Forensic Botany in the High School Classroom: Real-World Application of Molecular Techniques

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Motivation for module

In the Introductory Biology Classroom most students say they want to go into the medical field

Motivation for module

Many don't know what other options exist with a Biology degree outside of the medical field

Goal was to showcase some options




Question to the Audience

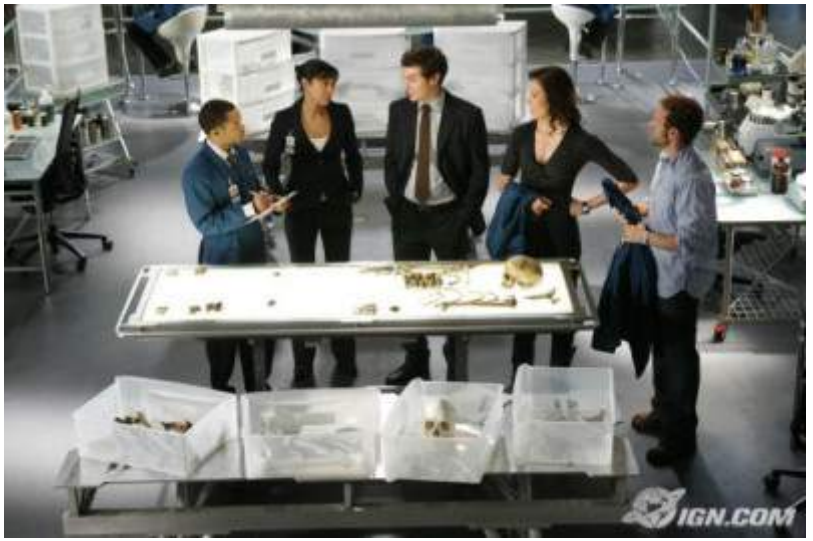
Has anyone else implemented a forensic module in the classroom? What did you do?

Goals

- Hands on exposure to common lab techniques
  - DNA extraction, PCR, gel electrophoresis, microscope work
- Team work
  - Work in groups of 4 to complete tasks
- Communication with scientists
  - Group leaders of graduate students and undergraduate students in the research lab

Real life set up

- Comparison of crime dramas to real life
  - CSI, Bones, Law and Order
- Ideas behind module came from an actual case I participated with



The crime

One of the students in the lab went missing

The crime

- A week later he was found in a remote area of campus, wrapped in blanket and tied up with *Vitis* vines

After the crime

- Four suspects were identified
  - Detained and personal belongings collected
- Coroner's report
  - Evidence of high levels of stress
  - Considerable wear on hands and wrist, most likely from extreme pipetting and typing on a computer
  - Evidence of frost bite on fingers, possibly from holding too many cold items

Suspect 1

Suspect 2

Do samples match any of the suspects?

Suspect 3

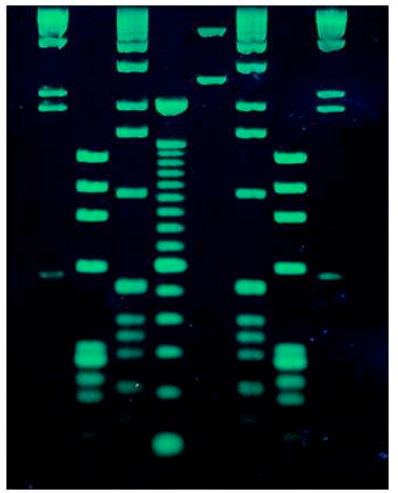
Suspect 4

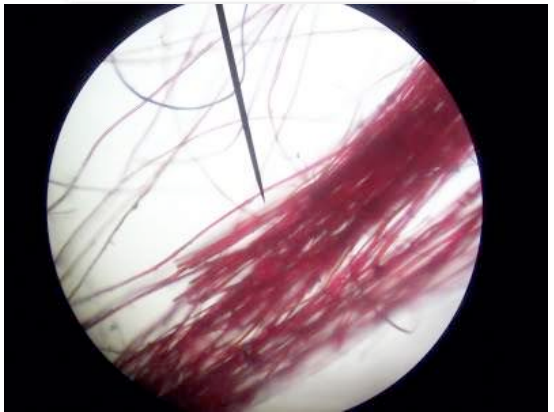
CSI Gainesville

THIS COULD BE YOU.

