

Solutions for Practice Dilution Problems, Bio 125, Winter 2006

1. *What do you know from the problem?*

You have: 10X TBE

You want: 1X TBE

How much? 10 L

We just need to dilute the stock we have to make the buffer we want. We can use the equation on page 3-7 to solve this problem, or we can realize that we need to dilute 10X ten times to get 1X. Here's both ways:

Using the equation on page 3-7:

$$v_1c_1=v_2c_2 \quad v_1=? \quad c_1=10X \quad v_2=10 \text{ L} \quad c_2=1X$$

$$(v_1)(10X) = (10 \text{ L})(1X)$$

$$v_1 = \frac{(10 \text{ L})(1X)}{(10X)} = \frac{(10 \text{ L})(\cancel{1X})}{(\cancel{10X})} = 1 \text{ L}$$

So, what does this mean? V_1 tells you how much stock (that's the 10X TBE) to add. You still need to determine how much water to add. This is where most students make it harder than it needs to be: you know how much stock you need (1 L), and you know the total volume of buffer you're making (10 L). Just subtract!

$$10 \text{ L} - 1 \text{ L} = 9 \text{ L}$$

So, mix together 1 L of the 10X TBE stock and 9 L of water to make 10 L of 1X TBE buffer.

Using the intuitive method, you could reason:

I have 10X stock, and I need to dilute it by a factor of 10. Since I want to make 10 L, I just divide 10 L by 10 to get 1 L ($10 \text{ L} \div 10 = 1 \text{ L}$). That's how much stock I should use. The rest ($10 \text{ L} - 1 \text{ L}$) should be water.

So, mix together 1 L of the 10X TBE stock and 9 L of water to make 10 L of 1X TBE buffer.

2. *What do you know from the problem?*

You have: 500 $\mu\text{g}/\text{ml}$ DNA

You want: 1 μg DNA

How much? 10 μl

This is similar to the previous problem, with two exceptions: (1) you now have an initial concentration which is in terms of weight per volume, and (2) you are not given the final concentration you are looking for, only a weight. You can use the equation on page 3-7 to solve the problem. (See below for a different method.)

$$v_1c_1=v_2c_2 \quad v_1=? \quad c_1=500 \mu\text{g}/\text{ml DNA} \quad v_2=10 \mu\text{l} \quad c_2=1 \mu\text{g}/10 \mu\text{l}=?$$

Remember that v_1 is what you are looking for in the end. You have everything you need to calculate c_2 in the problem. You know the final weight should be 1 μg , and it should be present in a volume of 10 μl , so divide 1 μg by 10 μl : ($1 \mu\text{g} \div 10 \mu\text{l} = 0.1 \mu\text{g}/\mu\text{l}$) to get the final concentration. Note that one of the requirements for the equation you're using is that c_1 and c_2 must be in the same units. In this case, we'll choose $\mu\text{g}/\mu\text{l}$, but you could do it equally well choosing $\mu\text{g}/\text{ml}$.

$$c_1 = \frac{(500 \mu\text{g DNA})}{1 \text{ ml}} \left(\frac{1 \text{ ml}}{1000 \mu\text{l}} \right) = \frac{(500 \mu\text{g DNA})}{1 \cancel{\text{ml}}} \left(\frac{1 \cancel{\text{ml}}}{1000 \mu\text{l}} \right) = 0.5 \mu\text{g}/\mu\text{l DNA}$$

Be aware that when you multiply a number by a conversion factor, like $1 \text{ ml}/1000 \mu\text{l}$, you are just multiplying by one, because $1 \text{ ml} = 1000 \mu\text{l}$. The key to setting these sorts of equations up is to make sure your units cancel in a way you want (imagine what your units would be if you multiplied $500 \mu\text{g}/\text{ml}$ by $1000 \mu\text{l}/\text{ml}$!).

So, here's what we're starting with now:

$$v_1c_1=v_2c_2 \quad v_1=? \quad c_1=0.5 \mu\text{g}/\mu\text{l DNA} \quad v_2=10 \mu\text{l} \quad c_2=0.1 \mu\text{g}/\mu\text{l DNA}$$

$$(v_1)(0.5 \mu\text{g}/\mu\text{l}) = (10 \mu\text{l})(0.1 \mu\text{g}/\mu\text{l})$$

$$v_1 = \frac{(10 \mu\text{l})(0.1 \mu\text{g}/\mu\text{l})}{(0.5 \mu\text{g}/\mu\text{l})} = \frac{(10 \mu\text{l})(0.1 \mu\text{g}/\mu\text{l})}{(0.5 \mu\text{g}/\mu\text{l})} = 2 \mu\text{l}$$

Again, v_1 tells you how much stock to add.
Remember to subtract this from v_2 .

