ASBMB
American Society for Biochemistry and Molecular Biology

BotanoTech: A Comparative Plant Genomics Module

Students imagine themselves as employees of a (fictitious) San Diego Biotech Company "Botanotech." The company develops anti-cancer medications and has identified a promising compound in broccoli (which actually is a source of at least one real anti-cancer drug currently in development called sinigrin). However, closely related plants may be a better source and the students' job for the week is to identify the closest relative of broccoli through genomic investigation.

Activity courtesy of HOPES 2012 Recipients
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Quick Guide and Materials:

- **Age Range:** Middle School
- **Preparation Time:** ~ 30 minutes
- **In Class Time:** ~ 4 hours
- **Duration:** 3 weeks
- **Materials List:** Vegetables, Thermocycler Gel electrophoresis set up, Standard lab supplies

www.asbmb.org/68activity/sdwgm

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Sample Activity Outline and Standards

Activity Outline

Class.Sequence	Activity	Timing
1.1	Intro to BotanoTech	5 min
1.2	Week 1 PowerPoint	10 min
1.3	Plant Observations	15 min
1.4	Hypothesis Writing and Discussion	10 min
1.5	DNA Extraction	40 min
2.1	Week 2 PowerPoint	10 min
2.2	Pipetting Lesson and Practice	15 min
2.3	PCR Introduction	15 min
2.4	PCR Reaction Set Up	10 min
2.5	Review	10 min
3.1	Week 3 PowerPoint	10 min
3.2	Gel Loading and Running	45 min
4.1	Week 4 PowerPoint	5 min
4.2	Gel Viewing	10 min
4.3	Results and Discussion Analysis	30 min

Activity Breakdown Below

Standards

CA 7th Grade Life Science Standards addressed by this module (most relevant standards in italic):

- *Students know the nucleus is the repository for genetic information in plant and animal cells.*
- Students know sexual reproduction produces offspring that inherit half their genes from each parent.
- Students know an inherited trait can be determined by one or more genes.
- *Students know plant and animal cells contain many thousands of different genes and typically have two copies of every gene. The two copies (or alleles) of the gene may or may not be identical, and one may be dominant in determining the phenotype while the other is recessive.*
- *Students know DNA (deoxyribonucleic acid) is the genetic material of living organisms and is located in the chromosomes of each cell.*
- *Students know both genetic variation and environmental factors are causes of evolution and diversity of organisms.*
- *Students know how independent lines of evidence from geology, fossils, and comparative anatomy provide the bases for the theory of evolution*
- *Students know how to construct a simple branching diagram to classify living groups of organisms by shared derived characteristics and how to expand the diagram to include fossil organisms.*

Materials

Week 1

Quantities for 4-6 classes

- 3 heads of cauliflower
- 4 heads of broccoli
- 3-4 bunches broccoli rabe
- Dried and fresh pea pods
- Student workbooks (www.asbmb.org/68activitymanuals/)
- 200-600 mL Glass beakers, 1/group
- Blender, 1/group
- Table salt, 1 container/group
- Strainer, 1/group
- Liquid detergent, 1/group
- Meat tenderizer, 1/class
- 1/8 tsp measuring spoon, 1/class
- Isopropyl alcohol, 1/group
- Wood sticks or skewers, 1/student
- Knife and paper plate, 1/group
- 15 mL conical, 1/group
- 15 mL spoon, 1/group
- 1 mL spoon 1/group

Week 2

- 2-20 µL pipettor (1 for every 2 students)
- Pipette tips
- Wax paper, 1/student
- 1.5 mL tube of colored water, 1/pipettor
- 0.5 mL PCR tube, 1/student
- 1.5 mL Eppendorf tubes, 1/pipettor
- Tube racks, 1/group
- Thermocycler

Week 3

- 2-20 µL pipettor (1 for every 2 students)
- Pipette tips
- Flinn Scientific Pipette Practice Kit (cat # FB1649)
- Low melting agarose (Promega)
- Gel electrophoresis units
- Electrophoresis power supply
- DNA size marker (Invitrogen 1kb plus ladder)
- 10x Gel Loading Buffer
- SYBRSAFE® stain (lifetechnologies.com cat. # S33102)
- Gel Running Buffer 1x TAE
- Plastic wrap

Week 4

- Transilluminator with orange filter cover (Invitrogen)

BotanoTech

Week One

Background and Preparation for the Teacher

Review: http://learn.genetics.utah.edu/content/labs/extraction/howto/
The protocol the students will use (in their notebooks) is an adaptation of this method. Some students will substitute other plants for the peas and the volumes are slightly changed. Also, because there is less DNA in the fresh plants than the dry peas, students will not pour the extract from the beaker into individual test tubes. They will add the alcohol directly to the beaker and pass the beaker around the group so each student can spool out some DNA.