Evidence

- Leaf samples
  - Comparative morphology
- DNA markers
  - Four different markers
- Thread samples
  - Hair and fiber samples

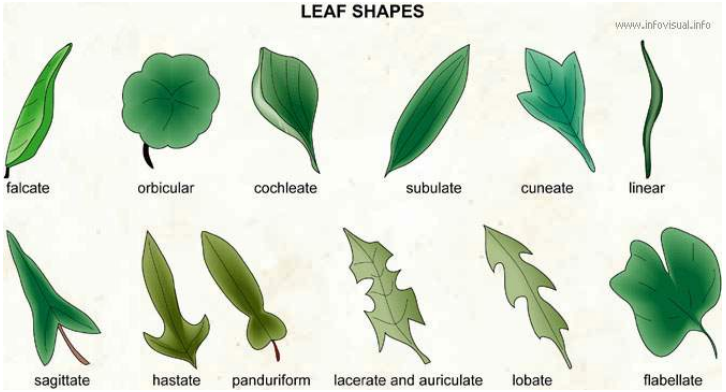


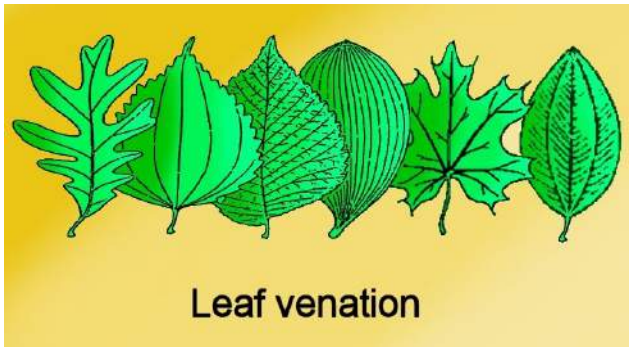


Leaf morphology

Comparative size

Leaf shape





Edges

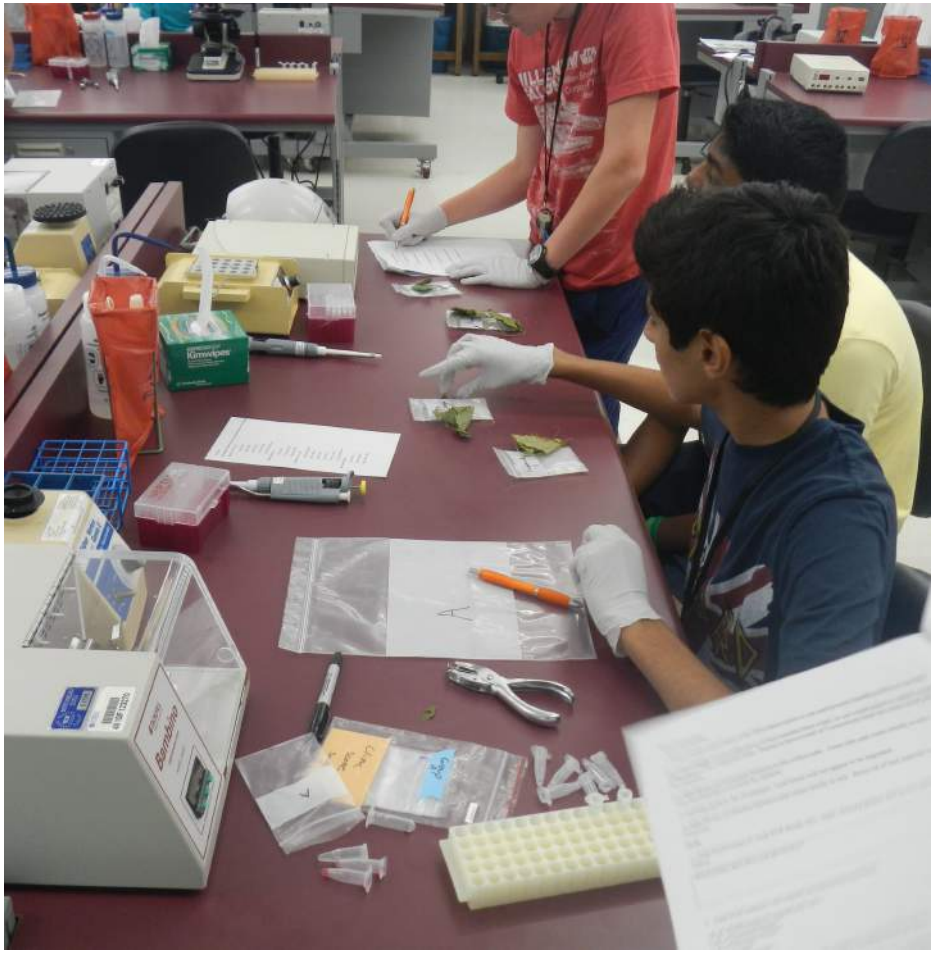
Groupings

Leaf Morphology

- Describe 4 suspects, and compare the crime scene to other samples
- Can your group pick a likely culprit?

Describe the leaf material from the Crime Scene
Describe the leaf material from Suspect 1
Describe the leaf material from Suspect 2

