LAB 8- pGLO TRANSFORMATION LAB NAME \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

In this lab you will perform a procedure known as genetic TRANSFORMATION. Genetic transformation occurs when a cell takes up and expresses a new piece of genetic material (DNA). The new genetic information often provides the organism with a new trait which is identifiable in organisms that have been transformed.  
  
**USES:**  
Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or drought resistance can be genetically transformed into plants. In bioremediation, bacteria can be genetically transformed with genes enabling them to digest oil spills. In medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by genetically transforming a sick person’s cells with healthy copies of the defective gene that causes their disease. Genes from human, animal, or plant DNA can be placed inside bacteria. For example a healthy human gene for the hormone insulin can be put into bacteria. Under the right conditions, these bacteria can make human insulin which can then be used to treat patients with diabetes.

**pGLO PLASMID:**One of the challenges in in studying molecular biology is that many of the events and processes are invisible. Scientists have “borrowed” the green fluorescent protein (GFP) gene naturally found in the jellyfish, *Aequorea victoria,* and causes the jelly fish to fluoresce and glow in the dark. Scientists at BIO-RAD have created a genetically engineered plasmid (pGLO) for use in biotechnology research. The pGLO plasmid contains an origin of replication, the modified GFP gene, a gene that confers resistance to the antibiotic ampicillin (amp) , and a special gene regulation system (*ara* operon) that can be used to control expression of the GFP protein in transformed cells. The gene for GFP can be switched on in transformed cells simply by adding the sugar arabinose to the cell’s nutrient medium.   
  
**TRANSFORMATION:**In this lab students will use a simple procedure to transform *Escherichia coli (E. coli*) bacteria with a gene that codes for Green Fluorescent Protein (GFP). The **addition of transforming solution (CaCl2)** and **HEAT SHOCK** are used to make the bacteria **COMPENTENT** and allow them to pick up the plasmid DNA. It is postulated that Ca+2 cations neutralize the repulsive negative charges on the phosphate backbone of the DNA and the phospholipids of the cell membranes allowing DNA to enter the cells. Heat shock increases the permeability of the cell membrane to DNA While the mechanism is not known, the duration of the heat shock is critical to the success of this process. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on antibiotic plates. Transformed cells will appear white (wild type phenotype) on plates without arabinose, and fluorescent green when arabinose is included in the nutrient agar.

IDENTIFY the following shorthand abbreviations used in this lab:

LB = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
Amp = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
Ara = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

pGLO = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

THINK ABOUT THESE QUESTIONS BEFORE COLLECTING THE DATA AND ANALYZING THE RESULTS.

1. On which plates would you expect to find bacteria most like the original non-transformed *E.coli* colonies you initially observed? WHY?

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2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? WHY?

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3. Which plates should be compared to determine if any genetic transformation has occurred? WHY?

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4. How does the addition of CaCl2 and “heat shocking” help facilitate the introduction of plasmids into the *E.coli*?

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5. What does “COMPETENT” mean? Why don’t all the bacteria take up the plasmid?

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RESULTS

-pGLO, LB -pGLO, LB/Amp + pGLO, LB/Amp + pGLO, LB/Amp/Ara

EXPLAIN what happened in each of the different dishes.   
Which bacteria can/can’t grow on each and WHY?

-pGLO LB: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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-pGLO LB/amp \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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+pGLO LB/amp\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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+ pGLO LB/amp/ara\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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QUESTIONS

1. What is a plasmid?

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2. How are plasmids used in genetic engineering? Give some examples.

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3. The pGLO plasmid contains the following genes. Explain their purpose.

Origin of replication\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

pGLO:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

ampR: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
 *ara* operon\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4 . If you were to put the pGLO plasmid DNA under a black light, would the DNA glow?  
 EXPLAIN your answer.  
  
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5. Why does the +pGLO / LB/amp plate not “glow” if the bacteria picked up the pGLO plasmid?

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6. Why is the pGLO gene attached to the *ara* operon?

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7. Explain how the arabinose operon works to induce the *E. coli* bacteria to express the “glow” gene?

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8. The *ara* operon is similar to which operon you learned about? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

9. What advantage would there be for an organism to be able to turn on or off particular genes in response to certain conditions?

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LAB 9- RESTRICTION ENZYME ANALYSIS OF DNA NAME \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

WHILE YOUR GEL IS RUNNING COMPLETE THE FOLLOWING:  
  
ACTIVITY I (pS113)  
  
 5’- AAAGTCGGAATTCACTGCATCGAATTCCCGGGGCTATATATGGAATTCGA-3’

1. What is the sequence of the complementary DNA strand? Draw it directly below the strand.

2. Assume you cut this fragment with the restriction enzyme *EcoR1*. The restriction site for EcoR1 is   
 5’-GAATTC-3’ , and the enzyme makes a staggered (“sticky end”) cut between G and A on both strands of the DNA molecule. **Show where this enzyme would cut the DNA strand above cut by EcoR1.**   
**How many fragments would be produced? \_\_\_\_\_\_\_\_\_\_.**

**READ** ACTIVITY II (pS114-115)  
1. Based on this information, can you make a prediction about the products of DNA from different sources cut with the same restriction enzyme?  
  
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2. Make a prediction about the RFLP patterns of identical twins cut with the same restriction enzymes? How about the RFLP patterns of fraternal (non-identical) twins or triplets?

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ACTIVITY III (pS115)  
  
WHY do DNA fragments migrate through the gel from the negatively charged pole to the positively charged pole?

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ADDITIONAL QUESTIONS  
  
MAKE A PREDICTION about how changing the following would affect the results of electrophoresis:  
  
 INCREASING/DECREASING VOLTAGE: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
 \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

LONGER/SHORTER RUNNING TIME:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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AMOUNT OF DNA USED: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
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REVERSAL OF POLARITY:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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Where were restriction enzymes originally found? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
  
Scientists have “borrowed” these enzymes for DNA technology, but what is there function in nature?  
  
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What is the function of the blue loading dye when running the DNA in the gel? THINK ABOUT WHAT WOULD HAPPEN IF you just added the DNA samples to the well without the loading dye and started the   
gel running?

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DNA is invisible to the naked eye. What did you do to allow the DNA bands in the gel to become visible after the current was turned off?

