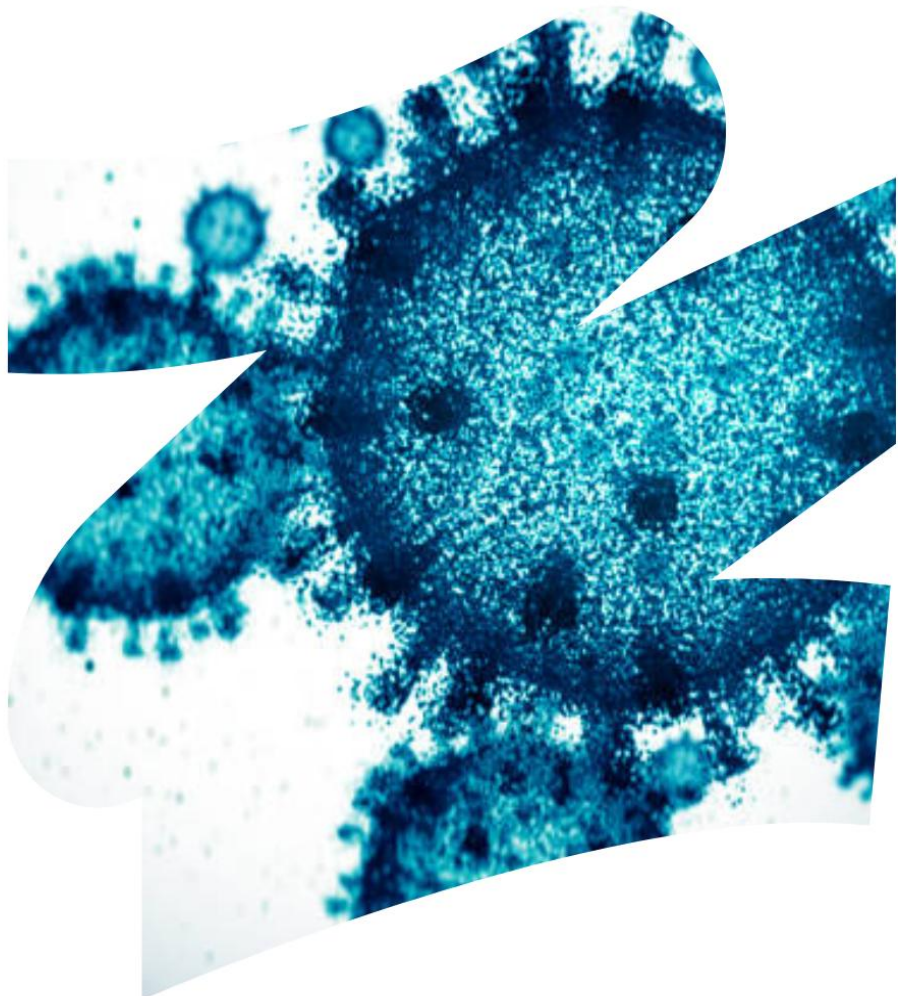




A NEW GENERATION COMPANY



SN Bacteria Genomic DNA extraction Teaching Kit

Solution Based

Innovation Speaks

SN Bacterial Genomic DNA Extraction Teaching Kit (Solution Based)

Product Code: SNLS –TK006

Number of experiments that can be performed: 5 Duration Experiment
Protocol: 1.5 hours Agarose Gel Electrophoresis 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Control DNA, Proteinase K and Bacterial Pellets at -20°C
- Store 6X Gel Loading dye at room temperature
- 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 solution based method).

Introduction:

Bacteria are unicellular microorganisms. Bacteria have one large circular chromosome which is located in the cytoplasm. Chromosomes consist of DNA molecules which carry most of their inherited information. Bacterial DNA is not enclosed in the nucleus; rather they form an irregular shaped body called nucleoid. Some bacteria also have small circular molecules of extra chromosomal DNA, known as plasmids which are capable of replicating independently.

