Part II: Standard Laboratory Techniques

Introduction: In addition to demonstrating the performance indicators relating to scientific inquiry described in NYS Living Environment Curriculum Standard 1, students need to develop proficiency in certain laboratory skills in order to successfully conduct investigations in biological science. Part II contains 2 labs (a dilution / solution lab and an electrophoresis lab). The dilution / solution lab should be completed in 1—40min. lab period and the electrophoresis lab will require 2 (one to run the gel and one for the assessment. Included also is an extension of the dilution / solution intended for AP students. With the extension you may need an additional period to complete the dilution / solution lab.

I. Overview: As a result of these lab experiences students will:

☒ Have a better understanding of how to make
   1. A percent solution
   2. A dilution of a stock solution

☒ Practice using graduated cylinders and balances for measuring

☒ Understand the uses of gel electrophoresis as a way to separate molecules of different sizes and charges.

☒ Become more familiar with lab safety techniques

II. Student prior knowledge and skills required

☒ Students should be able to measure using either a triple beam balance or electronic balance

☒ Students should have basic math skills to be able to solve for x in a simple proportion

III. Time required:

☒ 3 --- 40 minute lab periods

☒ (optional lab for AP may require more time)

IV. Advance preparation

☒ Within each lab is a more detailed list of materials / lab station but the minimum requirements for all of the labs in this sections include:

1. Calculators and pencils
2. 10X TBE buffer (Fisher # BP1333-1)
3. agarose
4. beakers and graduated cylinders
5. balance or scale and weigh paper
6. magnetic stir bars and plate with heat (or stir plate and a separate heat source)
7. DNA source (Edvotek)
8. Methylene blue
9. Electrophoresis chamber, casting tray with comb, and power source
10. P20 microliter pipettor with tips for loading DNA samples
11. Red and blue food coloring (for the optional extension lab)
12. Distilled water and tap water

V. What is expected from students

☒ Lab #3 handout (dilutions and solutions) –

1. students will solve mathematical equations using examples on the handout
2. students will make a 1%agarose solution and dilute a 10XTBE solution to make a 1XTBE solution to be used in electrophoresis lab

☒ Lab #3 (optional extension for AP)

1. Students will do dilution calculations

2. Students will make a series of dilutions and use standard solutions to assess their work

☒ Lab #4 (electrophoresis handout)

1. Students will pour a gel into a casting tray

2. Students will practice using a microliter pipettor to measure small volumes

3. Students will load DNA onto the gel

4. Students will make and record observations based on the results of the gel and answer simple questions in relation to gel electrophoresis.

5. Students will take a post lab test to assess understanding

VI. Assessment

☒ All student work should be incorporated into a student lab notebook or folder and graded based on level of completion, level of participation and level of understanding (based on post lab test).

Lab # 3: Solutions, Dilutions, and Concentrations Lab

Objective: Part of any lab includes the mixing of reagents to make solutions to work with. It is very important that the solutions you make are at the correct concentration for your experiment to be repeatable. (**Remember the way to make any experiment more valid or believable is for anyone to be able to repeat it and get the same results!**) Today we will practice making percent solutions. After completing this lab you should be able to:

☒ Make a percent solution
☒ Make dilutions of a stock solution

Part I (Calculations)

Introduction and input:

Percent solutions are based on the volume or weight of something in 100ml.

When mixing a solid with a liquid you use

1g of solid to make 100 ml of a 1% solution
10g of solid to make 100 ml of a 10% solution

When mixing a liquid with water use

1ml of the stock to make 100ml of a 1% solution
10ml of the stock to make 100ml of a 10% solution

Example #1

A solution that is 10% sucrose has 10 grams of sucrose in 100ml of solution.
Note: The sucrose will take up some space in the solution so the amount of water you add will be a little less than 100ml

Example #2

What if we wanted to make 500 ml of a 6% sucrose solution?
We will have to set up a proportion:

A 6% solution has 6 grams in 100ml or $\frac{6g}{100ml}$

Even if the volume is different than 100ml the ratio is the same so if we want to make 500ml we could set up a proportion $\frac{6\ g}{100\ ml} = \frac{x\ g}{500\ ml}$

$6 * 500 = x * 100$
$3000 = 100x$
$30 = x$

therefore you need 30 grams of sucrose to make 500 ml of a 6% solution

Problem #1

For next week's lab you will need 200 ml of a 1% agarose solution. Set up a proportion to determine how much agarose you will need.

1g/100ml = x/200
100x = 200
x = 2g
Therefore you will need 2 g of agarose

Note: Always check your answers! Do the numbers you got make sense for what you are doing. (For example if you are making more than 100ml of a 1% solution then your answer has to be greater than 1 g)

Example #3

A 70% ethanol solution would be made of 70ml of ethanol plus enough water to make 100ml
100ml – 70ml = 30ml of water
Therefore to make 100ml of 70% ethanol add
70 ml of pure ethanol and 30 ml of water.

