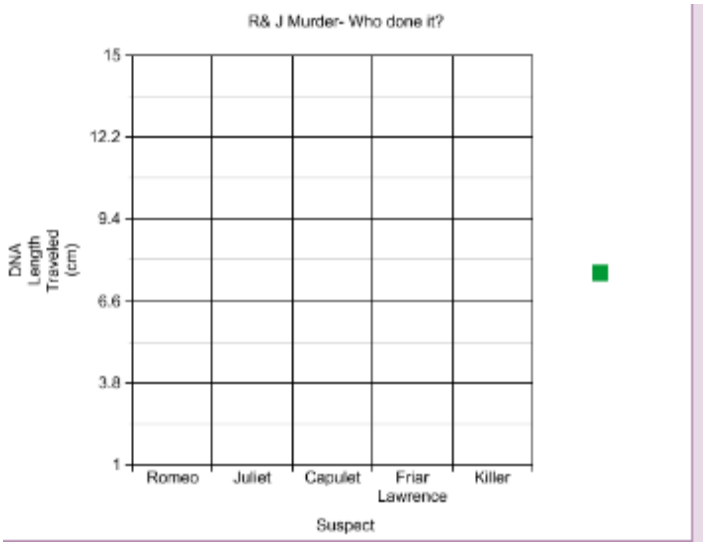


Suspect	Romeo	Juliet	Paris	Capulet	Montague	Lady Capulet	Friar John	Friar Lawrence
DNA Length (cm)								
Difference in length of DNA to killer (cm)								Same DNA length
Killer?	No	No	No	No	No	No	No	Yes

and Juliet DNA Lab

Neto's Annotated Instructions

Our Suspects:

Materials:

Procedures:

Pictures:

Purpose: The purpose of this lab is to determine which suspect's DNA matches that of the killer's.

Hypothesis: If Friar Laurence was the killer, then his DNA will travel the same length in the gel chamber as the killer's DNA, because their DNA is identical.

Materials:

- 2 teaspoons (10 ml) 0.9 percent salt water (2 teaspoons table salt in one quart/liter of water)
- disposable plastic cup
- large test tube (or any clear tube that can be sealed with a rubber or cork stopper) and (1 for each DNA sample you will collect)
- 1 teaspoon (5 ml) 25 percent mild detergent or dishwashing soap, e.g., Woolite or
- Palmolive (1 volume detergent or soap + 3 volumes water)
- 2 teaspoons (10 ml) 95 percent ethanol, chilled on ice
- small test tube (1 for each DNA sample you will collect)
- 5 µl methylene blue solution for each DNA sample (Neto pre-made 1 L of water to 2ml of methylene blue)
- Pipette and disposable pipette tips
- Baking soda
- Deionized water
- Glycerin
- Cold Ethanol 95% (Ask Neto when ready, it's in the freezer)

Identify the Independent and Dependent Variable:

- X: The suspect whose DNA is being tested
- Y: Which DNA matches that of the killer's

Procedure:

Constructing Gel Chamber:

1. Cut two 10 inch steel wires and fold them over a plastic container on one side. Position the two wires on opposite ends of the container, having them facing the same direction.
2. Cut out a "comb" out of styrofoam, with 8 teeth evenly spaced. There should be at least 2 millimeters of space between the bottom of the teeth and the bottom of the plastic box.
3. Make the buffing solution by combining 4 grams (g) of baking soda with one 400 mL of bottled water in one bowl and stir well.
4. Make a 1% agarose gel solution by combining 2 g of agar powder with 200 mL of buffer solution in a microwave-safe bowl.
5. Heat the agar solution on a hot plate to dissolve the powder. Remove from heat every 10-15 seconds to stir the solution.

