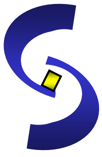

GenoSensor Corporation

GenoSensor Viral Infection Kit iso-S

Catalog # 3303

Version A
November 2019

User's Manual

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Literature Citation
When describing a procedure for publication using these products, please refer to them as the *GenoSensor Viral Infection Kit iso-S*.

Notes for Instructors

Kit Components and Storage Conditions:

Component	Storage
2X iso-S Master Mix	-20ºC
Sample A	-20ºC
Sample B	-20ºC
Sample C	-20ºC
Sample D	-20ºC
DNA Ladder	-20ºC

Preparation for Isothermal LAMP Reaction (for 6 teams)

1. Set heat block or water bath to 60 ºC. 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 iso-S 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 40µL of 2X iso-S Master Mix into each tube, store on ice.
4. Label 6 tubes each (24 total) "A, B, C, D" and aliquot 10µL of each DNA sample, store on ice.
5. In class, distribute 1 each "MM, A, B, C, D" tube / team.

Each package contains enough 2X iso-S Master Mix for 24 digest reactions, sufficient to cover all of the samples provided in the kit. Students will use 10 µL of 2X iso-S 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 Viral Infection kits are shipped at ambient temperature. 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 education 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 who are trained in laboratory techniques 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 Viral Infection Kit iso-S Overview

The GenoSensor Viral Infection Kit iso-S introduces common techniques used in DNA research and in medical diagnostics. The kit creates a scenario in which a virus has infected many people and caused a disease. The main symptom of the disease is high fever. Four patients come in a hospital emergency room and they all have high fever. The virus test needs to be done on those patients to diagnose who is infected with the virus. The specific virus gene is going to be tested using this kit and lab results will answer who (A, B, C, and D) has the virus. The test uses loop mediated isothermal amplification (LAMP) technology on all four samples. After completing the experiment students will understand the concepts behind gene detection, gene isothermal amplification, gel electrophoresis, and the genetic concepts driving the experiment.

Kit Components and Storage Conditions
(For 6 teams)

Component	Amount (24 rxns)	Storage
2X iso-S Maser 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
Sample D	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 (e.g., SYBR® safe, Gel Red)
- UV light box or “Gel Doc” equipment and program
- Permanent marker
- Gloves

Student Guide

Objective overview

1. Understand how DNA is responsible for genotypic differences between the test samples of interest.
2. Investigate techniques used in DNA technology: DNA sequence diversity and uniqueness, DNA amplification, isothermal amplification, and gel electrophoresis.
3. Investigate and understand the process for gel electrophoresis and data analysis.

In this lab you will examine an abridged version of a DNA amplification process. During the exercise you will learn to analyze and compare a number of DNA fragments to determine whether or not the tested DNA fragments are present in the samples. These fragments can be visualized through a process known as "gel electrophoresis" (iso-S kits) or in some situations by direct visualization (iso-B kits).

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 organisms by looking at the minute differences in their DNA. In this exercise, you will use several techniques to figure out if there is the DNA of your interest in any of the four samples.

DNA amplification is a common technique for life science research. Amplification has many purposes and one of them is to see if your sequence of interest is there. If you see amplification products, then you can say that the sequence you are interested is there.

Polymerase chain reaction (PCR) is a commonly used technique. What you are going to learn in this exercise is another technique called loop mediated isothermal amplification (LAMP).

LAMP is an isothermal nucleic acid amplification technique. In contrast to the PCR technology in which the reaction is carried out with a series of alternating temperature steps or cycles, isothermal amplification is carried out at a constant temperature, and does not require a

thermal cycler. This may be of use in the future as a low cost alternative to detect genes or certain diseases.

LAMP was initially developed by Notomi et al. (published in 2000). The technique exhibits high specificity and selectivity because of the use of multiple primers on the target base sequence, and can be completed in a short time (1 hr or less) due to the high amplification efficiency under isothermal conditions.

