GenoSensor Corporation

GenoSensor DNA Fingerprinting Kit I

Catalog # 4001

Version B
July 2015

User's Manual

GenoSensor DNA Fingerprinting Kit I Manual

Table of Contents

Literature Citation

When describing a procedure for publication using these products, please refer to them as the *GenoSensor DNA Fingerprinting Kit I*.

Notes for Instructors

Kit Components and Storage Conditions:

Component	Storage
2X Res. Dig. Master Mix	-20ºC
Sample A	-20ºC
Sample B	-20ºC
Sample C	-20ºC
Unknown DNA	-20ºC
DNA ladder	-20ºC

Preparation for Restriction Digest (For 6 teams)

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 Res. Dig. 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.
3. Label 6 tubes "MM" and aliquot 40 µL of 2X Res. Dig. Master Mix into each tube, store on ice.
4. Label 6 tubes each (24 total) "A, B, C, U" and aliquot 10 µL of each DNA sample, store on ice.
5. In class, distribute 1 each "MM, A, B, C, U" tube / team.

Each package contains enough 2X Res. Dig. Master Mix for 24 digest reactions, sufficient to cover all of the samples provided in the kit. Students will use 10 µL of 2X Res. Dig. Master Mix with 10 µL sample DNA for a total reaction volume of 20 µL.

Electrophoresis

- Electrophoresis reagents are not provided in the kit. Please refer to the Additional Required Materials list, on page 4.

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

- For light sensitive DNA dyes, 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.

- DNA ladder supplied is enough to load 3 lanes with 10 µL each.

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 table above. 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 who are and familiar with the principles of good laboratory practice should handle these products. Wear suitable protective clothing such as laboratory coats, safety glasses, and gloves. Exercise caution to avoid contact with skin or eyes: if contact should occur, wash immediately with water and follow your laboratory safety protocols. Safety Data Sheets for products are available upon request.

GenoSensor DNA Fingerprinting Kit I Overview

The GenoSensor DNA Fingerprinting Kit I 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, labeled Sample A, B, and C, one of which matches the "Unknown sample" representing criminal 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 students will be able to understand the concepts behind restriction digests, gel electrophoresis, and the genetic concepts driving the experiment.

Kit Components and Storage Conditions (For 6 teams)

Component	Amount (24 rxns)	Storage
2X Res. Dig. Master Mix	240 µL	-20ºC
Sample A	60 µL (6 rxns)	-20ºC
Sample B	60 µL (6 rxns)	-20ºC
Sample C	60 µL (6 rxns)	-20ºC
Unknown DNA	60 µL (6 rxns)	-20ºC
DNA ladder	30 µL	-20ºC

Additional Required Materials

- Heat Block or (heat plate, Beaker with de-ionized water; water bath, Tube floater; Thermometer)
- Microcentrifuge
- Microcentrifuge tubes (30)
- Vortexer
- Micropipettes (p10, p100)
- Pipette tips
- Tube Racks
- Electrophoresis equipment (gel box & power source)
- Electrophoresis supplies: agarose, TBE, DNA loading buffer, running buffer, gel dye (eg. SYBR safe, Gel Red)
- UV light box or "Gel Doc" equipment and program
- Permanent marker