6. When the solution is starting to bubble, remove it from the microwave. The solution should be translucent. Make sure to watch the agar solution carefully and remove it promptly from the microwave (or hot plate); when it gets hot it will easily bubble over.
7. Remove the steel wires from the chamber.
8. Insert the leaving Styrofoam comb into either end of the gel chamber, oximately 0.5 centimeters (cm) between the end of the box and the comb. Gently pour the agar solution into the gel chamber. Add just enough solution to the box so that the comb teeth are submerged approximately 0.5 cm.

Collecting and Extracting DNA:

9. Gather the DNA from each suspect by having them swish salty water in their mouth for one minute, and collect spit in separate cups. The salt will grind away epithelial tissue which contains DNA within.
10. Make a soap and water solution (enough for all 8 DNA samples) Using a large test tube containing 5 ml of liquid detergent to 15 ml of water (25% mild detergent)
11. Take 5 ml of each sample of DNA and place them in their own test tube. Be sure to label each test tube carefully.
12. Add 5 ml of mild detergent to each test tube.
13. Cap tube and gently rock it on its side for 2.5 minutes. The detergent will break open the cell membrane to release the DNA into the soap solution.
14. Open and slightly tilt the tube and pour 5 ml of the chilled 95 percent ethanol down the side of the tube so that it forms a layer on the top of the soapy solution. Do this for each of the 8 samples.
15. Use separate pipettes to grab approximately 0.25 mL of the DNA from the surface, and add it to the containers holding the 95 percent ethanol.
16. Using the pipette and disposable tips, add 0.25 mL methylene blue solution for each DNA sample
17. Add one drop of glycerin to each DNA/methylene blue solution.
18. Make new buffer solution (Same amount as in step 5)
19. Pour new buffer solution over the solidified gel.
20. Gently pull the comb out of the gel. Be sure not to remove the comb until sure that the agarose gel is completely set. The resulting wells will be used as reservoirs for the samples.
21. Using a butter knife, carefully cut a thin slice of the gel from the top and the bottom to make room for the electrodes.
22. Re-attach the stainless steel wire electrodes.
23. Using a micropipette, fill each well in the gel with a each suspects' DNA solution
24. Using the alligator clip leads, attach the battery pack to the wires resting on the gel chamber. The positive terminal of the battery pack should be connected to to the clip farthest away from the DNA samples.
25. Set electricity to 150 V after ensuring that it is properly arranged.
26. Observe bubbles forming around the electrodes in the buffer as the current passes through them.
27. If bubbles do not appear, recheck all electrical connections. Make sure the batteries are properly placed in series, and that the batteries are fresh and fully charged.

28. Record results (possibly use time-lapse.
29. Turn off electricity and clean-up lab.

Our Suspects:

- Friar Laurence
- Lady Capulet
- Montague
- Romeo
- Juliet
- Benvolio
- Capulet

Materials:

- 2 teaspoons (10 ml) 0.9 percent salt water (2 teaspoons table salt in one quart/liter of water)
- disposable plastic cup
- large test tube (or any clear tube that can be sealed with a rubber or cork stopper) and (1 for each DNA sample you will collect)
- 1 teaspoon (5 ml) 25 percent mild detergent or dishwashing soap, e.g., Woolite or
- Palmolive (1 volume detergent or soap + 3 volumes water)
- 2 teaspoons (10 ml) 95 percent ethanol, chilled on ice
- small test tube (1 for each DNA sample you will collect)

- 5 µl methylene blue solution for each DNA sample (Neto pre-made 1 L of water to 2ml of methylene blue)
- Pipette and disposable pipette tips
- Baking soda
- Deionized water
- Glycerin
- Cold Ethanol 95% (Ask Neto when ready, it's in the freezer)

