HiPer® Bacterial Genomic DNA Extraction Teaching Kit (Column Based)

Product Code: HTBM008E

Number of experiments that can be performed: 10

Duration of Experiment

Protocol: 1.5 hours

Agarose Gel Electrophoresis: 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of receipt
- Store Control DNA, Proteinase K and at -20°C
- Store the 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To extract and analyze genomic DNA from bacterial cells (using spin columns).

Introduction:

The term bacteria is a plural form of the Latin *bacterium*, meaning "staff" or "rod". Bacteria are amongst the most abundant prokaryotic organisms and have been on earth since almost 3.5 billion years. They have adapted to more living conditions than any other group of organisms. They inhabit air, soil, and water and exist in enormous numbers on surface of virtually all plants and animals. Most of the genetic information in a bacterial (or prokaryotic) cell is contained within the chromosome, where a single molecule of DNA is arranged as a double helix, usually in a closed loop. *Escherichia coli (E. coli)* are amongst the most commonly found bacteria. They are abundant in human and animal intestine and very easy to grow in the laboratory.

The isolation of genomic DNA from a bacterium (*E. coli*) generally comprises of three stages:

- Cultivation of the cells
- Disruption to release cell contents
- Purification of the DNA.

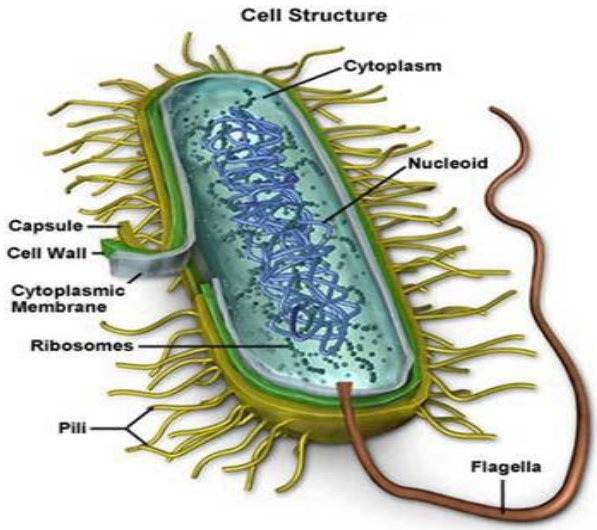


Fig 1: Structure and contents of a typical bacterial cell

HiPer® Bacterial Genomic DNA Extraction Teaching Kit (Column Based) provides a fast and easy method for purification of total DNA for reliable applications in PCR, library screening and sequencing etc. It is fast, simple and does not contain harmful organic compounds such as phenol and chloroform.

The DNA purification procedure using the miniprep spin columns comprises of three steps:

- Adsorption of DNA to the membrane
- Removal of residual contaminants
- Elution of pure genomic DNA

HiElute Miniprep Spin Column format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained.

Principle:

HiPer® Bacterial Genomic DNA Extraction Teaching kit (Column Based) simplifies isolation of DNA from bacteria by the spin-column procedure. Bacterial cells are grown in the medium till they reach log phase and are harvested by centrifugation. After harvesting, the bacterial cell wall is degraded by Proteinase K digestion and lysis. Following lysis, the DNA is allowed to bind to the silica-gel membrane of the HiElute Miniprep Spin column.

HiElute Miniprep Spin Column eliminates the need for alcohol precipitation, expensive resins, and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA isolation techniques. DNA binds specifically to the advanced silica-gel membrane while contaminants pass through. The adsorbed DNA is washed to remove trace salts and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer provided with the kit.

Kit Contents:

The kit can be used to extract genomic DNA from Gram negative bacteria.

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

Sr. No.	Product Code	Materials Provided	Quantity 10 expts	Storage
1	TKC011	Control DNA	0.11 ml	-20ºC
2	DS0015	Lysis Solution I	2.2 ml	R T
3	DS0010	Lysis Solution II	2.4 ml	R T
4	DS0031	Prewash Solution	6 ml	R T
5	DS0012	Wash Solution	6 ml	R T
6	DS0040	Elution Buffer	2.5 ml	R T
7	DS0013	Proteinase K Solution	0.25 ml	-20ºC
8	DS0003	RNase A Solution	0.25 ml	R T
9	DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	11 Nos.	R T
10	DBCA016	Collection Tubes (Uncapped), Polypropylene (2.0 ml)	11 Nos.	R T
11	PW1139	Collection Tube, Polypropylene (2.0 ml)	11 Nos.	R T
12	MB002	Agarose	4.8 g	R T
13	MI016	50X TAE	120 ml	R T
14	ML015	6X Gel Loading Buffer	0.05 ml	2-8 ºC

