Experiment Overview

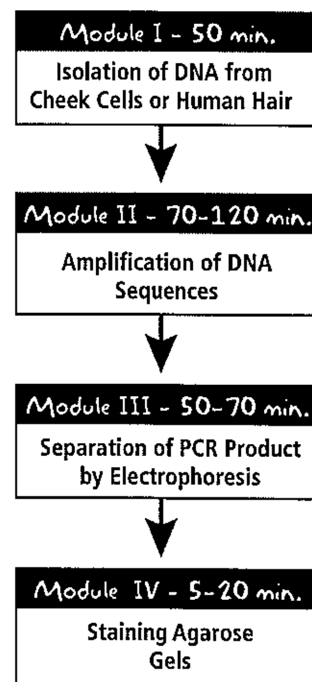
EXPERIMENT OBJECTIVE:

In this experiment, students will isolate their DNA and use the Polymerase Chain Reaction (PCR) with unique primers to amplify three different regions of their genome. Results are analyzed using agarose gel electrophoresis.

LABORATORY SAFETY:

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.
- Contaminated laboratory waste (saliva solution, cup, pipet, etc.) must be disinfected with 15% bleach solution prior to disposal. Be sure to properly dispose any biological samples according to your institutional guidelines.



NOTE: Experimental times are approximate.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

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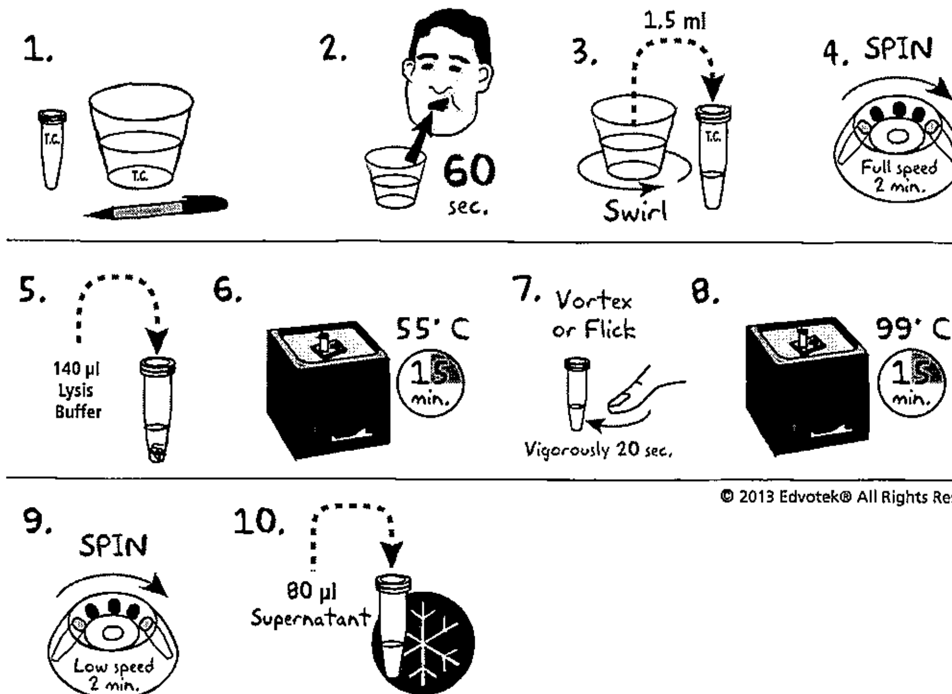
Module I-A: Isolation of DNA from Human Cheek Cells

Preferred Method



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Warning!
Students should use screw-cap tubes when boiling samples.

1. **LABEL** a 1.5 ml screw top microcentrifuge tube and a cup with your lab group and/or initials.
2. **RINSE** your mouth vigorously for 60 seconds using 10 ml saline solution. **EXPEL** the solution into cup.
3. **SWIRL** the cup gently to resuspend the cells. **TRANSFER** 1.5 ml of solution into the labeled tube.
4. **CENTRIFUGE** the cell suspension for 2 min. at full speed to pellet the cells. **POUR** off the supernatant, but **DO NOT DISTURB THE CELL PELLET**! Repeat steps 3 and 4 twice more.
5. **RESUSPEND** the cheek cells in 140 µl lysis buffer by pipetting up and down or by vortexing vigorously.
6. **CAP** the tube and **PLACE** in a waterbath float. **INCUBATE** the sample in a 55° C waterbath for 15 min.
7. **MIX** the sample by vortexing or flicking the tube vigorously for 20 seconds.
8. **INCUBATE** the sample in a 99° C waterbath for 15 min. Be sure to use screw-cap tubes when boiling DNA isolation samples.
9. **CENTRIFUGE** the cellular lysate for 2 minutes at low speed (6000 rpm).
10. **TRANSFER** 80 µl of the supernatant to a clean, labeled microcentrifuge tube. **PLACE** tube in ice.
11. **PROCEED** to Module II: Amplification of DNA Sequences.

