BotanoTech

Science Notebook

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Comparative Plant Genomics Study

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Introduction & Problem

Imagine yourself as a scientist working at BotanoTech Incorporated, a California-based **biotechnology** company whose mission is to develop anticancer medications. You have done experiments that suggest broccoli plants produce a chemical that may have anti-cancer properties. Now you want to determine if other plants may be a better source for this same chemical. You believe that other plants, perhaps ones that are closely related to broccoli, may produce this same anticancer chemical. You are uncertain which plants are most closely related, so your task this week is to answer the following question.

Question (to be answered by the end of the week)

Using **phenotypic** and **genotypic** information, which of the following plants are most closely related to broccoli?

1)_____2)_____3)_____

If you were to make a guess right now, which of the plants listed above do you think is most closely related to broccoli? Why?

_____

_____

_____

The following phenotypic traits will be used to further investigate the relationships among the plants:

1. _____

2. _____

3. _____

Research

Observations of Plant Phenotype

More information is needed to create your hypothesis. Observe the plant seedlings and draw the leaf shapes and margins (edges) in the spaces below. Note the leaf colors.

Plant 1: _____

Plant 2: _____

Plant 3: _____

Plant 4: _____

1) Which plant has leaves which are most similar to broccoli? Why?

Hypothesis

Based on my research, including observations of the plant phenotypes, I predict that the genotype(s) of the following plant(s) are most closely related to broccoli. Explain.

Research Notes:

Materials & Method

Comparison of Plant Genotypes

To study the genotype of each plant, you will first need to extract the DNA (**D**eoxyribo**N**ucleic **A**cid) from the nuclei of the plant cells.

Part 1: Genomic DNA Extraction

Step 1: Put the following items into a blender:
200 ml chopped_____ plant,
1 ml NaCl,
100 ml cold H_2O.
Blend using the medium setting, for 15 seconds.

Step 2: Pour the blended mixture through a strainer, into a glass beaker.
Add 15 ml liquid detergent.
Swirl the beaker to mix (Do not stir with a spoon.)
Let the mixture stand for 5 minutes.
(While waiting, clean the blender and the strainer.)

Step 3: Add one microspoon of enzyme powder to beaker.
Swirl gently.

Step 4: Tilt the beaker.
Slowly pour in isopropyl alcohol, down the side, until the volume of alcohol is equal to the volume of the plant mixture.
The alcohol will float on the denser plant mixture.

Step 5: Use a skewer to gently spool and then lift the stringy white DNA out of the beaker.
Transfer the DNA to a water-filled collection tube. For each type of plant, combine all of the DNA into a single tube for your group.

Step 6: Clean up
Put the strained plant mixture into the garbage, not into the sink.
Rinse all of the containers.

Materials & Methods (cont.)

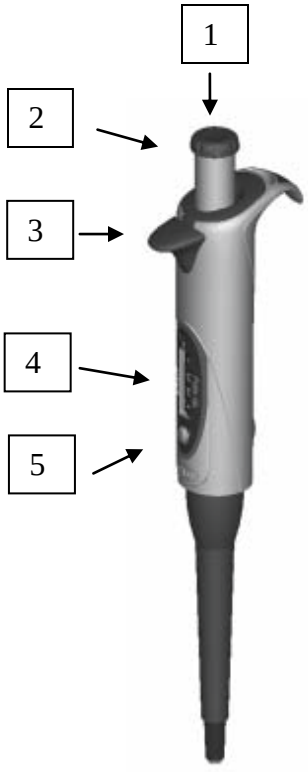
Introduction to Micropipettes

Precautions:

- Do not drop your pipette.
- Insert only the tip of the pipette into a liquid.
- Use a clean tip when inserting into a different solution.
- Do not lay a filled pipette on its side.

Anatomy of a pipette:

1. **Plunger button** -- Press to extract and dispense liquid. It has two "stops."
2. **Volume adjustment dial** -- While pressing the volume lock button (5), turn to adjust the volume in the display window (4).
3. **Tip ejector** – Press to eject used tips**.**
4. **Volume display window** – Shows the extraction volume.
5. **Volume lock button:** Press, while turning the adjustment dial (2), to adjust the extraction volume.



Pipette Practice

Extracting liquid

Install a clean tip.
Push plunger to the first stop.
Put only the tip into the liquid.
Slowly release the plunger.
Lift the pipette tip out of liquid.

Dispensing liquid

Lower the tip full of liquid to the bottom of a new tube.
Push the plunger to the second stop.
Pull the tip out of the liquid.
Release the plunger.
Remove the tip from the tube.
Eject the tip into a waste container.

Practice

1) Place wax paper over a Pipette Practice Template.
2) Practice placing drops of red water on the dots.

Materials & Methods (cont.)