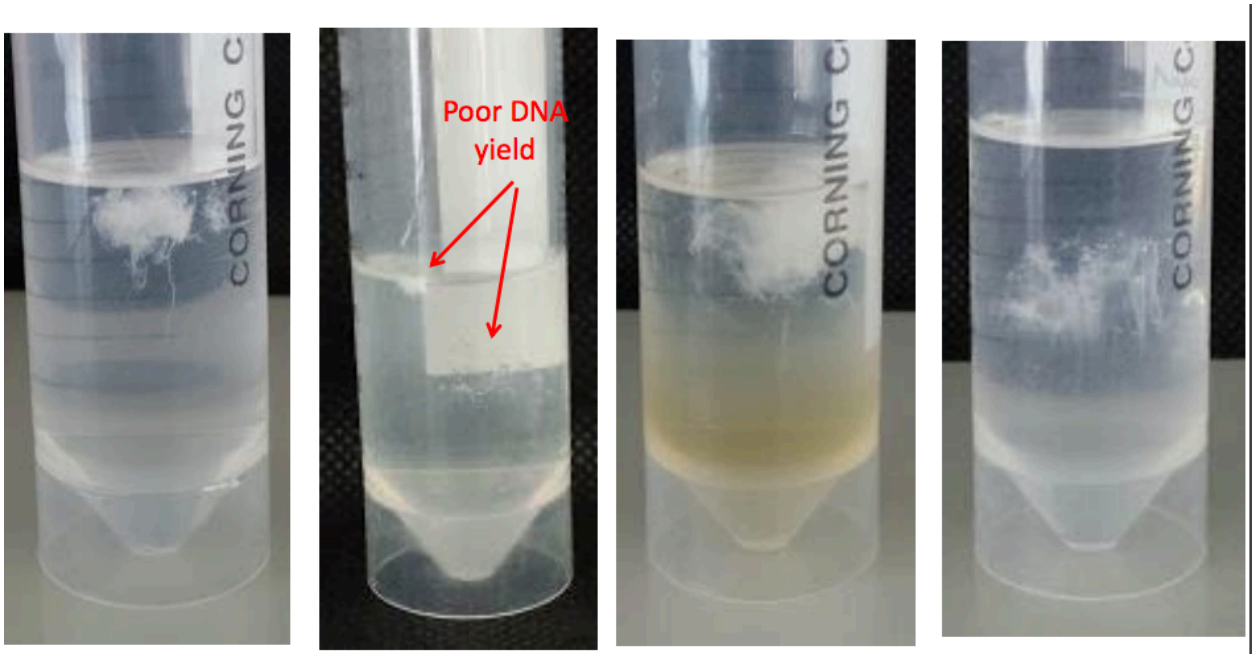
Procedures:

1. Cut two 10 inch steel wires and fold them over a plastic container on one side. Position the two wires on opposite ends of the container, having them facing the same direction. ✓
2. Cut out a “comb” out of styrofoam, with the teeth being evenly spaced. There should be at least 2 millimeters of space between the bottom of the teeth and the bottom of the plastic box. ✓
3. Make the buffing solution by combining 4 grams (g) of baking soda with 1 400 mL of bottled water in one bowl and stir well. ✓
4. Make a 1% agarose gel solution uby combining 2 g of agar powder with 200 mL of your buffer solution in a microwave-safe bowl. ✓
5. Heat the agar solution in a microwave (or hot plate) to dissolve the powder. Stop the microwave every 10-15 seconds to stir the solution. ✓

6. When you see that the solution is starting to bubble, 1 remove it from the microwave. The solution should be translucent. Make sure to watch the agar solution carefully and remove it promptly from the microwave (or hot plate); when it gets hot it will easily bubble over. ✓
7. Remove the steel wires from the chamber. ✓
8. Insert the Styrofoam comb into either end of the gel chamber, leaving approximately 0.5 centimeters (cm) between the end of the box and the comb. Gently pour the agar solution into the gel chamber. Add just enough solution to the box so that the comb teeth are submerged approximately 0.5 cm. ✓
9. Make your soap and water solution (enough for all your DNA samples) Using a large test tube containing 5 ml of liquid detergent to 15 ml of water (25% mild detergent) ✓
10. Take 5 ml of each sample of DNA that you plan on collecting and place them in their own test tube. Be sure to label each test tube carefully. ✓
11. Add 5 ml of your mild detergent to each test tube. ✓
12. Cap tube and gently rock it on its side for 2-3 minutes. The detergent will break open the cell membrane to release the DNA into the soap solution. Do not be too vigorous while mixing! DNA is a very long molecule. Physical abuse can break it into smaller fragments, a process known as shearing. ✓
13. Open and slightly tilt the tube and pour 1 teaspoon (5 ml) of the chilled 95 percent ethanol down the side of the tube so that it forms a layer on the top of your soapy solution.✓

