Understanding and Using Lab Stock Solutions – interpretation and calculations

Introduction: In molecular biology you generally start with a specific set of general instructions, called a Protocol. You can think of it as a recipe: it gives *the components* you have to add and *the amounts of each* (usually as a mass, a volume, or a percent of the total) that have to be in the final mixture. It also lets you know if there are *specific suppliers* you have to get materials from for the protocol to work properly, and any special precautions, such as the *order of addition* for components and if there are *intermediate actions* where you are supposed to do something before going on, like mixing the materials at a given point before adding the next ones. The *working temperature* is important: a common warning is that you must keep everything on ice (at 4°C) while you are setting up, so that the materials do not start to interact too soon, or start to break down.

Interpreting Reagent Concentration labels: The reagents are the starting materials you are given that you have to mix in the correct proportions for the final reaction to work. For most molecular biology reactions you are provided with what are called Stock Solutions, which are more concentrated versions of the chemicals you need, made to be very accurate and very pure. Because of their concentrated form, you have to figure out how to do the correct dilutions. In molecular biology, the concentration of the stock solution is usually given in one of five forms:

1. **A fold ('X') concentration**, such as 2X, 5X or 10X. Many buffers are provided in this form. To use a 2X buffer, half of the solution must be the buffer and the other half is available for your other reagents. To use a 5X buffer, $1/5^{th}$ of the final solution comes from this stock and you have the other $4/5^{th}$ for the other reagents. Similarly for the 10X buffer, $1/10^{th}$ of the final volume must be made up of the stock solution and you have $9/10^{th}$ for the other reagents. I have also seen 20X, 50X and 100X stock solutions.
    a. Note: when you thaw a stock solution there may be layers of different components (the water may separate from detergent, for example, when it is frozen), so you must always *vortex* (rapid mixer) the solution for 2-3 seconds and then spin it in a *minifuge* (a low-speed centrifuge- don't forget to put a balance tube on the opposite side) to collect droplets that splashed on the sides and lid- then store them properly (for example put on ice if it was originally frozen) until you are ready to use it.

    b. If I want to make 100ul of a 1X solution, how much of each stock do I add, and how much volume is left for the other components? (assume that you want a final concentration of 1X, although this might not always be true).

	Target Volume: 100ul			Target volume : 250ul		
Stock concentration	2X	5X	10X	2X	5X	10X
Volume Stock	100/2=50ul	100/5=20ul	100/10=10ul	250/2=125ul	250/5=50ul	250/10=25ul
Remaining volume	100-50= 50ul	100-20=80ul	100-10=90ul	250-125=125ul	250-50=200ul	250-25=225ul

2. **A molar concentration**, which is how many moles (molecules, not small furry animals) per liter of the material are in the solution. Salt solutions are commonly provided in these units, for example 1M NaCl means you have 1 mole (Avogadro's number of molecules is $6.023*10^{23}$) dissolved per liter. Since one mole of salt molecules weighs 58.44 grams, if you were directed to make the solution you would weigh out 58.44 grams, add water to a level just short of 1 liter, adjusting the volume to 1liter once the salt was dissolved. When a company supplies you with the 1M NaCl stock solution you don't have to make it yourself, but you do have to know how to use it correctly.
    a. If it is frozen, thaw it completely, vortex it, and quick-spin the tube, as noted above.
    b. Below I have an example for using 3 different concentrations of stock solution (0.1M, 1M and 5M) where you have a target volume (100ul) and a final desired concentration of the chemical (0.05M) – colors are to let you see where the values go for the calculation.

	Target volume: 100ul		
Stock Concentration	0.1M	1M	5M
Final desired concentration	0.05M	0.05M	0.05M
Volume Stock	100ul(0.05M) = 0.1M(Xul) or 50ul	100ul(0.05M) = 1M(Xul) or 5ul	100ul(0.05M) = 5M(Xul) or 1ul
Remaining volume	100ul - 50ul = 50ul	100ul - 5ul = 95ul	100ul-1ul = 99ul
Two steps:	100*0.05 = 5		
	5/0.1 = 50		

