Chlorophyll Extraction
(using spinach cells)

Grade Level: 3rd- 8th grade

Purpose/Outcome:
To extract chlorophyll from spinach leaves and utilize the extract to demonstrate various physical properties of plants and chlorophyll.
This experiment consists of four learning modules: Chlorophyll extraction, Chlorophyll magic, Observation of the leaf in the absence of chlorophyll, Making paint with extracted chlorophyll.

Relevance:.
Scientists use this extraction technique during the early stages of product development. Chlorophyll masks and hides details which are important to visualize during the analysis and assessment of the effectiveness some laboratory tissue staining tests.

Safety:
Perform this experiment only under the close supervision of a teacher or parent. Wear safety glasses/goggles to protect eyes from accidental splashes. Wear lab coat covering to protect clothes from staining. Avoid contact with open flames because the solutions contain alcohol. Always wash your hands when the experiment is complete.

IMPORTANT SAFETY NOTICE: This experiment was designed by MONSANTO employees -- not the MONSANTO COMPANY -- to demonstrate various scientific properties. You, the user, assume responsibility for repeating this experiment in a safe manner. You, the user, understand that it is critical to follow all safety precautions noted in the procedure below. You, the user, understand that the substances used and produced in this experiment are **NOT INTENDED FOR HUMAN OR ANIMAL CONSUMPTION** and that consumption or misuse may cause severe harm or death.

Supply List:

1) Spinach leaves make an excellent chlorophyll source but any green leaves will be ok. *Spinach leaves work best for this experiment because the chlorophyll is easily removed.*
2) Gallon bucket
3) Nitril exam gloves
4) 90% Isopropyl alcohol (aka Isopropanol or rubbing alcohol)
5) Two Glass test tubes with cap
6) Coffee filters
7) Petri dish
8) Squirt bottle
9) Disposable plastic bowls
10) Glitter of different colors
11) Paint brushes

12) Microscope
13) Test tubes (preferably glass or clear plastic)
14) Small flashlight with bright illumination
15) Standard office supply paper hole punch
16) Elmer's® all purpose school glue or any other school glue
17) Two 500ml squirt bottles
18) Paper for painting

Prep time/Activity Duration: 1 day prior to experiment/ 30 min

Procedure:
Part A of this procedure should be conducted 1 day prior to experiment.
Extracted chlorophyll is light sensitive and degrades quickly. It should be stored in a dark refrigerator used for experimental material only. Chlorophyll and leaf tissue derived from Section A will be used throughout this experiment. For best results, follow directions in the order listed.

A. Performing the Chlorophyll Extraction from whole leaf (performed by teacher 1 day prior):

1) Grind in a blender or finely tear the spinach leaves
   Save some of the whole leaves for use in section C.
2) Fill container with the pre-ground spinach leaves.
3) Put on gloves to prevent drying of skin by Isopropyl alcohol .
4) Add enough 90% Isopropyl alcohol to the container to cover the spinach leaves.
5) Let soak for at least an hour and as long as overnight in the 90% Isopropyl alcohol to allow the chlorophyll to leach out of leaves. If stored overnight, the container should be labeled and stored in an area not accessible to small children.
   Speed up the process by stirring with a wooden spoon. More chlorophyll is recovered with longer soaking time. If soaked overnight, cover and seal the labeled container with plastic and store in a refrigerator. Sufficient soak time will yield a dark green Isopropyl alcohol *solution.*
6) Visually verify that the Isopropyl alcohol solution is green by pouring 25mL into a small container.
   a. If solution is dark green, discard solution and proceed to step 8
   b. If solutions is light green, allow leaves to soak for 1 hour and repeat step a.
7) Place coffee filter over a bucket
8) Strain the Isopropyl alcohol from step 6 now containing chlorophyll onto coffee filter to separate liquid from the leaf debris.
   Save leaf debris generated in step 8 for use in Section E-Painting with chlorophyll
9) Repeat steps 1-9 until 1L of chlorophyll or desired amount has been extracted.
10) Pour 15ml of the strained 90% Isopropyl alcohol solution into a labeled glass container. Save for later use in Section D-Chlorophyll Magic. Keep in the dark until use.