Student Guide

Objective overview

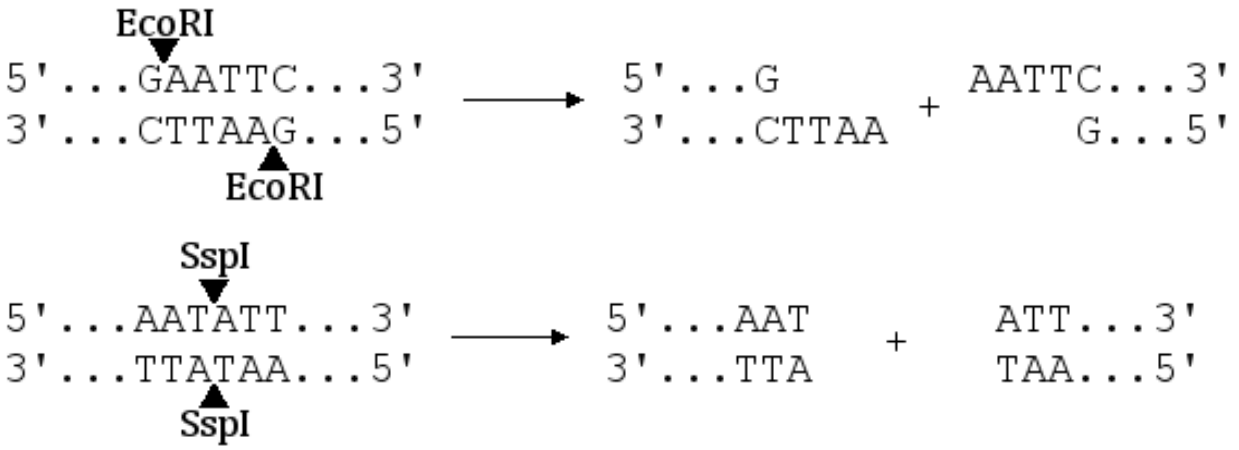
1. Understand how DNA is responsible for genotypic differences between individuals.
2. Investigate techniques used in DNA technology: DNA sequence diversity and uniqueness, DNA digests, restriction enzymes, and gel electrophoresis.
3. Investigate and understand the process for gel electrophoresis including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

In this lab you will examine an abridged version of a DNA digestion 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 a long double helix polymer that uses deoxyribose rings (sugars) and phosphate molecules as support in its backbone. Attached to the backbone are unique sequences of nucleotides which are often referred to as base pairs. There are two different types of nucleotides: purines and pyrimidines. Adenine (A) and Guanine (G) are both purines because they have two rings in their structures. Meanwhile, Thymine (T) and Cytosine (C) are pyrimidines because they have only a single ring in each of their structures. These nucleotides form a bond with their complementary base pair on the other strand of DNA. This is how the double helix structure is formed that resembles a spiral staircase. In a DNA molecule, A is paired with T and G is paired with C to form the double helix structure. Each individual will have different sequences of A, T, G, and C in their DNA. There are highly similar and yet unique sequences of DNA that are used to identify humans by looking at the minute differences in their DNA. In this exercise, you will use several techniques to figure out if the DNA in any of the three samples matches up with the DNA of the unknown sample.

Due to the vast size of the human genome, scientists have had to find several ways to fragment the DNA into smaller pieces so that they can work with it more easily. One of these 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, that when added to any DNA will break 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 different for each person - much like cutting out a paper snowflake.



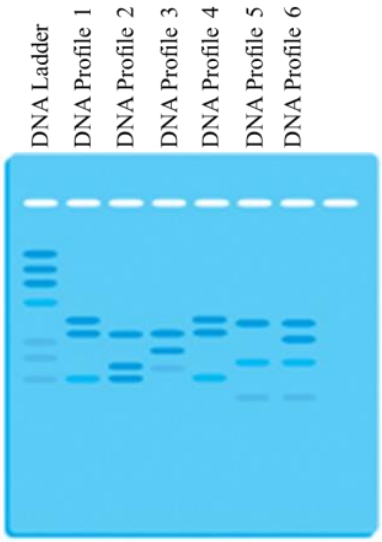
In order to compare the DNA profiles of individuals, we have to be able to separate these fragments and take a look at them. This can be accomplished through agarose gel electrophoresis, which uses a matrix within an electric field to separate these various segments of DNA. Since DNA is negatively charged, when it is placed in an electric field it will migrate from the cathode to the anode (negative side to positive side). The smaller fragments will migrate faster than the larger fragments. By staining the DNA with a special chemical and viewing it under a UV light, the DNA profiles, seen as bands, become visible. This allows us to analyze the differences and/ or the similarities between each one. Remember that each individual has different sizes and numbers of DNA fragments that are cut by the same restriction endonucleases. Therefore, the differences between the individuals' DNA profiles will be characterized by the separation patterns of the DNA bands seen in the gel.

Pre-lab questions

1. Describe how digesting DNA with enzymes allows scientists to differentiate individuals using their DNA.

2. In this lab, we will use small pieces of DNA instead of complete chromosomal DNA to simplify the DNA profile. If all of the chromosomal DNA in one human cell, containing 3 billion base pairs (bp), is cut into 4000 bp pieces, how many DNA pieces will be created?