A simple formula to use when mixing liquids to make a % solution is :

C = concentration
V = volume

What you start with = What you want to make
$C_1V_1 = C_2V_2$

Example #4

To make a 100ml solution of 10% ethanol from a stock that is 100% ethanol how much water would you add.

$$C_1V_1 = C_2V_2$$

$$100\%\ (x) = 10\%\ (100ml)$$

$$x = \frac{10(100)}{100}$$

$$x = 10ml$$

Therefore you would need to add 10ml of 100% ethanol to make the solution
The rest of the solution will be water

100ml – 10ml = 90ml of water

Problem #2

For next week's lab you need to make a 1% TBE solution from a stock that is 10% TBE. We will need to make 1L of solution (remember that 1L = 1000ml). How much of the 10% stock solution will we need to add and how much water will we need?

10%(x) = 1% (1000ml)
x = 1000/10
x = 100ml of the 10% solution

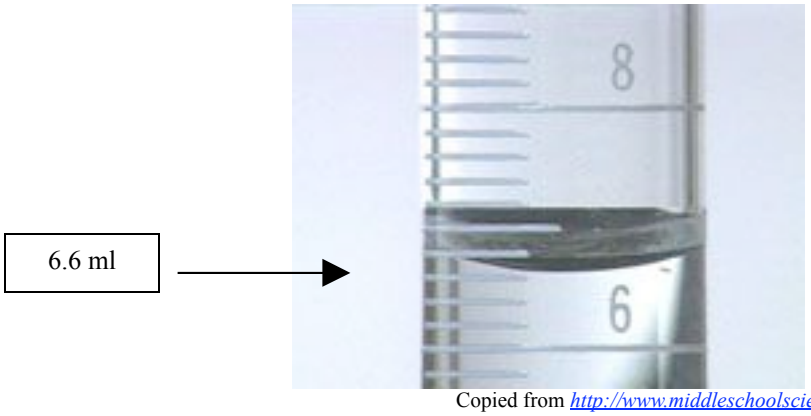
1000ml – 100ml = 900ml of water

Note: Always check your answers! Do the numbers you got make sense for what you are doing. (For example if you are only making 100ml of a liquid then your answer has to be less than 100ml)

Part II (Making solutions)

Introduction and Input: When scientists do experiments it is very important to write down everything that they do? Why? *So they or someone else can repeat it exactly the same way* When you do anything in this lab it will be important to write down everything you do. In this part of the lab we will make the solutions we talked about in part I of this lab. There are a few things we need to remember about weighing measuring and mixing chemicals.

- ☒ Always use a graduated cylinder when measuring volumes of liquids. (Note: beakers often have numbers on them but they are not accurate enough to use when measuring)
- ☒ Always measure from the bottom of the meniscus



Copied from http://www.middleschoolscience.com/meniscus.jpg

- ☒ When weighing solids use a triple beam balance or electronic scale
- ☒ Heat often helps solids go into solution
- ☒ Use extreme caution when heating liquids

1) Use pot holders or beaker tongs to handle hot glassware
2) Never put a stopper on a test tube while it is heating (pressure may build up → explosion)
3) Always tip a test tube or beaker away from your face to avoid breathing in fumes.
4) Wear protective goggles and clothing when handling hot liquids in case of splashing.

http://www.usoe.k12.ut.us/curr/science/phillips/safety.gif

- ☒ Always write down the exact measurements that you used and label all solutions

Materials at your lab station
Distilled water (1L)
1L and 500ml beakers
1L and 500ml graduated cylinders
Balance or scale
1 sheet weigh paper
scoop, spoon, or spatula to measure agarose
10X TBE (Need at least150ml)
Agarose (Need at least 2g)
1L Flask or bottle with a stopper
500ml Flask or bottle with a stopper
Magnetic stir bar and plate with heater
Pot holder for handling hot liquids
Sharpie and tape for labeling

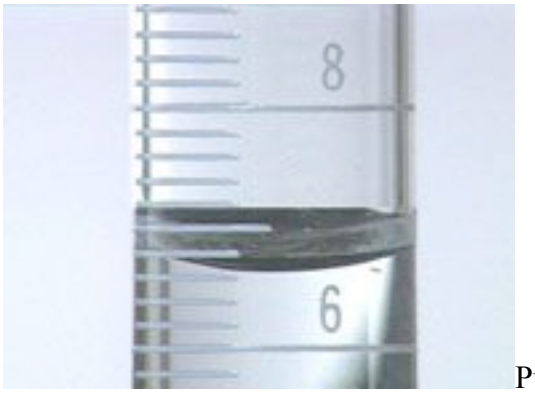
Solution #1 Make 1L of a 1X TBE solution

1) First add a stir bar to your beaker

Substance to add	Amount needed	Amount added
10X TBE	100ml	
Distilled Water	900ml	

2) Add both substances to the beaker
3) Mix with the stir bar
4) Then pour the solution into a flask labeled 1X TBE with the date and your group name (store at room temperature for later use)

Solution #2 Make 200ml of a 1% agarose solution in TBE
Note: It is important that you use 1X TBE to make this solution not water!!

