SHANKARANARAYANA LIFE SCIENCES
A NEW GENERATION COMPANY

SN PLASMID DNA Extraction Teaching Kit (Solution Based)

Product Code: SNLS-TK003

Number of experiments that can be performed: 20

Duration of Experiment: 3 days

Day 1: Revival of Host Day

Day 2: Inoculation of culture

Day 3: Plasmid Extraction and Agarose Gel Electrophoresis

Storage Instructions:

- The kit is stable for 12 months from the date of receipt
- Resuspension Solution at 4°C after adding RNase A
- Other kit contents can be stored at room temperature (15-25°C)

INDEX

Aim:

Isolation and purification of plasmid DNA (using solution-based method).

Introduction:

Plasmid is an extra-chromosomal DNA molecule different from the chromosomal DNA which is capable of independent replication. The term "Plasmid" was first introduced by theAmerican molecular biologist Joshua Lederberg in 1952. Plasmid size varies from 1 to over 1000 kilo base pairs (kbps). Plasmids are mostly circular and double-stranded. Plasmids are found in awide variety of bacterial species; but are sometimes found in eukaryotic organisms e.g., in *Saccharomyces cerevisiae*.
Plasmids typically have two important elements:

- An origin of replication
- A selectable marker gene (e.g., resistance to ampicillin)

Conformations of plasmid: Plasmid DNA may appear in one of the five conformations which are as follows:

- "Nicked Open-Circular" DNA has one strand cut.
- "Relaxed Circular" DNA is fully intact with both strands uncut, but has been enzymatically "relaxed" (super coils removed).
- "Linear" DNA has free ends, either because both strands have been cut, or because the DNA was linear in vivo.
- "Super coiled" (or "Covalently Closed-Circular") DNA is fully intact with both strands uncut, andwith a twist built in, resulting in a compact form.
- "Super coiled Denatured" DNA is like super coiled DNA, but has unpaired regions that make its lightly less compact.

The conformations listed above are in order of electrophoretic mobility from slowest to fastest;and for a given size, run at different speeds in the gel during electrophoresis.
Plasmid DNA Extraction Teaching Kit (Solution Based) provides a fast and easy method forpurification of total DNA for reliable applications in PCR, library screening and sequencing,restriction digestion etc

The DNA purification procedure using the precipitation method comprises following steps:

- Resuspension of bacterial cell pellet
- Lysis of cells
- Precipitation of Genomic DNAPrecipitation of Plasmid DNA
- Wash to remove residual contamination
- Elution of Plasmid DNA

Principle:

The alkaline lysis method for isolation of plasmid DNA from *E. coli* has been used for more than 20years. This method takes advantage of the physical difference between linear, closed and supercoiled DNA. The bacterial suspension is first exposed to a strong anionic detergent (i.e., SDS) at high pH which helps to rupture the cell wall by hyper lytic osmosis releasing the DNA (chromosomal and plasmid), proteins and other contents which are denatured. The strands of the closed circular plasmid DNA are not completely ruptured in this process as the plasmid has a highly supercoiled confirmation. On addition of neutralization solution, the proteins, polysaccharides and genomic DNA are precipitated, whereas the plasmid DNA remains in the solution. The plasmid DNA is then precipitated by addition of isopropanol. Subsequently, other contaminants are removed by addition of Wash Solution I and II. The pure plasmid is then eluted in Elution Buffer.

Kit Contents:

The kit can be used to perform plasmid DNA extraction from *E. coli* strain.

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

Sl.No	Materials Provided	Quantity			Storage
		5 expts	10 expts	20 expts	
1	Control DNA	0.025 ml	0.05 ml	0.10 ml	- 20°C
2	*E. coli* cells (with plasmid)	0.25 No.	0.5 No.	1 No.	2- 8°C
3	Resuspension solution	1.62 ml	3.25 ml	6.5 ml	2- 8°C
4	RNase A	0.0005 g	0.001 g	0.002 g	- 20°C
5	Lysis solution	1.62 ml	3.25 ml	6.5 ml	R T
6	Neutralization solution	1.87 ml	3.75 ml	7.5 ml	R T
7	Isopropanol	5 ml	10 ml	20 ml	R T
8	Wash solution	1.25 ml	2.5 ml	5 ml	R T
9	Elution Buffer	0.375 ml	0.75 ml	1.5 ml	R T
10	Agarose	1.5 g	3 g	6 g	R T
11	Luria Bertani (LB) Broth	2.5 g	5 g	10 g	R T
12	Agar Powder, Bacteriological	0.5 g	1 g	2 g	RT
13	Ampicillin	0.5x0.025g	1x0.025 g	2x0.025g	- 20°C
14	Collection Tubes, Polypropylene (1.5 ml)	10 Nos.	20 Nos.	40 Nos.	R T

15	5X TBE	60 ml	120 ml	240 ml	R T
16	6X Gel Loading Buffer	0.025 ml	0.05 ml	0.1 ml	2-8°C

NOTE: Reconstitute the lyophilized RNase A by adding 200µL of Nuclease free water.