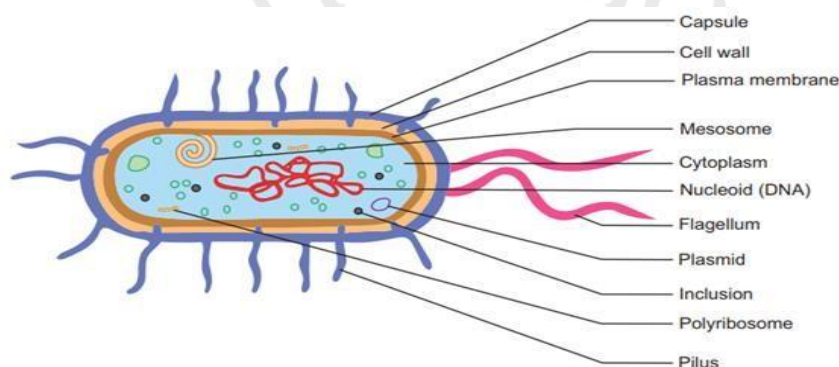


Fig 1: Structure and contents of a typical bacterial cell

SN Bacterial Genomic DNA Extraction Teaching Kit (Solution Based) provides a fast and easy method for purification of total DNA for reliable applications in PCR, library screening and sequencing etc.

The DNA purification using precipitation method involves the following steps:

- Resuspension of bacterial cell pellet
- Lysis of bacterial cells
- Precipitation of Genomic DNA
- Removal of residual contaminants by washing
- Elution of pure genomic DNA

Principle:

Many methods have been developed to extract and purify genomic DNA from bacteria. These methods invariably involve three steps:

- Growth of the bacterial culture
- Harvesting and lysis of the bacteria
- Purification of genomic DNA

The harvested bacterial culture is lysed and collected by centrifugation after which it is precipitated using isopropanol. The precipitated DNA is washed to remove contaminants, and the pure genomic DNA is eluted in

Elution Buffer. The purified genomic DNA can be used for immediate use in all molecular biology procedures such as digestion with restriction enzymes, cloning, PCR, in vitro translation, blotting and sequencing.

Kit Contents:

This kit can be used to perform genomic DNA extraction from Gram negative bacteria.

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

Sl. No.	Materials Provided	Quantity	Storage
		5 expts	
1	Control DNA	20 μ L	-20°C
2	Bacterial Cell Pellets	5 Nos.	-20°C
3	Lysis Solution	0.9 ml	R T
5	Isopropanol	4.5 ml	R T
6	Wash Solution	0.5 ml	R T
7	Elution Buffer	250 μ L	R T
8	Proteinase K Solution	100 μ L	-20°C
9	RNase A Solution	50 μ L	-20°C
10	Effendorf tubes	5 Nos.	R T
11	Agarose	1.5 g	R T
12	5X TBE	15 ml	R T
13	6X Gel Loading Dye	10 μ L	2-8 °C

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker

Reagents: Ethanol (96-100%), Ethidium bromide (10 mg/ml), Distilled water

Other requirements: U V Spectrophotometer, Tabletop microcentrifuge (with rotor for 1.5 ml tubes), Electrophoresis apparatus, Incubator, UV Transilluminator, Micropipettes, Tips, Vortex Mixer, Adhesive tape, Water bath or Heating block, Microwave/Burner/Hotplate.

Storage:

SN Bacterial Genomic DNA Extraction Teaching Kit (Solution Based) is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store

Control DNA, Bacterial Cell Pellets and Proteinase K Solution at -20°C . 6X Gel Loading Dye should be stored at $2-8^{\circ}\text{C}$. Other kit contents can be stored at room temperature ($15-25^{\circ}\text{C}$).

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 $55-65^{\circ}\text{C}$ until the precipitate dissolves completely, allow it to cool to room temperature before use.
5. Ensure that only clean & dry Eppendorf tubes and tips are used for the entire procedure.

Procedure:

1. Allow the bacterial cell pellet collection tube to thaw at room temperature.

2. Resuspension of pellet and cell lysis

Add 200 µl of Lysis Solution and resuspend the pellet by gentle pipetting.

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

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

4. Centrifuge at 10,000 rpm for 10 minutes. Transfer the supernatant into a new collection tube without disturbing the small white pellet.

5. Precipitation of genomic DNA

Add 0.8ml of isopropanol to the lysate and mix by gentle inversion till white precipitate is seen. Centrifuge at 10,000 rpm for 10 minutes. Carefully discard the supernatant without disturbing the white pellet.