1) 

Put a stir bar into a 500 ml beaker and add the following:

Substance to add	Amount needed	Amount added
1X TBE	150 ml	
Agarose	2g	

2) Mix the above ingredients on the stir plate with low heat until all of the agarose has dissolved.
3) Pour the liquid into the 500ml graduated cylinder (CAUTION: Liquid will be hot use pot holders and pour carefully!!!)
4) Add enough 1X TBE to bring the volume up to 200ml
5) Pour back into the beaker and stir with stir bar again until thoroughly mixed
6) When mixed carefully pour into the 500ml flask and label 1% agarose with the date and your group name (store at room temp for later use)(note agarose should become solid as it cools)

Part III (Dilution Laboratory) *Note: This additional lab was designed for AP students and might be difficult for regular ed.*

Introduction and Input: The purpose of this lab is for you to become familiar with how to make and calculate a series of dilutions.
Making a 1:10 dilution means that 1 part from your solution will be diluted with 9 parts water.

Example: If you were to make a 200ml of a 1:50 dilution of solution A you would use a proportion to determine how much of A to add

$1/50 = x/100$
$50x = 100$
$x = 2$ ml of solution A

If you need 2 ml of solution A you will need 98ml of water to make 100ml of solution

Practice:

Materials at your lab station
Tap water
Food coloring (red and blue)
P200 pipetor and pipet tips
100ml graduated cylinder
4 -100ml beakers
Sharpie for labeling

Procedure:
1) Add 200ul of red food coloring to 100ml of water and place in a beaker labeled solution A
2) Add 200ul of blue food coloring to 100ml of water and place in a beaker labeled solution 1
3) Make 200ml of a 1:10 dilution from solution A and label this solution B

Solution A add *20 ml*
Water add *180 ml*

Show your work:

What is the final dilution factor for this solution? Ans. *1:5000 dilution (note: solution A is a 1:500 x 10 gives you a final dilution of 1:5000)*

4) Make 100ml of a 1:50 dilution from solution 1 and label this solution 2

Solution 1 add *2 ml*
Water add *98 ml*

Show your work:

What is the final dilution factor for this solution? Ans. *1:25,000 dilution (note: solution 1 is a 1:500 x 50 gives you a final dilution of 1:25,000*

5) Make 100ml of a 1:20 dilution of solution B and label this solution C

Solution B add *5 ml*
Water add *95 ml*

Show your work:

What is the final dilution factor for this solution? *1:100,000 (note: solution B is a 1:5000 x 20 gives you a final dilution of 1:100,000*

6) Make 100ml of a 1:5 dilution from solution 2 and label this solution 3

Solution 3 add *20 ml*
Water add *80 ml*

Show your work:

What is the final dilution factor for this solution? *1:125,000 dilution of food coloring(note: solution 2 is a 1: 25,000 x 5 gives you a final dilution of 1:125,000*

7) Compare your solutions with the stock solutions in the front of the room.

Note for teachers: Prepare all of the solutions ahead of time and label them with the final dilution factor so that students can compare the colors they got from their dilutions with yours.

Lab #4: Gel electrophoresis

Objective:

In this lab students will run DNA fragments of different sizes on a gel and observe their migration patterns to determine whether smaller DNA fragments travel faster or slower towards a + charge than larger fragments. This is an inexpensive lab that can be done with minimal equipment and does not require the use of kits. This lab was designed for any high school level biology class (not just AP) and does not require prior knowledge of restriction enzymes). Prior knowledge of DNA structure is recommended.

Reagents and materials:

Gel

Most commonly used gels are made with polyacrylamide or agarose. (Polyacrylamide is toxic and should be avoided when working with students.) Agarose has a larger pore size and is used to separate nucleic acids, large proteins, and protein complexes. Agarose can be purchased from a wide variety of sources, can be used at varying concentrations, and is relatively inexpensive.

For these experiments I prepared a 1% agarose gel in 1X TBE buffer. *Note: It is important to make your gel with TBE buffer and not water. The salts in the TBE buffer are necessary for the current to flow evenly through the gel.*

Agarose (Fisher scientific #BP164-25 (25g)~$47.25) *(enough for 25(100ul size) gels)*

Buffer

While there are some bufferless gel systems on the market (see www.invitrogen.com), they are a little more expensive than normal gels that are run in a liquid buffer. Bufferless gels are faster than conventional gel electrophoresis methods and therefore might be of interest to teachers trying to run a gel within a 40 min. lab period. Currently a package of 18 gels can be purchased for about $135. This would also require a one time purchase of the power base.

Conventional gel electrophoresis labs require the use of a basic pH buffer. The most commonly used buffer is TBE (Tris/Borate/EDTA). Below is a recipe for TBE buffer but I recommend purchasing a ready to use 10X solution from Fisher scientific and diluting it 1 to 10 prior to use. It stores for extremely long periods of time (up to a few years) and is very reliable.

10X TBE buffer (Fisher scientific #BP1333-1, (1L bottle)~$35 **(enough for at least 25 runs depending on the size of your chamber).**

---OR---

To make a 1L batch of a 10X TBE solution

Amount (g)	Reagent	Molarity	FW	Source
107.8	Tris Base	0.89	121.10	Fisher BP152-1
55.0	Boric Acid	0.89	61.83	Fisher BP168-500
9.3	EDTA·$2H_2O$	0.025	372.20	Fisher BP120-500

Might have to heat up to get the EDTA into solution

pH should be (8.0-8.5) filter through a 0.45u filter and store at room temp.

