Bio-Tek
Tomorrow's BioScience Education Today

Classroom Biotechnology

DNA FINGERPRINTING SIMULATION USING DYES

Instruction Manual

Catalogue Number
FPKR

Store the dye components of this kit at 4°C.

Bio-Tek Services Pty Ltd – Tomorrow's BioScience Education Today
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BACKGROUND

DNA fingerprinting… what is it?

DNA fingerprinting, like real fingerprinting, is used to identify individuals in a large population where individuals need to be distinguished- such as criminal investigations and paternity disputes. It uses the fact that the DNA in higher organisms (eukaryotes) usually has large sections that do not seem to code for proteins. Some of these sections are made up of small sequences of bases that are repeated over and over again (5-50 repeats). These repeated sequences are called microsatellites and vary widely between individuals and the number of repeats also varies greatly between individuals.

DNA fingerprinting shows up the unique patterns in these repetitive sequences. These patterns are produced when restriction enzymes cut up DNA. The DNA fragments are run on a gel and then hybridised with radioactive probes.

Scientists extract the DNA from a sample of cells (such as blood, sputum or semen) and incubate the DNA with a restriction enzyme which cuts each side of a known repeated sequence. This cuts the DNA into pieces of many different lengths. The DNA fragments are separated according to size by gel electrophoresis. The separated fragments of DNA are transferred from the gel to a nitrocellulose filter after being chemically split into single strands. This process is called Southern Blotting after E.M. Southern, a scientist who developed the technique. The filter sheet is put in a liquid containing radioactive probes (DNA or RNA which is complementary to the sequences being looked for).

The filter is then placed on an X-ray film. After developing the film, a banding pattern corresponding to where the radioactive probe has bound to the DNA can be seen. The banding pattern differs from individual to individual. Most of these differences are in introns and other non-coding regions. Generally more than one probe is used to make it much less likely that two people will have the same pattern (except for identical twins, of course, as they inherit the same DNA).

Many more bands would result from the use of multiple probes.

The DNA fingerprinting technique needs to be carried out very carefully to be accurate. There are many sources of error such as other sources of DNA and other substances that may affect the process.

Other uses of DNA fingerprinting (profiling):

- Protecting endangered species- ensuring genetic diversity
- Ensuring compliance with international agreements- e.g. whaling
- Evolutionary patterns- using mitochondrial DNA
- Medical uses- tracking genetic diseases and monitoring survival of transplanted genes

CURRICULUM FIT

Due to the simple nature of this activity and the fact that no DNA or staining is required, it is ideal for inclusion in middle school programs as an introduction to the basic concepts of DNA Fingerprinting- a readily accepted forensic technique.

FURTHER ACTIVITIES

Recommended Prior Activities:

- Electrophoresis In a Butter Container
- Agarose Gel Electrophoresis Using Dyes

Recommended Concurrent Activities:

- Interpreting Autoradiograms

Recommended Subsequent Activities:

- Electrophoresis of DNA
- DNA Fingerprinting Simulation Using DNA
- Enzyme digests and Electrophoresis of DNA
- Southern Blotting

DNA Fingerprinting Simulation Using Dyes (Teacher Notes)

Background: DNA fingerprinting (more correctly termed profiling) is now used routinely to solve crimes. In recent years, news stories have reported how miniscule amounts of DNA have been used to identify individuals involved in incidents even many years in the past, as well as exonerate innocent people from incrimination.

Forensic scientists visualize DNA fingerprints using agarose gel electrophoresis. This is a separation technique that can resolve molecules based on charge, size, and shape. The activity that your students are about to undertake uses dyes to simulate DNA and allows them to assume the role of a forensic scientist.

Classroom time needed for this lab:

- 50 minutes (if agarose gels are poured before class)

You will need the following materials (For each team of 2-4 individuals):

- 1 x TAE buffer (diluted from a 50X stock solution)
- Tubes 1- 6: Samples taken from suspects. Tubes CS1 & CS2: Samples taken from the crime scene.
- Agarose gel- 1% (pre-poured).

You will need the following equipment:

- Micropipettes and tips or Pasteur pipettes- to load dye samples
- Microtubes (1.5ml)
- Electrophoresis units and power supplies
- Microwave oven

Setting up the classroom for this lab:
Distribute at each lab station (dependant on the number of gel tanks):

- 50 μl of each dye sample in labelled/coloured microcentrifuge tubes
- Micropipette and tips or Pasteur pipettes
- Electrophoresis unit and power supply
- 1X TAE buffer (500 mL)
- Pre-poured 1% agarose gel

Before the lab:

- Be sure to calculate how much agarose solution you will need. You can add a defined volume of water into the gel tray to determine the volume of agarose that would be needed for each tray (usually 25- 30 ml).
- Be sure to calculate how much 1X TAE you will need. Using a graduated cylinder, you can pour a defined volume of water into the electrophoresis unit to determine the volume of buffer that would be needed for each unit (usually 300- 500 ml).