Student Involvement

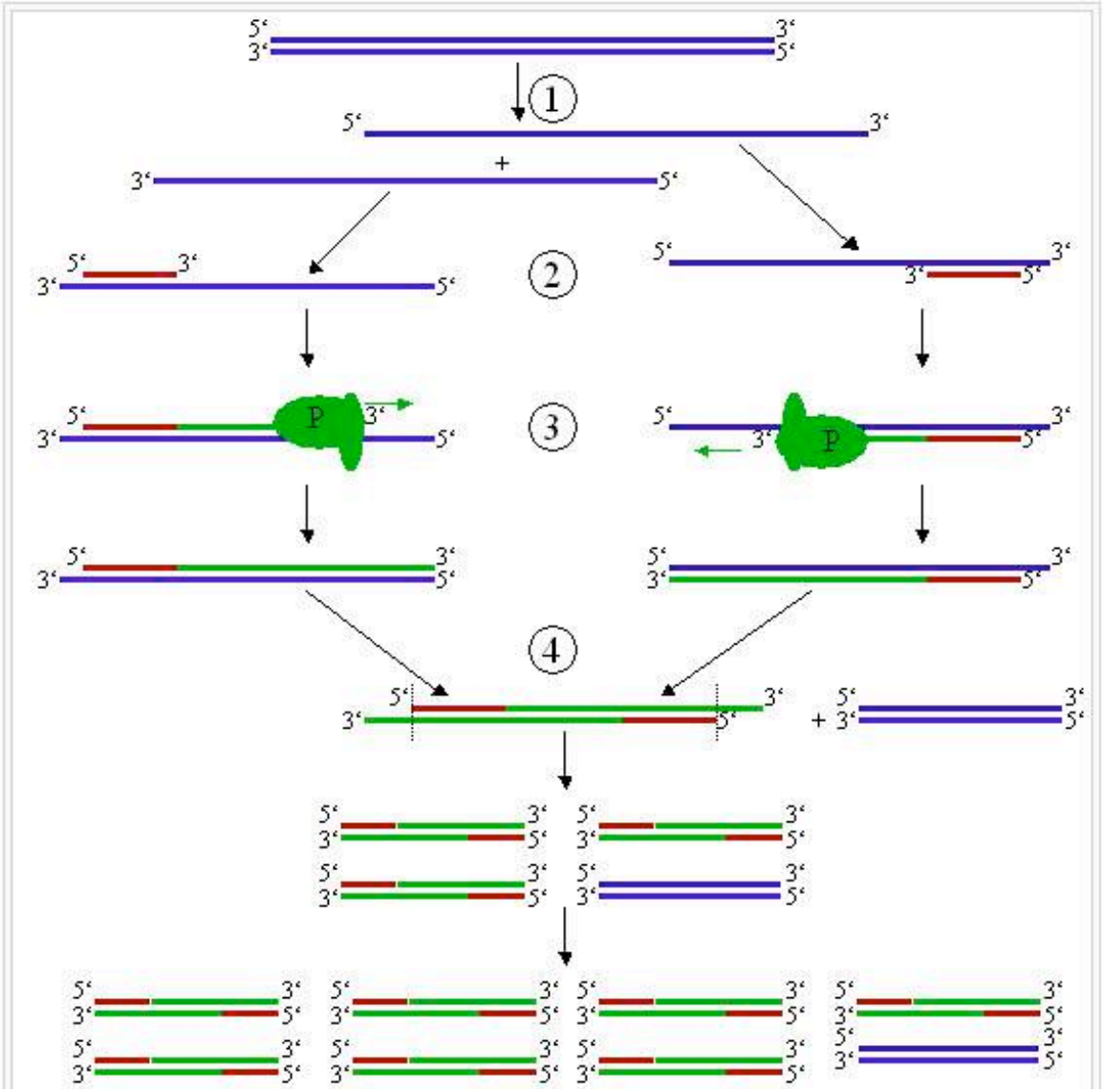


DNA extractions

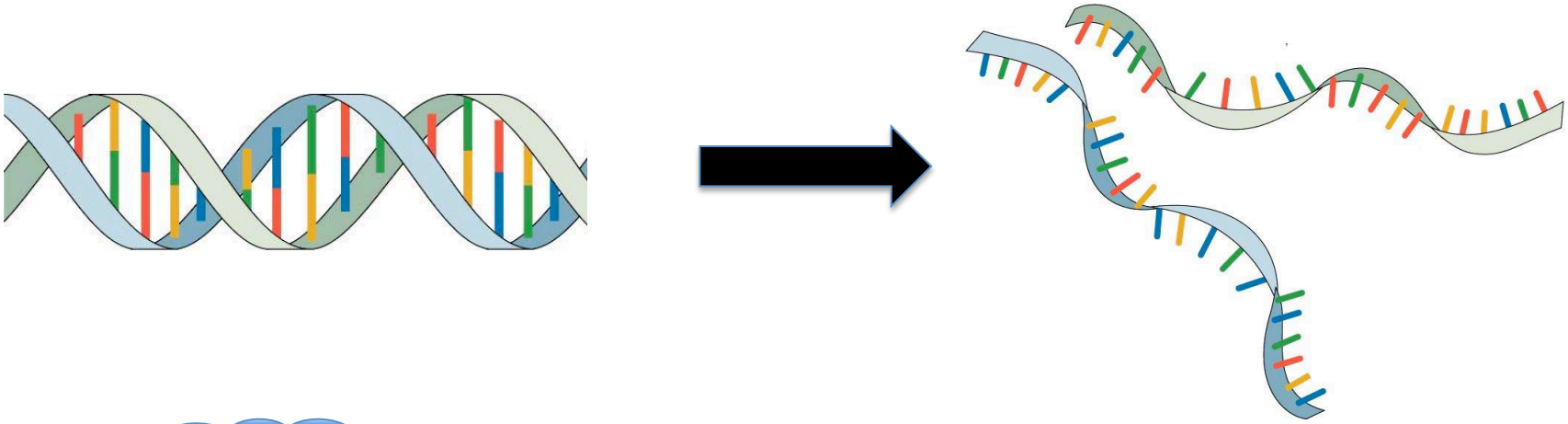
- Label tubes
- Take hole punch of leaf material
- Extraction solution
- Cook at 95°C for 10 minutes
- Dilution solution
- Sigma Extract-N-Amp Kits