DNA source

DNA ladders and markers are composed of DNA fragments that have been cut into various lengths. They can be purchased from a variety of sources. In my experience DNA is very stable for long periods of time and can be stored for more than 1 year as long as you avoid freeze/thawing by storing in a freezer that doesn't have a defroster. If one is not available you can store it at 4˚C in a regular refrigerator.

If you are using DNA that doesn't contain a loading dye, you will have to use one when you load the DNA. The loading dye weighs it down and allows you to visually monitor the speed of migration. There are many types of loading dyes to choose from. I recommend using a 10X solution of 0.25% bromophenol blue in 60% glycerol. *[Note: (0.125g bromophenol blue and 30ml glycerol → 50ml (you might have to add a drop of NaOH to make it blue)(50ml is more than you will need in a lifetime)]*

Most DNA ladders come with the dye already added so you can disregard this step if you are using a purchased ladder or marker containing a dye.

1) **1kb DNA step ladder (1000-10,000) → 10 bands (Fisher scientific #PRG6941 (90ug at 0.3ug/ml) $77) (Comes with a loading dye.)** *Enough for 300 lanes if you load 10ul/lane*
2) EDVOTEK will also sell you ready to load DNA from their kits. For these experiments I recommend using markers of different sizes for comparison.
   For 40 lanes each order the following DNA samples (DNA comes at a concentration of 0.15ug/ul. I recommend loading 10ul or 1.5 ug for good staining. The more you add the bigger the band will be, but 10ul will give you very clear bands (see sample results):
   EDVOTEK kit #130 special component C for $15 (C →1 band 3kb)
   And
   EDVOTEK kit #130 special component D for $15 (D→2 bands 3kb and 1kb)

Safety Note: When purchasing DNA for student use it is best to purchase DNA from non-human sources due to the possible contamination of viral DNA. The DNA from the EDVOTEK kit above has been isolated from plasmid DNA and is considered safe for student use.

DNA Staining

When choosing a stain for your DNA bands you should stay away from the more commonly used ethidium bromide or crystal violet as both are carcinogenic and should be avoided when working with students. As a nontoxic alternative I suggest using a 0.002% methylene blue solution. The problem with using methylene blue is that you have to add more DNA than you would have to if using ethidium bromide but the nice thing about it is that you can see the bands in regular light and take nice pictures using a normal digital camera. When using methylene blue it is necessary to add at least 200ug of DNA to each well.

Prepare a 0.2% stock solution of methylene blue solution in 0.1X TBE (0.2g methylene blue / 100ml 0.1XTBE). When staining your gel dilute the stock solution of methylene blue 1:100 in TBE buffer. 25g of methylene blue is $18.40 from **Sigma #MB-1**

Gel Electrophoresis Apparatus

Power supply

Any power supply that can run at 110v should be fine. A single gel running at 100volts will be ready within an hour. More expensive models that can run at 220 volts can be purchased for faster runs.
Carolina sells a 110v unit for $186 (ww-21-3672)
And a 220v unit for $228 (ww-21-3672B)

Chamber and casting trays

Chambers and trays can be purchased from many vendors. One possible source is Carolina biological supplies
The following chamber can hold 2 gels at one time capable of running 32 samples if you use 2 casting trays stacked on top of each other.
Carolina #ww-21-3668, $189
Extra casting trays and 8 well combs can be purchased
Carolina #ww-21-3655, $33 per set

Sample Equipment Costs:

Item	Vendor and catalog #	Price
Electrophoresis chamber	Carolina #ww-21-3668	$189.00
Extra casting trays and combs	Carolina #ww-21-3655	$ 33.00
Power source	Carolina #ww-21-3672	$186.00
	Total	**$408.00**

While initial setup cost of buying the electrophoresis chamber and power source is high most of the reagents are reusable and store for long periods of time. While it would be great to have many chambers and power sources – up to 32 students can participate with just one chamber and power source by stacking 2 gels on top of each other.

Sample reagent costs:

Item	Vendor and catalog #	Price	~ # of gels or lanes
25g Agarose	Fisher scientific #BP164-25	$47.25	25 gels
10X TBE buffer	Fisher scientific #BP1333-1	$35.00	25 gels
DNA ladder (optional)	Fisher scientific #PRG6941	$77.00	300 lanes
DNA Marker 1 band	EDVOTEK kit #130 special component C	$15.00	40 lanes
DNA Marker 2 bands	EDVOTEK kit #130 special component D	$15.00	40 lanes
Methylene blue (25g)	Sigma #MB-1	$18.40	Unlimited
	Total	**$207.65**	**20 gels**

Using all of the above reagents the total cost / gel is only about $10.38
Note: Prices reflect 2003 catalog prices from each of the following vendors:
Carolina Biological Supplies http://www.carolina.com/
Fisher Scientific www.fishersci.com 1-800 640-0640
Sigma www.sigma-aldrich.com 1-800-325-3010
Edvotek www.edvotek.com 1-800-338.6835

Lab prep for Teacher

1) Prepare a DNA sample for each student labeled with either a 1,2,or 3

Tube	DNA	Vol. DNA to add	Loading buffer to add
1	Hyper ladder (lots of fragments)	10.2ul	2ul
2	(C) 1 fragment (3kb)	10.2ul	---
3	(D) 2 fragments (1kb and 3kb)	10.2ul	---
Note: Because of the small volumes it is helpful to do a quick spin in a microcentrifuge after making these tubes if one is available.			