LAMP typically uses DNA polymerase, such as Bst DNA polymerase that is isolated from *Bacillus stearothermophilus*, for DNA amplification. The Bst enzyme has 5' → 3' DNA polymerase activity and strand-displacement activity. It synthesizes a new DNA strand while dissociating the hydrogen bond of the double stranded template DNA by itself, and DNA can be synthesized at a constant temperature. Bst is active at temperature 60 – 72 ^{o}C.

The assay commonly uses 4 primers recognizing 6 distinct regions designed to create continuous loop structures during DNA amplification. Each primer includes a target-specific section of nucleotides and “tags” of contiguous nucleotides that are not complementary to the target sequences but allow the formation of loop structures (figure 1).

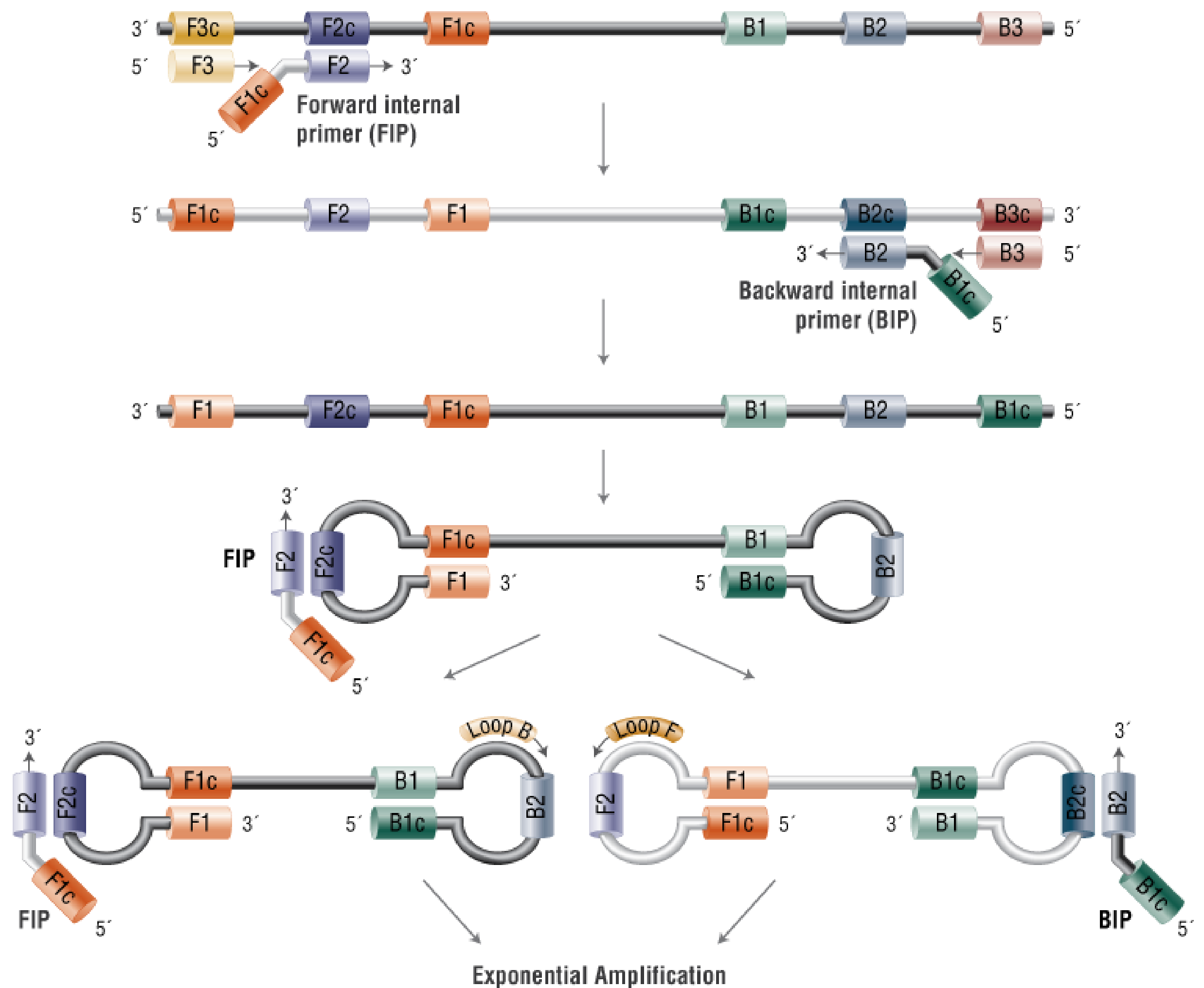


Figure 1. Four primers used are Forward initial primer (FIP), F3, Backward initial primer (BIP), and B3.