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What are some other methods that can be used to visualize DNA on gels?   
  
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Running a DNA “ladder” on each gel provides a size comparison for bands on the gel. EXPLAIN what other function ladders serve.

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AFTER DESTAINING  
Use a transparency sheet to mark the bands on your gel. Cut and paste this BELOW.

ANALYZING RESULTS  
Use the ideal gel show at the right to measure the distance (in cm) that each fragment migrated from the origin (the well).   
  
(HINT: For consistency, measure from the front end of each well to the front edge of each band, i.e., the edge farthest from the well.  
  
 Enter the measured distances into TABLE 1 below. (See \* and \*\* notes below the table for an explanation for why there are only six bands seen but more fragments.

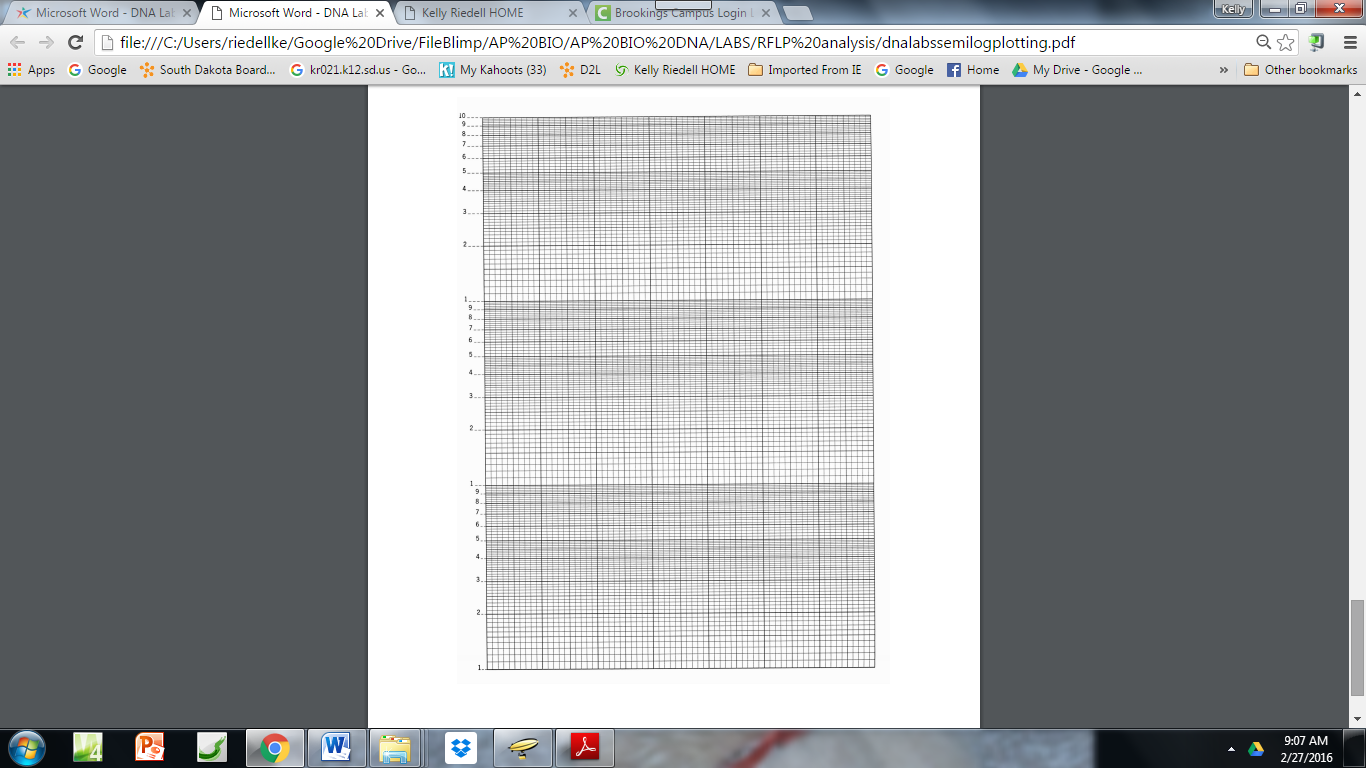
TABLE !. DNA FRAGMENT MIGRATION DISTANCE

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *HINDIII* | | *BAMHI* | | *EcoR1* | |
| Distance  Traveled | BP Length | Distance Traveled | BP length | Distance Traveled | BP Length |
|  | \*27,491 |  |  |  |  |
|  | \*23,130 |  |  |  |  |
|  | 9,416 |  |  |  |  |
|  | 6,557 |  |  |  |  |
|  | 4,361 |  |  |  |  |
|  | 2,322 |  |  |  |  |
|  | 2,027 |  |  |  |  |
|  | \*\* 564 |  |  |  |  |
|  | \*\* 125 |  |  |  |  |

\* For this “ideal” gel, assume that these two bands appear as a single band instead of resolving into separate bands

\*\* These bands do not appear on the ideal gel and likely will not be seen.

Plot the standard curve using data from DNA samples cut with *HINDIII* below. Connect the data points with a line of best fit. Use the standard curve graph to calculate the approximate sizes of *EcoR1* and *BamHI* fragments and fill in the data table on the previous page.



[Type a quote from the document or the summary of an interesting point. You can position the text box anywhere in the document. Use the Drawing Tools tab to change the formatting of the pull quote text box.]

[Type a quote from the document or the summary of an interesting point. You can position the text box anywhere in the document. Use the Drawing Tools tab to change the formatting of the pull quote text box.]

MAKE A PREDICTION  
Use the standard curve graph to predict how far (in cm) a 8,000 bp fragment would move?

A certain restriction enzyme digest results in DNA fragments of the following sizes:   
4,000 bp, 2,500 bp, 2,000 bp, 400 bp  
Draw a diagram that shows the results of their separation by gel electrophoresis. Show the starting point, positive and negative electrodes, and relative positions of resulting bands.

How might a mutation that alters a restriction enzyme recognition site change the pattern that can be seen on an electrophoresis gel?

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CELL RESPIRATION LAB NAME \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

In this experiment you will work with seeds that are living but DORMANT. A seed contains an embryo plant and a food supply surrounded by a seed coat. When the necessary conditions are met, germination occurs, and the rate of cellular respiration greatly increases. In this lab you will measure oxygen consumption during germination by measuring the change in gas volume in respirometers containing either germinating or non-germinating pea seeds at two different temperatures.