2) Set up a lab station for each gel to be run. Each lab station will need:
   100 ml 1% agarose
   500 ml 1x TBE
   1- 8 toothed plastic comb
   1- gel casting tray and chamber with power source
   roll of masking tape
   1- p20 ul pipettor and pipet tips
   2 ml 0.2% methylene blue
   centrifuge for quick spin of eppendorfs (optional but helpful)
   DNA samples (1 for each member of the class)

3) In a 40 minute period - students should be able to pour the gel, load the samples and begin to run the gel. As the gel will probably take about an hour to run you may have to do the staining yourself. (You can let the gel sit in the stain overnight in the fridge or just for an hour at room temperature. Follow with de-staining as needed)
4) Analysis of data: Bands should be easily seen and a digital image can be produced by taking a picture with any digital camera.
5) Safety concerns:

a) When using the hot agarose be sure to use pot holders and remove all caps or loosen them before putting into the microwave. Pressure will build up as you heat the solution.
b) When swirling the hot liquid steam will be released so it will be necessary to keep the bottle pointed away from your body.
c) Some gel casting trays are made of a less durable plastic and require cooling of the gel before pouring. Be sure to follow manufacturers advice before pouring hot gel. It may be necessary to wait until gel is luke warm (but still liquid)
d) If students are staining you may want to have them wear protective gloves and lab coat to prevent getting any of the stain on items of clothing
e) Use caution with all electrical wires and outlets, and avoid contact with the chamber until the power source has been disconnected

Lab #3: Gel electrophoresis

Objective: Gel electrophoresis is a technique used to separate proteins and nucleic acid fragments based on the size and charge of the molecules. The mobility of a molecule through an electrical field will depend on the strength of the field, the net charge, size and shape of the molecule, and the concentration and temperature of the gel.

In this lab you will run DNA fragments of different sizes on a gel and observe their migration patterns to determine whether smaller DNA fragments travel faster or slower towards a + charge than larger fragments.

Materials / lab station:

Pot holders
100 ml 1% agarose
1- Gel casting tray
1- Electrophoresis chamber with power source (note: 2 lab groups can share 1 chamber and power source)
roll of masking tape
1- p20 ul pipetor and pipet tips
centrifuge for quick spin of eppendorfs (optional but helpful)
DNA samples (1 for each member of the class)

2-3 ml 0.2% methylene blue
1 spatula and one tupperware container
} For staining procedures if done during class

Procedure:

1) Make sure the lid on the agarose bottle is loose before putting it in the microwave oven and melt the 1% agarose solution in a microwave (1-3min.) so that it is completely liquid. Set the microwave oven for 1 min. swirl, than add 30seconds swirling each time, until the solution has completely melted **(make sure you handle the hot agarose carefully using pot holders as it will be very hot!)**
2) If your gel casting tray doesn't have removable ends you may need to put masking tape around the edges so that you can pour the gel in. Make sure there are no gaps for the hot gel to squeeze though.
3) Insert the comb and pour the hot gel into the casting tray filling it up to the top of the teeth-marks of the comb (note some casting trays will melt if the solution is too hot so you may need to wait until it cools follow instructions from the manufacturer).
4) Wait for the gel to harden (this should take about 20 min.)

5) During this time each student will practice using the ul pipetor with water and receive a DNA sample:
   a) All samples labeled #1 contain DNA ladders (pieces of DNA that have been cut up into 10 different size fragments of known lengths using specific restriction enzymes)
   b) Samples labeled #2 contain a marker that contains a fragment of DNA that is 3kb long
   c) Samples labeled #3 contain 2 fragments one that is 1kb and one that is 3kb

Question # 1: What do you think will happen to each of the samples when we run them on the gel? Hint: DNA has a slightly negative charge.

Answers will vary but could include: DNA will move towards the + electrode

Smaller fragments will migrate farther on the gel than the larger ones

6) Remove the tape when the gel is solid and put into the electrophoresis chamber (be sure that the comb is closer to the negative (black) end
7) Fill the chamber with 1X TBE buffer making sure the gel is completely submerged and carefully remove the comb
8) Each student can now load 10ul of his/her sample in each well in order from 1-3
9) Attach the power-source cables into the chamber (being extremely careful to plug black into black and red into red)
10) Set the power-source at 100volts and start

Question #2. Describe what is happening.