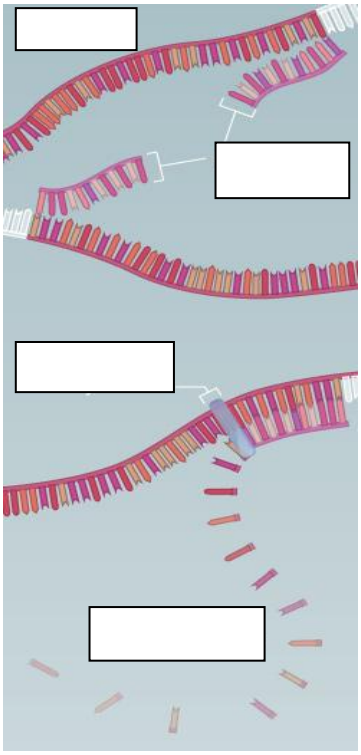
Part 2: PCR reaction

PCR (Polymerase Chain Reaction) will be used to make copies of specific sections of each plants genomic DNA. You will then compare the sections to determine how closely the plants are related.

1. The number on my PCR tube is _____.
2. The DNA is from a _____ plant.
3. Using a clean pipette tip, put _____ µl of **PCR mix** into your PCR tube.
4. Using another clean pipette tip, put _____ µl of **genomic DNA** from the appropriate plant species into the tube.
5. Place the tube in the Thermal Cycler or into a tube rack provided by teacher.

Questions

View the PCR Virtual Lab (http://learn.genetics.utah.edu/content/labs/pcr/), and complete the following, and answer questions 1-5 on page 8.



Match the following terms with their definitions and label each component of the PCR mixture in the diagram (use the letters A-D):

_____DNA polymerase

_____Primers

_____ Nucleotides

_____Genomic DNA template

A. DNA that contains the target sequence that will be replicated using PCR.

B. An enzyme that copies the DNA sequence.

C. A mixture of 4 nucleotides (A,G,C, and T) that will be polymerized into the replicated DNA sequence.

D. A short DNA sequence that allows the enzyme to bind and initiate polymerization.

Questions

1. What words do the letters in “PCR” represent?

   _____

2. What does “**A**ll **T**igers **C**an **G**row” represent?

   _____

   _____

3. After isolating the plants genomic DNA, why is it necessary to do PCR?

   _____

   _____

   _____

   _____

4. What is the function of the DNA polymerase enzyme?

   _____

   _____

   _____

   _____

5. * Bonus Question *

   Circle the section on the DNA template where the example primer would bind.

3’ 5’
ATTGCGCTAACGTCAGTCGATGGCTCGGAACTCGTTCGTCGATCTTCCTGCTATTCAT

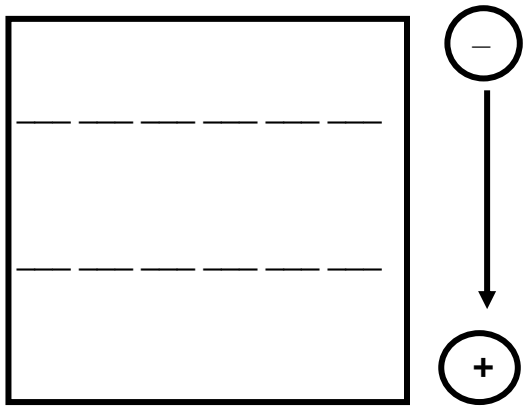
Primer: CGATT
5’ 3’

Materials & Method (cont.)

Part 3 Gel Electrophoresis

1. Pipette _____ µl of **gel loading buffer** into your PCR product. Stir with tip.
2. Pipette _____ µl of your **sample** (gel loading buffer + PCR product) into the specified well on the gel.
3. Record, on the gel diagram, where each sample and marker is loaded.

Gel electrophoresis box diagram



Key
Label the gel diagram (left) with the following markers and number:

X	Empty well
M	Size marker
#	Sample number

Questions

View the Gel Electrophoresis Virtual Lab (http://learn.genetics.utah.edu/content/labs/gel/), and answer the following questions:

1. After replicating sections of plant DNA using PCR, what is the purpose of performing gel electrophoresis?

_____

_____

2. What do you load into the well of the gel?

_____

_____

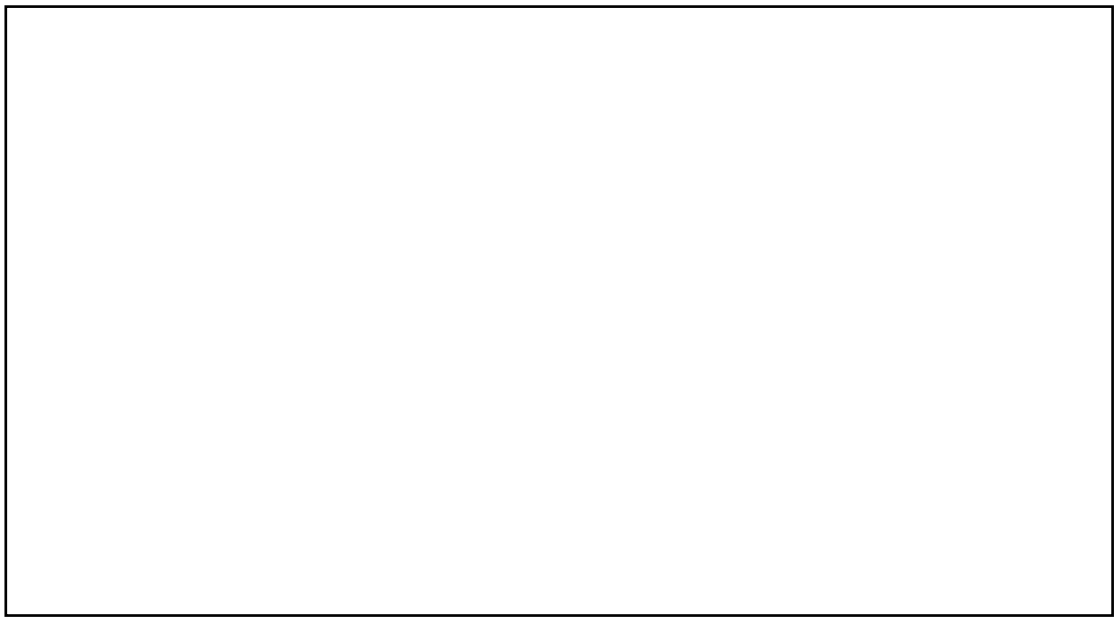
3. What causes the DNA fragments to migrate through the gel?

_____

_____

Results

Attach a photo of your gel in the box below. Label each lane with the name of the plant and marker.



1. Do any of the plants have the same banding patterns? If so, which plants?

_____

_____

2. Which plants have *some* matching bands?

_____

_____

3. Do any plants have no bands in common?

_____

_____

Conclusion

Based on your analysis of plant genotypes (PCR and gel experiments)…

1. Which plant is most closely related to broccoli? Why?

_____

_____

_____

_____

2. Which plants do not seem to be related to broccoli? Why?

_____

_____

_____

_____

Revisiting Your Hypothesis

Review your hypothesis (pg. 4) predicting the closest relative of broccoli based on phenotypic information. Given what you know now from your genotypic analysis, was it correct or incorrect? Why?

_____

_____

_____

_____

_____

_____

_____

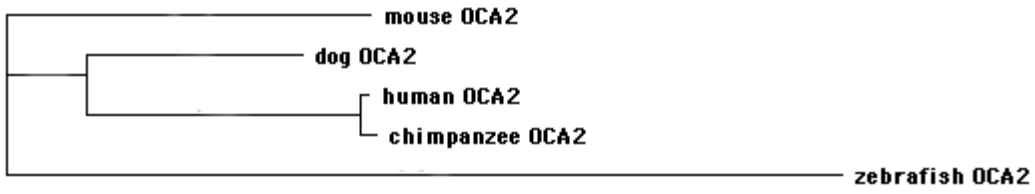
_____

Conclusion (cont.)

Draw a **phylogenetic tree** in the box below to show what you believe now to be the relatedness of the plants.

The length of each horizontal line shows the degree of similarity (short line means very similar).

Example:



Phylogenetic Tree

Glossary

Alleles: alternative versions of a gene. Diploids have two alleles for every gene, one inherited from mother and one from father. Alleles may be functionally different, but sequences of different alleles within a single species are extremely similar (e.g. nucleotide sequences >99% identical)

Artificial selection: Human intervention in animal or plant reproduction to ensure that certain desirable traits are represented in successive generations

Biotechnology (Biotech): the manipulation of living organisms or their components to produce useful, usually commercial, products

DNA replication: a biological process that occurs in all living organisms yielding copies of their DNA

Gene: a linear segment of DNA containing the information for building a specific protein

Genome: the entirety of an organism's hereditary information encoded in its DNA. All the DNA in a single organism

Genotype: genetic makeup of an organism (genotype determines phenotype)

Microliter (µl): a unit of capacity equal to one millionth of a liter

Mutations: change in the DNA sequence (genotype) that sometimes changes the phenotype of the organism that inherits it

Natural Selection: organisms with phenotypes that are most successful in their environment survive and leave offspring. Sometimes called “survival of the fittest”

Phenotype: outward characteristics of the organism (appearance, behavior, etc.) determined by its genotype

Phylogenetic tree: a branching diagram or "tree" showing the evolutionary relationships among various species based upon similarities and differences in their physical and/or genetic information

Phylogeny: classification of organisms based on evolutionary relatedness