CELLULAR RESPIRATION is the release of energy from organic compounds by metabolic chemical oxidation in the mitochondria within each cell. Cellular respiration involves a series of enzyme-mediated reactions. The equation below shows the complete oxidation of glucose. Oxygen is required for this energy-releasing process to occur.  
  
 C6H12O6 + 6 O2 → 6CO2 + 6 H2O + 686 kilocalories of energy/mole of glucose oxidized

The equation above shows there are three ways cellular respiration could be measured:  
 1. Consumption of O2  
 2. Production of CO2  
 3. Release of energy

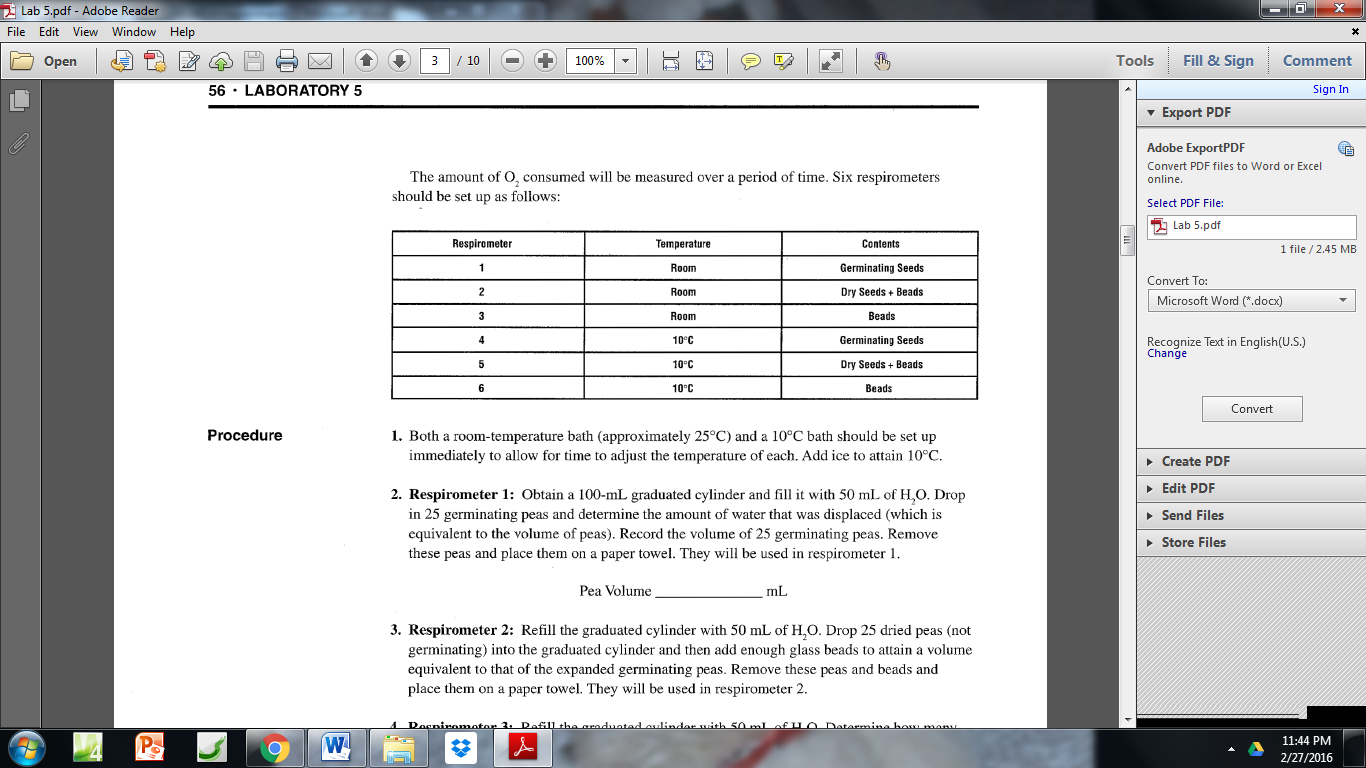
In this experiment, the relative volume of O2 consumed by germinating and non-germinating (dry) peas at two different temperatures will be measured.

RESPIROMETERS  
During cellular respiration, O2 is consumed and CO2 is released. In this experiment, CO2 produced will be removed by potassium hydroxide (KOH) and will form solid potassium carbonate (K2CO3).

CO2 + 2 KOH → K2CO3 + H2O

During respiration, the gas volume in the respirometer will be reduced because oxygen is being consumed and the CO2 produced is being converted to a solid. The net result is a decrease in gas volume and a related decrease in pressure in the tube. Since the CO2 is being removed, the change in the volume of gas in the respirometer is directly related to the amount of oxygen consumed. If water temperature is kept constant and the volume of gas inside the respirometer decreases, the water will move toward the region of lower pressure moving into the tube. The vial with glass beads alone will permit detection of any volume changes due to atmospheric pressure changes or temperature changes.