PCR

- Polymerase Chain Reaction
- Amplify small section of DNA, makes many many copies
- Will use common markers



How PCR works



Melting

Annealing

Extending

PCR steps

- Follow along with the protocol to conduct PCR with 2 separate genetic markers

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	

PCR steps continuted

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 _____

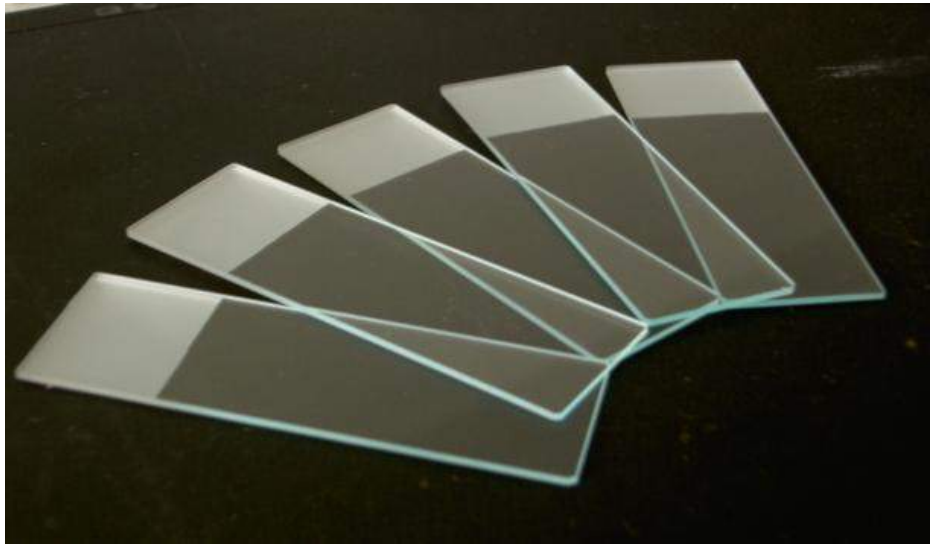
- Ready Mix
- Forward Primer
- Reverse Primer
- Water

PCR specifics

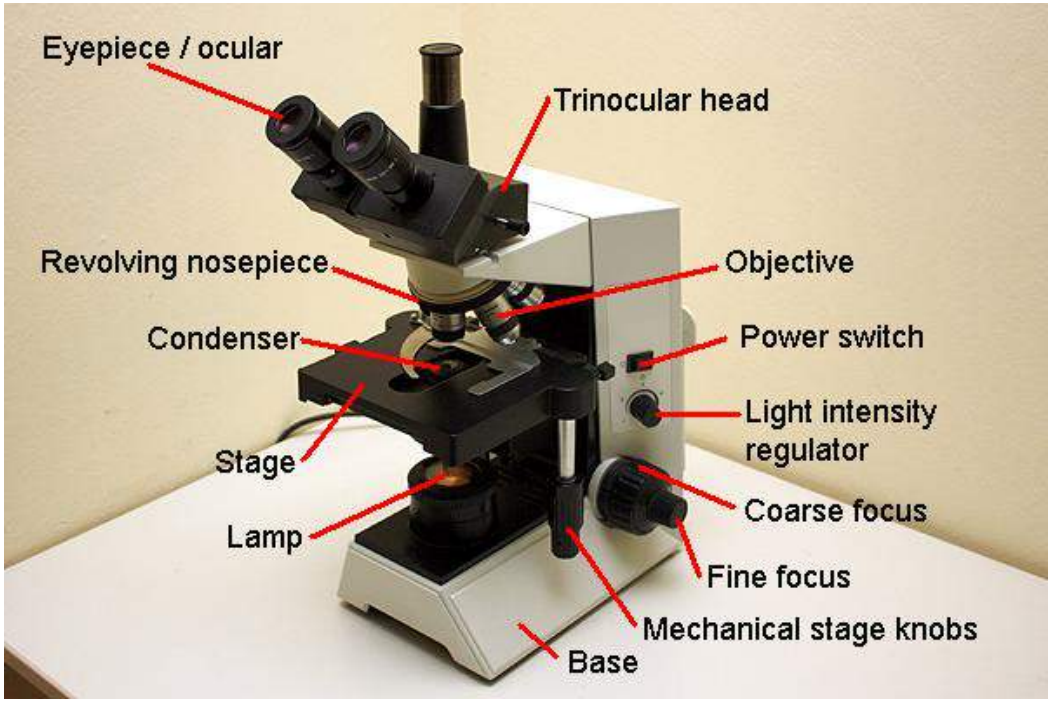
- Primers used were microsatellite markers published for grapevine
  - Were specific to actual case the module was based on
- Many options here, will talk more about that later on