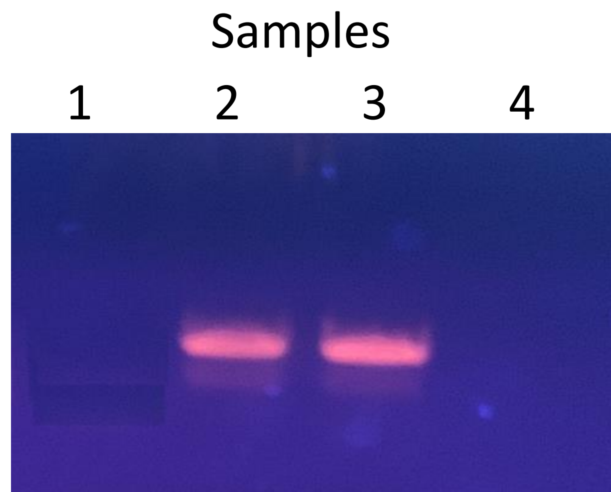
The detection of the amplification product is indirectly determined by a number of methods: (1) by the visualization of turbidity, (2) by visualization of dye color due to the incorporation of nonspecific DNA dyes, such as SYBR-Green I dye and Syto 9 Green Fluorescent stain, or (3) by visualization under UV light after gel electrophoresis and incorporated with specific DNA dyes. GenoSensor's kit iso-B uses the (2) method, and GenoSensor's kit iso-S uses the (3) method by learning the gel electrophoresis technique.

Pre-lab questions

1. Describe why we amplify DNA.

2. Describe what DNA amplification techniques you have read about. If you know more, can you tell the pros and cons?

3. Below is an example of a gel electrophoresis. Which one has DNA amplification?



Full Protocol

Lab Setting

Materials are enough for 6 groups.

Reagent Preparation

Refer to “Notes for Instructors 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 iso-S Master Mix. Can you see the enzymes?

Part 1: iso-S 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 iso-S Master Mix, which contains the Bst enzyme along with the iso-S buffer, into each of the four DNA sample tubes labeled "A, B, C, and D" (already containing 10 μL of each DNA).

Iso-S Reaction Mixtures			
DNA Samples		2X iso-S Master Mix	Total Reaction Volume
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
Sample D [D]	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 60 °C for ~20 minutes in a water bath or heat block.
4. Spin briefly (~10 seconds) to pool condensation that has collected on the cap and then immediately place tube in ice until ready for next step.
5. 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, answer the following questions:

1. After combining the 2x iso-S master 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 amplified or altered in any way by the addition of the 2X iso-S mix? Explain.

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

Part 2: Agarose Gel Electrophoresis Protocol

General Procedure, detailed directions given by instructor

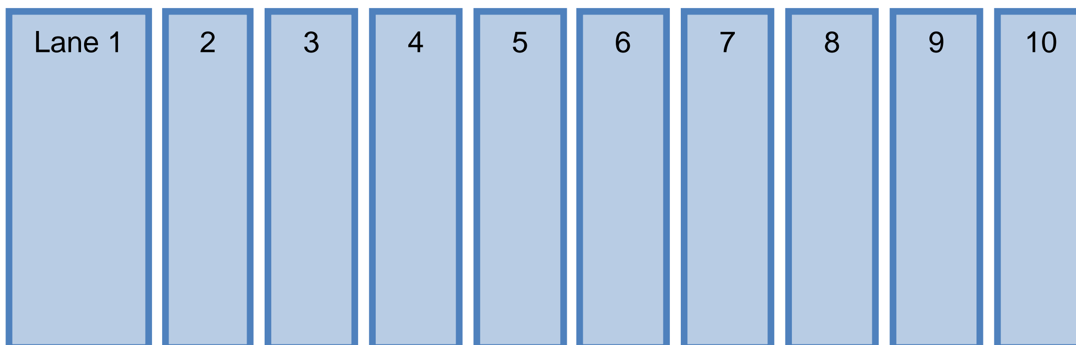
1. Prepare 1% agarose.
2. For staining, use a DNA dye which is added directly to the molten agarose. For light sensitive dyes, keep the gel in the dark during gelation. This can be done by performing in a dark room or placing a box over the gel.
3. Set up electrophoresis apparatus and pour in the 1%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

