HiGenoMB™
Unzipping Genes

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Product Information

HiPer® DNA Molecular Size Determination Teaching Kit

Product Code: HTBM014

Number of experiments that can be performed: 10

Duration of Experiment

Protocol: 1.5 hours
Observation and result: 30 minutes

Storage Instructions

- The kit is stable for 12 months from the date of manufacture
- Store the DNA Marker and Test samples at -20°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To determine the molecular size of three linear double stranded (ds) DNA fragment.

Introduction:

Agarose gel electrophoresis method is used to measure the molecular size of DNA and RNA molecules. For separation of very large DNA molecules (1000-2000 kb), pulsed field gel electrophoresis method has to be employed. The migration rates of DNA molecule depend upon their respective molecular weight. This simple and fast method is frequently used to determine the size of a linear DNA fragment by comparing its mobility to a DNA fragment marker of known size.

Principle:

Negatively charged DNA molecules are separated on agarose gel matrix according to their molecular weight upon electrophoresis. The position of DNA in the agarose gel is visualized by staining the gel with low concentration of a fluorescent intercalating dye like Ethidium bromide. Smaller molecules move faster and migrate farther than larger ones because the migration rates of DNA molecules are inversely proportional to the logarithms of the molecular weights. This method is frequently performed to determine the size of an unknown DNA fragment by comparing it with DNA ladders of known size. A standard curve can be obtained by plotting the molecular size of the fragments of the marker against the reciprocal of their respective mobility. The relative mobility (R_f) of the DNA ladder depends upon the log of its relative molecular weight. The R_f value can be determined after dividing the distance traveled by the DNA by distance traveled by tracking dye. The molecular size of the test sample can be obtained from the standard curve. The mobility rate of DNA molecules vary from one experiment to another. So, the control DNA ladder should always be loaded on the same gel.

Kit Contents:

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	MB002	Agarose	4.8 g	R T
2	ML016	50X TAE	120 ml	R T
3	MBT051	1 kb DNA ladder	0.035 ml	-20°C
4	TKC061	Test sample 1	0.11 ml	-20°C
5	TKC062	Test sample 2	0.11 ml	-20°C
6	TKC063	Test sample 3	0.11 ml	-20°C

Materials Required But Not Provided:

Glasswares: Conical flask, measuring cylinder, beaker.
Reagents: Distilled water, Ethidium bromide (10 mg/ml)
Other requirements: Electrophoresis apparatus, UV Transilluminator, micropipettes, tips

Storage:

HiPer® DNA Molecular Size Determination Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. DNA Marker and samples should be stored at -20ºC. Other kit contents can be stored at room temperature (15-25ºC).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. **Preparation of 1X TAE:** To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water*. Mix well before use.

   * Molecular biology grade water is recommended (Product code: ML024).

Procedure:

1. Prepare gel tray by sealing the ends with adhesive tape. Place comb in gel tray about 1 inch from one end of the tray and position the comb vertically such that the teeth are about 1-2 mm above the surface of the tray.

2. To prepare 50 ml of 0.8% agarose solution, measure 0.4 g agarose into a glass beaker or flask and add 50ml 1X TAE buffer. Heat the mixture on a microwave or hot plate by swirling the glass beaker/ flask occasionally, until agarose is dissolved completely (Ensure that the lid of the flask is loose to avoid buildup of pressure).

3. Allow solution to cool down to about 55-60°C. Add 0.5 µl Ethidium bromide, mix well and pour the gel solution into the gel tray to a depth of about 5mm. Allow the gel to solidify for about 30 minutes at room temperature.

4. To start the run, carefully remove the adhesive tape from both the ends of the gel tray, place the tray in electrophoresis chamber, and fill the chamber (just until wells are submerged) with 1X TAE electrophoresis buffer and gently remove the comb.

5. Load 3 µl of the ready to use DNA ladder onto well 1. Load 10 µl of each DNA samples onto well 2, 3 and 4.

6. Connect the power cord to the electrophoretic power supply according to the convention: Red-Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA current until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

7. Electrophoresis apparatus should always be covered to protect against electric shocks. Avoid use of very high voltage which can cause trailing and smearing of DNA bands in the gel, particularly with high-molecular-weight DNA.

8. Monitor the temperature of the buffer periodically during the run. If the buffer becomes heated, reduce the voltage. Melting of an agarose gel during electrophoresis is a sign that the voltage is too high, that the buffer may have been incorrectly prepared or has become exhausted during the run.

9. Switch off the power supply once the tracking dye from the wells reaches 3/4th of the gel which takes approximately 45 minutes. Observe the gel on a UV transilluminator.

Safety:

➢ **Precaution:**

UV light can damage the eyes and skin. Always wear suitable eye and face protection when working with a UV light source. UV light damages DNA. If DNA fragments are to be extracted from the gel, use a UV source of lower intensity and minimize exposure of the DNA to the UV light.