STEP 4:
If cell pellet size is not large enough, repeat steps 3 - 4 until you have a large size pellet. For best results, make sure your cell pellet is at least the size of a match head.

STEP 7:
If a vortex is not available, mix samples by flicking the tube vigorously for 20 seconds.

OPTIONAL STOPPING POINT:
The extracted DNA may be stored at -20°C for amplification at a later time.

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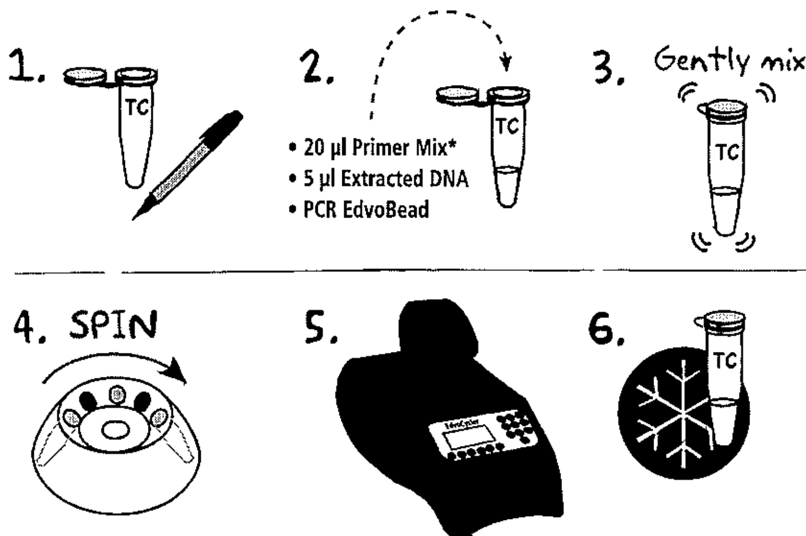
Module II: Amplification of DNA Sequences



NOTES AND REMINDERS:

This kit includes enough DNA for 5 control reactions. At least one control reaction should be performed per class to confirm that PCR was successful.

If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with wax to prevent evaporation. See Appendix B for guidelines.

*Add one of the following primer solutions: D1S80, PV92, or Mitochondrial

1. **LABEL** a 0.2 ml PCR tube with the sample and your initials.

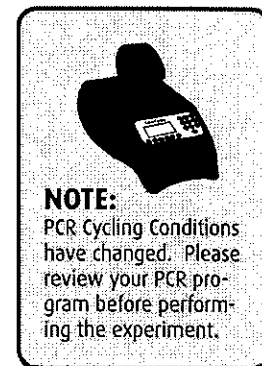
2. **ADD** 20 µl Primer Mix (D1S80, PV92, or Mitochondrial), 5 µl extracted DNA (or control DNA) and the PCR EdvoBead™ to the labeled 0.2 ml tube. At least one control reaction should be performed per class to confirm that PCR was successful.

3. **MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved.

4. **CENTRIFUGE** the sample for a few seconds to collect the sample at the bottom of the tube.

5. **AMPLIFY** DNA using the following PCR cycling conditions:

	D1S80	PV92	Mitochondrial
Initial Denaturation	94° C for 4 minutes		
Denaturation	94° C for 30 sec.	94° C for 30 sec.	94° C for 60 sec.
Annealing	65° C for 30 sec.	65° C for 30 sec.	55° C for 60 sec.
Extension	72° C for 30 sec.	72° C for 60 sec.	72° C for 120 sec.
Number of Cycles	35	32	25
Final Extension	72° C for 4 minutes		



NOTE: PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.

6. After PCR, **ADD** 5 µl of 10x Gel Loading Solution to the sample. **PLACE** tubes on ice. **PROCEED** to Module III: Separation of PCR Products by Electrophoresis.

OPTIONAL STOPPING POINT:
The PCR samples may be stored at -20° C for electrophoresis at a later time.

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(*) Gels will have already been prepared for you - (*)
Remove rubber bumpers & plastic comb. from tray

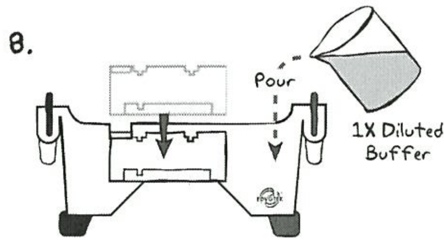
Module III: Separation of PCR Products by Electrophoresis

Includes EDVOTEK's All-NEW EdvoQuick™ DNA Ladder

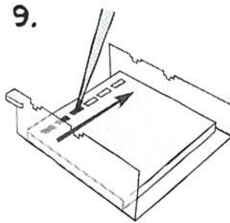
- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes: 2640, 1400, 1100, 700, 600, 400, 200

8.

