DNA Fingerprinting/ Electrophoresis NAME\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Paternity Testing BLOCK\_\_\_\_\_\_\_

Georgia Performance Standards: SCSh2; SCSh3; SCSh4; SCSh5; SCSh6; SCSh8; SB2f

**Background Information**

DNA fingerprinting is a recently developed method that allows for the identification of the source of unknown DNA samples. DNA fingerprinting involves the electrophoretic analysis of DNA fragments generated by restriction enzymes. No two individuals have exactly the same pattern of restriction enzyme recognition sites. There are several reasons for this fact. A large number of alleles exist in the population. Alleles are alternative forms of a gene. Alleles result in alternative expression of genetic traits which can be dominant or recessive. Chromosomes occur in matching pairs, one of maternal and the other of paternal origin. The two copies of a gene (alleles) at a given chromosomal locus constitute an individual’s unique genotype. It follows that alleles have differences in their base sequences which consequently creates differences in the distribution and frequency of restriction enzyme recognition sites. Other differences in base sequences between individuals can occur because of mutations and deletions. Such changes can create or eliminate a recognition site. Variations in the lengths between these recognition sites are known as restriction fragment length polymorphism (RFLP). RFLP’s are a manifestation of the unique molecular genetic profile, or “fingerprint”, of an individual’s DNA.

This experiment will use gel electrophoresis to determine paternity identification. In such a determination, DNA samples obtained from the mother, the child and possible fathers are fingerprinted. A child’s DNA is a composite of its parents’ DNA. Therefore, comparison of DNA fragmentation patterns obtained from the mother and child will give a partial match. Bands in the child’s fingerprint that are not present in the mother’s must have been contributed by the father.

In this hypothetical case, DNA was extracted from samples obtained from two possible fathers of a child. Their DNA was cleaved with the same restriction enzyme in separate reactions. The objective is to analyze and match the DNA fragmentation patterns after agarose gel electrophoresis and determine if Father 1 or Father 2 is the biological parent of the child.

**Materials**

DNA samples for electrophoresis gel electrophoresis apparatus with trays/combs

Agarose power supply

1X TAE buffer water bath (65°C)

10-100 µl pipet Methylene Blue Stain

yellow tips Flask

.5-10ul pipet plastic trays

clear tips illuminator

Electronic Balance paper towels

Forceps gel rocker

**DNA samples to be tested:**

**Lane**:

A Standard DNA Fragments

B Mother DNA cut with Restriction Enzyme

C Child DNA cut with Restriction Enzyme

D Father 1 DNA cut with Restriction Enzyme

E Father 2 DNA cut with Restriction Enzyme

**Procedure- Gel Electrophoresis**

1) Obtain gel poured in the casting tray, micropipet calibrated to 10 microliters & electrophoresis box and take to your station. (\*\*note- if using the smaller boxes, calibrate the pipeter to 0.8 ul and use the clear tips)

2) If using old trays, take tape off casting tray & place in electrophoresis box (black to black/DNA side on the black side). If using new trays, just place in electrophoresis chamber.

3) Obtain the buffer and pour into gel box. Completely cover the gel with the buffer. Gently pull out combs and make sure there are no well 'dimples'.

3) Obtain your DNA samples and draw out what sample you will load in what well on a piece of paper. SEE ABOVE!

4) Load samples into the appropriate wells, using a new pipet tip for each DNA sample. \*\*Important- do not cross contaminate samples!! (REMEMBER : yellow tips for the 10-100ul pipets used for larger boxes; clear tips for 0.5-10 ul pipets used for smaller boxes). Eject used tips in bleach solution.

5) Cover gel box (black to black and red to red) and plug into voltage box (2-4 gel boxes per voltage box). If you are using the Biorad boxes, plug into the “green” Biorad voltage source that can accommodate up to 4 boxes. If using the Carolina boxes (that had the tape), use the black voltage source.

6) Turn voltage to ~140 and watch to make sure the blue dye line does NOT run off of the gel. Probably will take 25-30 minutes. When the dye gets half way down the gel, it’s time to disconnect voltage.

**Staining the Gel**

1) Turn off voltage box and unplug.

2) Take top off gel box.

3) Remove casting tray containing the gel.

4) Gently push the gel into the staining tray.

5) Cover with DNA fast-blast stain for 15 min, gently agitating every now and again. \*\*Depending on the time factor, may use a more concentrated stain and leave it on for 3 minutes, gently agitating

6) When the time is up, pour stain into specified container.

7) Cover stained gel with DIO and gently agitate for 15 min.

\*You may need to continue with your destaining if the gel is very dark still. May have to be rocked overnight

8) Put gel on plastic wrap on the illuminator (light box) and record data.

9) Pour buffer back into the buffer container and clean up.

10) Wipe your station down with disinfectant

**Electrophoresis Lab Questions**

1. What procedures must have occurred before electrophoresis could be performed?
2. How does electrophoresis sort molecules?
3. What is the purpose of the…
   1. Agarose Gel?
   2. Buffer?
   3. Electrodes?
4. Why do micropipets have to be used in this lab?

5. Why is it advantageous to use multiple restriction enzymes on a fragment rather than just one?

6. Who is the father? How do you know? Be Specific!