The Power of Sunlight:

Investigations in Photosynthesis & Respiration

Student's Guide

In this module, you will join a research team to ask questions about ***photosynthesis*** and ***cellular respiration***, then carry out experiments to help answer these questions. You'll create a team blog where you'll post your ideas and share your data with a plant biologist, and share what you learned about photosynthesis and respiration with your classmates. Here's what's in store:

- **Get re-acquainted with plants:** You will look at what happens to plants in the light and dark and generate ideas about how they use photosynthesis and respiration to grow.
- **Laboratory investigations**: You will work in teams to brainstorm research questions and develop a plan for an experiment. You will learn a specific experimental method and may read through a manual to find other methods allowing you test a range of possible research questions. You can blog to get feedback about your experiment and ideas from a scientist.
- **Think like a scientist:** Once you finish your lab investigations, teams will develop a storyboard that will be used to present their findings. As you look at data from all of teams, the class will work together to make sense of it all – to explain how different environmental and plant characteristics might affect a plant's photosynthesis and respiration.

What does this have to do with me?

Do you eat? A bowl of granola is a world of plants – oats, wheat, nuts, and fruit. In that juicy hamburger, the mustard, catsup, lettuce and bun all come from plants. The burger was once a cow that transformed its food – grain or grasses – into meat. Even the fries – oh those fries! - are potatoes cooked in vegetable oil – plants again.

The food we eat comes from a plant's harvested energy from the sun that has been converted by it or by the animal that ate it into carbohydrates, fats, and proteins. How does that happen? Will our food sources be affected as the planet warms? Will the wheat in our bread and other crops tolerate periods of drought and other environmental stresses? How might overuse of fertilizers affect the soils in which crops grow?

In this investigation, you will examine how plants use their own biochemical "solar panels" to collect energy from the sun and convert it into food through photosynthesis and respiration. You'll also be asking questions about how the environment and a plant's characteristics might affect the efficiency of these two processes – and the future of food on our planet.

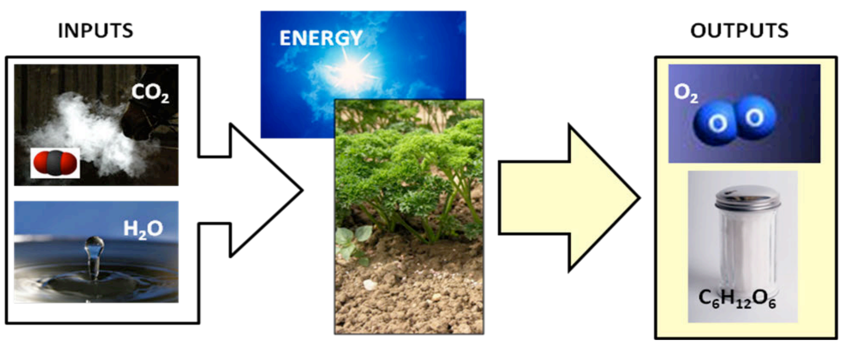
Background: Photosynthesis and Cellular Respiration in Plants

A PlantingScience Resource

Living things that carry out **photosynthesis** play an incredibly important role in supporting life on Earth. Photosynthesis is the key biochemical process responsible for capturing energy from the Sun and channeling it into other living systems, while producing oxygen as one product. Plants and other photosynthetic organisms, such as algae, microbes, and some protists, use photosynthetic products to fuel the cellular processes needed to live and grow. Humans and other animals can't do this. We depend on photosynthetic organisms as our energy sources, either directly as food, or indirectly as food for the animals we eat.

How does photosynthesis work?

Photosynthesis transforms the carbon from carbon dioxide (CO_2) in the air into carbohydrates. This process, called *carbon fixation*, is a physico-chemical reaction and requires energy to occur. The energy comes from the Sun in the form of sunlight. Water, which is needed for the reaction to proceed, is transformed into oxygen gas (O_2) as a product of photosynthesis. The overall process can be visualized in terms of inputs, energy and organisms, and outputs:



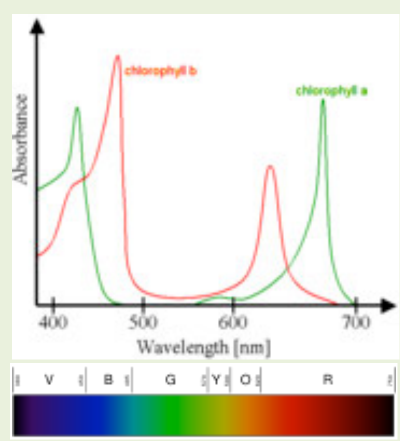
Photosynthesis can also be summarized in a simple biochemical equation:

6 CO_2	+	6 H_2O	+	light	→	$C_6H_{12}O_6$	+	6 O_2
carbon dioxide		water		energy		carbohydrate		oxygen

Photosynthesis takes place in cellular organelles called *chloroplasts*. Inside chloroplasts, green-colored chlorophylls and other plant pigments capture light energy during a phase of photosynthesis called the *light reactions*. The captured light energy is used to transform water molecules into oxygen, which also releases electrons and hydrogen ions. These are bound to NADP+ to form NADPH, a temporary storage form of energy. In the second phase of photosynthesis, the *Calvin cycle*, captured light energy and hydrogen in NADPH is used to fix carbon dioxide, forming the sugar glucose and regenerating NADP+. The light energy that was captured from sunlight is thus partially stored in the chemical bonds of the sugar. By photosynthesis in green tissues, plants produce usable forms of energy and carbon that they need to live and grow.

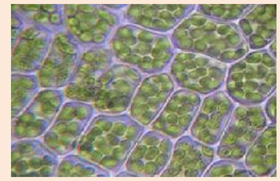
What makes plants green?

The colors we see are wavelengths of light reflected. Plants are green because their chlorophylls reflect green light. However, chlorophylls capture other wavelengths of light and use the energy for photosynthesis. Land plants have two major forms of chlorophyll, and distinct families of algae produce three other types. Chlorophylls *a* and *b*, found in land plants, absorb light energy in the wavelengths shown below.



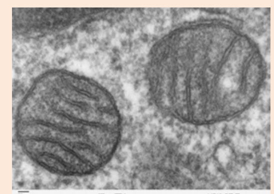
Sugar produced by photosynthesis can be used further or stored. Glucose is the chemical energy source for cellular respiration and also provides chemical structures for synthesis of new proteins, carbohydrates, and fats needed to make cells, leaves, roots, flowers and seeds, or spores. If more sugar is produced than the plant can use, glucose molecules are linked together to form starch and stored temporarily or long-term storage, such as during the winter.

Chloroplasts



Inside each plant cell (purple borders) are many green oval bodies. These bodies, called chloroplasts, are the organelles that capture light for photosynthesis.

Mitochondria



Mitochondria, such as the two shown here, carry out two of the three phases of cellular respiration. The fingerlike projections or cristae are folds in the inner membrane, which separates the intermembrane space and the large matrix.

Cellular respiration is another critical biochemical process for life on Earth. All cells require a daily supply of energy to live. Plants and other organisms can recover the solar energy stored in the molecular bonds of glucose by breaking down the sugar. Energy can then be stored in the bonds of ATP, which is used for a variety of processes that a cell must carry out to live. Cellular respiration is the most efficient way that glucose can be broken down to generate energy for other cellular reactions. The process requires oxygen and releases carbon dioxide, water, and ATP. Cellular respiration can be summarized as:

$$\underset{\text{carbohydrate}}{\mathbf{C_6H_{12}O_6}} + \underset{\text{oxygen}}{\mathbf{6\ O_2}} \rightarrow \underset{\text{carbon dioxide}}{\mathbf{6\ CO_2}} + \underset{\text{water}}{\mathbf{6\ H_2O}} + \underset{\text{energy}}{\mathbf{32\ ATP\ \&\ heat}}$$

The ATP made during cellular respiration can be thought of as a transfer of solar energy from the molecular bonds of glucose to bonds between phosphate groups in ATP. Breaking the phosphate bonds releases the stored energy, allowing a wide variety of other reactions to proceed.

Cellular respiration occurs in three phases. During *glycolysis*, one molecule of glucose in the cell's cytoplasm is broken down over a series of reactions to form two three-carbon molecules. This phase requires two ATP but produces four, for a net gain of two ATP. The three-carbon molecules then move into cellular organelles called *mitochondria*. Because they produce the bulk of a cell's ATP, such organelles have been called the "powerhouse" of the cell. After crossing both the outer and the inner mitochondrial membrane, the three-carbon molecules are transformed into two-carbon molecules in the mitochondrial *matrix*, each producing one molecule of carbon dioxide and one of NADH.

