Name: _____ Period: _____

Cellular Respiration Lab

GOAL: measure and compare respiration rates of dormant and germinating seeds in cold and warm environments.

Cellular Respiration:

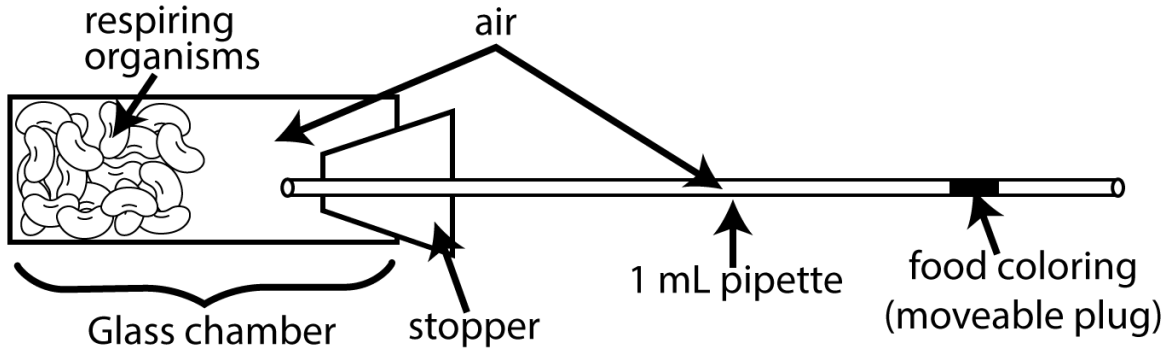
$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ENERGY$$

How could we measure this?

- _____
- _____

In this lab, we look at _____. We do this *volumetrically*, using a respirometer

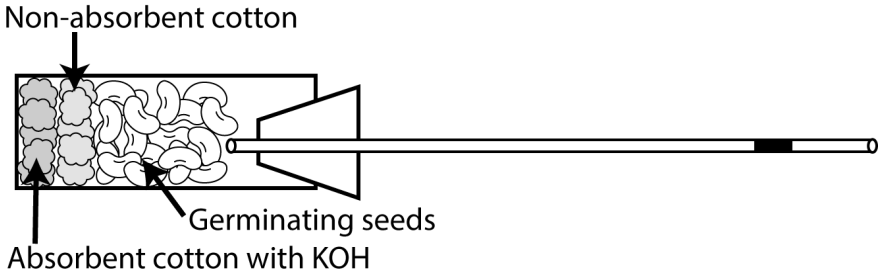
Respirometer design # 1:



Chamber with air, which contains respiring organisms (such as seeds). The tip of the tube is open, so water can enter.

Thought experiment: Assume that the seeds in the respirometer above are respiring. What would happen to the a) O_2 content of air? _____ b) The CO_2 content of air? _____ c) What would happen to seeds? _____ d) What would happen to the volume of air inside chamber and tube? _____

Respirometer design # 2: To see how much O_2 is removed by respiration you have to remove the CO_2 that is produced by cellular respiration: We do this by modifying our set-up as follows:



We soak the absorbent cotton with KOH (potassium hydroxide). KOH reacts with CO_2 as follows:

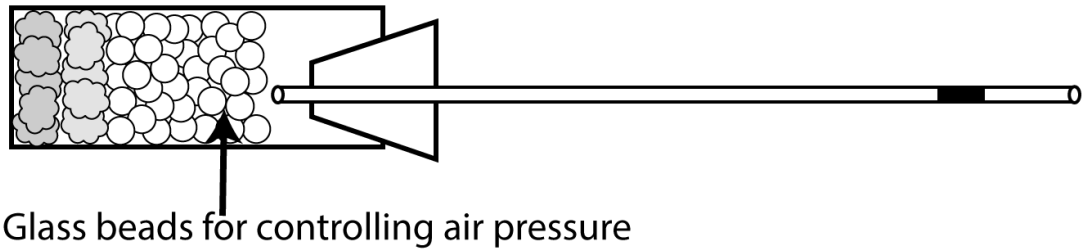
$$2KOH + CO_2 \rightarrow K_2CO_3 + H_2O$$

K_2CO_3 (potassium carbonate) is a solid precipitate (not a gas) so it has very little volume. Liquid water also has very little volume). What we've done is soak up all the CO_2 produced by respiration. What happens now: As respiration proceeds_____

_____

STILL ONE PROBLEM: Remember PV=nRT (where P = pressure, V = volume, n = # of molecules of gas, R is the gas constant, and T is temperature)? Gas volume is affected by temperature, and temperature is one variable in this experiment. For example, if we put our respirometer in cold water, what would happen to the volume of gas in the chamber? What would happen to our food coloring plug?