6. Wash

Add 500 µl of Wash Solution (100 µl stock solution +400 µl ethanol) and resuspend the pellet by gentle pipetting. Centrifuge at 10,000 rpm for 10 minutes. Carefully discard the supernatant without disturbing the white pellet. Air dry the pellet for 10 minutes at room temperature (15-25°C).

7. Repeat the step 6 again.

8. DNA Elution

Resuspend the pellet in 50 µl of Elution Buffer and incubate at 55°C for 10 minutes. Centrifuge at 10,000 rpm for 10 minutes and transfer the supernatant containing pure genomic DNA into a new collection tube.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short term storage (24- 48 hours) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) 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 TBE:

To prepare 50 ml of 1X TBE buffer, add 10 ml of 5X TBE Buffer to 40ml of sterile distilled water*. Mix well before use.

Preparation of Agarose gel:

To prepare 30 ml of 1% agarose gel, add 0.3 g of agarose in 30 ml 1X TBE buffer in a glass beaker or flask. Heat the mixture in a microwave/burner/hot plate by 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 down to 55 - 60°C. Add 0.5 µl 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 dye to 10 µl of DNA sample. Mix well by pipetting and load the sample into the well. Load the Control DNA after extracting the DNA sample.

Electrophoresis:

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

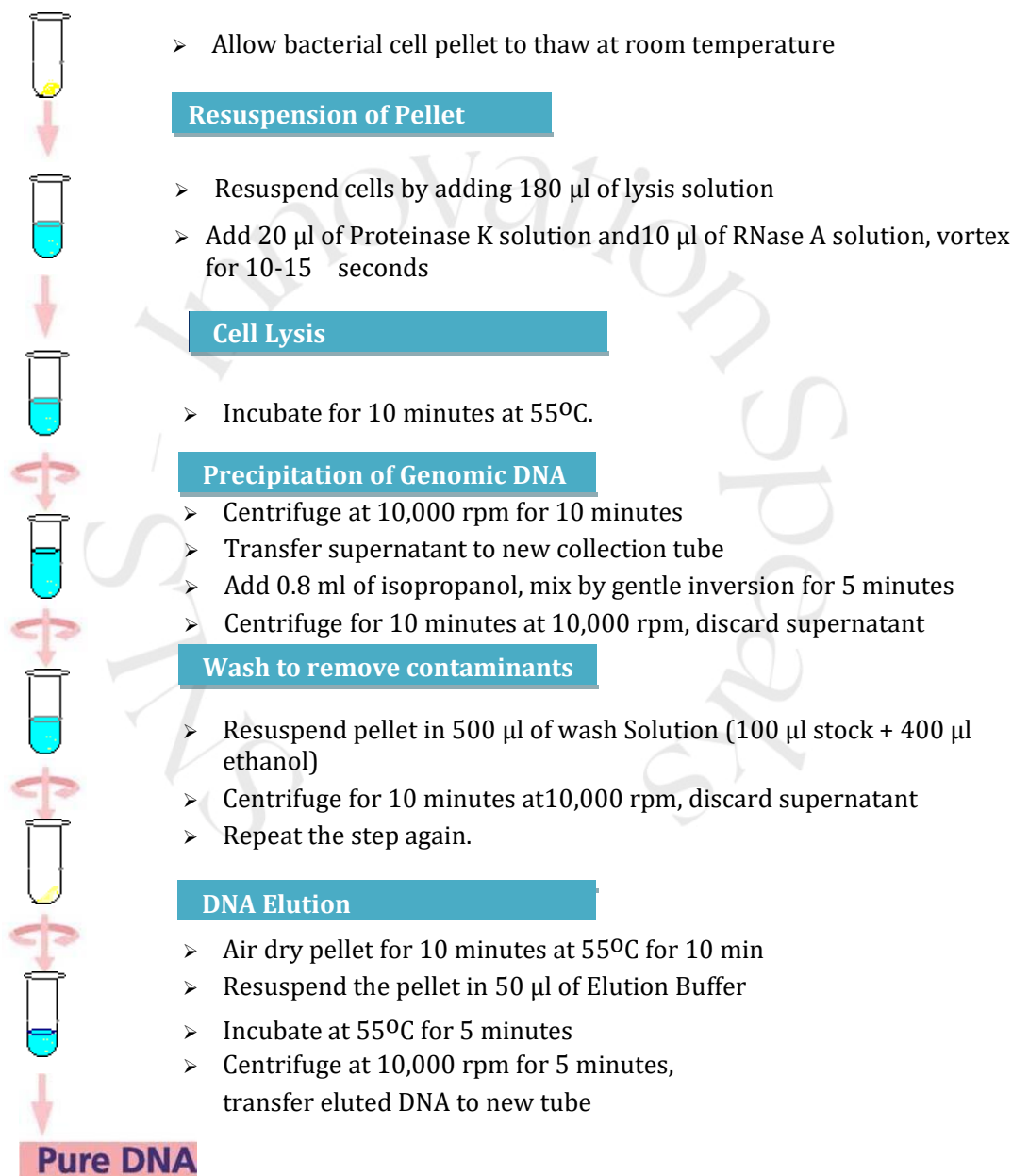
* Molecular biology grade water is recommended.