Fiber and Hair samples

- Each suspect should have one or two samples, plus there are samples from the crime scene
  - We used Fisher Scientific Lyle and Louise: Strands of Evidence Hair and Fiber Analysis (Cat#S94586)



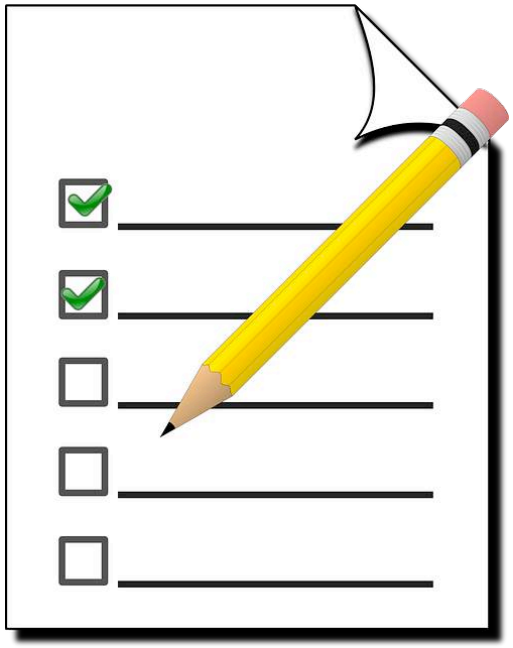
How many samples do you have from the Crime Scene?
How many samples do you have from Suspect 1?
How many samples do you have from Suspect 2?
How many samples do you have from Suspect 3?
How many samples do you have from Suspect 4?



Student Participation

Morning wrap up

- DNA extractions
- PCR up and running
- Leaf morphology identified and recorded
- Slides mounted and recorded



Afternoon activities

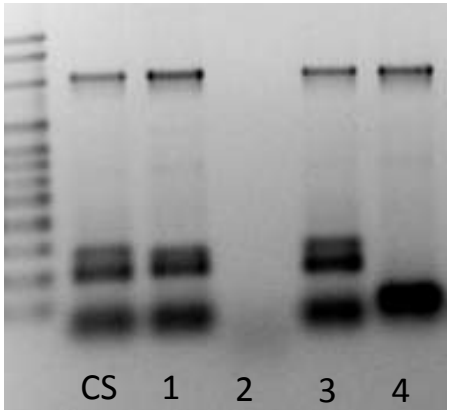
- Running gels
- Comparing bands
- Talk about real world projects and options that research can offer

