How to dilute solutions

A. Why dilute?

Here are two situations that arise repeatedly in molecular biology labs:

1. You have a stock solution of some compound, let's say an antibiotic, and you want to add the compound to growth medium, at a much smaller concentration than the stock solution.
2. You have a tube of very concentrated bacteria, perhaps a billion cells per milliliter. You want to put a few hundred of them on a petri plate, so that the colonies that arise will be easily distinguishable.

In both cases, the way out of the problem is to dilute the original solution. If you work in a lab, you need to know how to do this.

B. Methods of calculating dilutions

1. DILUTION FACTOR METHOD (fast, but requires inspiration): First, figure out the factor by which the original solution must be diluted. Second, divide the final volume of the desired solution by that factor, yielding the volume required of the original solution.

   EXAMPLE: Suppose you need to make a 3 ml solution of growth medium supplemented with 50 μM of the antibiotic ampicillin from a stock solution of 5 mM ampicillin. The dilution factor is :

   (5 mM) / (50 μM) = (5000 μM) / 50 μM) = 100

   so you need to dilute:

   (3 ml) / 100 = (3000 μl) / 100 = 30 μl

   of the stock solution to a final volume of 3 ml.

2. CONSERVATION OF PARTICLES METHOD (slow but foolproof... almost): Presume that the particles to be diluted can neither be created nor destroyed. If you use a pipet to deliver some stock solution to a larger volume of water to make the final solution, then the number of particles in the pipet is the same as the number of particles in the final solution (Fig. 1). Since they're equal:

   # of particles in pipet = # of particles in final solution

   # of particles in pipet = (Concentration in pipet) × (Volume in pipet) $= \mathbf{C_{pipet}} \times \mathbf{V_{pipet}}$

   # of particles in final solution =
   (Concentration in final solution) × (Volume in final solution) = $\mathbf{C_{final}} \times \mathbf{V_{final}}$

   So $\mathbf{C_{pipet}} \times \mathbf{V_{pipet}} = \mathbf{C_{final}} \times \mathbf{V_{final}}$

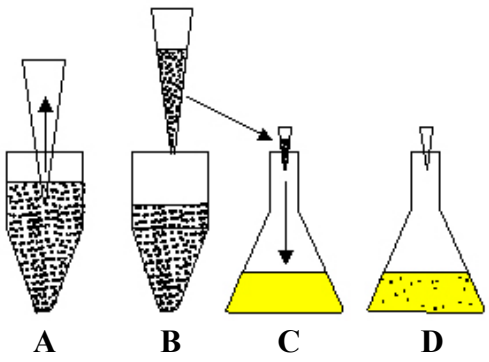

Fig. 1: Conservation of particles method to calculate dilutions. (A-B) Suck up a volume ($\mathbf{V_{pipet}}$) into a pipet tip. The concentration ($\mathbf{C_{pipet}}$) of particles in the tip is the same as the concentration ($\mathbf{C_{stock}}$) in the original tube. **(C-D)** Deliver the particles to a fresh tube of liquid of volume $\mathbf{V_{final}}$. The number of particles in the final solution is the same as the number of particles in the pipet tip.

The concentration in the pipet is the same as the concentration in the stock solution it sampled, the concentration in the final solution is what you're aiming at, and the volume in the final solution you know. The only unknown is the volume of the stock solution in the pipet. So:

$$\mathbf{V_{pipet}} = \mathbf{C_{final}} \times \mathbf{V_{final}} / \mathbf{C_{stock}}$$

Using the previous example:

$$\mathbf{V_{pipet}} = (50\ \mu M) \times (3\ ml) / (5000\ \mu M) = 30\ \mu l$$

So you must add 30 µl of the stock solution to a final volume of 3 ml. ALWAYS check to make sure the units come out right!

SQ3. Starting with an *E. coli* culture of $5 \cdot 10^8$ cells/ml, describe how you would obtain 2 ml of a diluted culture, containing $2 \cdot 10^7$ cells/ml.