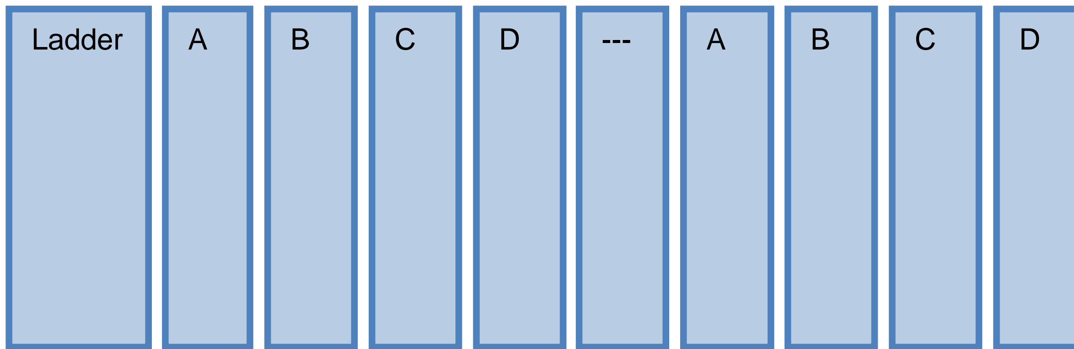
Amplified Samples		Loading Volume
Sample A [A]	20 μL	10 μL or more
Sample B [B]	20 μL	10 μL or more
Sample C [C]	20 μL	10 μL or more
Sample D [D]	20 μL	10 μL or more
DNA Ladder		10μL

5. Record which wells hold which samples.

Recommended Gel Loading:

Load two teams/gel





6. Run at ~120V for ~20 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. Examine the band patterns. The DNA ladder can be used as a band size reference.

Reference: For gel electrophoresis method, visit https://www.youtube.com/watch?v=QEG8dz7cbnY

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).

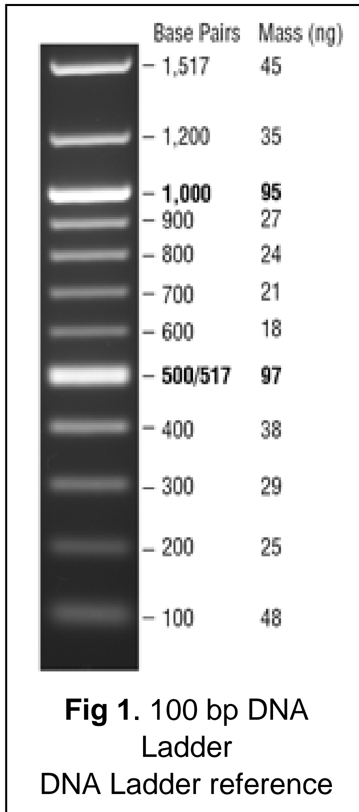


Fig 1. 100 bp DNA Ladder
DNA Ladder reference

Results and Discussion

Take a look at the bands visible from your samples on the gel. Which sample(s) is the answer to the question from this exercise?

Who were infected?

Can you see amplified DNA before running the samples on the gel? Why?

Can you see amplified DNA after running the samples on the gel? Why?

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

Summarize the process of DNA amplification and DNA gel electrophoresis using the correct terminology.

Troubleshooting

Problem	Possible causes	Solutions
Incomplete or no amplification of DNA	2X iso-S 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 60 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

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