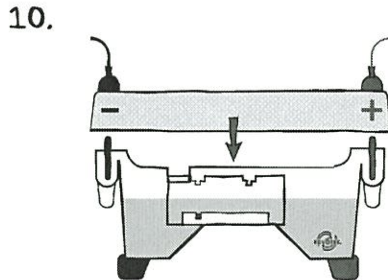


9.

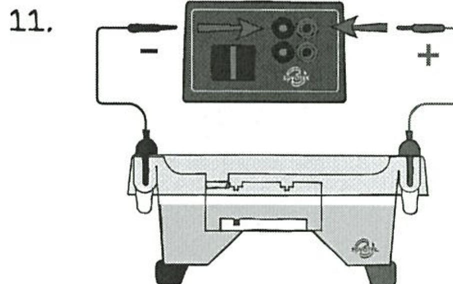


Wear gloves and safety goggles

10.



11.



Reminder:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** the entire volume (30 µl) into the well in the order indicated by Table 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

Table 1: Sample Table

Lane	Recommended	Sample Name
1	EdvoQuick™ DNA Ladder	
2	Control DNA*	
3	Student #1	
4	Student #2	
5	Student #3	
6	Student #4	

** Optional, or additional student sample.*

Table B: 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table C: Time and Voltage Guidelines (1.5% - 7 x 14 cm Agarose Gel)

Volts	Recommended Time: Minimum	Maximum
150	45 min.	60 min.
125	55 min.	1 hour 15 min.
70	2 hours 15 min.	3 hours

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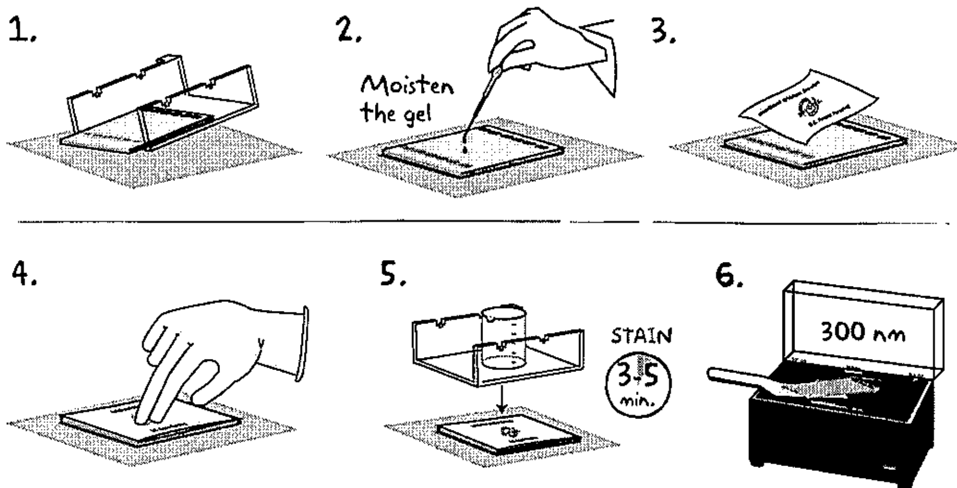
Module IV-A: Staining Agarose Gels with InstaStain® Ethidium Bromide

Preferred Method



1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface. **DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.**
2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, **REMOVE** and **DISCARD** the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). **PLACE** the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 2 cards to stain a 7 x 14 cm gel.
4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for 3-5 minutes.
6. **REMOVE** the InstaStain® Ethidium Bromide card(s). **VISUALIZE** the gel using a mid-range ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

Wear gloves and UV safety goggles

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!

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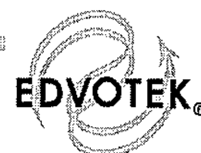

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

VNTR HUMAN DNA TYPING

1. Compare your D1S80 PCR product with those of the rest of the class. Did any students have genotypes similar to yours? How could you explain such similarities?
2. What is polymorphic DNA? How is it used for identification purposes?
3. What is CODIS? How is it used to solve crimes?
4. What is an STR? A VNTR? Which (STR or VNTR) is predominantly used in law enforcement? Why?

ALU-HUMAN DNA TYPING

1. Compare your Alu genotype with those of your classmates. Did anyone else have a similar result? If so, what are some possible explanations?
2. What is "selfish DNA"? How are Alu elements thought to replicate? What is the function(s) of Alu elements?
3. Could dimorphic Alu elements be used for DNA identification (i.e., in criminal investigations)? Why or why not?

MITOCHONDRIAL DNA ANALYSIS

1. What are the three energy-producing sets of chemical reactions that take place inside the mitochondrion?
2. How are mitochondria different from other organelles inside the cell?
3. Is it possible for a child to be healthy if his/her father is affected with a mitochondrial disease? From an unaffected mother? Why or why not? What might be some symptoms of such a disease?
4. If a crime scene sample is too degraded for normal DNA profiling, are any further analyses possible? If so, what assay(s) could be performed?

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