10 μl final volume – 2 μl of 500 $\mu\text{g}/\text{ml}$ DNA = 8 μl of buffer

So, mix together 2 μl of the 500 $\mu\text{g}/\text{ml}$ DNA stock solution and 8 μl of buffer (no DNA) to get 10 μl of buffer containing 1 μg of DNA.

Another way to do this problem is to determine what volume of stock solution (the 500 $\mu\text{g}/\text{ml}$) will give you 1 μg of DNA. This seems obvious to some people, but not to everyone. Do what you understand.

$$(1 \mu\text{g DNA}) \left(\frac{1 \text{ ml}}{500 \mu\text{g DNA}} \right) = (1 \mu\text{g DNA}) \left(\frac{1 \text{ ml}}{500 \mu\text{g DNA}} \right) = 0.002 \text{ ml}$$

And then convert ml to μl :

$$(0.002 \text{ ml}) \left(\frac{1000 \mu\text{l}}{1 \text{ ml}} \right) = (0.002 \text{ ml}) \left(\frac{1000 \mu\text{l}}{1 \text{ ml}} \right) = 2 \mu\text{l}$$

10 μl final volume – 2 μl of 500 $\mu\text{g}/\text{ml}$ DNA = 8 μl of buffer

So, mix together 2 μl of the 500 $\mu\text{g}/\text{ml}$ DNA stock solution and 8 μl of buffer (no DNA) to get 10 μl of buffer containing 1 μg of DNA.

3. *What do you know from the problem (& the previous problem)?*

You have: 500 $\mu\text{g}/\text{ml}$ DNA,
which we found in #2 equals 0.5 $\mu\text{g}/\mu\text{l}$

You want: 1 μg DNA per 10 μl ,
which we found equals 0.1 $\mu\text{g}/\mu\text{l}$
How much? 600 μl

There are many ways to solve this problem, depending on how you did #2. Here is one:

$$v_1 c_1 = v_2 c_2 \quad v_1 = ? \quad c_1 = 0.5 \mu\text{g}/\mu\text{l DNA} \quad v_2 = 10 \mu\text{l} \quad c_2 = 0.1 \mu\text{g}/\mu\text{l DNA}$$

$$(v_1)(0.5 \mu\text{g}/\mu\text{l}) = (600 \mu\text{l})(0.1 \mu\text{g}/\mu\text{l})$$

$$v_1 = \frac{(600 \mu\text{l})(0.1 \mu\text{g}/\mu\text{l})}{(0.5 \mu\text{g}/\mu\text{l})} = \frac{(600 \mu\text{l})(0.1 \mu\text{g}/\mu\text{l})}{(0.5 \mu\text{g}/\mu\text{l})} = 120 \mu\text{l}$$

Again, v_1 tells you how much stock to add
Remember to subtract this from v_2 .

600 μl final volume – 120 μl of 500 $\mu\text{g}/\text{ml}$ DNA = 480 μl of buffer

So, mix together 120 μl of the 500 $\mu\text{g}/\text{ml}$ DNA stock solution and 480 μl of buffer (no DNA) to get 600 μl of buffer containing 0.1 μg of DNA per ml of buffer.

4. *What do you know from the problem?*

You have: 0.5 M Sorenson buffer pH 7.5
0.25 M EDTA
full-strength (100%) β -mercaptoethanol

You want: 50 mM Sorenson buffer pH 7.5
25 mM EDTA
1% β -mercaptoethanol
How much? 40 ml

This problem adds a layer of complexity by requiring multiple components in the final buffer solution. This will not cause problems as long as the initial volumes of all the components do not add up to more than the total final volume (40 ml, in this case). Work through each component methodically.