Answers will vary but could include: buffer starts to bubble, dye is moving towards the + electrode

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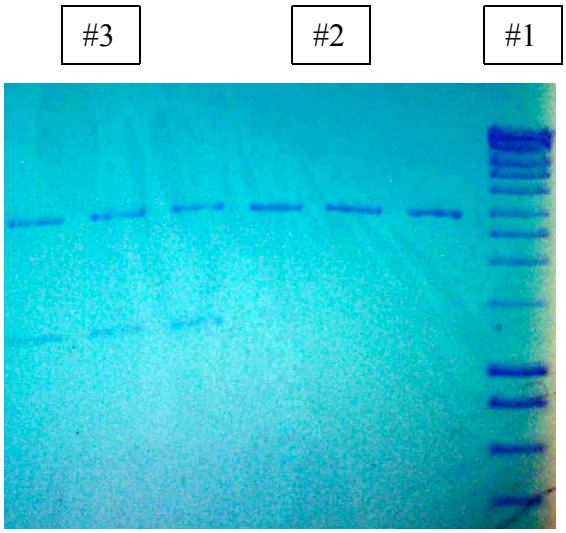
11) Run the gel until the loading dye band is about ½ inch from the bottom of the gel then turn it off and unplug the apparatus. (30 min. – 1 hour)

12) Staining the DNA ---
   a) Dilute the 0.2% methylene blue solution 1:100 in TBE buffer (you will need about 200ml so take 2ml methylene blue in about 98ml 0.1%TBE buffer)
   b) Remove the gel from the chamber with a spatula and put into a small tupperware container containing the 0.2% methylene blue solution
   c) Gel can be left to stain overnight at 4ºC or for 1 hour at room temp.

13) Destaining the gel---
   a) To better visualize the bands it might be necessary to destain by putting the gel into a fresh tupperware container full of distilled water
   b) The water can be changed several times until the bands are clear.

DAY 2

Results: Draw a picture of what your gel looks like being sure to label the lanes



This gel was obtained using 10ul or 1.5ug of Edvotek DNA / well. Using more DNA will give you bigger bands but for these purposes 1.5 ug works well. #3 represents component D and #2 represents component C from Edvotek kit #130

Conclusions: Based on your results which fragments travel the farthest through the gel and why?

The smaller the fragment the further the band traveled due to less resistance. Changing the concentration of the gel itself will change the rate of migration. A gel with less agarose will allow larger fragments to migrate faster. To test this theory students could design a lab using gels of varying concentrations while keeping all other parameters the same. They could then measure and record the distance traveled in a set amount of time.

Lab # 3: Solutions, Dilutions, and Concentrations Lab

Objective: Part of any lab includes the mixing of reagents to make solutions to work with. It is very important that the solutions you make are at the correct concentration for your experiment to be repeatable. (**Remember the way to make any experiment more valid or believable is for anyone to be able to repeat it and get the same results!**) Today we will practice making percent solutions. After completing this lab you should be able to:

- ☒ Make a percent solution
- ☒ Make dilutions of a stock solution

Part I (Calculations)

Introduction and input:

Percent solutions are based on the volume or weight of something in 100ml.

When mixing a solid with a liquid you use

1g of solid to make 100 ml of a 1% solution
10g of solid to make 100 ml of a 10% solution

When mixing a liquid with water use

1ml of the stock to make 100ml of a 1% solution
10ml of the stock to make 100ml of a 10% solution

Example #1

A solution that is 10% sucrose has 10 grams of sucrose in 100ml of solution.
Note: The sucrose will take up some space in the solution so the amount of water you add will be a little less than 100ml

Example #2

What if we wanted to make 500 ml of a 6% sucrose solution?
We will have to set up a proportion:

A 6% solution has 6 grams in 100ml or $\frac{6g}{100ml}$

Even if the volume is different than 100ml the ratio is the same so if we want to make 500ml we could set up a proportion $\frac{6\ g}{100\ ml} = \frac{x\ g}{500\ ml}$

$6 * 500 = x * 100$
$3000 = 100x$
$30 = x$

therefore you need 30 grams of sucrose to make 500 ml of a 6% solution

Problem #1

For next week's lab you will need 200 ml of a 1% agarose solution. Set up a proportion to determine how much agarose you will need.
Show all work:

Note: Always check your answers! Do the numbers you got make sense for what you are doing. (For example if you are making more than 100ml of a 1% solution then your answer has to be greater than 1 g)

Example #3

A 70% ethanol solution would be made of 70ml of ethanol plus enough water to make 100ml

100ml – 70ml = 30ml of water

Therefore to make 100ml of 70% ethanol add
70 ml of pure ethanol and 30 ml of water.

A simple formula to use when mixing liquids to make a % solution is :

C = concentration
V = volume

What you start with = What you want to make

$C_1V_1 = C_2V_2$

Example #4

To make a 100ml solution of 10% ethanol from a stock that is 100% ethanol how much water would you add.

$$C_1V_1 = C_2V_2$$

$$100\%\ (x) = 10\%\ (100ml)$$

$$x = \frac{10(100)}{100}$$

$$x = 10ml$$

Therefore you would need to add 10ml of 100% ethanol to make the solution
The rest of the solution will be water

100ml – 10ml = 90ml of water

Problem #2

For next week's lab you need to make a 1% TBE solution from a stock that is 10% TBE. We will need to make 1L of solution (remember that 1L = 1000ml). How much of the 10% stock solution will we need to add and how much water will we need?