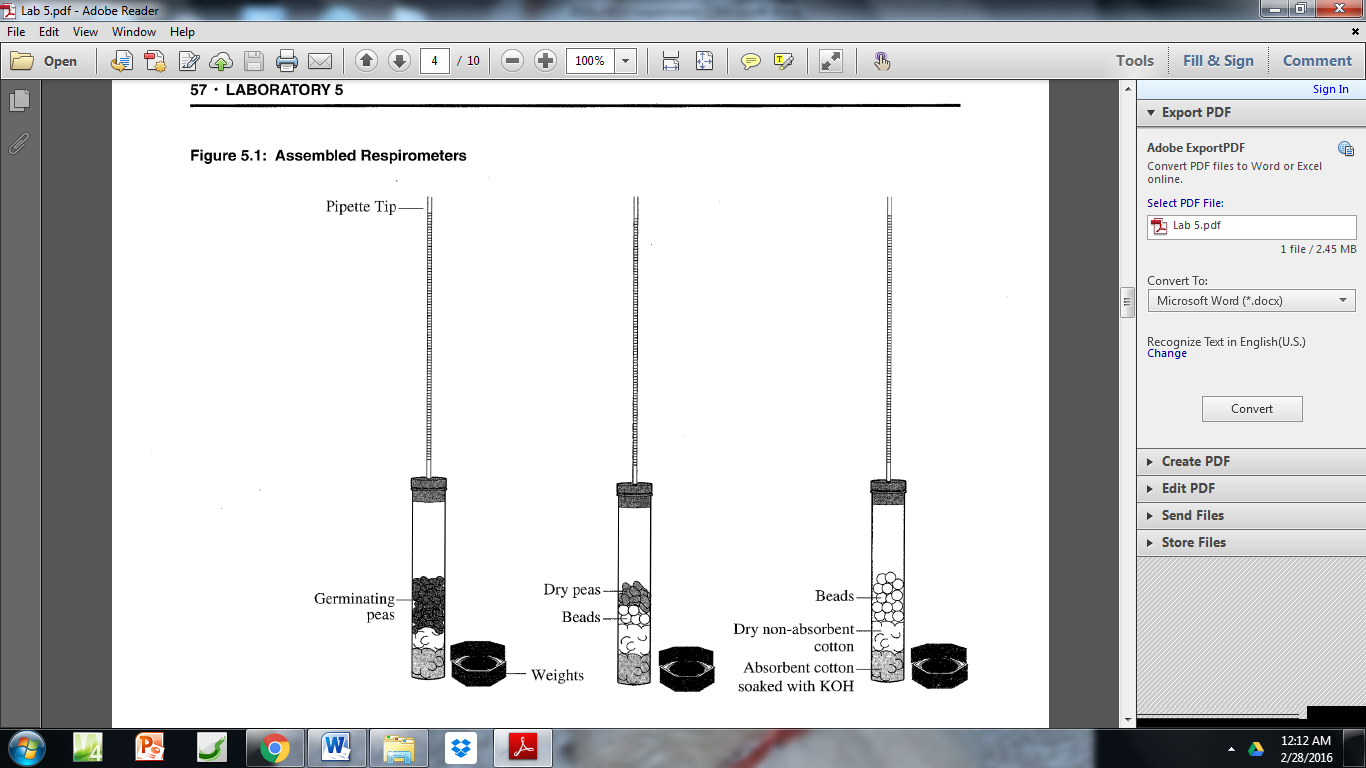
EXPERIMENTAL DESIGN  
The amount of O2 consumed will be measured over a period of time. SIX respirometers should be set up as follows:

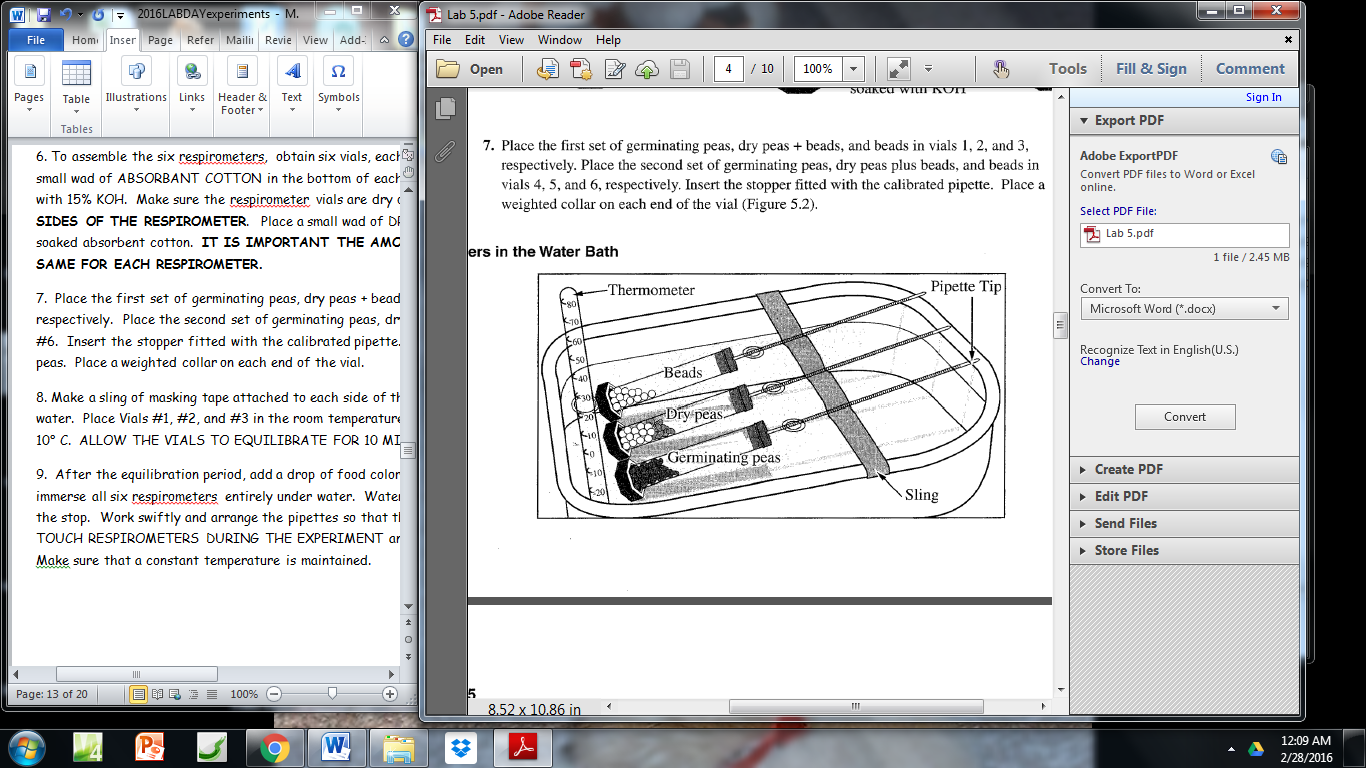


1. RESPIROMETER #1: Fill a 100-mL graduated cylinder with 50 mL of H2O. Drop in 20 germinating peas and determine the amount of water that was displaced. This is equivalent to the volume of peas. Record the volume. Remove the peas and place them on a paper towel.

