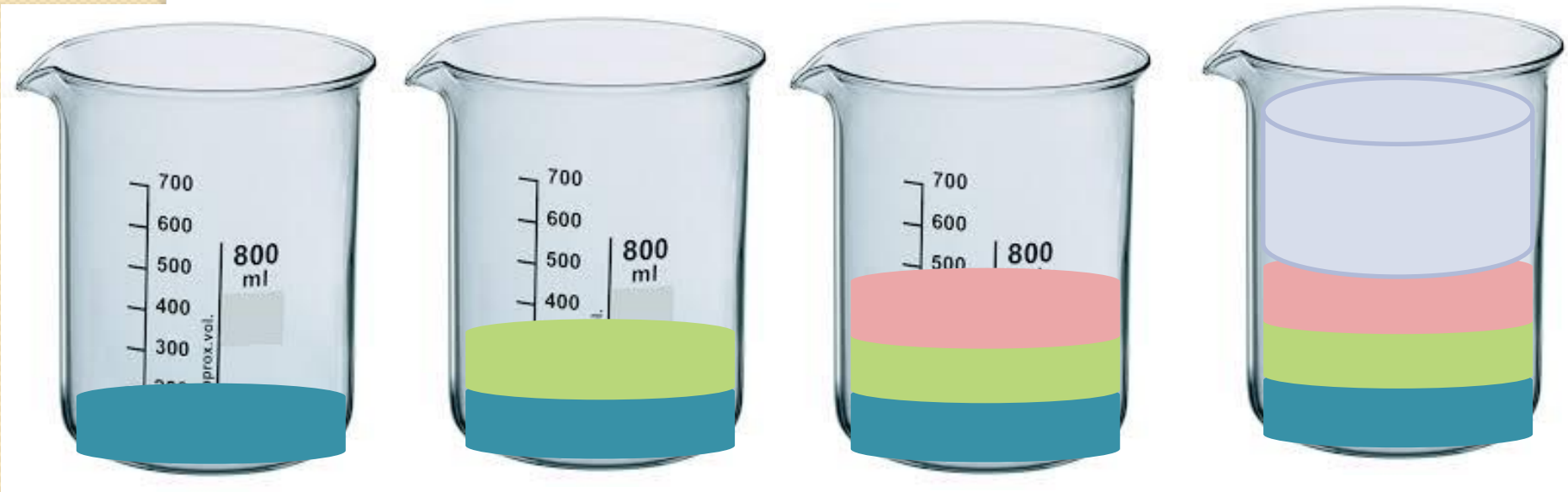
Lab Skills: Making Lab reagents from Stock Solutions

B3 Summer Science Camp at Olympic High School 2016

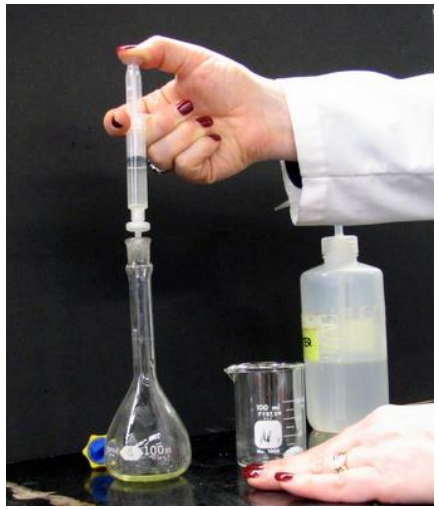
Introduction - 1

- When you make a solution from several components , each solute is diluted by the others, plus extra dilution from any solvent you add (like water).
- Add 200ml of 50% glycerol and 100ml of 5M NaCl
- Add 200ml of '10X' Triton 100.
- Add 400ml of water.
- What are the concentrations of each solute at each step?
  - Does it matter that the units don't match?
  - Could you make 60 ml as easily as 600 ml?
  - Would it be easier to measure the individual solutes each time?



Introduction - 2

- Using carefully-made stock solutions and good technique in removing the intended amounts from the stock bottle helps you achieve more reproducible measurements and therefore more reproducible results.