Note: Always check your answers! Do the numbers you got make sense for what you are doing. (For example if you are only making 100ml of a liquid then your answer has to be less than 100ml)

Part II (Making solutions)

Introduction and Input: When scientists do experiments it is very important to write down everything that they do? Why?

_____When you do anything in this lab it will be important to write down everything you do. In this part of the lab we will make the solutions we talked about in part I of this lab. There are a few things we need to remember about weighing measuring and mixing chemicals.

- ☒ Always use a graduated cylinder when measuring volumes of liquids. (Note: beakers often have numbers on them but they are not accurate enough to use when measuring)
- ☒ Always measure from the bottom of the meniscus

Copied from *http://www.middleschoolscience.com/meniscus.jpg*

- ☒ When weighing solids use a triple beam balance or electronic scale
- ☒ Heat often helps solids go into solution
- ☒ Use extreme caution when heating liquids

6.6 ml

1) Use pot holders or beaker tongs to handle hot glassware
2) Never put a stopper on a test tube while it is heating (pressure may build up → explosion)
3) Always tip a test tube or beaker away from your face to avoid breathing in fumes.
4) Wear protective goggles and clothing when handling hot liquids in case of

http://www.usoe.k12.ut.us/curr/science/phillips/safety.gif

- ☒ Always write down the exact measurements that you used and label all solutions

Materials at your lab station
Distilled water (1L)
1L and 500ml beakers
1L and 500ml graduated cylinders
Balance or scale
1 sheet weigh paper
scoop, spoon, or spatula to measure agarose
10X TBE (Need at least150ml)
Agarose (Need at least 2g)
1L Flask or bottle with a stopper
500ml Flask or bottle with a stopper
Magnetic stir bar and plate with heater
Pot holder for handling hot liquids
Sharpie and tape for labeling

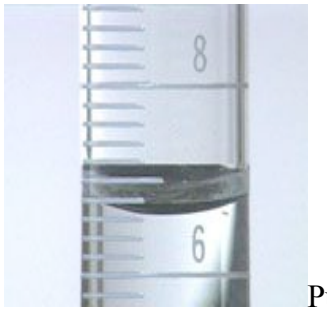
Solution #1 Make 1L of a 1X TBE solution

1) First add a stir bar to your beaker

Substance to add	**Amount needed**	**Amount added**
10X TBE	100ml	
Distilled Water	900ml	

2) Add both substances to the beaker
3) Mix with the stir bar
4) Then pour the solution into a flask labeled 1X TBE with the date and your group name (store at room temperature for later use)

Solution #2 Make 200ml of a 1% agarose solution in TBE
Note: It is important that you use 1X TBE to make this solution not water!!

1) 

Put a stir bar into a 500 ml beaker and add the following:

Substance to add	**Amount needed**	**Amount added**
1X TBE	150 ml	
Agarose	2g	

2) Mix the above ingredients on the stir plate with low heat until all of the agarose has dissolved.

3) Pour the liquid into the 500ml graduated cylinder (CAUTION: Liquid will be hot use pot holders and pour carefully!!!)
4) Add enough 1X TBE to bring the volume up to 200ml
5) Pour back into the beaker and stir with stir bar again until thoroughly mixed
6) When mixed carefully pour into the 500ml flask and label 1% agarose with the date and your group name (store at room temp for later use)(note agarose should become solid as it cools)

Part III (Dilution Laboratory)

Note: This additional lab was designed for AP students and might be difficult for regular ed.

Introduction and Input: The purpose of this lab is for you to become familiar with how to make and calculate a series of dilutions.
Making a 1:10 dilution means that 1 part from your solution will be diluted with 9 parts water.

Example: If you were to make a 200ml of a 1:50 dilution of solution A you would use a proportion to determine how much of A to add

1/50 = x/100
50x = 100
x = 2ml of solution A

If you need 2 ml of solution A you will need 98ml of water to make 100ml of solution

Practice:

Materials at your lab station
Tap water
Food coloring (red and blue)
P200 pipetor and pipet tips
100ml graduated cylinder
4 -100ml beakers
Sharpie for labeling

Procedure:
1) Add 200ul of red food coloring to 100ml of water and place in a beaker labeled **solution A**

2) Add 200ul of blue food coloring to 100ml of water and place in a beaker labeled **solution 1**

3) Make 200ml of a 1:10 dilution from solution A and label this **solution B**
Solution A add _____
Water add _____

Show your work:

What is the final dilution factor for this solution? Ans. _____

4) Make 100ml of a 1:50 dilution from solution 1 and label this **solution 2**

Solution 1 add _____
Water add _____

Show your work:

What is the final dilution factor for this solution? Ans. _____

5) Make 100ml of a 1:20 dilution of solution B and label this **solution C**

Solution B add _____
Water add _____

Show your work:

What is the final dilution factor for this solution? _____

6) Make 100ml of a 1:5 dilution from solution 2 and label this solution 3

Solution 2 add _____
Water add _____

Show your work:

What is the final dilution factor for this solution?_____

Compare your solutions with the stock solutions in the front of the room.