Materials Required But Not Provided:

Glass wares: Conical flask, Measuring cylinder, Beaker
Reagents: Ethanol (96-100%), Ethidium bromide (10 mg/ml), Distilled water
Other requirements: UV Spectrophotometer, Tabletop microcentrifuge (with rotor for 2.0 ml tubes), Electrophoresis apparatus, Incubator, UV Transilluminator, Micropipettes, Tips, Vortex Mixer, Adhesive tape, Water bath or Heating block, Microwave/Burner/Hotplate

Storage:

HiPer® Bacterial Genomic DNA Extraction Teaching Kit (Column Based) is stable for 12 months from the date of receipt without showing any reduction in performance. On receipt, store control DNA and Proteinase K at -20°C and 6X Gel Loading Buffer at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Thaw all refrigerated samples before use.
3. Preheat a water bath or heating block to 55°C.
4. Thoroughly mix the reagents. Examine the solutions for any kind of precipitation, if any solution (other than enzymes) forms a precipitate warm at 55-65°C until the precipitate dissolves completely, allow it to cool down to room temperature before use.
5. Ensure that only clean & dry eppendorf tubes and tips are used for the procedure.

Procedure:

Day 1: Revival of Bacterial culture

1. Take a loopful of Gram negative bacterial culture and streak onto LB agar plate

2. Incubate overnight at 37°C.

Day 2: Inoculation of Culture

1. Pick up a single colony from LB agar plate and inoculate in 10 ml of LB broth

2. Incubate the test tube overnight at 37°C.

Day 3: Genomic DNA Extraction

1. Harvest Cells:

Take 1.5 ml of the overnight grown culture into a collection tube and centrifuge the cells at 13,000 rpm for 1 minute. Discard the supernatant containing culture medium.

2. Resuspension of cell pellet and lysis

Add 180 µl of Lysis Solution I and resuspend the pellet by gentle pipetting.

3. Add 20 µl of Proteinase K solution to the above collection tube, vortex thoroughly for 10-15 seconds, and incubate for 30 minutes at 55°C.

4. Add 20 µl of RNase A solution to the above collection tube, vortex thoroughly for 10-15 seconds, and incubate for 5 minutes at room temperature (15-25°C).

NOTE: This step helps in getting RNA-free genomic DNA.

5. Add 200 µl of Lysis Solution II, vortex thoroughly for about 15 seconds, and incubate at 55°C for 10 minutes.

NOTE: A homogeneous mixture is essential for efficient lysis.

5. Prepare for binding

Add 200 µl of ethanol (95-100%) to the lysate and mix thoroughly by gentle pipetting.

NOTE: A white precipitate may form on addition of ethanol. This precipitate does not interfere with the DNA isolation procedure or with any subsequent application. It is essential to apply all of the precipitate to the HiElute Miniprep Spin column.

6. Load lysate into the HiElute Miniprep Spin Column

Transfer the entire lysate obtained from step 5 into the HiElute Miniprep Spin column for binding the DNA. Centrifuge at 10,000 rpm for 1 minute. Discard the flow-through liquid and place the spin column in the same 2.0 ml collection tube.

NOTE: Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents onto the column. If the solution has not completely passed through the membrane, centrifuge again at 13,000 rpm until all the solution has passed through. Centrifugation at high speed will not affect the yield or purity of the DNA.

7. Prewash

Add 500 µl of Prewash Solution to the HiElute Miniprep Spin column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

8. Wash

Add 500 µl of Wash Solution to the column and centrifuge for 3 minutes at 14,000 rpm to dry the column. Discard the flow through and place the column in the same collection tube. Centrifuge the column for an additional 1 minute at 14,000 rpm to remove the traces of Wash Solution.

9. DNA Elution

Pipette 200 µl of the Elution Buffer directly into the column without spilling to the sides. Incubate for 1 minute at room temperature. Centrifuge at 10,000 rpm for 1 minute to elute the DNA.

NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer, then centrifuge. Elution with volumes less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short term storage (24-48 hours) of the DNA, 2-8ºC is recommended. For long-term storage, -20ºC or lower temperature (-80ºC) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

Agarose Gel Electrophoresis:

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.