PEA VOLUME = \_\_\_\_\_\_\_\_\_\_\_ mL

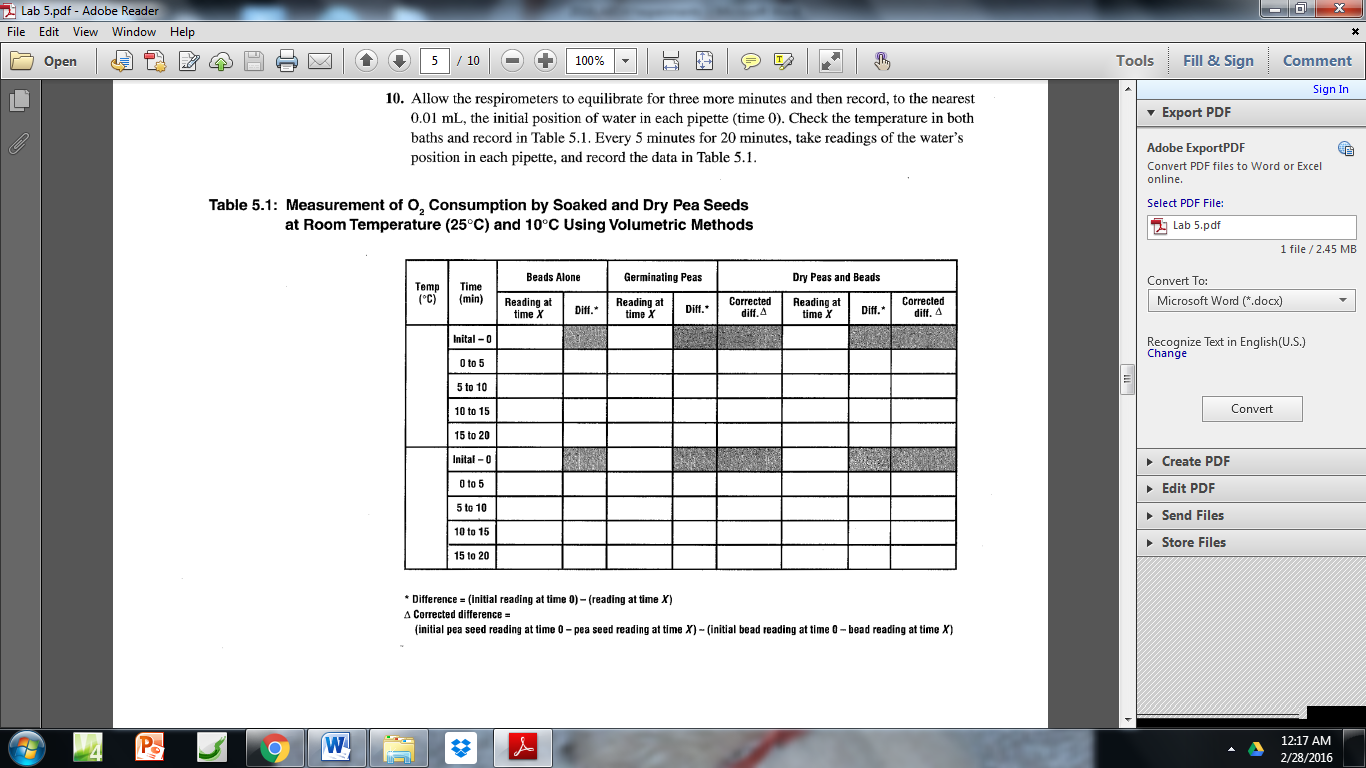
2. RESPIROMETER #2: Refill the graduated cylinder with 50 mL of H2O. Drop in 20 **DRY** peas. Add enough glass beads to attain a volume equivalent to the germinating peas. Remove these peas and beads and place them on a paper towel.

3. RESPIROMETER #3: Refill the graduated cylinder with 50 mL of H2O. Add glass beads to attain the volume equivalent to the germinating peas. Remove the beads and place them on a paper towel.   
  
4. REPEAT THE PROCEDURES ABOVE TO PREPARE A SECOND SET OF GERMINATING PEAS, DRY PEAS PLUS BEADS, AND BEADS FOR USE IN RESPIROMETERS #4, #5, AND #6.  
  
5. To assemble the six respirometers, obtain six vials, each with an attached stopper and pipette. Place a small wad of ABSORBANT COTTON in the bottom of each via. Using a dropper, saturated the cotton with 15% KOH. Make sure the respirometer vials are dry on the inside. **DO NOT GET KOH ON THE SIDES OF THE RESPIROMETER**. Place a small wad of DRY RAYON cotton balls on top of the KOH-soaked absorbent cotton. **IT IS IMPORTANT THE AMOUNTS OF COTTON AND KOH BE THE SAME FOR EACH RESPIROMETER.**