Lab #3: Gel electrophoresis

Objective: Gel electrophoresis is a technique used to separate proteins and nucleic acid fragments based on the size and charge of the molecules. The mobility of a molecule through an electrical field will depend on the strength of the field, the net charge, size and shape of the molecule, and the concentration and temperature of the gel.

In this lab you will run DNA fragments of different sizes on a gel and observe their migration patterns to determine whether smaller DNA fragments travel faster or slower towards a + charge than larger fragments.

Materials / lab station:

Pot holders
Microwave oven
100 ml 1% agarose
1- Gel casting tray
1- Electrophoresis chamber with power source (note: 2 lab groups can share 1 chamber and power source)
roll of masking tape
1- p20 ul pipetor and pipet tips
centrifuge for quick spin of eppendorfs (optional but helpful)
DNA samples (1 for each member of the class)

2-3 ml 0.2% methylene blue
1 spatula and one tupperware container
} For staining procedures if done during class

Procedure:

1) Make sure the lid on the agarose bottle is loose before putting it in the microwave oven and melt the 1% agarose solution in a microwave (1-3min.) so that it is completely liquid. Set the microwave oven for 1 min. swirl, than add 30 seconds swirling each time, until the solution has completely melted **(make sure you handle the hot agarose carefully using pot holders as it will be very hot!)**
2) If your gel casting tray doesn't have removable ends you may need to put masking tape around the edges so that you can pour the gel in. Make sure there are no gaps for the hot gel to squeeze though.
3) Insert the comb and pour the hot gel into the casting tray filling it up to the top of the teeth-marks of the comb (note some casting trays will melt if the solution is too hot so you may need to wait until it cools follow instructions from the manufacturer).
4) Wait for the gel to harden (this should take about 20 min.)

5) During this time each student will practice using the ul pipetor with water and receive a DNA sample:
   a) All samples labeled #1 contain DNA ladders (pieces of DNA that have been cut up into 10 different size fragments of known lengths using specific restriction enzymes)
   b) Samples labeled #2 contain a marker that contains a fragment of DNA that is 3kb long
   c) Samples labeled #3 contain 2 fragments one that is 1kb and one that is 3kb

Question # 1: What do you think will happen to each of the samples when we run them on the gel?

_____

_____

6) Remove the tape when the gel is solid and put into the electrophoresis chamber (be sure that the comb is closer to the negative (black) end
7) Fill the chamber with 1X TBE buffer making sure the gel is completely submerged and carefully remove the comb.
8) Each student can now load 10ul of his/her sample in each well in order from 1-3
9) Attach the power-source cables into the chamber (being extremely careful to plug black into black and red into red)
10) Set the power-source at 100volts and start

Question #2. Describe what is happening.

_____

_____

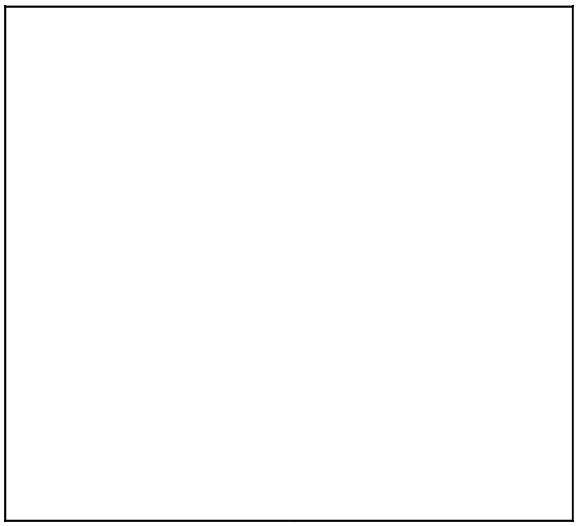
11) Run the gel until the loading dye band is about ½ inch from the bottom of the gel then turn it off and unplug the apparatus. (30 min. – 1 hour)

12) Staining the DNA ---
   d) Dilute the 0.2% methylene blue solution 1:100 in TBE buffer (you will need about 200ml so take 2ml methylene blue in about 98ml 0.1%TBE buffer)
   e) Remove the gel from the chamber with a spatula and put into a small tupperware container containing the 0.2% methylene blue solution
   f) Gel can be left to stain overnight at 4ºC or for 1 hour at room temp.

13) Destaining the gel---
   d) To better visualize the bands it might be necessary to destain by putting the gel into a fresh tupperware container full of distilled water
   e) The water can be changed several times until the bands are clear.

DAY 2

Results: Draw a picture of what your gel looks like being sure to label the lanes



Conclusions: Based on your results which fragments travel the farthest through the gel and why?

Post Lab Quiz

1) What should you use when measuring liquids? _____

2) If you have a solid that won't go into solution what can you do to help it?
_____

3) If we have a 10X stock solution and we want to make 200 ml of a 1X solution we will need to add _____ ml of the stock solution and we will have to add _____ml of water. (Show all work for full credit.)

4) List 3 safety procedures that should be used when heating a liquid in a test tube.
_____

_____

_____

5) Draw a picture of a gel containing a DNA sample containing fragments that have been run under a current for 30min. The fragments are 1kb, 3kb and 10kb long. Label each of the fragments in the order that they would appear from top to bottom.