Preparation of Agarose gel: To prepare 50 ml of 0.8% agarose gel, add 0.4 g agarose to 50 ml 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate, swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool to about 55-60ºC. Add 0.5µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

NOTE: 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.

Loading of the DNA samples: To prepare sample for electrophoresis, add 2 µl of 6X gel loading buffer to 10 µl of DNA sample. Mix well by pipetting and load the sample onto the well. Load the Control DNA after extracting the DNA sample.

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

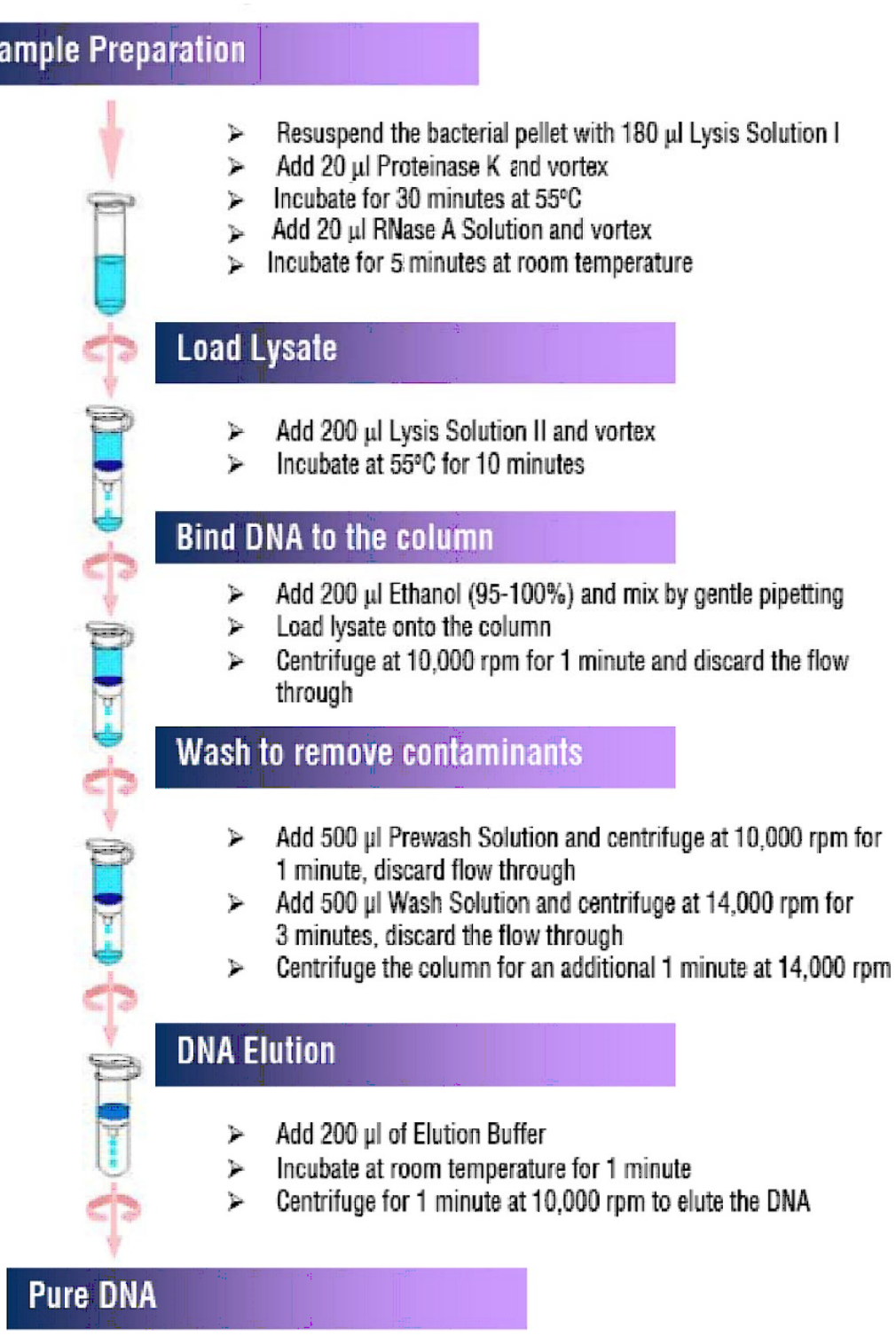
* Molecular biology grade water is recommended (Product code: ML024).

Quantitation of DNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct background absorbance. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm.