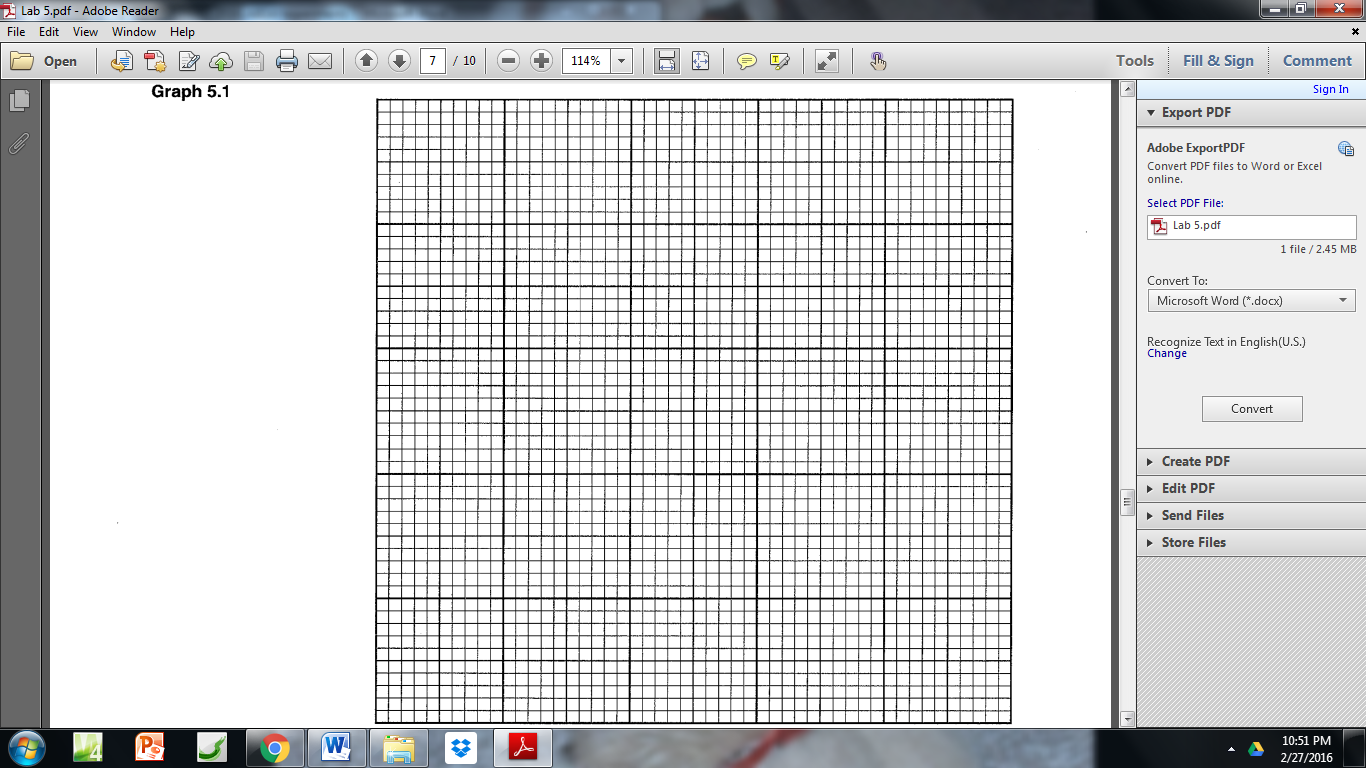
6. Place the first set of germinating peas, dry peas + beads, and beads in vials #1, #2, and #3, respectively. Place the second set of germinating peas, dry peas + beads, and beads in vials #4, #5, and #6. Insert the stopper fitted with the calibrated pipette, making sure the end of the pipette doesn’t poke into the peas. Place a weighted collar on each end of the vial.

7. Make a sling of masking tape attached to each side of the water baths to hold the pipettes out of the water. Place Vials #1, #2, and #3 in a room temperature water bath. Place vials #4, #5, and #6 in the 10° C water bath. ALLOW THE VIALS TO EQUILIBRATE FOR 10 MINUTES

9. After the equilibration period, add a drop of food coloring to the tip of each respirometer and immerse all six respirometers entirely under water. Water will enter the pipettes for a short distance the stop. Work swiftly and arrange the pipettes so that they can be read through the water. DON’T TOUCH RESPIROMETERS DURING THE EXPERIMENT and KEEP the TANKS AS STILL AS POSSIBLE> Make sure that a constant temperature is maintained.

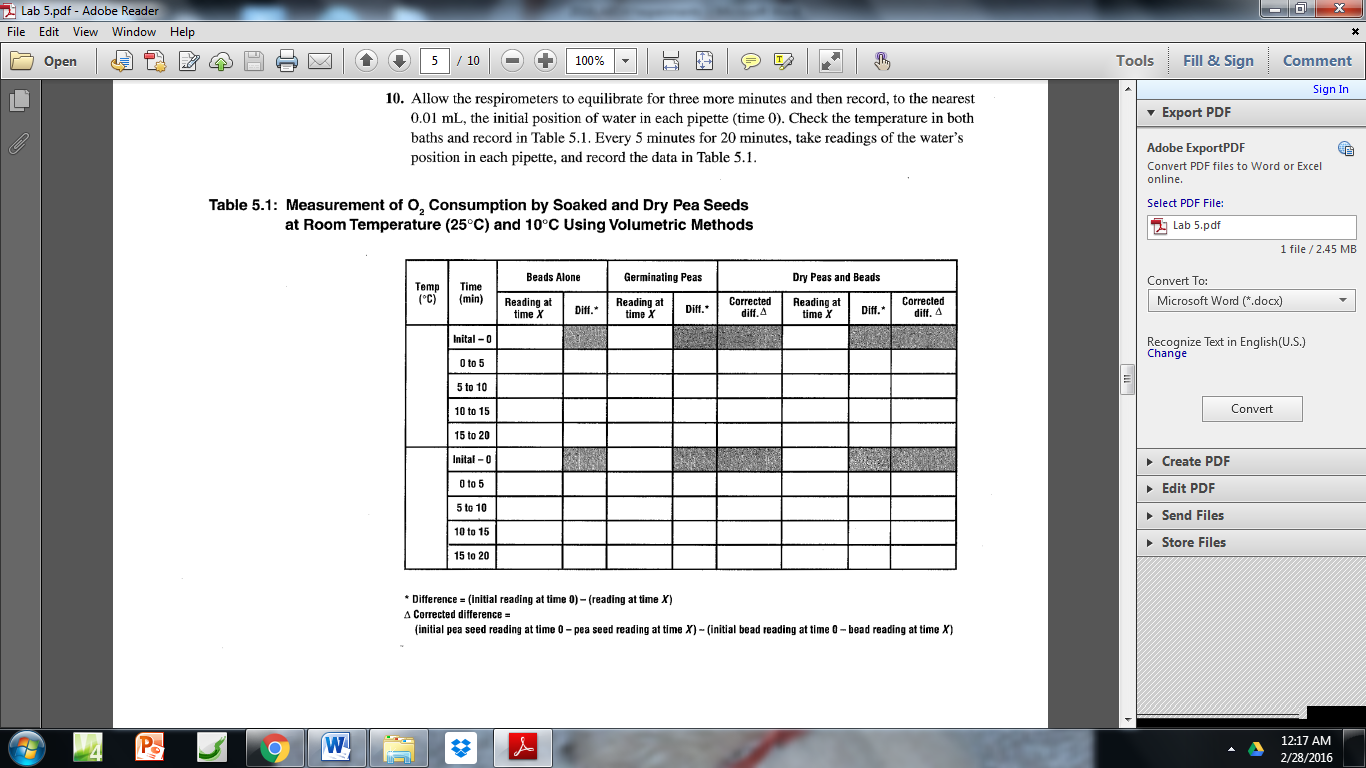
10. Allow the respirometers to equilibrate for 3 minutes, then record to the nearest 0.01 mL the initial position of the water in each pipette at TIME ZERO. Check the temperature in both baths and record in the table below. Every 5 minutes for 20 minutes, take readings of the water’s position in each pipette and record in the date table below.  
  
