PLANT MORPHOLOGY AND DNA EXTRACTION

1. Select four plants for morphological and genetic analysis. Record their names below.

Plant Number	Plant Name	Description of Plant
1		
2		
3		
4		

2. Observe and describe your four plants. As a group, identify floral characters you can use in order to group them by similarity. Which plants are most similar and why? Draw and describe below. Do you think the morphological relationships you describe also indicate genetic relatedness?

3. Label four 2.0mL collection tubes with your group identifier and the plant id number.
4. To remove tissue samples, you will use either a single hole punch or forceps and scissors. Rinse these items with 70% ethanol prior to use and between handling different samples.
5. Cut or punch a 0.5cm piece of leaf material from a small, young leaf. Place into the appropriately labeled 2.0mL collection tube.
6. Repeat steps 3 and 4 for each plant to have a total of four extractions from different species.
7. Add **100µL of Extraction Solution** to each collection tube. Close tubes and flick briefly to mix. Make sure leaf material is covered by solution.
8. Incubate all samples at 95°C for 10 minutes. (There will be little change in the appearance of the leaf tissue.)
9. Add **100µL of Dilution Solution** and flick briefly to mix. You do not need to remove the leaf tissue.
10. Use sample immediately to prepare PCR or place at 4°C until ready for use.

PCR

1. Identify the following reagents at your student workstation, or collect from a common station:

Tube Label	Contents	X
PCR Mix	REDExtract-N-Amp PCR Ready Mix (Contains $MgCl_2$, dNTPs, Taq Polymerase)	
Water	Sterile water	
Forward	Forward primer	
Reverse	Reverse primer	
Positive	Positive control	
1-4	Your plant DNA samples from the previous procedure	

2. Label six 0.2mL PCR tubes: 1-4, +, and -. Include your group identifier on each as well.
3. Prepare your PCR master mix in a clean 1.5mL microcentrifuge tube using the chart below. You will perform 6 PCR reactions (4 leaf extraction DNA samples, positive control, and negative control). To ensure you have enough PCR master mix, you will prepare for 7 reactions. Be sure to change your tip between each reagent.

1 PCR Reaction	7 PCR Reactions	X
10µL REDExtract-N-Amp Ready Mix		
2µL of Forward Primer		
2µL of Reverse Primer		
2µL of Water		
16µL Master mix per reaction		

4. Mix the PCR master mix by flicking briefly. Tap tube or centrifuge to bring all contents to the bottom.
5. Add 16µL PCR master mix to each of the six PCR tubes.
6. Add 4µL DNA to the appropriately labeled PCR tube.
7. Mix briefly by flicking tube and use the mini-centrifuge to collect all components at the bottom of the tubes.
8. Place all six PCR reactions in the thermocycler programmed with the following conditions:

Step 1: Initial denaturation at 95°C for 3 minutes
Step 2: Denaturation at 95°C for 1 minute
Step 3: Annealing at 52°C for 1 minute
Step 4: Extension at 72°C for 1.5 minutes
Go to step 2; repeat 34 times
Step 5: Final extension at 72°C for 10 minutes
Step 6: Hold at 4°C indefinitely

GEL ELECTROPHORESIS

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

1. Remove and discard comb from the E-Gel® cassette.
2. In the first 6 wells, add 10µl water.
3. In wells 7-12, add 20µl of water.
4. Then, in wells 1-6, add 10µl of PCR product according to the chart below. Be certain to change your pipet tip between each sample.

Well #	1	2	3	4	5	6	7	8	9	10	11	12
What to add to the well	10µl water + 10µl PCR Pos	10µl water + 10µl PCR Neg	10µl water + 10µl PCR Sample 1	10µl water + 10µl PCR Sample 2	10µl water + 10µl PCR Sample 3	10µl water + 10µl PCR Sample 4	20µl water	20µl water	20µl water	20µl water	20µl water	20µl water

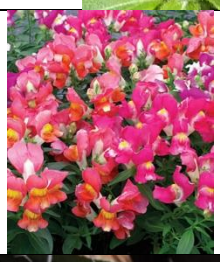
Run gel

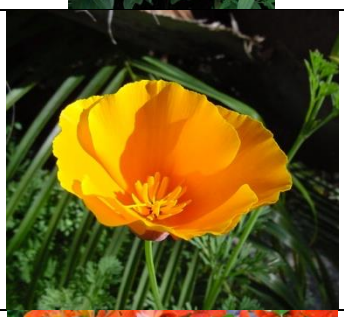
1. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
2. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping, and unplug the E-Gel® PowerBase™.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

Flowers used in module

<table>
<tr><td colspan="2">  </td><td><u>Hummingbird pollinated flowers</u></td></tr>
<tr><td>Maltese Cross</td><td>  </td><td rowspan="3">Describe at least three characteristics these plants share.

Now consider the hummingbird. What characteristics does it posses that allows it to utilize these flowers?</td></tr>
<tr><td>Cardinal climber</td><td>  </td></tr>
<tr><td>Petunia</td><td>  </td></tr>
</table>

<table>
<tr><td colspan="3">Bee pollinated flowers  </td></tr>
<tr><td>Snapdragon</td><td>  </td><td rowspan="4">Describe at least three characteristics these plants share.

Now consider the bee. What characteristics does it posses that allows it to utilize these flowers?</td></tr>
<tr><td>California Poppy</td><td>  </td></tr>
<tr><td>Dwarf, Empress of India</td><td>  </td></tr>
<tr><td>Blue Daze</td><td>  </td></tr>
</table>

Butterfly pollinated flowers

Pentas		Describe at least three characteristics these plants share.
Lantana		Now consider the butterfly. What characteristics does it posses that allows it to utilize these flowers?
Vinca		

Based on the characteristics you have identified above, can you predict which organism(s) pollinate these flowers? What evidence supports your prediction?

Unknown

1) Blue Flax

2) Nicotiana

Characteristics Chart

Complete the following table of characteristics for your plants by coding 0 (absence) or 1 (presence) for the listed characters. Visit other groups until you have observed and recorded characteristics for all species.

Species	Character 1 Color (Red)	Character 2 Color (Blue)	Character 3 Flower size	Character 4 Tubular flower	Character 5 Symmetry	Character 6 Petal lobes fused	Character 7 Stamens exserted	Character 8 Horizontal Orientation	Character 9 Individual flowers	Character 10 Scent	# of traits
	0= not red 1=red	0=not blue 1=blue	0=small 1=large	0=not tubular 1=tubular	0=radial (wheel) 1=bilateral (mirror images)	0=not fused 1=fused	0=not exserted past petals 1=exserted	0=not horizontal flowers =horizontal flower	0=flower clusters 1=individual flowers	0=no scent 1=scent	
Maltese Cross											
Cardinal climber											
Petunia											
Snapdragon											
California poppy											
Empress of India											
Blue Daze											
Pentas											
Lantana											
Vinca											
Blue Flax											
Nicotiana											

Distance Matrix

Use the information from your table of characteristics above to record the number of differences between each species.

Species	Maltese Cross	Cardinal climber	Petunia	Snapdragon	California poppy	Empress of India	Blue Daze	Pentas	Lantana	Vinca	Blue Flax	Nicotiana
Maltese Cross	X											
Cardinal climber		X										
Petunia			X									
Snapdragon				X								
California poppy					X							
Empress of India						X						
Blue Daze							X					
Pentas								X				
Lantana									X			
Vinca										X		
Blue Flax											X	
Nicotiana												X