3. Below is an example of a gel electrophoresis of 6 different DNA profiles. Which profiles seem to come from the same individual? Justify your answer.



4. Predict several problems that could occur and explain how they would interfere with the production of an accurate DNA profile (digestion and gel).

Full Protocol

Lab Setting

Materials are enough for 6 groups.

Reagent Preparation

Refer to “Notes for Instructors – Preparation for Restriction Digest” on Page 2.

Pre-Experiment Observations

1. Describe the DNA samples (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 2X Res. Dig. Master Mix. Can you see the enzymes?

Part 1: Restriction Digest Protocol

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

1. Using a NEW pipette tip for each sample, pipette 10 µL of the 2X Res. Dig. Master Mix, which contains the restriction enzymes SspI and EcoRI along with the restriction digest buffer, into each of the four DNA sample tubes labeled "A, B, C, and U" (already containing 10 µL of each DNA).

Restriction Digest Reaction Mixtures			
DNA Samples		2X Res. Dig. Master Mix	Total Reaction Volume
Unknown DNA [U]	10 µL	10 µL	20 µL
Sample A [A]	10 µL	10 µL	20 µL
Sample B [B]	10 µL	10 µL	20 µL
Sample C [C]	10 µL	10 µL	20 µL

2. Carefully pipette the mixture up and down to mix thoroughly. Tightly cap each tube. Alternatively, mix the components by gently flicking the tubes with your finger. Arrange the tubes in a microcentrifuge machine and spin for 5 seconds to force all liquid to the bottom of the tubes. (Be sure the tubes are in a BALANCED arrangement in the rotor).
3. Incubate the tubes at 37°C for ~45 minutes in a water bath or heat block.
4. STOPPING POINT-If there is no time to continue, store samples @ 4°C until following lab period.

Part 1: Questions

While waiting for the samples to be digested by the endonucleases, answer the following questions:

1. After combining the 2x Res. Dig mix with the DNA samples, was there any visible change or any sign of reactivity?

2. Was there any evidence indicating that 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 given by instructor

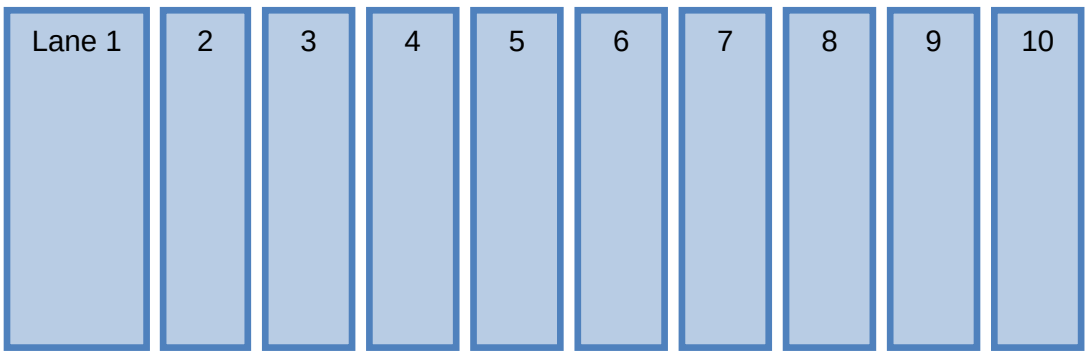
1. Prepare 1% agarose.
2. For staining, use a DNA dye which is added directly to the molten agarose. For light sensitive dyes, keep the gel in the dark during gelation. This can be done by performing in a dark room or placing a box over the gel.
3. Set up electrophoresis apparatus and pour in the 1% molten agarose with DNA dye for gelation.
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 on the agarose gel. If gel well volume will accommodate more than 10 µL, a higher volume is preferred.