B. Performing the Chlorophyll Extraction from leaf punch (performed by student)

1) Use hole punch tool to clip (2) leaf samples into Petri dish
2) Apply enough Isopropyl alcohol to cover leaf punch with squirt bottle and swirl.
   This step gradually removes the chlorophyll from the leaf punch revealing other details and colors within the leaf sample.
3) Pour off the Isopropyl alcohol and repeat step 2 until the leaf punch appears transparent. Two repetitions are usually sufficient.
4) Save Petri Dish containing leaf punches for Section C.

C. Viewing the Leaf without chlorophyll:

1) Place Petri dish containing leaf punches created in section B under microscope
2) Carefully focus to view your sample.
3) Draw what you see. Try to identify some of the parts. Use web link below for assistance.
   a. http://www.ftexploring.com/photosyn/chloroplast.html

D. Chlorophyll Magic:

Chlorophyll is visible as a green color. However, when white light is shined on extracted chlorophyll other colors become visible.

1) Pick up a tube of chlorophyll extracted in part A by teacher 1 day prior (do not open).
2) Turn lights off in the room if possible or conduct this experiment in dark area of the classroom.
3) Shine the flashlight on the tube.
4) What color(s) do you see? (see discussion for explanation)
   i. Red/violet

E. Painting with Extracted Chlorophyll:

1) Add approximately 15ml (about 2 tablespoons) glue to disposable bowls.
2) Add approximately 5ml of Isopropyl alcohol solution containing chlorophyll and stir. Add more Isopropyl alcohol solutions if necessary so that the glue "thins" and becomes more like paint consistency?
3) Add a little of the leaf debris saved from Section A for color.
   Leaf debris should be finely ground. Use a mortar and pestle to grind further.
4) Paint a picture.
5) Add a little glitter for sparkle.

Clean-up/Waste Disposal: Care should be taken not to ingest any of the solutions used in this procedure. If you wish to dispose of it, simply pour it down a drain or place in trash.

Discussion:

Section VII part A

1) What does Isopropyl alcohol do to the leaf that extracts the Chlorophyll?
   The Isopropyl alcohol breaks open the cell making the cell leaky or more permeable. This allows the chlorophyll to escape from the cell into the solution.

Section VII part B

2) Why does the chlorophyll make a leaf appear green?
   a. Chlorophyll absorbs light of red and blue very well and reflects green light. This is the color that we see.

Section VII part C

3) What is the purpose of the epidermal cells of the leaf?
   a. Epidermal cells allow exchange of gases. Yes, plants breathe too. Plants breathe in carbon dioxide (CO_2) and release oxygen (O_2). Humans breathe O_2 and release CO_2.

Section VII part D

4) Why can we see Chlorophyll as red after extraction?

Chlorophyll is a photoreceptor made up of two molecules: chlorophyll a, chlorophyll b. As a photoreceptor, it absorbs light energy from a source such as the sun. Once captured, light energy breaks molecular bonds within the chloroplast and turns it into a chemical energy that the plant needs to grow.

Light energy, measured in nanometers (nm), comes in different lengths. These wavelengths are part of the visible light spectrum which is actually a combination of different wavelengths from 700-400nm. Wavelengths appear to humans as different colors: red (700-650nm), yellow (650-600nm), green (600-500nm), blue/violet (500-400nm). Chlorophyll has the unique ability to separate and absorb energy from some wavelengths and fluoresce others. Fluorescence occurs when a chemical absorbs one wavelength and emits light of a different wavelength.

Chlorophyll is seen as green (600-500nm) because it reflects this color. However, it absorbs quite well in the red light spectrum (700-650nm). When chlorophyll is contained in the plant, it absorbs red light energy. When chlorophyll is removed from the plant and other components, the red light is re-emitted or fluoresced.

Related websites:
Monsanto Biotech Knowledge Center: http://www.biotechknowledge.com,
Monsanto Home Page: http://www.monsanto.com

http://users.erols.com/jkimball.ma.ultranet/BiologyPages/C/Chlorophyll.html
http://users.erols.com/jkimball.ma.ultranet/BiologyPages/L/Leaf.html
http://imagers.gsfc.nasa.gov/ems/visible.html
http://www.ch.ic.ac.uk/local/projects/steer/chloro.htm

Acknowledgements:
http://www.cheminst.ca/ncw/experiments/epigments.html
http://www.organicworldwide.net/solvents.html
http://scifun.chem.wisc.edu/chemweek/EtOH/EtOH.html
http://www.ftexploring.com/photosyn/chloroplast.html