GenoSensor Corporation

GenoSensor DNA Fingerprinting Kit

Catalog # 4000's

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User Manual

GenoSensor DNA Fingerprinting Kit Manual

Table of Contents

Literature Citation

When describing a procedure for publication using these products, we would appreciate that you refer to them as the GenoSensor DNA Fingerprinting Kit.

Notes for Instructors

Kit Components and Storage Conditions:

Component	Storage
2X Master Mix	-20ºC
Suspect A	-20ºC
Suspect B	-20ºC
Suspect C	-20ºC
Criminal DNA	-20ºC
DNA ladder	-20ºC

Preparation for Restriction Digest

Set heat block or water bath to 37ºC

Thaw 2x Master Mix on ice. Before opening tube, **spin 10 sec at 6,000 rpm or greater in a microcentrifuge. Vortex 10 seconds, then spin again for 10 seconds.**

Aliquot the 2X Master Mix as necessary *after* doing the above preparation.

Each package contains enough 2X Master Mix for 24 digest reactions, sufficient to cover all of the suspect and criminal samples provided in the kit. Use 10 ul of 2X Master Mix with 10 ul sample DNA for a total digest volume of 20 ul.

Electrophoresis

Electrophoresis reagents are not provided in the kit. Please refer to the required materials list.

Best results are obtained by adding DNA dye (i.e. Gel Red or Sybr Safe) to molten agarose.

Avoid exposing the agarose gel to light. It is best to store and run the gel in a dark room, or cover the gel with a box during gel polymerization and the whole electrophoresis process.

There is enough DNA ladder to load 3 lanes with 10 ul.

Shipping, Storage and Safety

Shipping and Storage

GenoSensor DNA Fingerprinting kits are shipped on dry ice. Components should be stored at temperatures shown in the above table. At proper storage conditions, components are stable for 1 year from the date received. Expiration dates are also noted on product labels.

Safety Warnings and Precautions

This product is intended for research use only. It is not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Consider all chemicals as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Exercise caution to avoid contact with skin or eyes: if contact should occur, wash immediately with water (Material Safety Data Sheet for products is available upon request).

GenoSensor DNA Fingerprinting Kit Overview

The GenoSensor DNA Fingerprinting Kit introduces common techniques used in DNA research and in forensic analysis. The kit creates a crime scene scenario utilizing three different plasmids to represent three suspect samples, one of which matches an additional sample labeled as "Criminal DNA" representing DNA collected from the scene of the crime. The goal of the experiment is to identify which of the suspects is the culprit by performing restriction digests with two restriction enzymes (EcoRI and SspI) on the four samples. After completing the experiment, one should be able to understand the concepts behind restriction digests, gel electrophoresis, and the genetic concepts driving the experiment.

Kit Components and Storage Conditions
(for a lab of 24 students)

Component	Amount (30 rxn's)	Storage
2X Master Mix	240 ul (24 rxns)	-20ºC
Suspect A	60 ul (6 rxns)	-20ºC
Suspect B	60 ul (6 rxns)	-20ºC
Suspect C	60 ul (6 rxns)	-20ºC
Criminal DNA	60 ul (6 rxns)	-20ºC
DNA ladder	30 ul	-20ºC

Additional Required Materials

1. Heat Block or (heat plate, Beaker with de-ionized water; water bath, Tube floater; Thermometer)
2. Microcentrifuge
3. Microcentrifuge tubes
4. Vortex
5. Micropipettes (p10, p200, p1000)
6. Pipette tips
7. Tube Racks
8. Electrophoresis equipment
9. Electrophoresis supplies: agarose, TBE, DNA loading buffer, running buffer, gel dye (eg. Sybr safe, Gel Red)
10. UV light box or "Gel Doc" equipment and program