 O2 CONSUMPTION BY GERMINATING AND NON-GERMINATING DRY PEAS at 10° C and 25° C.

GRAPH the results from the CORRECTED DIFFERENCES column for the germinating peas and dry peas at both room temperature and at 10° C.

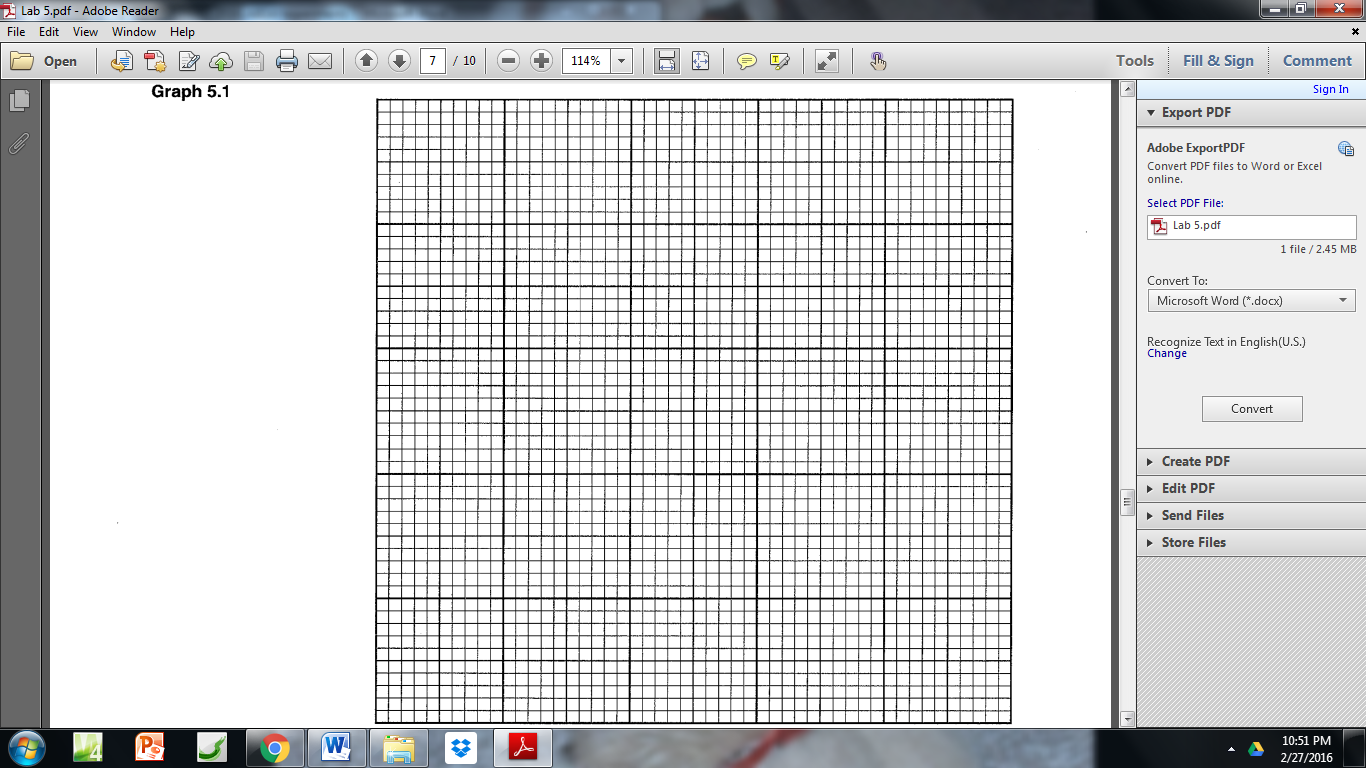


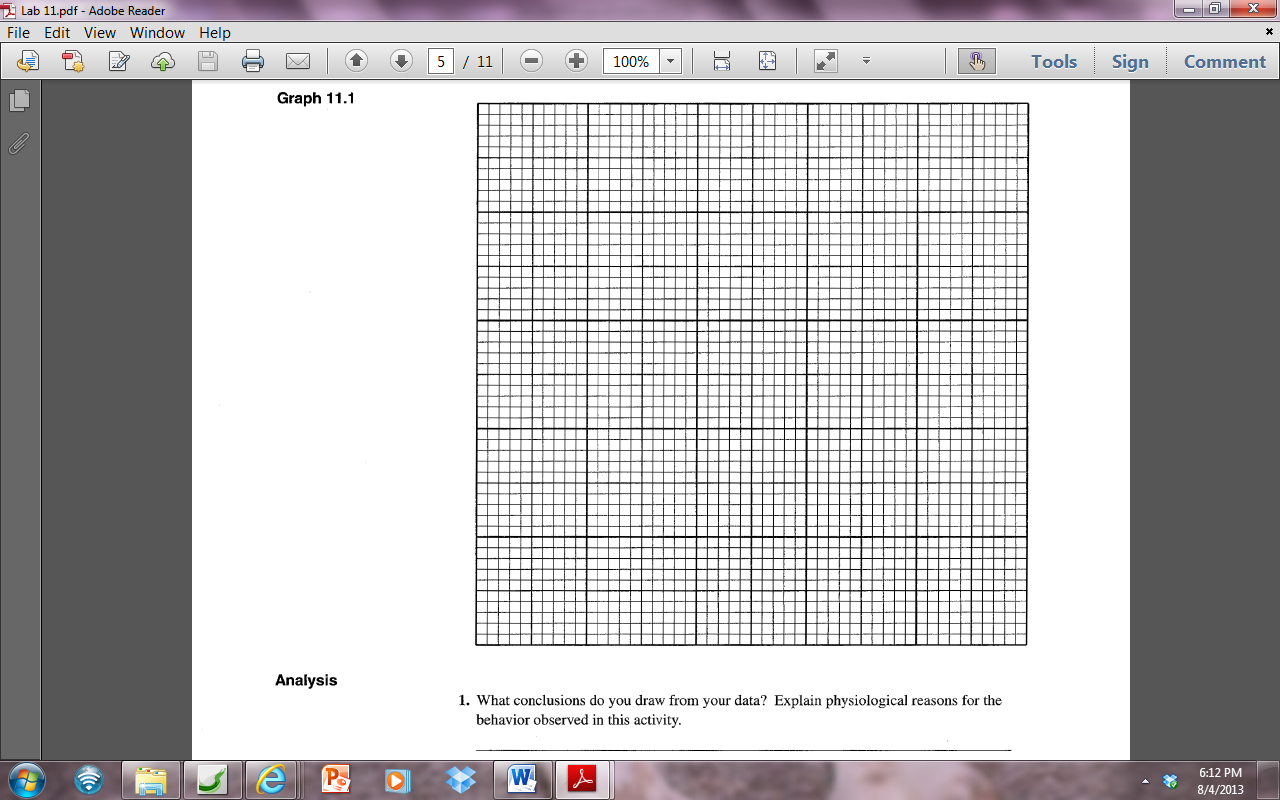
DESIGN YOUR OWN EXPERIMENT

|  |  |  |  |
| --- | --- | --- | --- |
| Title of Experiment: | | | |
| Hypothesis (place in the “If…, then…” format) | | | |
| Independent Variable | | | |
| Levels of Independent Variable |  |  |  |
| Dependent Variable | | | |
| Controlled Factors (List at least 5) | | | |
| Explanation of why this is a controlled experiment | | | |
| Show how you will record initial data | | | |
| How will you display data for your report | | | |

MODIFY THE DATA TABLE TO FIT YOUR EXPERIMENTAL DESIGN

GRAPH YOUR RESULTS



QUESTIONS

1. In this activity, you are investigating both the effect of germination versus non-germinating AND warm temperature versus cold temperature on respiration rate. WRITE an H0 and HA for both of these variables being tested in this experiment

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2.This activity uses a number of controls. Identify at least three of the controls and describe the purpose of each control?