Running gels or gel electrophoresis

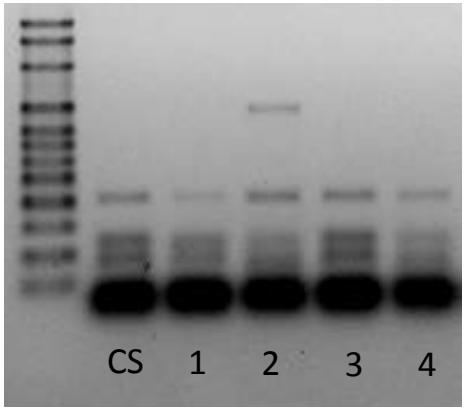
Student participation

Results you may have seen

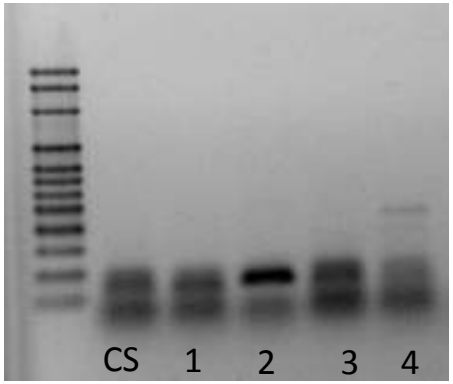
Primer 1



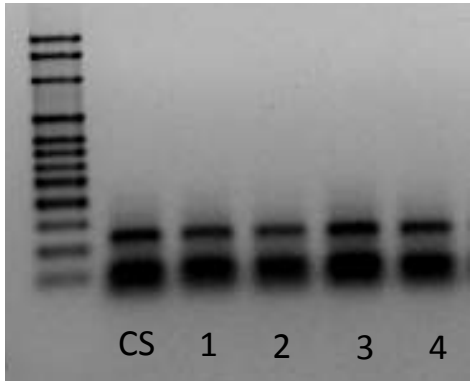
Primer 2



Primer 3



Primer 4



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?

Who did it and your support?

Group A:

Group B:

Group C:

Group D:

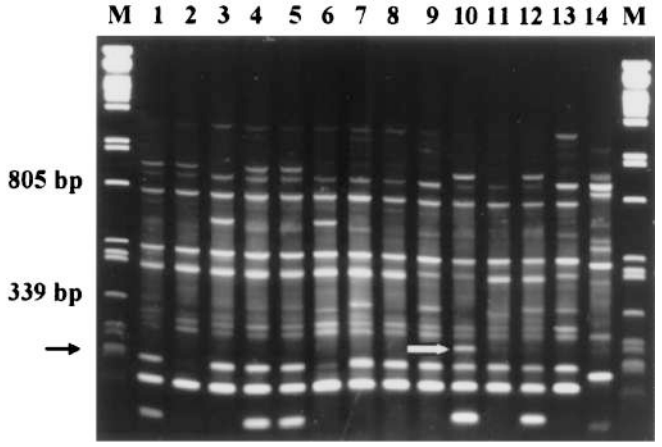
Group E:

Group F:

Variations

- Plant material could be any plants you find outside or at a nursery

- Primers for other genes can be utilized from NCBI, also use of restriction enzymes for distinct bands



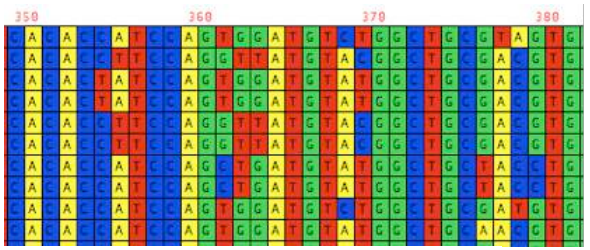
Variations

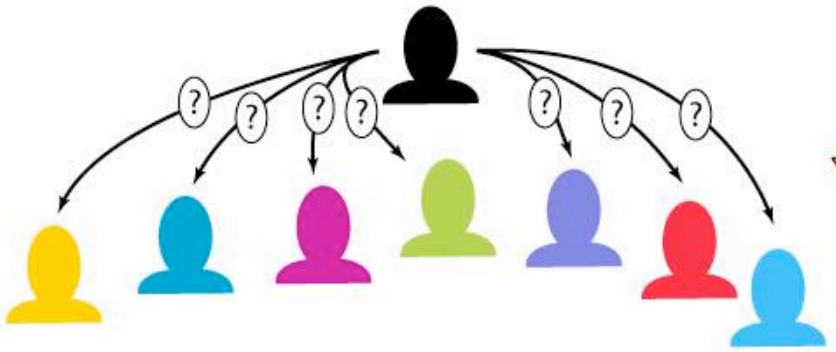
- Fibers/hair samples could be from students/specific suspects