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor

Flowchart:


Sample Preparation
Resuspend the bacterial pellet with 180 µl Lysis Solution I
Add 20 µl Proteinase K and vortex
Incubate for 30 minutes at 55°C
Add 20 µl RNase A Solution and vortex
Incubate for 5 minutes at room temperature
Load Lysate
Add 200 µl Lysis Solution II and vortex
Incubate at 55°C for 10 minutes
Bind DNA to the column
Add 200 µl Ethanol (95-100%) and mix by gentle pipetting
Load lysate onto the column
Centrifuge at 10,000 rpm for 1 minute and discard the flow through
Wash to remove contaminants
Add 500 µl Prewash Solution and centrifuge at 10,000 rpm for 1 minute, discard flow through
Add 500 µl Wash Solution and centrifuge at 14,000 rpm for 3 minutes, discard the flow through
Centrifuge the column for an additional 1 minute at 14,000 rpm
DNA Elution
Add 200 µl of Elution Buffer
Incubate at room temperature for 1 minute
Centrifuge for 1 minute at 10,000 rpm to elute the DNA
Pure DNA

Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV Transilluminator and calculate the yield and purity using UV Spectrophotometer.

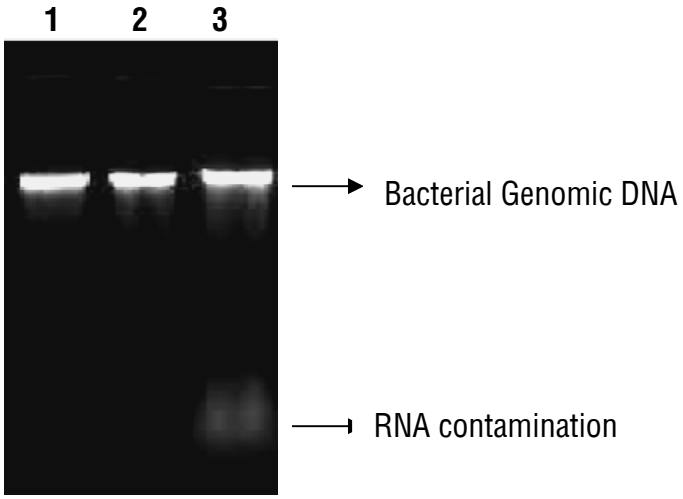


Fig 2: Gel picture of isolated bacterial genomic DNA

Lane 1: Control DNA
Lane 2: Extracted bacterial Genomic DNA
Lane 3: Bacterial Genomic DNA with RNA contamination

Table 2: Absorbance of the extracted plasmid DNA at 260 nm and 280 nm

Sample	Dilution Factor	A_{260}	A_{280}	A_{260}/A_{280}	Concentration (µg/ml)
1					
2					
3					

Calculate the concentration of isolated DNA using following formula:

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor

Interpretation:

The data in Lane 1 and 2 demonstrates that highly purified bacterial genomic DNA has been obtained with no visible RNA contamination when electrophoresed on agarose gel. If RNA contamination is present, one would see a faint and smeary RNA band below the genomic DNA as shown in lane 3 since RNA being of lower molecular weight runs faster than the genomic DNA. RNA contamination is observed when the RNase treatment has not been carried out properly.
An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. If the A_{260}/A_{280} ratio is 1.6-1.9, then the isolated DNA sample is considered to be pure. If higher A_{260}/A_{280} ratio is observed it indicates the possibility of RNA contamination.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Poor / Lower yield of genomic DNA	Incomplete lysis of cells	The sample and the lysis solution should be mixed thoroughly by pulse-vortexing
		Lysate/Ethanol mixture is not homogenous	Vortex the tubes for at least 5-10 seconds in order to obtain a homogenous solution before applying it to the column
		DNA Elution is incomplete. Eluate contains residual ethanol from the wash	DNA yield can be improved by incubating the Elution Buffer for 5 minutes at room temperature (15-25°C) after it is added to the column. Ethanol from the final wash should be eliminated completely before eluting DNA. Spin the tubes for longer time to remove the traces of ethanol from the column completely
2	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is too high	RNA contamination	Ensure that the sample is properly mixed after adding of RNase A Solution
3	Shearing of DNA	Improper handling of genomic DNA	All pipetting steps should be executed as gently as possible. Wide orifice pipette tips are recommended to eliminate shearing of the DNA to a large extent
4	Downstream applications are inhibited	Traces of Wash Solution present in the final genomic DNA preparation	Spin the column for additional 1 minute at 14000 rpm after the final wash step

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