The second phase of cellular respiration, the *citric acid cycle*, also occurs in the matrix. Each two-carbon molecule is joined to a four-carbon molecule, then broken down over a series of reactions to yield two additional CO_2, 3 more NADH, 1 $FADH_2$, and 1 GTP. The GTP is easily converted to ATP, and the original four-carbon molecule is regenerated upon completing the cycle.

The final, *ATP synthesis* phase of cellular respiration occurs in the inner mitochondrial membrane. NADH and $FADH_2$ produced in the citric acid cycle transfer their electrons to proteins within this membrane, releasing hydrogen ions. The energy from the electrons helps pump hydrogen ions across the inner membrane, producing a pH gradient between the matrix and *intermembrane space* of the mitochondria. Along with four hydrogen ions, every four electrons used are finally donated to an oxygen molecule, converting it into two water molecules. Meanwhile, hydrogen ions pumped into the intermembrane space return to the mitochondrial matrix through the protein *ATP synthase*, which joins an ADP and phosphate ion to form ATP. Up to 28 molecules of ATP can be formed from one molecule of glucose in this final phase.

What Happens With Photosynthesis and Cellular Respiration Inside Leaves During the Day and at Night?

In this **guided investigation**, you will explore photosynthetic and respiratory activity in light and in the dark. You will make small “leaf disks” from leaves and place them under conditions of light and dark. If you put the leaf disk in bright light, you are re-creating a condition that plants encounter in nature – bright daytime sunlight. Putting the leaf disk in very dim light or darkness is similar to deep shade or nightfall. A leaf disk represents the whole leaf, and it has the same cellular structure. Leaves consist of different layers of cells, and the cells come in different shapes, sizes, and specialized roles. Spaces between cells allow gases to move through the leaf. Carbon dioxide, the gas used in photosynthesis, occupies some of these spaces.

Here, you will first practice the leaf disk floatation method. Infiltrating leaves with a liquid can be a tricky, so it’s best to practice before carrying out an experiment. After practicing the method, you will use it to think like a scientist. Given what you know about plants so far, which light conditions will allow photosynthesis to occur? What about respiration? Your team will test your expectations in an experiment using the leaf disk floatation method. What you learn from the experiment is more important than whether the results match your expectations. You will come up with ideas to explain your data and share your findings with the class, much like scientists presenting their research.

Part I. Practicing the Method

Step 1. Understand why the method is used and what it means for a method “to work.”

- You’re trying to get this method to work because the whole class will use it to answer the research questions:

Does photosynthesis happen more quickly in the light or in the dark? What about respiration?

- You may also use the method later in an independent investigation, to help answer your own research question.
- Getting a method to work means that:
  - You understand the science behind it.
  - You’ve tried it out and can convince yourself and others that it works.
  - You are comfortable with handling the materials and procedures.

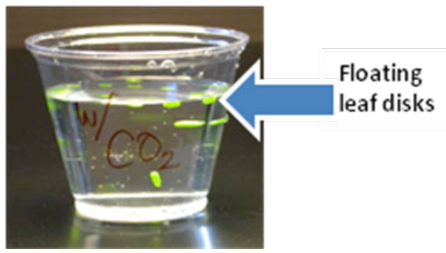
Why do scientists test methods? Isn’t this a waste of time?

Scientists often use trials to get a technique “up and running” before they launch an experiment. Materials are often expensive and plant samples might be rare, collected only at a certain time of year, or from a distant place. A scientist may also have slightly different equipment than what was originally used to develop a method.

This is a bit like trying out a new recipe before making it for guests. Recipes aren’t fool-proof. If one comes out too salty or overcooked the first time, you can make changes to the recipe on the second trial. When it’s time to cook for your guests, you’ll have a recipe that looks and tastes good.

Step 2. Make sense of the scientific basis for the method.

- Baking soda is sodium bicarbonate. When baking soda is put into water, it dissolves to form carbon dioxide. Plants can use this carbon dioxide for photosynthesis.
- You will prepare leaf disks by infiltrating them with a baking soda solution. Leaf disks normally float because of the gases inside, but infiltration removes most of the gases and allows them to sink.
- You will put each set of leaf disks into a cup of baking soda solution, then place one cup in the dark and the other under a bright light.
- If an infiltrated leaf disk produces a gas, such as oxygen or carbon dioxide, the gas will accumulate inside the leaf disk, causing it to float as pictured below.



Step 3. Practice and plan ahead!