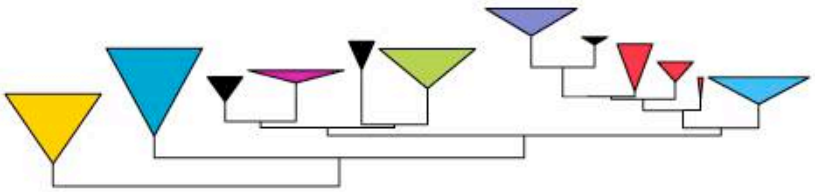
- Black light/UV light to look at pollen from different plants

Variations continued

- Could use ITS or other universal primers, and simulate DNA sequencing to incorporate bioinformatics
  - Specific programs talked about in next presentation



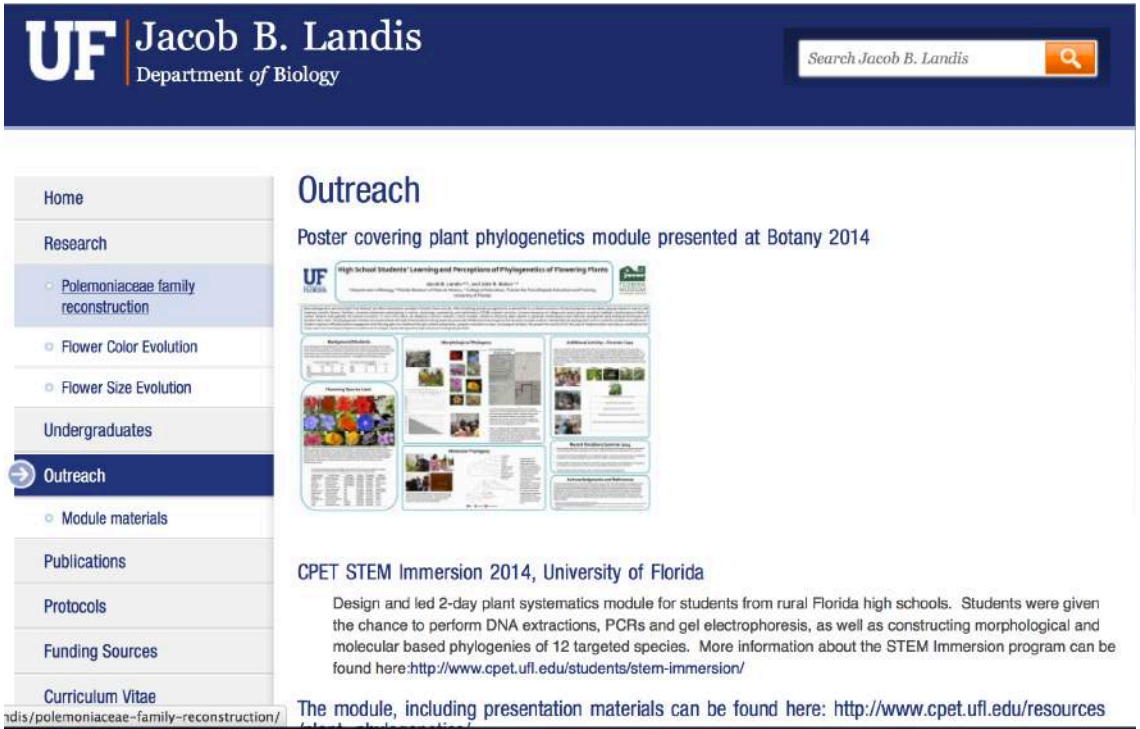




Module materials

Example presentations and handouts found on my University of Florida Website

http://people.clas.ufl.edu/jblandis/module-materials/



Acknowldegements

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- Members of the Soltis lab for helping with the module implementation
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Questions

Would be happy to help modify this for the classroom

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