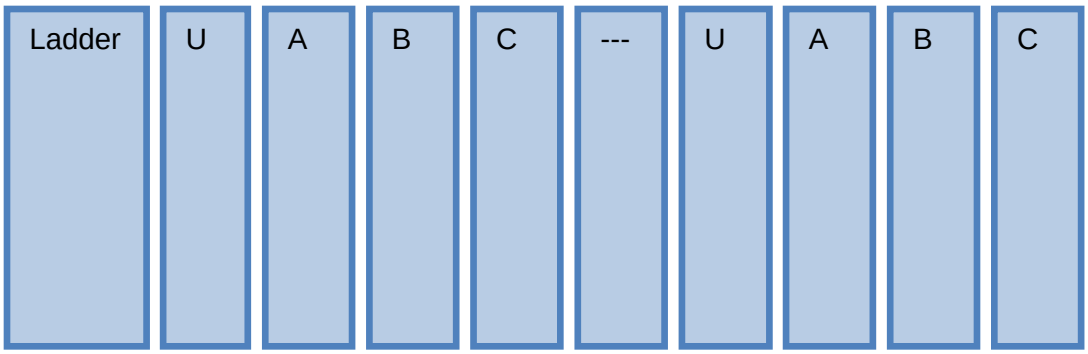
Sample Gel Loading Setup			
Digested DNA Samples		Loading Mix	Total Volume
Unknown DNA [D]	20 µL	5 µL	25 µL
Sample A [A]	20 µL	5 µL	25 µL
Sample A [B]	20 µL	5 µL	25 µL
Sample A [C]	20 µL	5 µL	25 µL
DNA Ladder			10µL

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

Recommended Gel Loading:

<u>Load two teams/gel</u>





6. Run at ~120V for ~30 minutes and stop before loading dye runs off of gel. Depending on the DNA dye used, caution may need to be taken to reduce exposure of gel to light
7. Visualize under UV light exposure 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 at different speeds. What size fragments (large vs. small) would you expect to move toward the opposite end of the gel and travel a longer distance most quickly? Explain.

3. What do you see moving through the gel? (Tricky question).

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 an EcoRI cut (alone) and indicate their sizes. Draw them on a gel to indicate where you expect them to be after electrophoresis.

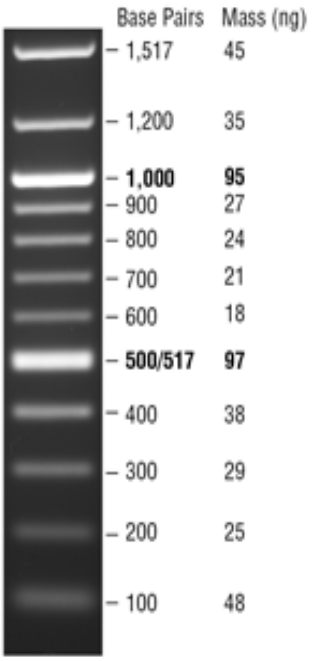


Fig 1. 100 bp DNA Ladder
DNA Ladder reference

Results and Discussion

Take a look at the bands visible from your samples on the gel. Refer to your gel map to identify which lanes contained the samples and which contained the unknown DNA sample. Do any of the samples match with the unknown DNA sample on the gel?

Looking at the bands in relation to one another is quick and useful, but what would be a more accurate way to infer band size the distance traveled? (Hint: DNA ladder)

Compare the results from your team with those of other teams. Describe similarities and differences.

Summarize the process of DNA digestion using restriction enzymes and DNA gel electrophoresis using the correct terminology.

Describe a new experiment you could perform using the DNA restriction enzyme digestion method and agarose gel electrophoresis.

Troubleshooting

Problem	Possible causes	Solutions
Incomplete or no digestion of DNA	2X Res. Dig. 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 has stabilized 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.

GenoSensor Corporation
4665 S. Ash Avenue
Suite G-18
Tempe, Arizona 85282
Tel: 1-480-598-5378
Fax: 1-480-755-3319
Email: tech_service@GenoSensorcorp.com
Web: www.GenoSensorcorp.com

Limited Warranty

GenoSensor is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about a GenoSensor product or service, please contact our Technical Service at tech_service@GenoSensorcorp.com. GenoSensor warrants that all of its products will perform according to the specifications stated on the certificate of analysis. This warranty limits GenoSensor Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. GenoSensor reserves the right to select the method(s) used to analyze a product unless GenoSensor agrees to a specified method in writing prior to acceptance of the order. GenoSensor makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore GenoSensor makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service. GenoSensor assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.