- Read through **Part II** below and the full leaf disk floatation method (pp. 7-9) to understand all of the experiment.
- At a minimum, all team members should try their hand at infiltrating the leaf disks **at least once**.
  - It's okay to use fewer leaf disks for your practice.
  - If it doesn't work at first, try again!
- Think about ways to streamline the procedure for the experiment.
  - Who will do each part of the work to ensure that all the data is collected properly?
  - How can the materials be set up to quickly transfer leaf disks from syringe to cup?

Part II. Testing the Question

Step 1. Predict what will happen in your experiment.

- Try to answer the questions:
  - What do I expect to happen to the disks in each cup and why?
  - What do I think is happening biologically that makes my answers credible?
  - The answers to these questions make up your *research prediction*.
- See the **Student Roadmap though an Investigation's** "Research Question" to learn more about *research predictions* and how to develop one as a team.
- Fill in the **Experimental Design** worksheet to record your thinking.

Step 2. Set up a data collection sheet.

- Figure out what your data will be:
  - What will you record, and how often?
  - Where will you record it, and in what format?
  - What materials do you need to record it?
  - Who will record it?

Be Prepared!

When you're in the middle of running an experiment, your hands are busy and you're paying attention to details that may need to be recorded. You won't have time to think about creating data tables on the spot!

- Use a notebook page or a computer file to record your data, e.g., in a hand-drawn table or an Excel file with labeled columns.

Step 3. Carry out the experiment.

- Follow the **full** procedure described in the Leaf Disk Floatation Method instructions on pp. 7-9.
- Collect the data as you planned in *Step 2*.

Step 4. Make sense of what happened.

- See the **Student Roadmap through an Investigation**'s "Making Sense of Findings" for a guide to help develop explanations about what you observed.
- For this experiment, key questions include:
  - Do you think the method worked or not? How can you tell?
  - How do the results compare with your predictions?
  - How do the results from leaf disks in the light compare with those from leaf disks in the dark?
- Write out a **well-reasoned, convincing** argument to tell someone how you can tell if the method was working and what the overall results mean. Include **your data** and **what you think you understand** about plants, photosynthesis, and respiration.

- You may want to use the **Making Sense of the Data** worksheet to record your team's ideas.
- Plan to discuss your ideas with the whole class in a Storyboard Discussion. Your teacher will help guide this process.

MEASURING PHOTOSYNTHESIS AND RESPIRATION USING A LEAF DISK FLOATATION METHOD

Purpose: Measuring how quickly photosynthesis or respiration is occurring in leaves or cotyledons.

How the Method Works: The method uses leaf disks cut from the plants you choose to study (see photo). When leaf disks are put into water, they usually float. In this method, leaf disks are filled with a liquid solution so that they sink. The solution contains dissolved carbon dioxide. When the leaf disks are exposed to conditions in which photosynthesis occurs, they consume carbon dioxide from the solution, produce oxygen gas, and begin to float. This method cannot easily identify the type of gas produced by the leaves; however, carbon dioxide will tend to dissolve into the water. Oxygen gas can also be consumed due to cellular respiration.

Technical Skill: Medium.

Time Required: One class session (45 minutes or more).

Materials:

- Plant leaves or whole plants
- Baking soda (sodium bicarbonate)
- Liquid dish or hand soap
- Water (room temperature)
- Citrate-phosphate buffer (optional, if using enough baking to change the pH in your cups; see **PlantingScience Power of Sunlight Toolkit** for instructions)
- pH paper
- Single-hole paper punch, plastic straw, No. 3 cork borer, or scissors
- 10 mL or larger disposable syringes, without needles
- Clear, wide-mouth plastic cups or petri dishes
- Sharpie marker
- Light source that can be placed various distances from leaf disks or with adjustable intensity (40 W or more; this could be a light bank with adjustable shelves or height used by the whole class)
- Ruler
- Aluminum foil, box, black cloth, or something to cover a cup to create darkness
- Stop watch, timer, watch or clock with a second counter

Procedures:

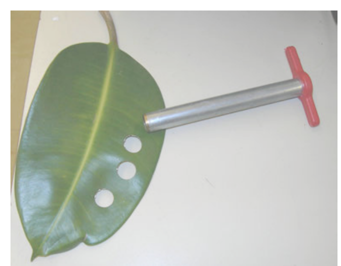
Step 1. Set up your materials and solutions. (5 minutes)

- Gather your materials: a syringe, 2 or more plastic cups, water or citrate-phosphate buffer, baking soda, a hole punch or straw, and (if not shared by the whole class) a light source.

