PATERNITY CASE: WHO IS THE FATHER OF MY KITTENS?

Scenario:

Mary's white cat "Honey" was lost for 2 days about three months ago. She now has 4 kittens (see photo 1). Mary wants to know if the two neighboring cats ("Tom" in photo 2 and "Butch" in photo 3) could be the father. To analyze their DNA fingerprints, Mary has collected hair follicles from each adult cat and kitten, extracted DNA, amplified DNA using polymerase chain reaction.

You will be running the agarose gel electrophoresis on these "DNA" samples to determine the genetic father of each kitten.

Photo 1. Honey and kittens Cream, Molasses, Ginger, Sugar

Photo 2. Tom (male)

Photo 3. Butch (male)

HYPOTHESIS:

Looking at the photos, who might be the father of each kitten?

KITTEN	POTENTIAL DAD	EXPLAIN REASONING
Cream		
Molasses		
Ginger		
Sugar		

PROCEDURE:

Note: Your teacher will have an agarose gel, 1X SB buffer and the gel electrophoresis chamber ready.

1. Doublecheck that the gel has been immersed (covered by buffer). Make sure that the wells are closer to the negative electrode, since "DNA always runs to Red" (positive electrode).

2. Obtain the "DNA samples" – there are seven microfuge tubes labeled P- V.
3. Using a P-20 Pipettor and a pipet tip, measure 10 uL from Tube P and transfer into the first well of the agarose gel.

4. Using a new tip for each sample, transfer 10 uL of each sample into new wells of the gel. Be sure to keep track of your sample loading, if you do not follow the table below.

5. If there were any problems with the loading (punctured gel, not enough sample), be sure to write in the NOTE column.

GEL LOADING ORDER

Well	Tube	DNA Sample (10 uL)	NOTE
1	P	Tom (male)	
2	Q	Cream (kitten)	
3	R	Molasses (kitten)	
4	S	Honey (female)	
5	T	Ginger (kitten)	
6	U	Sugar (kitten)	
7	V	Butch (male)	

6. Cover the chamber tightly but prevent sloshing, and plug in the electrodes into the power supply (match colors).

7. Run the gel at 130 V for about 15-30 minutes, until you see good separation of the various colored bands.
(Note: pure DNA is clear and needs to be stained to visualize, we have substituted colored bands for easier viewing).

ANALYSIS

1. Use color pencils to record the band patterns (color the appropriate blocks) in the Data Table below.

Data Table. Colored "DNA" Bands Separated by Agarose Gel Electrophoresis

Tube	P	Q	R	S	T	U	V
Band	Tom (Male)	Cream	Molasses	Honey (Female)	Ginger	Sugar	Butch (Male)
Blue							
Blue							
Pink							
Orange							
Orange							
Yellow							

2. Carefully consider each band of all four kitten samples and determine whether the band matches Tom, Honey or Butch. For the Kitten samples (columns QRTU) in the Data Table, write (on top of the colored blocks) who each band matches -- Tom, Honey, Butch.

3. For each gel band of a kitten sample, the band must match a band either from the genetic mother or the genetic father.

Is there a DNA band found in a kitten that does not match Tom, Honey or Butch?_____

What does that infer? _____

CONCLUSION

KITTEN	FATHER based on Visual	FATHER based on DNA	Conclusion
Cream			
Molasses			
Ginger			
Sugar			