WHAT CAN WE DO?
Solution: *Another* respirometer w/ glass or plastic beads that we use to monitor pressure changes.

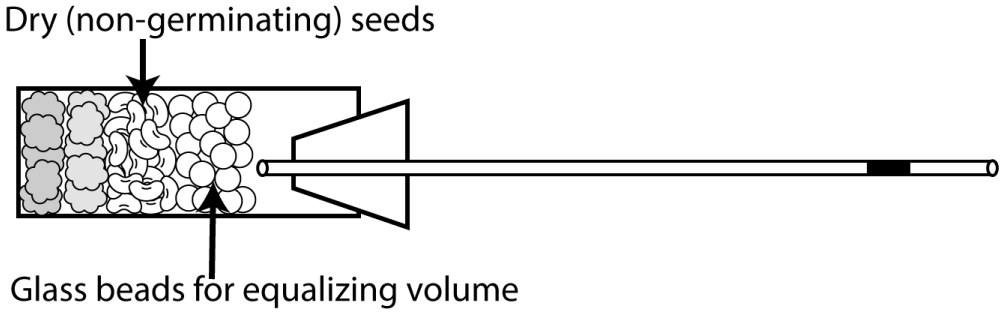


Difference = (initial reading at time 0) – (reading at time X)
Corrected difference = difference from respiration (column b) – difference from beads (column d)

SAMPLE DATA (to demonstrate how to determine the corrected difference)

Time (min)	**Beads**		**Germinating Beans at 20℃**		
	Reading (a)	Difference (b)	Reading (c)	Difference (d)	Corrected Difference
Initial	0.93	----------	0.91	----------	----------
0-5	0.91		0.84		
0-10	0.90		0.77		
0-15	0.90		0.71		
0-20	0.90		0.64		

Finally, if we want to compare respiration in dry seeds and germinating seeds, we need to account for the fact that the volume of a single germinating seed (which has been soaked in water) will (because of osmosis) be much greater than the volume of a dry seed. If we want to do a one to one comparison, we need to equalize the number of oxygen molecules, and we do that by equalizing the volume by adding glass beads to the dry beads. Here's what it looks like:



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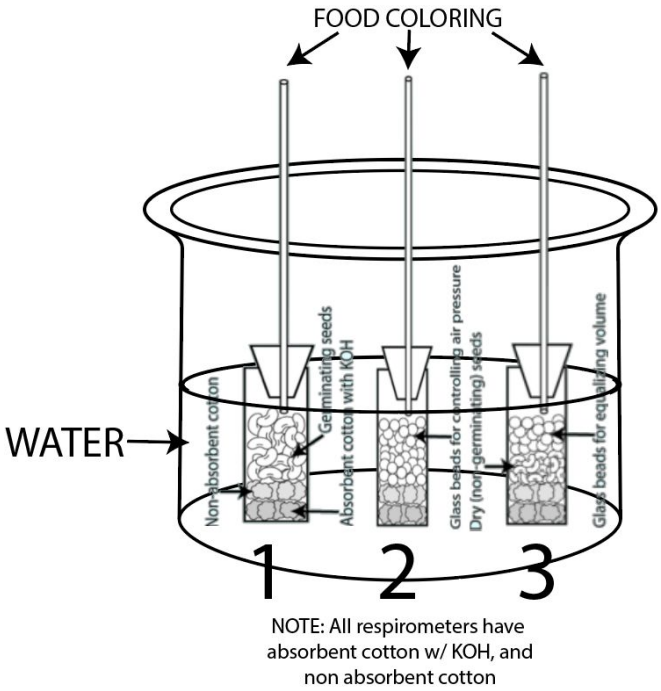
Cellular Respiration Lab: Setup and Data Sheet

We're comparing **four** things:

Respiration rate of germinating peas in at tap water temperature	Respiration rate of germinating peas at ice water temperature
Respiration rate of dormant peas at tap water temperature	Respiration rate of dormant peas at ice water temperature

…and we're trying to control for air pressure changes.



NOTE: All respirometers have absorbent cotton w/ KOH, and non absorbent cotton

BIG PICTURE:
Each team of four sets up 3 respirometers, either in ice water or in room temperature water.

