Forensic Science

Group:

Background information

One of the graduate students in the Department of Biology on the campus of the University of Florida went missing. A week later, a crime scene was discovered on a remote part of campus. The body was found wrapped in a blanket and tied up with a vine later identified as a species of *Vitis*. Fiber samples and plant material was collected from the crime scene. Based on the investigation, four suspects were identified as possibly being involved. Each suspect had his/her personal effects searched and collected.

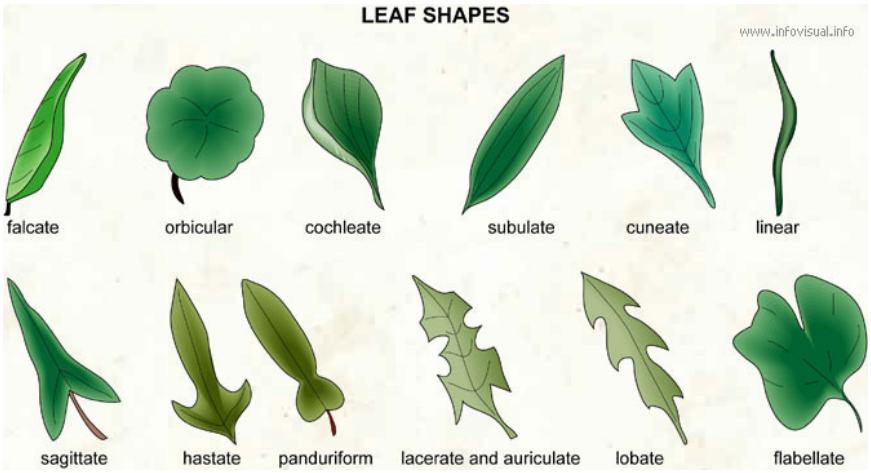
Each group is supplied with collected evidence and charged with trying to find out which suspect(s) is responsible for the murder. To do this, three pieces of evidence will be used: leaf morphology of plants collected, DNA evidence of plant material, and fiber analysis.

Leaf Morphology

In the space provided please describe aspects of the leaf material recovered from the crime scene and the suspect. Some examples of things to focus on:

Comparative leaf size

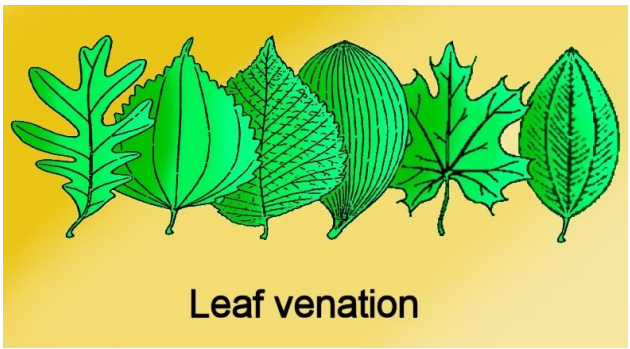
Overall shape



Edge of leaf (smooth versus teeth)

Single leaf or connected

Direction of leaf veins



Anything else that stands out

Describe the leaf material from the Crime Scene
Describe the leaf material from Suspect 1
Describe the leaf material from Suspect 2
Describe the leaf material from Suspect 3
Describe the leaf material from Suspect 4
Do any of the leaves from suspects look like the Crime Scene sample?
Based just on leaf morphology, who do you think committed the crime?

DNA Extraction

1. Rinse forceps and scissors with 70% ethanol prior to use and between handling different samples

2. Cut a roughly 0.5 cm wide piece of leaf material from a leaf, or use a hole punch to obtain a leaf disc. Place into a 2 mL collection tube. Repeat to have a total of 5 extractions from the crime scene and all of the suspects.

3. Add 100 µL of Extraction Solution to the collect tube. Close tube and vortex briefly to mix. Make sure leaf material is covered by solution.

4. Incubate at 95°C for 10 minutes. Leaf tissue will not appear to be degraded.

5. Add 100 µL of Dilution Solution and vortex briefly to mix. Removal of leaf material is not necessary.

PCR

1. Find REDExtract-N-Amp PCR Ready Mix, water, forward primer and reverse primer at your work station.

Which primer pairs does your group have?

2. Each PCR reaction will contain the following quantities:

10 µL REDExtract-N-Amp Ready Mix
2 µL of Forward Primer
2 µL of Reverse Primer
2 µL of water
4 µL of leaf extract
20 µL total reaction

3. Calculate the amount of reagents needed to conduct PCR for the 5 extractions + a negative control (total of 6 reactions).