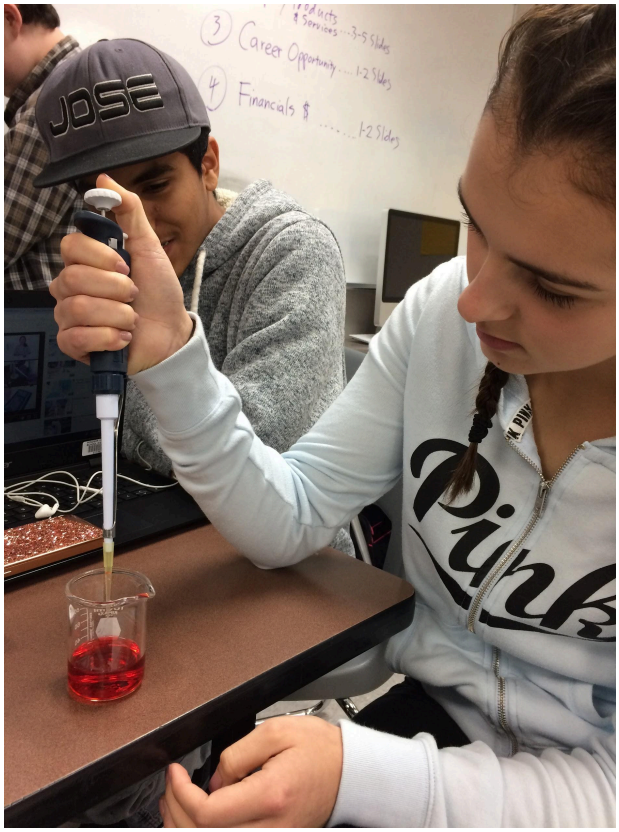
14. Allow tube to stand for 1 minute. Results should look similar to Sample #1.
15. Place a thin acrylic or glass rod into the tube✓
16. Twirl the rod in one direction to wind the DNA strands onto the rod. Be careful to minimize mixing of the ethanol and soapy layers. If too much shearing has occurred, the DNA fragments may be too short to wind up, and they may form clumps instead. You can try to scrape these out with the rod.✓
17. After you have wrapped as much DNA onto the rod as you can, remove the rod and scrape/shake the DNA into a small tube containing the rest of the 95 percent ethanol. Your DNA should stay solid in this solution. ✓
18. . Using the pipette and disposable tips, add 5 µl methylene blue solution for each DNA sample✓
19. Add equal amount of glycerin to DNA/methylene blue solution. ✓
20. Make new buffer solution (The buffer should be a 1% solution of baking soda. To make this, combine 2 grams (g) of baking soda with 200 mL of bottled water in one of your bowls and stir well. (If you don't have a kitchen scale, 2 g of baking soda is approximately ½ teaspoon.) ✓
21. Pour your buffer solution over the solidified gel. Add enough buffer to submerge the gel. ✓
22. Gently pull the comb out of the gel. Be sure not to remove the comb until you are ysure that the agarose gel is completely set. The resulting wells will be used as reservoirs for your samples.✓

23. Using the butter knife, carefully cut a thin slice of the gel from the top and the bottom to make room for the electrodes. ✓
24. Re-attach the stainless steel wire electrodes. ✓



Sample #1 Sample #2 Sample #3 Sample #4

Pictures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