Have blenders and DNA extractions materials set up at stations (1 blender and 1 vegetable/station). Recommend 4 students/station. If there is sufficient time before class, or the day before, the vegetables for the DNA extractions can be divided among classes/groups to ease prep between consecutive classes. The 15 ml "DNA collection" tubes can also be pre-filled with water to facilitate the extraction process.

Class Time Overview

1. Intro to Botanotech, distribution of notebooks
2. Day 1 PowerPoint
3. Plant observations (discussion of veggies, individual time)
4. Students write hypotheses
5. Class discussion of hypotheses
6. DNA extraction

DNA Extraction Procedure

1. Read steps together while students write pertinent information in their notes (p. 7). Point out materials for each step.
2. Students then perform the extraction as a group at one of the blender setups. Note that students using dried peas should use only 50 ml rather than 200 ml as specified in protocol. For broccoli and rabe, instruct students to use mainly the floral parts and not a lot of stalk tissue (for better DNA yields). For all fresh veggies be sure they chop them up into very small pieces before putting into the blender so they can get a fine grind and better DNA yields so they can see visible precipitation.
3. While mixture sits for 5 minutes, teacher should demonstrate the addition of Alcohol and how to "spool" the DNA and transfer to DNA collection tube.
4. Stress the importance of adding alcohol slowly, dribbling it down the inside wall of the beaker so that it floats on top of the water layer – this will produce a visible DNA precipitate at the interface that extends up into the alcohol layer and can be spooled out. If they mix the alcohol and veggie water layers, they won't see precipitation.

5. After alcohol has been added to beaker, have students pass it around and take turns carefully spooling DNA from the alcohol layer onto skewer. Dip skewers into labeled DNA collection tube containing water.
6. This DNA collection tube will be saved for the next day's PCR
7. Left over blender extracts should be poured into a garbage can or compost– not down sink drain, because pretty chunky still.
8. Students do not need to clean blenders if the next class will use them for the same plant. Only the last class of the day should clean.
9. Each class should rinse the beaker that contained the extract and alcohol.
10. Collect one DNA sample from each group labeled for re-distribution next week.

Week Two-- Day One

Background and Preparation for the Teacher

Review PCR Animation: http://learn.genetics.utah.edu/content/labs/pcr/
See description of RAPD PCR at the end of the teacher guide

To prepare for Pipette practice session:

1. Prepare a solution of dilute red food coloring (approx. 1:10 with water). Put ~1 ml of this solution in each Eppendorf tube (need one per pipette)
2. Distribute: pipettors, tips, waste containers, racks with tubes of food coloring
3. Distribute pipetting templates (1/student), cover with wax paper and tape it down (students can do this themselves)

To prepare for student PCRs (don't do this until practice session is over to avoid students mixing things up):

1. Label 1.5 ml Eppendorf tubes "PCR Mix" and add approx. 300 µl of water to each tube (1 per pipette)
2. Distribute prelabeled PCR tubes – one per student, matching the species they extracted DNA from (red = rabe, blue = broccoli, green = cauliflower, magenta = pea)

Class Time Overview

1. Day 2 PowerPoint slides 1-9 (review of yesterday, definitions, introduce concept that we are using PCR to compare parts of the genome, introduce micropipettes).
2. Pipette lesson, beginning with a few minutes demo for class.
3. Pipetting practice for students. Students use colored water to practice depositing 5 and 15 µl volumes onto dots on a template. These are the same volumes they will need to use later for setting up their PCR reactions.
4. When this is done, collect all materials to avoid confusion with components of PCR reaction.

5. Return to PowerPoint to introduce PCR.
6. Students set up PCR. Distribute materials needed for PCR reactions (PCR tubes, PCR mix, genomic DNA). To facilitate distribution of correctly labeled tubes to each person, ask students to raise their hand if they had broccoli and give them all tubes with blue stickers, etc. for each plant. Go over information about what volumes of what to mix together to assemble the reaction (students need to fill in blanks in their books with the correct information).
7. Clean up
8. During final 20 minutes, students can use Netbooks to review PCR animation at http://content.dnalc.org/content/c15/15475/19_polymerase_chain_reaction.mp4 and do this interactive PCR activity: http://learn.genetics.utah.edu/content/labs/pcr/ This provides additional background needed to answer PCR questions in their note books.