3. **A mass-per-volume concentration**. This is common for reagents such as proteins and chemically synthesized DNA molecules (but other stocks may also come that way). The units are usually millgrams per milliliter (mg/ml) or micrograms per microliter (ug/ul).
    a. Note: sometimes you are supplied with a reagent that uses mass-volume units and you get a protocol that requires you to use molar units (or vice versa). You can interconvert if you are given the molar mass of the chemical. If I have a solution that is 100micromolar (uM) of a type of *30-nucleotide strand of DNA (an oligonucleotide)* is and I want to know what the mass per microliter is I do the following:
        i. 100uM = 100micromoles per liter or $10^{2}*10^{-6}$ moles/L = 10^{-4}mol/L.
    b. The molecular weight of my oligonucleotide is 9978.7 gm/mol.
    c. 100uM → 10^{-4}mol/L* (9978.7 gm/mol) = 0.99787gm/L (the mol units cancel out).
    d. I asked about the mass per microliter. A liter has 10^{6} microliters, so multiply 0.99787gm/L*1L/10^{6} microliter (the L cancel out) and I have $0.99787*10^{-6}$ gm/microliter. Another way to write 0.99787 is $9.9787*10^{-1}$. If I combine these I have $9.787*10^{-1}$gm*10^{-6} /ul or $9.787*10^{-7}$ gm/ul. Since 10^{-9} is a nanogram, I can also

take 9.787*10^{-7} gm/ul *10^{9}ng/gm (the gm cancels out) say that 997.87ng/ul is 100uM for this oligonucleotide. Making the units cancel out this way to get to the type of information I want is called dimensional analysis.

e. There are oligonucleotide calculators on line that make this particular conversion easier to check, but this is not available for proteins, so far as I know.
   i. http://mbcf.dfci.harvard.edu/docs/oligocalc.html
   ii. http://www.promega.com/a/apps/biomath/index.html?calc=ugmlpmolul

4. **A percent concentration** – in this case you have to know the units: that is, if you are looking at mass-mass or mass-volume or volume-volume proportions. In molecular biology this is commonly used when two liquids are combined (volume-volume).
   a. For example, an 80% solution of ethanol and water uses volumes, because both components are liquid. The first named component is the one that has the indicated percent, if there are only two, and if the total percents do not add up to 100%, you assume that last component is water.
      i. To make 100ml of 80% ethanol (in water) I take 0.8(100ml) = 80ml and measure out that much of my pure stock (100% ethanol). For the rest of the volume, I subtract the ethanol volume (80ml) from my total volume (100ml) and get 20ml of water that I need to add. I combine them, cap or seal the container, mix thoroughly and then use.
         1. If there are multiple components, make sure the fractions add up to 1 (for 100%), so 10% toluene, 20% ethanol and 70% benzene means that you multiply the final volume you want to make by 0.1, 0.2 and 0.7 respectively. If I want 5 ml I will use 0.1*5ml =0.5ml of toluene, 0.2*5ml= 1ml of ethanol, and 0.7*5ml=3.5ml of benzene. I can double check that 0.1+0.2+0.7 = 1.0 (the percents add up) and the 0.5ml+1ml+3.5ml=5ml, my targeted final volume.
      ii. Note: some liquids interact, and the volume will change when they are mixed – that means you need to measure them separately, not add on to the other in the same measurement device. The ones you will be most likely to run into in molecular biology are solutions of alcohol in water (methanol, ethanol and isopropanol).
   b. Sometimes solid salts are dissolved in a liquid solvent as a mass-to-volume percent instead of a molar (moles/L) solution. So a 20% solution of KCl (in water - again, if the liquid is not named you assume it is water) means that if we want 100ml of final solution, we weigh 20 gm of KCL, put it in a container that will hold the total volume, add most of the water and mix until the salt dissolves, then adjust the final volume to 100ml with water. This makes sense for aqueous solutions because the mass of water is about 1gm/ml, so you will have added about 80ml of water (some salts also interact with water and you might add slightly more or less than 80gm of water to get 100ml of final volume).
      i. You could also have a mass-mass percent, in which you would weigh out the 20gm of KCl and then add 80gm of water.
      ii. Note – this sounds the same as the mass per volume concentration method in part 3, and you could convert between them, calling this a 0.2gm/ml KCl solution instead of a 20% KCl solution.