www.dartmouth.com

Introduction - 3

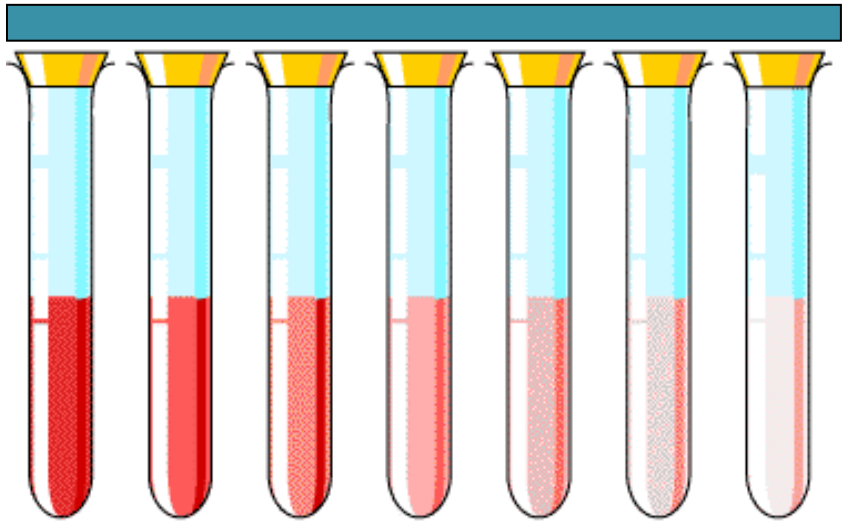
- Don’t go into the Stock bottle more than once per set of reactions/assays you are setting up
  - If you need 5 ml for 5 tubes, get a clean (big enough) container, remove a little more than the 5x5=25ml to that container and then set up the 5 tubes from there (the extra is usually ~10% more).
- Use a completely clean pipetter/tip each time you remove something from the stock bottle, or pour from the stock bottle into your secondary container.

Making Stock solutions

- A stock solution is made up of one or more dissolved chemicals that are at a higher concentration than you need for your processes.
- There are different ways to express the concentration of the stock – the most common in Molecular Biology are
  - Molar: the moles per liter, based on the grams per more of each chemical
  - The mass per volume, such as 2mg/ml
  - The percent, usually based on grams of solute per 100ml of solvent, unless you have two liquids, then it is ml per total of 100 ml
  - The ‘X’ of fold-concentration, meaning how much you would have to dilute it to get the desired amount (usually 1X is the target or recommended amount).

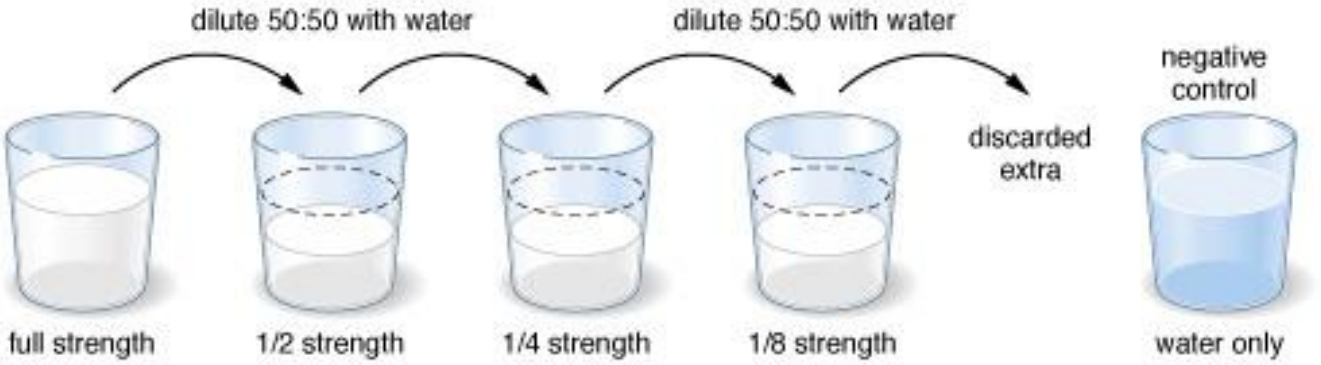
Serial Dilutions

- This is a assay technique, usually used to make sure the concentration of your unknown is in the range for your measurement instrument to give an accurate and precise reading.



Serial Dilution – concept of the steps

Hypothesis: If the original bleach solution is diluted repeatedly with water, the bleaching effect will lessen as concentration decreases.