During the lab:

- If necessary, demonstrate to students how to use a micropipette- they are a precision instrument so students should treat them accordingly.
- Sample separation patterns can be permanently recorded with a digital camera. Be aware that dyes will diffuse within the gel over time, and examination or photography should take place shortly after cessation of electrophoresis.

After the lab:

- Portions of the gel which are free of dyes can be broken/cut off and placed in a container for re-use and the used portion of the gels can be disposed of in the general refuse. Unfortunately, the dyes in the gel diffuse rapidly, so the banding patterns will disappear overnight if the gels are kept.
- Unused gels can be stored in the refrigerator in a zip lock plastic bag.

Results:

Students should draw a diagram of the banding patterns of the samples which show all lanes are different except for lanes 4 (CS1) & 6 (suspect 4).

Analysis:

1. Which suspect(s) would appear to have been at the crime scene?

   Suspect 4 (Lane 6).

2. Does this mean that they are the person(s) who committed the crime? (In other words, should a court of law convict them on this evidence alone?). Explain.

   No. They could have been at the scene of the crime at any time other than when the crime was committed. Other incriminating evidence needs to be uncovered.

3. Is all the crime scene evidence accounted for?

   No. Crime Scene 2 sample (Lane 5) does not match any of the suspects.

4. What advice would you now give to the police?

   They should continue their search for further suspects.

Safety Guidelines

Agarose Gel

Warning! If a microwave oven is used to melt the agarose gel, ensure that the gel is placed in an *unsealed* container. If a microwave oven is not available, a boiling water bath or hotplate may be used instead. The gel must be swirled as it melts to prevent charring. The use of a Bunsen burner to melt agarose is **not** recommended.

Electrical Supply

Warning! Any gel electrophoresis equipment is designed to be used at specific voltages. Under no circumstances should these voltages be exceeded! Refer to manufacturers/ supplier's recommendations.

TAE Buffer

When prepared, diluted and used as directed, these buffers present no serious safety hazards. Used buffer can be washed down the drain.

DNA Fingerprinting Simulation Using Dyes (Student Notes)

Background: DNA fingerprinting (more correctly termed *profiling*) is now used routinely to solve crimes. In recent years, news stories have reported how miniscule amounts of DNA have been used to identify individuals involved in incidents even many years in the past, as well as exonerate innocent people from incrimination.

Forensic scientists visualize DNA fingerprints using agarose gel electrophoresis. This is a separation technique that can resolve molecules based on charge, size, and shape. The activity that you are about to undertake uses dyes to simulate DNA and allows you to play the role of a forensic scientist.

Aim: To use agarose gel electrophoresis to compare samples found at a crime scene and those taken from suspects to enable you to make a positive ID!

Materials: (For each team of 2-4 individuals)

- Micropipettes and tips to load samples.
- Various samples in microtubes:
  - Tubes 1- 6: Samples taken from suspects.
  - Tubes CS1 & CS2: Samples taken from the crime scene.
- Electrophoresis unit and power supply.
- Agarose gel (1%, pre-poured)
- 1X TAE buffer for electrophoresis units.

Method:

Preparing the agarose gel.

1. Your teacher has prepared a 1% agarose gel for you by dissolving the agarose in 1xTAE buffer (using a microwave oven), pouring it and allowing it to cool and solidify.

2. Place the agarose gel in the electrophoresis tank. **Carefully** remove the comb. Add 1X TAE buffer to the electrophoresis unit until the gel is just submerged. Ensure that buffer enters the wells.

Loading the samples.

1. Record on the data sheet where you will load the samples (suspects and crime scene).

2. Load 10 μl of the samples into the wells (placing a black object under the electrophoresis unit will make the wells easier to see and load). Place the lid on the tank.

3. Ensure that the power supply is turned off. Connect the electrophoresis unit to it (red to red, black to black). Plug the power supply in and turn it on at the wall.

4. Set the voltage to 125 V and turn on the power supply. The samples will start resolving towards the positive pole.

5. Electrophorese the samples for ~10-15 minutes, which should give good banding patterns. Turn off the power supply, disconnect the electrophoresis unit and remove its lid.

6. Carefully remove the casting tray (with the gel intact) and place it on paper towel on the bench.

Results:

1. Draw a diagram below of the banding pattern of the samples.

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Analysis:

1. Which suspect(s) would appear to have been at the crime scene?

2. Does this mean that they are the person(s) who committed the crime? (In other words, should a court of law convict them on this evidence alone?). Explain.

3. Is all the crime scene evidence accounted for?

4. What advice would you now give to the police?