5. **A Units per volume concentration** (used only for enzymes) – a unit is how many enzyme molecules will carry out a certain number of chemical reactions in a certain amount of time – this expresses the enzymes activity. Because different enzyme preparations may differ in how many molecules give the same activity, and what we care about with enzymes is the activity and not the concentration, you will find enzymes are provided in 'concentrations' or Units per microliter rather than Mass or Molecules or Fold or Percent.
    a. DNA polymerases that are heat stable are used in the polymerase chain reaction. They are commonly provided in a 'concentration' of 5U/ul.
        i. If I want to add 1Unit of enzyme to a PCR reaction, I will have to measure 0.2ul into the reaction. And I will have to take the volume into account when I am making up the mixture.
        ii. Note: Enzymes are commonly provided in glycerol-containing solutions to help stabilize them at low temperatures. Glycerol is very viscous (thick and sticky), so you have to pipette slowly and carefully, both up and down, when you are measuring enzyme solutions from their stocks, and rinse the tip up and down in your final solution many times.

Putting it all together for the components required to carry out three types of reactions:

Note: A protocol is written to handle a certain number of reactions, and usually you add a certain amount of extra, because no matter how careful you are the tiny volumes cannot be pipetted perfectly accurately and you would rather have a little extra than come up short and not be able to process one of your samples. A rule of thumb is to make at least 10% more than you think you need, or one complete extra reaction mixture if you are only doing a couple.

Note: for all enzymatic reagents, once they are *completely thawed*, do a *quick-spin* in a minifuge for 5 seconds before *storing on ice*, unless directed to keep them at room temperature.

Example 1: To add components needed to extract DNA from frozen leaf tissue.

Because every leaf has a different mass, and it is the proportion of the solution to the mass that gives you high-quality DNA extraction, you have to do this calculation right at the time you are doing the experiment.

1. Weigh the mass of the frozen leaf on a microbalance, immediately move it to the mortar containing dry ice – this will preserve the tissue while you prepare the other components:
2. For each 1gm of leaf add
    a. 0.25gm of sorbital (a solid)
    b. 0.25gm of PVPP (a solid)
    c. 2.5 ml of 2X Grinding Buffer
    d. 50ul of Tween 20
    e. 50ul of beta-mercaptoethanol (stinky)
3. After grinding for 15 minutes, filter into a 15ml tube, find the volume
    a. Add an equal volume of 2X CTAB buffer
4. After 45 minute incubation,

a. add an equal volume of $CHCl_3$

5. After centrifugation, remove the top layer, determine the volume
   a. Add 70% of the volume in Isopropanol

Sample calculations depending on your starting material:

Component	Per gram of tissue or ml of resulting liquid	2gm leaf tissue	3gm leaf tissue
Sorbitol (solid)	0.25 gm	2gm*0.25gm/gm = 0.5gm	3gm * 0.25gm/gm
PVPP (solid)	0.25gm	0.5gm	0.75gm
1X Grinding Buffer (stock solution)	2.5ml per gram	5ml	7.5ml
Tween 20 (100%)	50ul per gram	100ul	150ul
Beta-mercaptoethanol (100%)	50ul per gram	100ul	150ul
2X CTAB buffer	Equal volume to what is measured	~3.0ml (measure)	~4.5ml
CHCl3 (100%)	Equal volume to what is measured	~6ml (measure)	~9ml
Isopropanol (100%)	70% volume to what is measured	Recover ~5ml, add 5*0.7=3.5ml	Recover ~8ml, add 8*0.7=5.6ml

Here you can see some typical calculations, and the type of calculations you will have to do on the fly.

Example 2: To carry out the Polymerase Chain Reaction – The Master Mix concept.

You are going to set up 2 PCR reactions with your DNA in them, 1 positive control with DNA we give you, and 1 negative control, with water (to check for contamination). This is 4 reactions. Since you are supposed to do extra, make enough reagent mix for 5 reactions.

The only thing that is different in these reactions is the DNA template, so you can make one mix of all the other components (remembering to factor in the volume for the DNA), then aliquot the amount you need into each tube and finally add the DNA. You will do less pipetting so it saves time and also means there will be less variability.