Quantitation of DNA:

Spectrophotometry analysis and agarose gel electrophoresis will reveal the concentration and 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 A₂₆₀ x dilution factor

Flow chart:



Observation and Result:

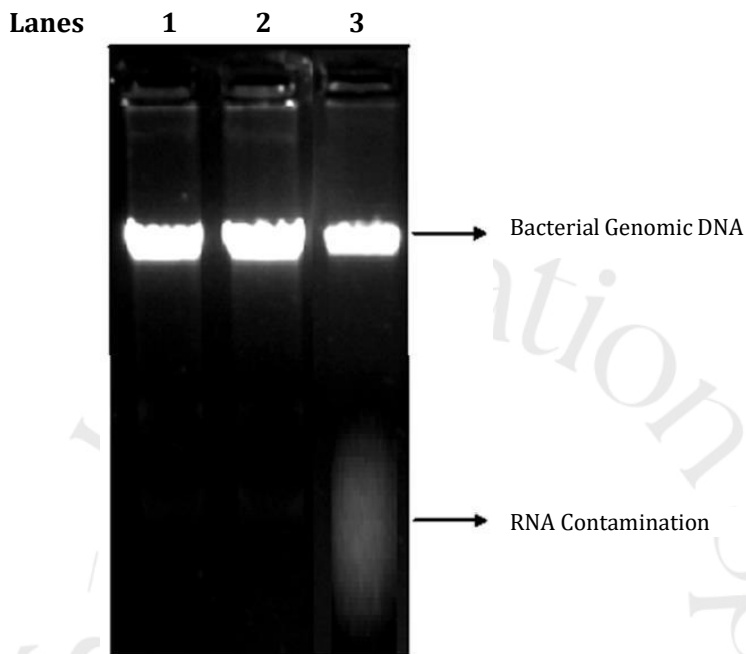


Fig 2: Gel image of isolated bacterial genomic DNA

Lane 1: Control DNA

Lane 2: Extracted bacterial Genomic DNA

Lane 3: Bacterial Genomic DNA with RNA contamination

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

Sample	Dilution Factor	A260	A280	A260/A280	Concentration (µg/ml)
1					
2					
3					

Calculate the concentration of isolated DNA using following formula: Concentration of DNA sample (µg/ml) = 50 x A260 x dilution factor

Interpretation:

The data in lanes 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 band 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 A260/A280 ratio is 1.6-2.0, then the isolated DNA sample is considered to be pure. If higher A260/A280 ratio is observed it indicates the possibility of RNA contamination.

Troubleshooting Guide:

Sl. 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
2	Purity of the DNA is lower than expected; A260/A280 ratio is too high	RNA contamination	Ensure that the sample is properly mixed after addition of RNase A Solution
3	DNA is sheared	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	After the washing steps the eluate should not come in contact with the column. Spin the column for 1 minute at 14,000 rpm, after emptying the collection tube



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SN Research Unit



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Contact Us -

☎ +91- 80959 61994 / 99005 05076

🌐 www.snlifesciences.in

✉ sales@snlifesciences.com / drmondal@snlifesciences.com

🏠 No.57/90, 3rd Road, 4th Phase Bommasandra Industrial Area,
Bengaluru - 560099

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