Introduction

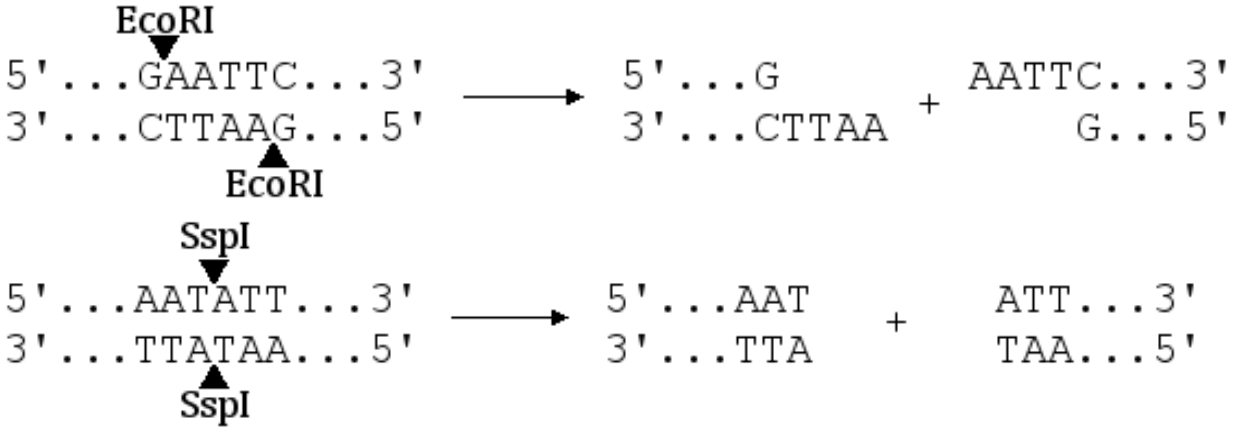
Objective overview

1. Understand how DNA is responsible for genotypic differences between individuals.
2. Investigate techniques used in DNA fingerprinting: DNA sequence diversity and uniqueness, gel electrophoresis, DNA restriction enzymes
3. Investigate and understand the process for gel electrophoresis including analyzing your data.

In this lab you will examine a simplified version of a DNA fingerprinting process. During the exercise you will learn to analyze and compare a number of DNA fragments to determine whether or not they are from the same individual. These fragments can be visualized through a process known as "gel electrophoresis."

DNA is long double helix polymer that is made up by different combinations of the 4 nucleotides: Adenine (A), Thymidine (T), Cytosine (C), and Guanine (G). In a DNA molecule, A is paired with T and G is paired with C to form the helix structure. Each individual will have different sequences of A, T, G, and C in their DNA. There are constant regions and variable regions in human DNA and we can use the variable regions to identify humans by their DNA. In this exercise, you will use several techniques to figure out if the DNAs of several suspects match the DNA collected at the crime scene.

Because DNA is a very long polymer, scientists have found several ways to fragment the DNA in order to work with it more easily. One of the techniques is to use restriction enzymes. In 1968, Dr. Werner Arber at the University of Basel, Switzerland and Dr. Hamilton Smith at the Johns Hopkins University, Baltimore, discovered a group of enzymes in bacteria, which when added to any DNA will result in the breakage of the sugar-phosphate backbone bond within a specific sequence of nucleotide bases called a recognition site. These enzymes cause the double strand of DNA to break within the recognition site and the DNA molecule becomes fractured into two pieces. These molecular scissors or "cutting" enzymes are restriction endonucleases. The two endonucleases you are going to use today are called EcoRI and SspI. The following figure depicts the recognition site of these endonucleases. Since each individual's DNA is unique, the fragmented DNA profile created by these two enzymes will be very different from each other.



In order to compare the DNA profiles of individuals, we have to find a way to separate these fragments and to visualize the DNA profile. This can be accomplished by agarose gel electrophoresis, a separation of DNA through a matrix within an electric field. Since DNA is negatively charged, when it is put under an electric field it will migrate from the cathode to the anode. When the DNA fragments are put through a matrix within an electric field, the smaller fragments will migrate faster than the bigger fragments. Lastly, the DNA profile can be visualized by staining the DNA and viewing it under UV light. Remember that each individual has a different fragmented DNA profile cut by the same restriction endonucleases, thus, each individual will have a different DNA separation pattern on the gel.