Stock available	Target final concentration
5X buffer with $MgCl_2$	1X buffer with Mg^{++}
10mM dNTP	0.8mM
10uM forward primer	0.2uM
10uM reverse Primer	0.2uM
10mg/ml BSA (also 10ug/ul)	2ug/ul
10% glycerol	1.00%
DNA polymerase @5Units/ul	1Unit per reaction= 0.2ul
DNA (10ng/ul)	50ng
Water	to required volume

I first need to decide what my reaction volume will be: for PCR the most common volumes are 25ul and 50ul. The calculations for 25 ul are shown below – I have not shown the volumes for the DNA and water, because the DNA concentration is sample dependent, and the amount of water added depends on the volume the DNA is in.

Stock available	Target final concentration	Calculating per reaction addition with V_{total} 25ul	Actual per-reaction volume (ul)
5X buffer with $MgCl_2$	1X buffer with Mg^{++}	25ul*1X=Yul*5X	5
10mM dNTP	0.8mM	25ul*0.8mM = Yul*10mM	2
10uM forward primer	0.2uM	25ul*0.2uM=Yul*10uM	0.5
10uM reverse Primer	0.2uM	25ul*0.2uM=Yul*10uM	0.5
10mg/ml BSA (also 10ug/ul)	2ug/ul	25ul*2ug/ul=Yul*10ng/ul	5
10% glycerol	1.00%	25ul*0.01=Yul*0.1	2.5
DNA polymerase @5Units/ul	1Unit per reaction= 0.2ul	0.2ul	0.2
DNA (10ng/ul)	50ng	50ng÷10ng/ul	5
Water	to required volume	25ul-components-DNA	4.3

I decided to dilute all of my DNA samples to 10ng/ul because it makes the calculations for multiple reactions easier - 50ng means adding 5ul of DNA as shown above. Otherwise I have to figure out a separate volume of DNA and water to add to each reaction, which can lead to mistakes.

Now you will note that since I am adding the same volume of water to each reaction, I can also include that in my Master Mix.

Stock available	Target final concentration	Calculating per reaction addition with V_{total} 25ul	Actual per-reaction volume (ul)	Master Mix: Volume needed for 5 reactions (125 - 5*5ul DNA = 100ul working volume). Use 25-5=20ul per reaction
5X buffer with $MgCl_2$	1X buffer with Mg^{++}	25ul*1X=Yul*5X	5	25
10mM dNTP	0.8mM	25ul*0.8mM = Yul*10mM	2	10
10uM forward primer	0.2uM	25ul*0.2uM=Yul*10uM	0.5	2.5
10uM reverse Primer	0.2uM	25ul*0.2uM=Yul*10uM	0.5	2.5
10mg/ml BSA (also 10ug/ul)	2ug/ul	25ul*2ug/ul=Yul*10ng/ul	5	25
10% glycerol	1.00%	25ul*0.01=Yul*0.1	2.5	12.5
DNA polymerase @5Units/ul	1Unit per reaction= 0.2ul	0.2ul	0.2	1
DNA (10ng/ul)	50ng	50ng÷10ng/ul	5	don't add
Water	to required volume	25ul-components-DNA	4.3	21.5
			25	100

The final thing to note is the best order in which to add the components: the enzyme is sensitive to the salt concentration, so it should be added last to the Master Mix. Enzymes also don't like to be vortexed, so the components should be vortexed before adding the enzyme, then pipetted up and down 5-6 times, then given a quick spin, and stored on ice until you are ready to put 20ul in each reaction tube, after which you will add the DNA.

Add in this order:
Water
5X buffer
dNTP
Primer-f
Primer - rev
BSA
Glycerol
Vortex and quick-spin
Add enzyme
Pipette up and down Quick-spin
Store at 4C
Aliquot 20ul into 4 tubes
Add DNA to 3 tubes, water to th

If my examples are confusing, the following might be more helpful:
http://www.mgel.msstate.edu/pdf/solutions.pdf

Practice (also will be needed in the next two labs)

Make yourself a table of the amounts of reagents you will need if you process 1gm of leaf tissue.

Make yourself a table of the reagents you will need to make a PCR Master Mix if you are going to make 6 PCR reactions that are 50ul each.