SEED TYPE: _____

SET UP

1. For respirometer 1: Get _____ germinating seeds (we'll decide based on which type of seed). Measure volume by displacement of water method. Record volume here: _____mL
2. For respirometer 2: Use beads to match the volume of germinating seeds. Set aside.
3. For respirometer 3: Use same number of *dried* (non-germinating) seeds. Measure their volume, using the displacement method. Add beads to match volume of germinating beans. Set aside.

Assemble respirometers

1. Place a small wad of **absorbent** cotton in the bottom of the chamber.
2. Using a 1ml plastic pipette, saturate the absorbent cotton using 1 mL of 10% KOH. Note: the KOH is a strong base. **Be careful.** Wear eye protection and rinse your hands off if any gets on your hands.
3. Add a small wad of non-absorbent cotton to protect your seeds.
4. Add *germinating seeds only* to respirometer 1.
5. Add *beads only* to respirometer 3.
6. Add *dry seeds and beads (to equalize volume)* to respirometer 2

Allow for *equilibration*: 1) Place respirometers in cups (one with ice, one without) Wait 3 minutes. Place a drop of food dye in the tip of your respirometer. Don't disturb. Allow 3 or four minutes before first reading. Things have equilibrated when the respirometer with beads (no organisms) has a relatively constant reading for a few minutes.

Keep temperature constant. During the course of the experiment, gently add ice to the ice cup as needed so that its temperature remains constant.

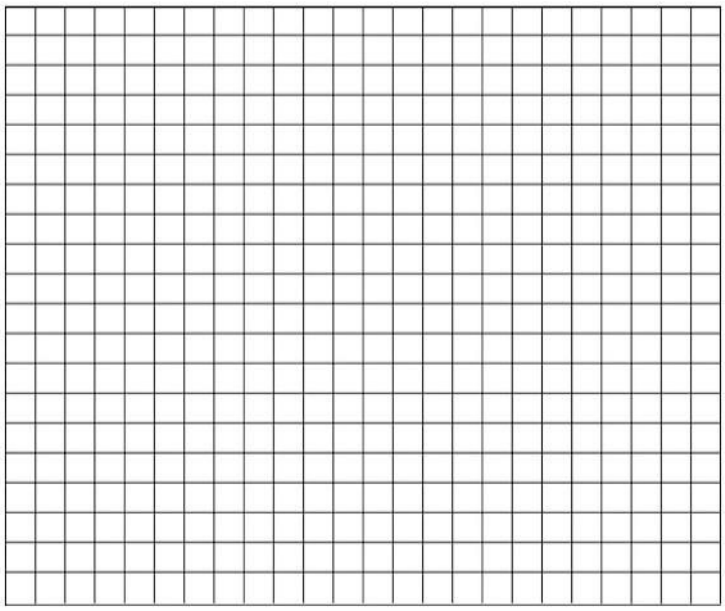
DATA COLLECTION TABLE

℃	Time (min)	Beads		Germinating Seeds			Dry Seeds and Beads		
		(a) Reading	(b) Difference)	(c) Reading	(d) Difference	(e) Corrected Difference	(f) Reading	(g) Difference	(h) Corrected Difference
25	Initial (0)		--------		------	---------		---------	---------
	0 to 5								
	0 to 10								
	0 to 15								
	0 to 20								
10	Initial (0)		-------		-------	---------		---------	---------
	0-5								
	0-10								
	0-15								
	0-20								

Table 1: Team Data for :

Calculate the tate of oxygen consumption over 20 minutes. You're calculating mL oxygen/minute. Use the space below to calculate: record your results in the right column below

Four lines go on this graph: Use error bars to show standard error for each plotted point.