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3. DESCRIBE and EXPLAIN the relationship between the amount of O2 used and time.

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4. What is the purpose of adding KOH to the respirometers in this experiment?

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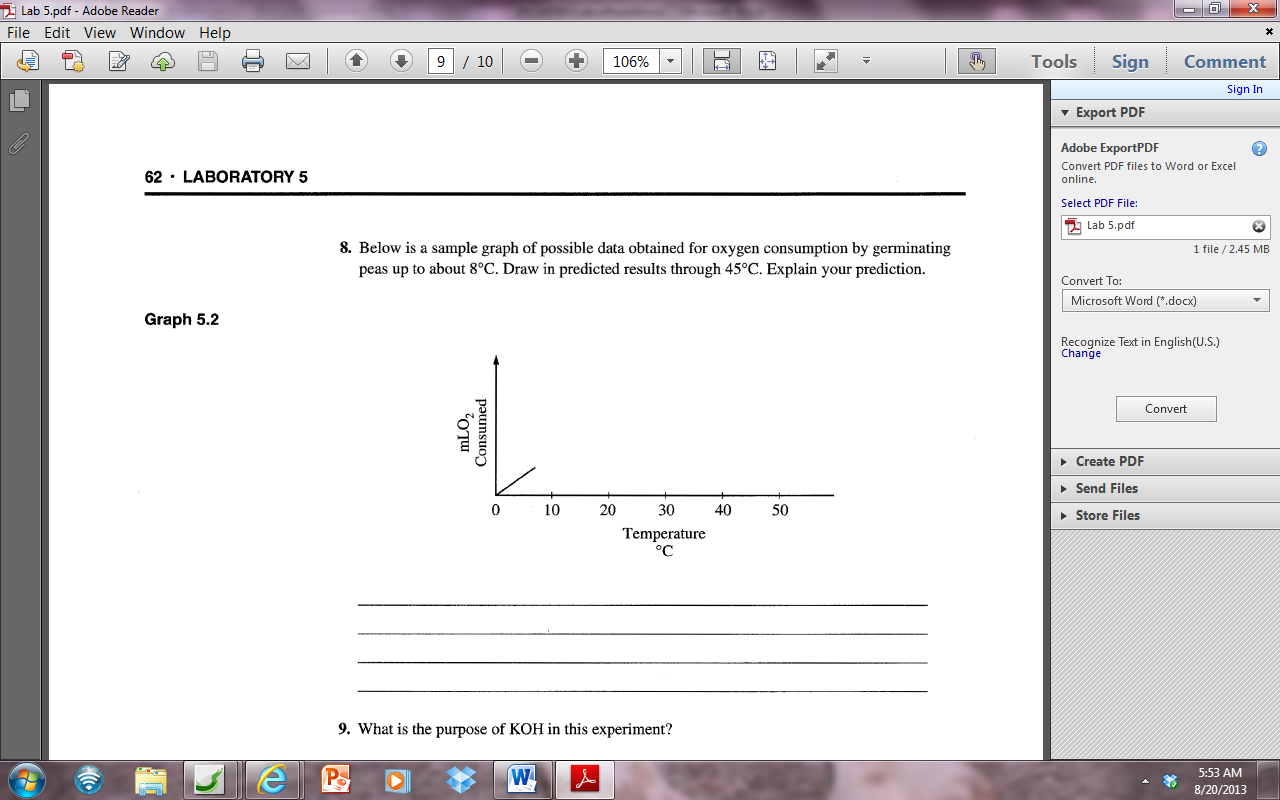
5. Why is it necessary to correct the readings using the changes in the “beads only” vial?

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6. The graph below is a sample graph of possible data obtained for oxygen consumption by germinating peas up to about 8◦ C. Draw in predicted results through 45° C. (=113° F) EXPLAIN your prediction.



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7. If you used the same experimental design to compare the rates of respiration of a 25 g reptile and a 25 g mammal at 10◦C , what results would you expect. EXPLAIN your reasoning.

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8. If respiration in a small mammal were studied at both room temp (21◦C) and 10◦C, what results would you predict? EXPLAIN your reasoning.

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9. EXPLAIN why the colored water moved into the respirometer pipettes.

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