Final dilution factor (DF) = DF1 * DF2 * DF3

Dilution Factors 2X example

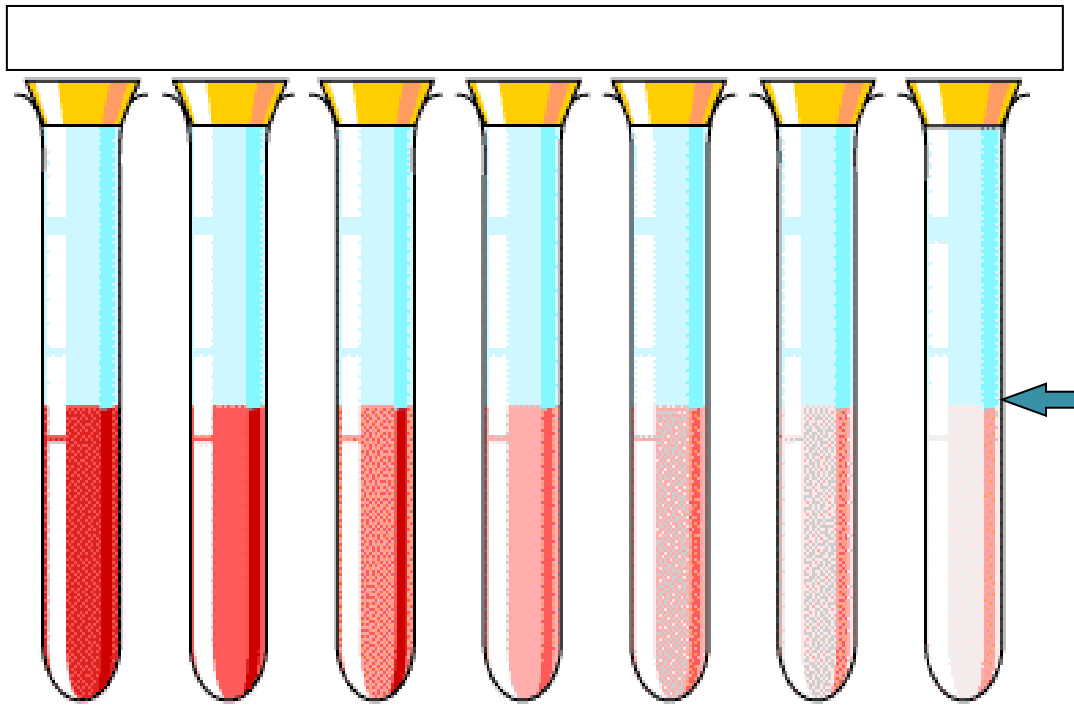
- Original Solution to Diluted Solution

  1: 2 = 1 to 2 (final) dilution

  - To know how much solvent to add subtract the starting volume (1) from the final volume (2): 2-1=1.
  - 1 + 1 = 2 dilution factor
- Note – for some lab methods the naming convention is different
  - 1:1 is a dilution of one part starting solution plus one part solvent, the FOLD dilution is 1+1 = 2X
- This is convenient because it does not matter what volumes you want, the same calculation works for microliters to liters (or ounces and gallons).

2X *Serial* Dilution Example

1:2 1: 2 1:2 1:2 1:2 1:2



Final Dilution after 6 steps that are all the same fold change?

Final dilution factor: 2 x 2 x 2 x 2 x 2 x 2 = ?

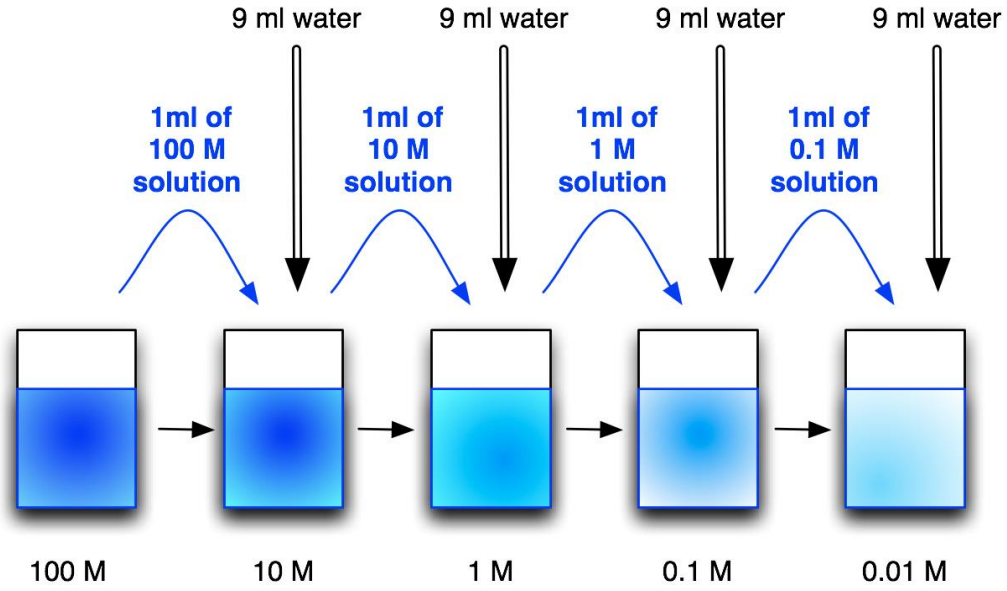
Dilution Factor for 10X

- Original Solution to Diluted Solution

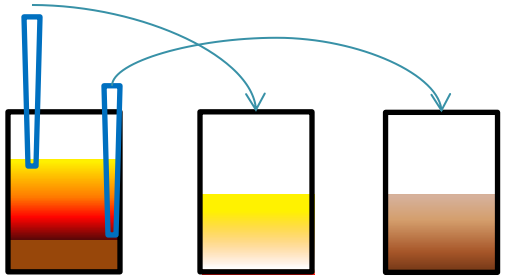
  1: 10 = 1 to 10 dilution

- Combine 1 volume of original solution + 9 volumes of the solvent
- 1 + 9 = 10 dilution factor

Mixing is essential: Diluting/dispensing for serial dilutions



If each solution is completely mixed before taking out 1ml to make the next solution in the series then your set will differ by a factor of 10 each time.



If the solution is not mixed then you get different amounts of the components depending on where the tip goes into the starting solution – all of the resulting samples will be different, and in a series you won't know what factor they differ by.