Reagent	Total amount needed for Master Mix
Ready Mix	
Forward Primer	
Reverse Primer	
Water	

4. Make a master mix for each primer pair that you received by adding the appropriate reagents into a 1.5 eppendorf tube. After each reagent is added, check it off on the list below to ensure each PCR has the appropriate material

Primer Pair _____

- Ready Mix
- Forward Primer
- Reverse Primer
- Water

Primer Pair _____

- Ready Mix
- Forward Primer
- Reverse Primer
- Water

5. Before adding your master mix to the PCR tubes, add in 4 µL of DNA extract into a labeled PCR tube. You will need 2 PCR tubes for each DNA sample, since we are running two different markers for each sample. After all of the DNAs are added, and water for the negative controls, add in your made up master mix. You will need to add 16 µL of this mix to each PCR tube.

6. Mix briefly and then centrifuge to collect all components at the bottom of the tube.

7. Place in thermocycler and run under the following conditions:

Initial denaturation at 95°C for 3 minutes
35 cycles of Denaturation at 95°C for 30 seconds
Annealing at 50°C for 30 seconds
Extension at 72°C for 45 seconds
Final extension at 72°C for 10 minutes
Hold at 4°C indefinitely

Prepare gel

1. Plug PowerBase™ into an electrical outlet.
2. Remove gel cassette from package
3. Insert the gel (with comb in place) into the base right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel cassette in the PowerBase™. A steady, red light will illuminate if the gel cassette is correctly inserted.

Load prepared samples

Well #	1	2	3	4	5	6	7	8	9	10	11	12
What to add to the well	20µl of PCR Crime Scene first primer	20µl of PCR Suspect 1 first primer	20µl of PCR Suspect 2 first primer	20µl of PCR Suspect 3 first primer	20µl of PCR Suspect 4 first primer	Negative control first primer	20µl of PCR Crime Scene second primer	20µl of PCR Suspect 1 second primer	20µl of PCR Suspect 2 second primer	20µl of PCR Suspect 3 second primer	20µl of PCR Suspect 4 second primer	Negative control second primer

1. Remove and discard comb from the E-Gel® cassette.
2. In the first 6 wells, load gel by adding 20 µl of PCR product of their first primer to each well. Product of the second primer will be added in wells 7-12.

Run gel

1. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
2. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping, and unplug the E-Gel® PowerBase™.
3. Remove the gel cassette and analyze your results by viewing on one of the transilluminators.

Gel Results

What do you see when you visualize the gel?
What does the Crime Scene sample look like?
Do any of the suspect samples look similar to the Crime Scene sample? If so, which one(s)?
Based only on the DNA results, who do you think committed the crime?

Hair and Fiber samples

Removing Fibers from Collect Tape

1. Place the collection tape into a beaker with around 400 mL of water. The evidence from this tape was collected from crime scene on the UF campus.

2. Gently agitate the tape to dissolve the adhesive.

3. When the fibers have detached, remove them from the beaker and place on paper towel/kimwipe to dry.

How many samples do you have from the Crime Scene?
How many samples do you have from Suspect 1?
How many samples do you have from Suspect 2?
How many samples do you have from Suspect 3?
How many samples do you have from Suspect 4?

Preparing slides

1. Paint a small amount of casting medium on to the center of the slide.

2. Place a hair or fiber sample into this drop of casting medium. The casting medium will act as a glue to permanently attach the strand to the slide.

3. Carefully place a cover slip over the strand and medium. Press gently on the slip to ensure the coverslip seals completely.

4. Label the slide with the appropriate person it came from (crime scene or which suspect).

5. Repeat until you have all hair and fiber samples prepared.

6. To visualize the slide, place onto microscope stage. Adjust and focus the scope to examine the mounted stranded. **MAKE SURE TO START OUT AT 4X, GET THE MATERIAL INTO FOCUS, THEN MOVE UP TO A HIGHER MAGNFICATION IF NECESSARY**.

7. Record notable characters of each sample such as:

Is the sample hair or fiber?

Color?

Shape?

Describe samples from crime scene.
Describe samples from Suspect 1.
Describe samples from Suspect 2.
Describe samples from Suspect 3.
Describe samples from Suspect 4.
Do any of the samples from the suspect match those found at the crime scene?
Based just on the fibers, who do you think committed the crime?

Overall results

Based solely on leaf morphology, who is the likely criminal?
Based solely on DNA, who is the likely criminal?
Based solely on the fiber analysis, who is the likely criminal?
Do all three pieces of evidence tell the same story? If not, how do they differ?
Based on all of the data combined, which suspect (if any) would your group charge with the crime?