- Make your **bicarbonate + soap solution**.
    1. Add a pinch of baking soda to your plastic cup.
    2. Add water or citrate-phosphate buffer to about 6 cm deep.
        - If you plan to test more than two treatments, make a larger volume of solution.
    3. Stir to dissolve thoroughly.
    4. Add a drop of liquid soap to the solution and stir thoroughly, but do not make suds.
    5. Divide the solution into 2 cups, and use a marker to label them so that you can tell them apart.
        - If you plan to test more than two treatments, make one cup for each treatment.
        - You may wish to use pH paper to measure the pH of the solution if you are not using citrate-phosphate buffer.
- Figure out where you will place your **light source** relative to your cups. Make sure the electrical cord can reach your cup. A light bulb can be placed as close as 2 cm from the cup.

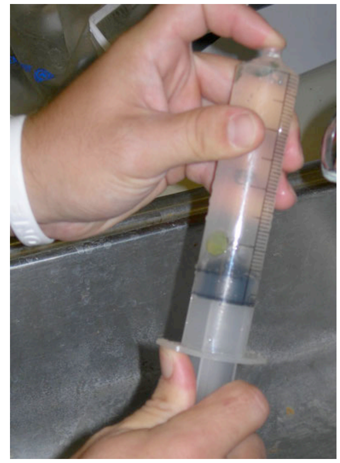
Step 2. Prepare your leaf disks. (10 minutes)

- To make your leaf disks:
    1. Select enough leaves to make about 25 leaf disks.
        - This method works best with healthy plant material. If your research question is about differences between healthy and unhealthy tissue, you can select plant material differently.
        - If you want to test more than two conditions, make enough leaf disks so that you have at least ten per treatment.
    2. Use a paper hole punch, a plastic straw, or a cork borer to punch out your leaf disks.
        - Avoid the heavy veins.
        - If you have an index card or small piece of paper handy, put the disks onto the card. This will make it easier to pour all the disks into the syringe.
        - *Alternatively*, you can cut square leaf "disks" using scissors.



- Remove the gases inside the leaf disks by infiltrating them with the bicarbonate + soap solution:
    1. Remove the plunger from the syringe.
    2. Pour about 12 leaf disks into the barrel of the syringe.
    3. Tap the barrel on the table or in your hand, so that the leaf disks move toward the narrow end where a needle would normally go.
    4. CAREFULLY push the plunger back into the syringe.
        - Avoid damaging the leaf disks.
        - Push the plunger nearly all the way into the barrel - about $1/10^{th}$ of the way from the tip.
    5. Place the syringe tip into the bicarbonate + soap solution and pull up some of the solution until the syringe is about 1/3 full.
    6. Tap the syringe to drop the leaf disks down into the liquid in the syringe as much as possible.
    7. Holding the syringe with the tip upwards, cover the open tip with your finger. Pull back on the plunger a bit with your finger over the open tip. You will feel a vacuum pulling on your fingertip. This will pull the gas out of the leaf disks and replace it with bicarbonate + soap solution.

        - You will probably notice your leaf disks darkening in color and/or sinking as they become infiltrated with the solution.
    8. Hold the vacuum for about 10 seconds. Gently release.
    9. Pull a vacuum 3 more times, or until all leaf disks sink in the solution.
        - If you have done this 5 times and some leaf disks are still floating, add more soap to the solution and try again.
    10. Once all leaf disks have all sunk, they are ready for testing.
    11. Pour the infiltrated leaf disks into one of your cups with bicarbonate + soap solution.



- Infiltrate your second set of 12 leaf disks as described above, then pour them into the second cup of bicarbonate + soap solution.
    - If you have additional sets to test, repeat until all leaf disks have been infiltrated. Each set should be kept in its own cup.
- Adjust the volume of solution in each cup by eye so that they have the same amount of liquid in them. About 3 cm deep is sufficient.
- All leaf disks should be at the bottom of the cups.

Step 3. Run your experiment. (30 minutes)

1. Place your cups in the appropriate spots for testing.
    - For the light/dark guided investigation:
        - One cup should be very close to your light source, about 2-5 cm away
        - The other cup should be in the dark (covered with foil, under a box – be creative!)
    - Do not turn on the light source yet!
2. Measure the distance between the light and the top of the liquid in your cup or the side of your cup, depending on whether your light is over the cup or to the side of your cup.
3. Record the number of leaf disks in each cup. Note if any are already floating.
4. Treatment begins when you turn the light on.
    - For the guided investigation, "light" and "dark" are the two treatments.
    - Start a timer or stopwatch when you begin the treatment to keep accurate data.
5. At **one minute** intervals after you begin treatment, count the number of leaf disks that are floating in each cup and record these numbers in the data sheet you prepared beforehand.
6. Continue recording data until all of the leaf disks are floating.
    - After 25 minutes, you can stop collecting data.
7. If you have time, test what happens if you put a cup with **floating** leaf disks in the dark. Collect data for 10 minutes by taking notes on what happens after each minute.