TEAM DATA
Rate of oxygen consumption between 0 and 15 minutes in mL O_2/minute

germ. warm: _____

germ. cold: _____

non-germ. warm: _____

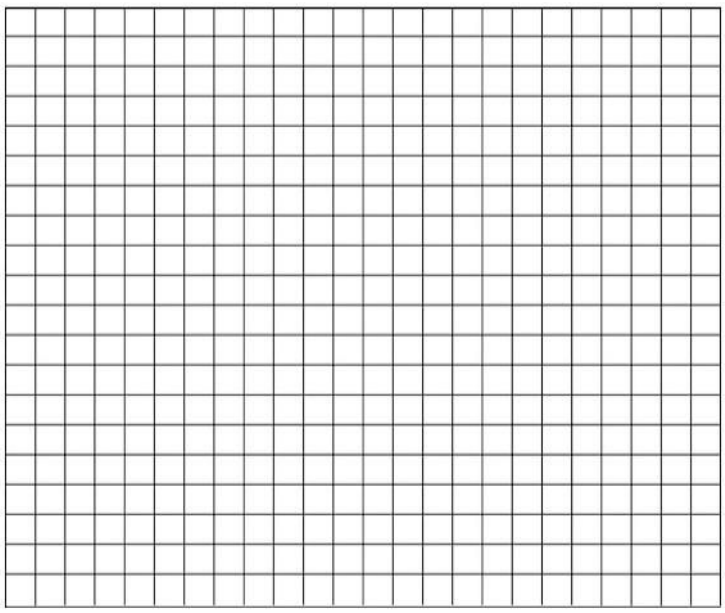
non-germ. cold: _____

Table 2: Class Data for :

Calculate the rate of oxygen consumption over 15 minutes. You're calculating mL oxygen/minute

	Average Rate in mL oxygen/minute	*Standard Error*
Germinating seeds in tap water		
Germinating seeds in ice water		
Non-germinating seeds in tap water		
Non-germinating seeds in ice water.		

Four lines go on this graph: Use error bars to show standard error for each plotted point.



CLEAN UP Instructions

1. take apart respirometers
2. used seeds, used cotton go into trash
3. re-assemble kits into baskets:
    a. -3 pipettes w/ stoppers
    b. -3 respirometer chambers
    c. -3 tiny cups
    d. –tweezers/forceps
    e. -bags w/ absorbent and non-absorbent cotton
    f. -graduated cylinders
    g. -bag or tube w/ plastic (or glass) beads
4. place kit on lab table

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Cellular Respiration Lab Analysis

Sample Data Using Peas (Everyone: complete this table. If your group's data is unworkable, graph this graph 2.

°C	Time (min)	Beads		Germinating Peas			Dry Peas and Beads		
		(a) Reading	(b) Difference)	(c) Reading	(d) Difference	(e) Corrected Difference	(f) Reading	(g) Difference	(h) Corrected Difference
25	Initial (0)	0.93	--------	0.91	--------	---------	0.92	---------	---------
	0 to 5	0.91		0.84			0.89		
	0 to 10	0.90		0.77			0.87		
	0 to 15	0.90		0.71			0.87		
	0 to 20	0.90		0.64			0.85		
10	Initial (0)	0.95	-------	0.92	--------	----------	0.91	---------	---------
	0-5	0.94		0.88			0.90		
	0-10	0.92		0.85			0.87		
	0-15	0.93		0.83			0.86		
	0-20	0.93		0.80			0.85		

Answer the questions below on another sheet of paper

1. Explain the overall setup of the respirometer we used in this lab.
    a. How did it work?
    b. Why did we need to have KOH?
    c. Why did the food coloring move toward the the respirometer's chamber?
    d. Why was it necessary to correct the readings from the seeds with readings from the beads?
2. Think of the overall setup of this lab. What controls were used?
3. DESCRIBE and EXPLAIN the relationship between the amount of O_2 consumed and time.
4. What is *dormancy*? Explain what's happening in germinating seeds that's causing them to have a higher rate of respiration than dormant seeds.
5. Draw a sample graph of oxygen consumption for germinating seeds that starts at 5 degrees C and gradually heats up to 50 degrees C. Explain your prediction.
6. Before answering this next question, think for a minute about how the metabolisms of reptiles and mammals are different. For one thing, energy requirements are different: for example, an 80 kg alligator can survive on about 100 food calories/day (or less), while an 80 kg person needs about 2000 food calories /day. Additionally, think about the relationship between body temperature and environmental temperature in each type of animal. Now, assume that we had a really big respirometer—big enough to hold a fence lizard and a field mouse of equal mass. If we compared their respiration rates at 10 degrees C, what results would you expect? Draw a graph of your anticipated results, and explain your reasoning.
7. Using the same really big respirometer, compare the respiration rate of a mouse at 10 degrees C and a mouse at 20 C. Assume the mice have the same mass. Draw a graph of your anticipated results. Explain your reasoning.
8. Your teacher has prepared four trays of germinating peas. In tray 1, the peas were just watered an hour ago. In tray 2, they've been germinating for 24 hours. In tray 3, they've been germinating for 48 hour, and in tray four, for 72 hours. Using respirometers, design a controlled experiment to test the rates of cellular respiration in these different groups of peas. Explain how you would set up your expermiment, and propose a hypothesis of what the results might be.