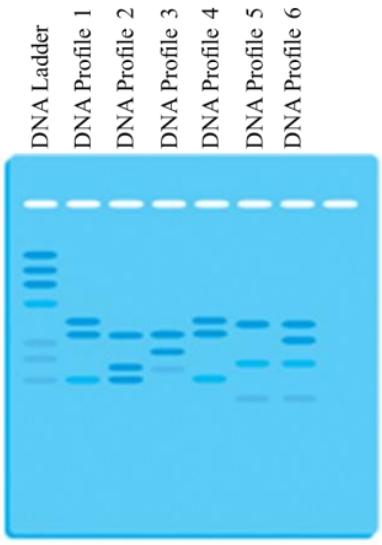
Pre-lab questions

1. What is DNA profiling?

2. In this lab, we will use small pieces of DNA instead of chromosomal DNA to simplify our DNA profile. If you cut a human's chromosomal DNA, containing 3 billion base pairs (bp) into 4000 bp pieces, how many DNA pieces would you get?

3. If the whole human chromosomal DNA of 3 different individuals are subjected to the EcoRI and SspI enzyme mix, and then those samples are separated by DNA gel electrophoresis, would you be able to compare the DNA profiling? Explain.

4. Below is an example of a gel electrophoresis of 6 different DNA profiles. Which of them do you think come from the same individual? Explain.



5. When working with DNA, what do you think is a critical factor? Explain.

DNA Fingerprinting – Protocol

Preparation

1. Set heat block or water bath to 37ºC. For a heat block, it is recommended to add water or sand to ensure proper heat transfer. For a water bath, be sure tubes are tightly sealed and not fully submerged to avoid contamination.
2. Thaw 2x Master Mix on ice. Before opening tube, **spin 10 sec at 6,000 rpm or greater in a microcentrifuge. Vortex 10 seconds, then spin again for 10 seconds.**

Pre-Experiment Observations

1. Describe the samples of DNA (physical properties: color, viscosity, etc…). Can you see the DNA?
2. Is there any observable difference between the samples of DNA?
3. Describe the appearance of the restriction endonuclease mix? Can you see the proteins?

Part 1: Restriction Digest Protocol

Keep the enzyme mix, all samples and reaction mixtures on ice when not in use.

1. Pipette 10 µL of the 2X Master Mix, which contains the enzymes SspI and EcoRI along with the restriction digest buffer, to four separate, fresh and labeled microcentrifuge tubes.
2. Using a NEW pipet tip for each sample, pipet 10 µL of each stock suspect DNA sample and the criminal sample into separate tubes from step 1. Pipet up and down carefully to mix well.

   Note: Change tips whenever you switch reagents, or, if the tip touches any of the liquid in one of the tubes accidentally. When in doubt, change the tip! DNA goes in the tube before the enzyme. Always add the enzyme last.

Restriction Digest Reaction Mixtures			
DNA Samples		EcoRI/SspI 2x Master Mix	Total Reaction Volume
Criminal [C]	10 µL	10 µL	20 µL
Suspect A [SA]	10 µL	10 µL	20 µL
Suspect B [SB]	10 µL	10 µL	20 µL
Suspect C [SC]	10 µL	10 µL	20 µL

3. Tightly cap on each tube. Mix the tube contents. Mix the components by gently flicking the tubes with your finger. Arrange the tubes in a microcentrifuge and spin for 5 seconds to force all the liquid to the bottom of the tubes. (Be sure the tubes are in a BALANCED arrangement in the rotor).
4. Incubate the tubes at 37oC for ~45 minutes in a water bath or heat block.

Part 1: Questions

While waiting for your samples to be digested by the endonucleases, consider these following questions:

1. When you mix the DNA with the endonuclease mix, was there any visible change or any sign of reactivity?

2. Can you see any evidence to indicate your samples of DNA were fragmented or altered in any way by the addition of the endonuclease mix? Explain.

3. In the absence of any visible evidence of change, is it still possible that the DNA samples were fragmented? Explain.

4. After the incubation period, are there any visible clues that restriction enzymes altered the DNA in any of the tubes? Explain.

Part 2: Agarose Gel Electrophoresis Protocol

General Procedure, detailed directions as given by instructor

1. Prepare 0.8 – 1% agarose.
2. Set up electrophoresis apparatus and pour the 1% molten agarose for gelation.
3. For staining, use a DNA dye which is added directly to the molten agarose. For light sensitive dyes, keep the gel in the dark during gelation, either by performing in a dark room or placing a box over the gel.
4. Mix sample with loading dye according to instructor directions to ensure that the sample will sink to the bottom of the well and properly enter the agarose gel. Use at least 10 µL of digested DNA product to visualize results by electrophoresis on agarose gel. If gel well volume will accommodate more than 10 ul, a higher volume is preferred.