Sorenson buffer:

$$v_1 c_1 = v_2 c_2 \quad v_1 = ? \quad c_1 = 0.5 \text{ M Sorenson buffer} \quad v_2 = 40 \text{ ml} \quad c_2 = 50 \text{ mM Sorenson buffer}$$

Convert for standard units (mM or M doesn't matter; we'll choose mM):

$$c_1 = 0.5 \text{ M} = (0.5 \text{ M}) \frac{(1000 \text{ mM})}{1 \text{ M}} = (0.5 \text{ M}) \frac{(1000 \text{ mM})}{1 \text{ M}} = 500 \text{ mM Sorenson buffer}$$

$$v_1 c_1 = v_2 c_2 \quad v_1 = ? \quad c_1 = 500 \text{ mM Sorenson buffer} \quad v_2 = 40 \text{ ml} \quad c_2 = 50 \text{ mM Sorenson buffer}$$

$$(v_1)(500 \text{ mM}) = (40 \text{ ml})(50 \text{ mM})$$

$$v_1 = \frac{(40 \text{ ml})(50 \text{ mM})}{500 \text{ mM}} = \frac{(40 \text{ ml})(50 \text{ mM})}{500 \text{ mM}} = 4 \text{ ml Sorenson buffer pH 7.5}$$

Now move on to the next component:

EDTA:

$$v_1 c_1 = v_2 c_2 \quad v_1 = ? \quad c_1 = 0.25 \text{ M EDTA} \quad v_2 = 40 \text{ ml} \quad c_2 = 25 \text{ mM EDTA}$$

Convert for standard units (mM or M doesn't matter; we'll choose M this time):

$$c_2 = 25 \text{ mM} = (25 \text{ mM}) \left(\frac{1 \text{ M}}{1000 \text{ mM}} \right) = (25 \text{ mM}) \left(\frac{1 \text{ M}}{1000 \text{ mM}} \right) = 0.025 \text{ M}$$

$$v_1 c_1 = v_2 c_2 \quad v_1 = ? \quad c_1 = 0.25 \text{ M EDTA} \quad v_2 = 40 \text{ ml} \quad c_2 = 0.025 \text{ M EDTA}$$

$$(v_1)(0.25 \text{ M}) = (40 \text{ ml})(0.025 \text{ M}) \quad v_1 = \frac{(40 \text{ ml})(0.025 \text{ M})}{0.25 \text{ M}} = \frac{(40 \text{ ml})(0.025 \text{ M})}{0.25 \text{ M}} = 4 \text{ ml EDTA}$$

And the final component:

β -mercaptoethanol:

$$v_1 c_1 = v_2 c_2 \quad v_1 = ? \quad c_1 = 100\% \beta\text{-mercaptoethanol} \quad v_2 = 40 \text{ ml} \quad c_2 = 1\% \beta\text{-mercaptoethanol}$$

$$(v_1)(100\%) = (40 \text{ ml})(1\%) \quad v_1 = \frac{(40 \text{ ml})(1\%)}{100\%} = \frac{(40 \text{ ml})(1\%)}{100\%} = 0.4 \text{ ml } \beta\text{-mercaptoethanol}$$

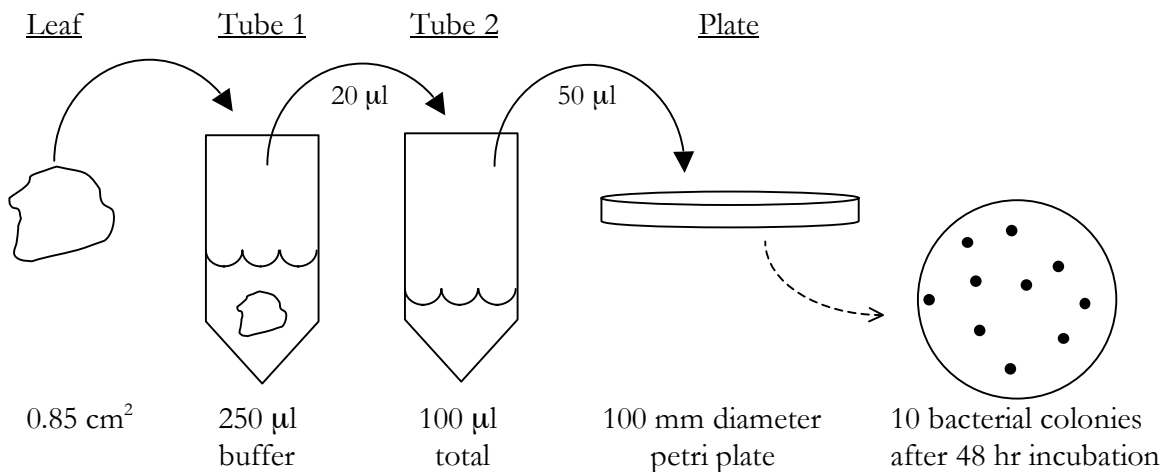
It works equally well to call 100% "1" and 1% "0.01". You'll have no units to cancel in that case.