Week Two-- Day Two

Background and Preparation for the Teacher

Review http://learn.genetics.utah.edu/content/labs/gel/

1. Prepare 3% agarose gels. This can be done day before if gels can be cooled and covered with small amount of buffer and plastic wrap or in morning before class (needs to set for at least 1hr)
2. Prepare DNA Ladder (optional). To the tube containing 250 µl Invitrogen 1kb plus DNA ladder (250 µg), add 500 µl 10X gel loading buffer (blue juice) and 4.25 ml (4250 µl) distilled water. Mix well. Load 15-20 µl per well. Note: this would be enough for >250 gel lanes!
3. For each group:
    a. Prepare small tubes of Flinn practice gel loading dye (1 per practice gel)
    b. Place practice gels in tubs at student workbenches and cover top of gel with water (Flinn practice gels float so it doesn't do any good to fill up the tub)
    c. Give each group: pipette, tips, rack with tube of practice loading solution, waste beaker for tips and tubes
    d. Prepare aliquots of real 5X gel loading dye but do not distribute yet, to avoid confusion with practice gel loading dye! This actually matters.
4. Set up gel boxes and power supplies around the side benches of room so they can continue running after one class without being in the way of the next. Use a Post It note to label them with group names and period, so later classes do not disturb and teacher or assistant will know when to turn it off.

Class Time Overview

1. PowerPoint Day 3: Gel Electrophoresis (Slides 1-6)
2. Practice Loading a gel
   Demonstrate how to load a gel—Emphasizing not poking the gel!!
   Push plunger down slowly and keep the plunger down until the pipette has been pulled out. Give students ~5 minutes to work with the practice gels loading as many wells as they have time for (20 µl of Flinn practice gel loading dye)
3. Loading real gel (Slides 7-8)
   a. Remove practice loading dye and practice gels
   b. Distribute real PCR reactions and aliquots of real 10X gel loading dye
   c. Students add 5 µl of 10X loading buffer to their PCR reaction tube
   d. Load marker lanes. Run gel at manufacturers specification.

Tip: Close supervision of gel loading is important in spite of the practice they have just had. Emphasize importance of not poking tip into gel, dispensing slowly and making corrections in pipette position as needed to make sure most of the sample gets into well. To save time, assistant can add gel loading buffer to samples, and then keep them in a rack near the gel electrophoresis unit. Then students can just come up one by one, claim their tube, and load their sample.

4. While students are waiting for their turn to load the gel they can answer question in their journal from yesterday and today and view http://learn.genetics.utah.edu/content/labs/gel/

Post Lesson

1. Turn off power supply when gel is finished running. (When blue dye band reaches next row.)
2. Wrap each gel in plastic wrap and place on top of gel box for students to view next day.
3. Prepare copies of gel photograph for all students to glue in their workbook the next class.

Week Three

Background and Preparation for the Teacher

See information in previous teacher guides and PowerPoint slides about PCR in general, and RAPD PCR in particular

1. Set transilluminator(s) so students can see the bands on the gel.
   Generate a sample results photo for each student in advance

Tip: Gel should ideally be photographed as soon as it completed it's run, but we found it still looks good if wrapped in plastic wrap and stored till the next day. With class, discuss the demo result already in the PowerPoint presentation, and gave them each a print of this for their notebooks. They copied down the labeling from the PowerPoint slide. This seemed to work very well.

1. Day 4 PowerPoint : RAPD PCR (Slides 1-5)
2. Have students view the gel and/or put under document camera
3. Distribute pre-printed images. While this is happening, display demo result slide and have students label their gel photos similarly.
4. Lead class discussion going over questions on pg. 10 of their notebooks (have them write their answers down), referring to demo results. Alternatively, leave it to students to do this independently looking at their own gel images if they are all good)
5. Have students answer questions pages 11 and 12 of their notebooks including drawing phylogenetic tree. Show slide 7 if they are having trouble drawing tree.
6. Go over last few PowerPoint slides reviewing names and relationships between plants used in the module.