➢ **Disposal of Ethidium bromide waste:**

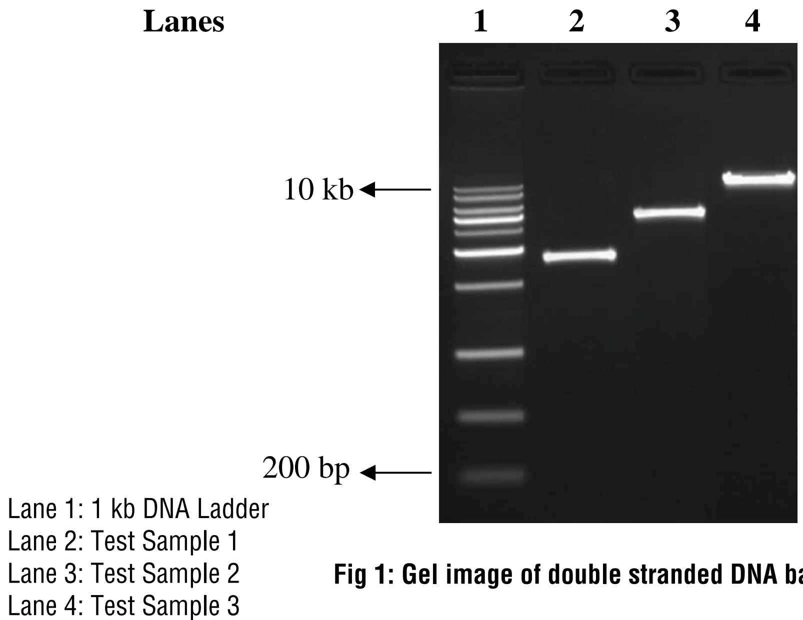
All items that were in contact with Ethidium bromide must be disposed off in the designated waste container (marked with "Ethidium bromide waste") within the GelDoc area, including gels, tissue paper used to clean the table, and nitrile gloves.

➢ **Hazard:**

Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves is recommended.

Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV transilluminator.



Lane 1: 1 kb DNA Ladder
Lane 2: Test Sample 1
Lane 3: Test Sample 2
Lane 4: Test Sample 3

Fig 1: Gel image of double stranded DNA bands observed

Measure the distance (in cm) traveled by each of the10 bands of 1 kb DNA ladder and the test samples (1, 2, 3) from the well.

Calculate the R_f value using the formula:

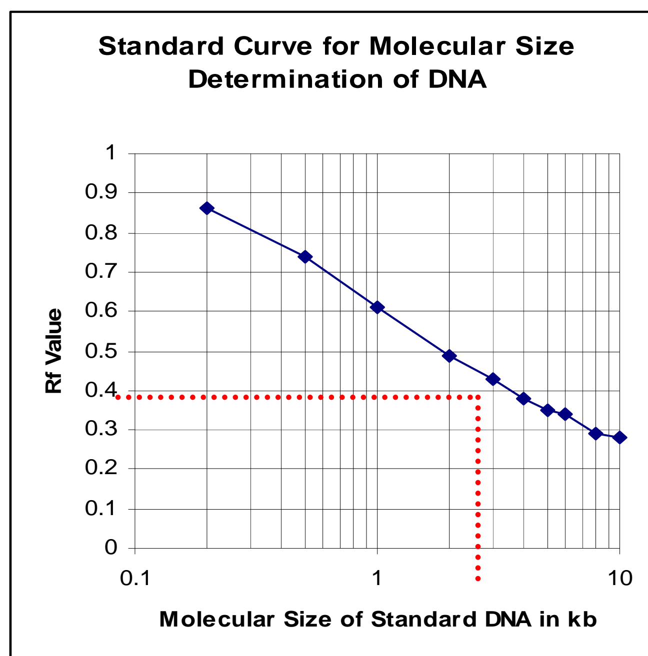
$$R_f = \frac{\text{Distance traveled by DNA molecule}}{\text{Distance traveled by the dye}}$$

For example, if the dye has migrated to a distance of 6.7 cm, following are the R_f values of the 1 kb DNA marker and test sample 1.

Table 2: R_f value of standard DNA Marker

Sr. No.	Molecular size of standard (in kb)	Distance traveled by DNA (in cm)	R_f Value
1	0.2	5.8	0.86
2	0.5	5.0	0.74
3	1	4.1	0.61
4	2	3.3	0.49
5	3	2.9	0.43
6	4	2.6	0.38
7	5	2.4	0.35
8	6	2.3	0.34
9	8	2.0	0.29
10	10	1.9	0.28
11	Test Sample 1	3.0	0.44

Record your observation as in Table 2. Plot the R_f values of each of the bands of the marker against its corresponding molecular size to construct a standard graph on a semi-log graph sheet.



Calculate R_f value of test samples and obtain the corresponding molecular size from the graph. Record the molecular size of the test samples as follows:

Table 3: Molecular Size of Test Samples

Test Sample	Molecular size in kb	Distance travelled by DNA (in cm)	R_f Value
1			
2			
3			

Interpretation:

By plotting the R_f values against the respective molecular size of a DNA marker in a semi-log graph sheet one can get the standard graph. This standard graph helps to determine the molecular size of any linear DNA band provided the DNA and the marker are electrophoresed on the same gel.

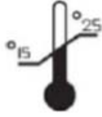
Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Faint or no band seen on the gel	Insufficient quantity of DNA loaded on the gel	Load exact amount of DNA as per the procedure
		Inaccurate amount of Ethidium bromide added to the gel	Always add proper amount of Ethidium bromide as given in the procedure
		DNA electrophoresed off the gel	Electrophorese the gel for less time, use a lower voltage
		DNA does not run in the proper direction	Always connect the cords of the electrophoretic unit as per convention: Black- Cathode, Red- Anode
2	Smeared DNA bands	DNA got degraded	Avoid nuclease contamination
		Improper electrophoresis condition	Ensure that the wells are completely submerged in the running buffer
		Gel Running Buffer reused several times.	Prepare fresh TAE buffer, dilute it as per instructions
3	Improper gel solidification	Gel not melted properly	Prepare gel as per the procedure. Ensure that the agarose is dissolved completely
		After preparation, gel kept for too long at room temperature	Add Ethidium bromide at right temperature after gel preparation and immediately pour the gel. Do not let the gel to solidify before pouring

Please refer disclaimer Overleaf.

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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