To determine how much water to add, simply subtract all the components from the total volume:

$$40 \text{ ml} - 4 \text{ ml buffer} - 4 \text{ ml EDTA} - 0.4 \text{ ml } \beta\text{-mercap.} = 31.6 \text{ ml water}$$

So, mix together 4 ml of the 0.5 M Sorenson buffer pH 7.5, 4 ml of the 0.25 M EDTA, 0.4 ml β -mercaptoethanol, and 31.6 ml water to get 40 ml of buffer containing 50 mM Sorenson buffer pH 7.5, 25 mM EDTA, and 1% β -mercaptoethanol.

5. What do you know from the problem? Whew! There's a lot of numbers there. You might find it helpful to draw out a diagram of what is known:



Each colony on the plate represents millions of bacteria; each colony is a clone of a single bacterial cell which came from Tube 2. The first thing to realize is that the diameter of the petri dish actually is not relevant to the problem; if the plate were larger or smaller, there would still be only 10 colonies on the plate, as long as you are putting 50 μl from Tube 2 on it.

One way to solve this problem (probably the most direct way in this case) is to simply work backwards from the plate and determine how many bacteria are present at each step.

Plate: 10 bacterial colonies represent 10 bacteria present in 50 μl from Tube 2.

Tube 2: 100 μl total, so $2(10 \text{ bacteria}/50 \mu\text{l}) = 20 \text{ bacteria}/100 \mu\text{l} = 20 \text{ bacteria in the tube}$

Tube 1: All bacteria in Tube 2 came from Tube 1, so there must have been 20 bacteria in the 20 μl from Tube 1. That's the same as 1 bacteria/ μl in Tube 1. Since there are 250 μl in Tube 1, there were a total of $(250)(1) = 250$ bacteria in Tube 1 initially.

Leaf: There were 250 bacteria on the leaf (because all the bacteria in Tube 1 came from the leaf). The size of the leaf is 0.85 cm^2 . Therefore, there must have been $(250)/(0.85) = 294.12$ bacteria/ cm^2 on the leaf.

There were 294 bacteria per square centimeter of leaf.

Another way to solve this problem is to break it up into different questions and solve each one in turn. This technique might be more generally applicable to other dilution problems involving plating.

Questions: 1. In Tube 1, how many square centimeters of leaf are there per μl ?
2. In Tube 1, how many bacteria are there per μl ?
3. On the leaf, how many bacteria are there per square centimeter?

Solutions: 1. In Tube 1, there is 0.85 cm^2 leaf per 250 μl buffer, or $(0.85/250) = 0.0034 \text{ cm}^2/\mu\text{l}$.
2. To determine how many bacteria there are per μl in Tube 1, we again need to work backwards from the plate. The plate contained 10 colonies, which came from 50 μl of the contents of Tube 2. $(10 \text{ bacteria})/(50 \mu\text{l}) = 0.2 \text{ bacteria}/\mu\text{l}$, the concentration of bacteria in Tube 2. To calculate the concentration of bacteria in Tube 1, we need to know the relationship between Tube 1 and Tube 2. The dilution that we performed to make Tube 2 (20 μl from Tube 1 + 80 μl buffer) is called a "5X" dilution: the initial solution (from Tube 1) is now five times more dilute. Because the concentration of bacteria in Tube 1 is five times (5X) greater than the concentration in Tube 2, and we know the concentration in Tube 2 is 0.2 bacteria/ μl , we can calculate the concentration in Tube 1: $5(0.2) = 1$ bacterial cell/ μl .
3. Now all we have to do is use the answers from question 1 and question 2 to arrive at the answer to the problem:

$$\frac{1 \text{ bacterial cell}/\mu\text{l}}{0.0034 \text{ cm}^2/\mu\text{l}} = \frac{1 \text{ bacterial cell}/\mu\text{l}}{0.0034 \text{ cm}^2/\mu\text{l}} = 294.12 \text{ bacteria}/\text{cm}^2$$

There were 294 bacteria per square centimeter of leaf.

Be aware that there are many ways to solve dilution problems, and you will need to find a way that works for you. If you use a very intuitive strategy, just make sure it will work in situations where you don't have nice, round numbers and even ratios. No matter which way you solve a problem, the best thing to do first is to figure out what you know and make sure you understand what your goal is. The best thing to do last is look at your answer and see if it seems reasonable (e. g. you should have only volume units for volumes and you should be using smaller amounts of stock solutions to make up larger volumes of working solutions).

If you have questions, do not hesitate to contact your TA or lab instructor for help. You might also try talking to several other students in the course for help; multiple perspectives and strategies might be beneficial.