TBE buffer
3. 8ml of 5% acrylamide (the bis is implied) in 1X TBE buffer

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4. 10ml of 6% acrylamide with6M urea in 1X TBE buffer

5. In order to polymerize (solidify) a polyacrylamide gel I add 0.5ul of TEMED per ml of acrylamide working solution and 6ul of 10% APS per ml of acrylamide working solution. How much TEMED and how much 10% APS do I add to the acrylamide solutions I made above?

6. Purchased 10X PCR buffer has a specific concentration of MgCl2 in it, but changing the concentration of Mg is one way to optimize the results. In order to titrate for the correct amount of Mg I need to make a PCR buffer that lacks Mg. How would I do the following?

    a. Make 1ml each four 10X PCR buffer solutions : each has {50mM Tris-HCl ph8.3, 100mM KCl, 10ug/ml BSA} and vary the MgCl2 from 2.5mM, 3.0mM,3.5mM, 4.0mM.

    b. To set up the four PCR reactions that optimize the Mg concentration I will use 4 reaction tubes. In each I need to end up with a final volume of 20 ul. I made my series of 10X PCR buffers in part (a) above. My sample is at 100ng/ul and I need to add 10ng to each reaction. The polymerase enzyme needs building blocks, the dNTPs – my stock is at 10mM in water and I need a final concentration of 0.2mM. The PCR primers are at a 10X concentration, in water. My enzyme is at 5 Units/ul and I need 1 Unit per reaction tube. The enzyme should be added last.

        How much of each reagent do I add to each tube, and in what order? (10X buffer, DNA, primers, dNTPs, water, polymerase)?

7. I want to precipitate a DNA sample – this means it falls out of solution and I can collect it into a pellet at the bottom of the tube by centrifugation, remove the solution from the tube and then resuspend the pellet in a smaller volume (possibly of a different buffer) in order to concentrate the DNA. There are several standard procedures for doing this. If my sample is in 50ul volume, what do I have to add to make the following solutions and what will the final volumes be?

    a. Add 0.6 volumes of isopropanol. How much do I add? and what it the final percent isopropanol?

    b. Add 0.1 (one-tenth) volume of 3M NaOAc to the sample, calculate the new volume, for the new volume add 2.5 volumes of 95% EtOH. How much NaOAc do I add? How much ethanol do I add? What is the final concentration of NaOAc? What is the final concentration of ethanol?

    c. Add 0.2 volume of 7.5M NH_4OAc, calculate the new volume, then add 2.5 Volumes of 95% EtOH. How much NH_4OAc do I add? How much ethanol do I add? What is the final concentration of NH_4OAc? What is the final concentration of ethanol?

8. I want to make a 2% agarose gel in 0.5X TBE buffer. I need 50 ml total volume. Describe two ways to make this mixture using the reagents listed. Note that agarose has to be heated to be a solution (it is essentially Jello, so it is a solid at room temperature) and it is very viscous, so it is

difficult to measure. Normally you would start with the powder, add buffer and water and heat them together – so describe this process. However, if you happen to run out of powder but you have material left from previous experiments you can re-use it. Note that the buffer concentration has changed, so you must manage that change as well as the change in agarose concentration.

9. In hybridizing microarrays, we use a solution that contains {3X SSC, 0.5M KCl, 0.5% SDS, 5ug/ml salmon sperm DNA, 5% glycerol}. Describe how to make 100ul of this solution using the stock reagents listed above. Note that Molecular Biology Grade H2O must be used in making up this solution.