Troubleshooting:

Several common problems in getting this method to work can be easily solved:

- Hairy leaves tend to trap gas bubbles and may be very difficult to infiltrate, so you will probably want to avoid these kinds of leaves in your experiments.
- Additional soap is usually helpful for other types of leaves.

- Air bubbles may get trapped on the surface of leaf disks. Try tapping the syringe or cup to dislodge any bubbles if leaf disks float unexpectedly.
- Cut edges of leaf disks can be sticky, causing them to adhere to a syringe or cup. Tap to release them.

How might a plant's environment affect its photosynthesis and cellular respiration?

In this **independent investigation**, you will examine how a plant's photosynthetic and respiratory activities are influenced by environmental variables. You have already looked at how the presence and absence of light influences these two processes. Here you get a chance to explore more broadly!

Step 1. Brainstorm a Research Question.
Use the **Student Roadmap through an Investigation**'s "Explore – Getting Started" and "Research Question" to help you come up with your own *research question*. The main requirements are that your question must be (a) related to environment, photosynthesis, and respiration, and (b) testable using one or more experiments. As you work through the guide, you can complete the **Brainstorming Page** worksheet to help you get your ideas down on paper. To finalize the question you select for study, complete the **About Our Research Question** worksheet.

Your question might be tested using the leaf disk floatation method you've already used, or it might involve a new method. A book of additional lab methods, the **PlantingScience Power of Sunlight Toolkit**, is available for you to explore. If your teacher has the supplies available, you can use one of these methods to ask different sorts of research questions about photosynthesis and respiration. In fact, it's best to explore the toolkit before you commit to a definite line of experiments. A list of methods described in the toolkit is on the next page.

Step 2. Design your experiment(s).
Use the **Student Roadmap through an Investigation**'s "Research Question" and "Planning Your Study" to help develop an experiment testing the research question you have chosen. "Tips for Making Predictions" will help you think about what you expect to happen in the experiment. Your team can also fill out the **Experimental Design** worksheet to keep track of your *research predictions* and *experimental design*. Along with the two worksheets from *Step 1*, this third worksheet will be part of your *research plan*.

Step 3. Blog your ideas.
Talk with your scientist about your ideas on how a plant's environment might affect photosynthesis and respiration. Get feedback on your research plan, and if needed, make changes to it before starting the experiment(s).

Step 4. Carry out your experiment(s).
The class will have at least two periods to work on experiments, so feel free to explore and perfect your protocols if needed. Your scientist may be able to provide feedback if you have trouble. If you find interesting results that lead to a new question and your teacher has the supplies on hand, you can even do a follow-up experiment.

Step 5. Visualize and analyze your data.
During and after each experiment, record and photograph, illustrate, or graph your data. Summarizing the data will help you see trends and patterns in your observations and help you make sense of it. A good analysis can also open up more questions that might lead to new experiments.

Step 6. Make sense of your data.
Fill out the **Making Sense of the Data** worksheet to help you figure out how your data might connect environmental conditions, photosynthesis, and cellular respiration. You will have to share your findings with the class in a Storyboard Discussion, so be able to explain what you put in this worksheet.

The following additional experimental methods are available in the

PlantingScience Power of Sunlight Toolkit:

- **Measuring Cellular Respiration Using a Respirometer**
- **Measuring Photosynthesis and Respiration Using a Computer-Based Probe**
- **Monitoring pH to Assess Photosynthesis and Respiration of Aquatic Plants**
- **Identifying Starch in Plant Leaves Using an Iodine Staining Method**
- **Visualizing Plant Cells and Chloroplasts Using a Microscope**
- **Identifying Chlorophyll and Other Plant Pigments**
- **Visualizing and Counting Stomates Using the Epidermal Peel Method**
- **Quantifying Fresh and Dry Mass of Plants**
- **Preparation of Citrate-Phosphate Buffer for Maintaining pH**

If you think that one or more of the above methods might help your team test a research question that interests you, ask your teacher which ones can be used.