Sample Gel Loading Setup			
Digested DNA Samples		Loading Mix	Total Volume
Criminal [C]	20 µL	5 µL	25 µL
Suspect A [SA]	20 µL	5 µL	25 µL
Suspect B [SB]	20 µL	5 µL	25 µL
Suspect C [SC]	20 µL	5 µL	25 µL

5. Load samples with loading dye into the gel. Record which wells hold which samples.

Recommended Gel Loading:

Lane 1	2	3	4	5	6	7	8	9	10
Ladder	C	SA	SB	SC	---	C	SA	SB	SC

6. Run at ~120V for ~30 minutes and stop before loading dye has run off gel. Depending on the DNA dye used, caution may need to be taken to reduce exposure of gel to light
7. Visualize under UV and record the results manually or by photography
8. Compare the bands. The DNA ladder can be used as a band size reference.

Part 2: Questions

While waiting for your samples to electrophorese, answer these questions with your group members.

1. The electrophoresis apparatus creates an electrical field with positive and negative poles at the ends of the gel. DNA molecules are negatively charged. To which electrode pole of the electrophoresis field would you expect DNA to migrate?

2. After the DNA samples are loaded into the sample wells, they are "forced" to move through the gel matrix. What size fragments (large vs. small) would you expect to move toward the opposite end of the gel most quickly? Explain.

3. Can you see the DNA moving while the gel is under the electric field? Explain.

4. The sequence of a DNA fragment is shown below. Use it to answer the following questions.

   5'-GTGAATTAATATTAAATATTGGGAATCCTTGGGAATTCGTACA-3'
   3'-CACTTAATTATAATTTATAACCCTTAGGAACCCTTAAGCATGA-5'

   a. How many EcoRI and SspI restriction sites are there in the sequence?

   b. How many pieces of DNA would result from cutting this DNA fragment with EcoRI alone? SspI alone? Both EcoRI and SspI together?

   c. Write out the sequences of the possible DNA fragments from a EcoRI cut (alone) and indicate their sizes. Which fragment will migrate the farthest distance in the gel? Which fragment will travel the shortest distance?

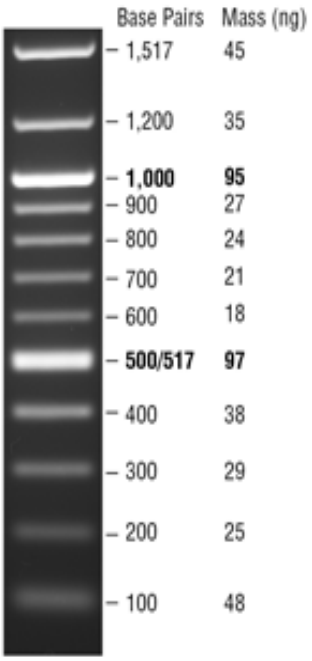


Fig 1. 100 bp DNA Ladder
DNA Ladder reference

Results and Discussion

Take a look at the bands visible in your samples on the gel. Recall which lanes contained the suspect samples and which contained the criminal sample. Do any of the suspect samples match with the criminal sample on the gel?

Visually analyzing the bands in relation to each other is quick and useful, but how would you better quantitatively measure each band's position for comparison?

Other members of your class will have done their own digests using the same samples. Compare your results with theirs. Do your results agree?

What did you learn today?

Troubleshooting

Problem	Possible causes	Solutions
Incomplete or no digestion of DNA	2X Master Mix not properly prepared	It's vital that the Master Mix be properly thawed, spun down and vortexed before use to ensure the enzyme and all components are properly mixed
	Heat block/Water bath/Heating source temperature incorrect	Be sure the heat source used for incubation sits stably at 37 degrees Celsius
	Incubation time too short	Shorter times should work, but if you're having trouble, increase the incubation times
Weak bands/faint signal	DNA Dye degradation during preparation	Light sensitive dyes should be kept in the dark during gel preparation. Prepare in dark room or place a box over the electrophoresis apparatus during gelation and electrophoresis
	Expired, contaminated or degraded DNA dye	Verify that the DNA dye has not degraded in storage, been contaminated or expired

Technical Service

For more information or technical assistance, please call, write, fax, or email.

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