- After reconstitution store at -20˚.

Materials Required But Not Provided:

Glassware: Conical flask, Measuring cylinder, Beaker

Reagents: Distilled water, Ethidium bromide (10 mg/ml)

Other requirements: UV Spectrophotometer, Tabletop microcentrifuge (with rotor for 2.0 ml tubes), Electrophoresis apparatus, Incubator, UV Transilluminator, Micropipettes, Vortex Mixer, Shaker, Tips, Adhesive tape, Microwave/ Hotplate/ Bunsen, sterile loop.

Storage:

SN Plasmid 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 the Control DNA and Ampicillin at - 20ºC. Store *E. coli* cells (with plasmid), 6X Gel Loading Buffer and Resuspension solution at 2- 8ºC. Other kit contents can be stored at room temperature (15- 25ºC).

Important Instructions:

Read the important instructions before starting the experiment. Thaw all refrigerated samples before use. Thoroughly mix the reagents. Examine the solutions for any kind of precipitation. If any solution (except Resuspension Solution) forms a precipitate warm at 55-65˚C, until the precipitate dissolves completely, allow it to cool to room temperature before use.

Ensure that clean & dry Eppendorf tubes and tips are used for the procedure.

Ampicillin Preparation: Dissolve 25 mg of the Ampicillin antibiotic in 500 µl of sterile distilled water to prepare a stock concentration of 25 mg/500 µl. Store at -200C.

Preparation of Luria Bertani Broth (10 ml): Dissolve 0.25 g of Luria Bertani broth in 10 ml of distilled water and autoclave.

Preparation of LB (Luria Bertani) agar plates with ampicillin (50 ml): Dissolve 1.25 g of LB agar in 50 ml distilled water to cool down to 40-45ºC. Add 50 µl of ampicillin into it and pour on sterile petri-plates.

Procedure:

Day 1: Revival of Host

- Open the vial containing culture and resuspend the cells with 0.25 ml of LB broth.
- Pick up a loopful of culture and streak onto LB agar plate with ampicillin.
- Incubate overnight at 37°C.

Day 2: Inoculation of Culture

Pick up a single colony from LB agar plate and inoculate in 10 ml of LB broth containing 10µl ampicillin.
Incubate the test tube overnight at 37ºC.

Day 3: Plasmid Extraction

Harvest Cells

Take 1.5 ml of the overnight grown culture into a collection tube and centrifuge the cells at 13,000 rpm for 3minutes. Discard the supernatant culture medium.

NOTE: For good plasmid yields, the $O.D_{600}$ of the culture should be around 3.0×10^6 cells/ml.

Resuspend Cells :

Thoroughly Resuspend the cell pellet in 250 µl of Resuspension Solution and mix well by gentle vortexing till no cell clumps are visible

Lysis Cells

Add 250 µl of Lysis Solution to lyse the cells. Mix thoroughly by gently inverting the tube 4- 6 times.

NOTE: Do not vortex the tubes as it may result in the shearing of genomic DNA which may contaminate the plasmid DNA. Do not allow this lysis reaction to exceed for more than 5 minutes. Neutralize

Add 350 µl of Neutralization Solution and immediately mix thoroughly by inverting the tube 4-6times.

NOTE: On addition of Neutralization Solution, genomic DNA will precipitate out. The mixture should become cloudy and the precipitation should be homogeneous.

Centrifuge the sample at 13,000 rpm for 10 minutes to obtain a compact white pellet.

NOTE: A compact white pellet of genomic DNA will form. If the supernatant is not clear, transfer the supernatant to a fresh tube and spin for an additional one minute at 13,000 rpm to remove the interfering salts/precipitates completely.

Precipitation of plasmid DNA
Carefully transfer the supernatant containing plasmid DNA to a new collection tube. Add 1 ml of isopropanol to precipitate plasmid DNA and mix by gentle inversion for 5 minutes.

Centrifuge at 13,000 rpm for 15 minutes. White pellet of plasmid DNA will be seen, sticking to the sides of the tube. Discard the supernatant and invert the vial on blotting paper to drain out left over supernatant.

First Wash
Resuspend the pellet by adding 500 µl of Wash Solution (400 µl ethanol+100ul stock washsolution) and centrifuge at 13,000 rpm for 3 minutes.

Second wash
Add 500 µl of Wash Solution (400 µl ethanol+100ul stock wash solution) and centrifuge at 13,000rpm for 3 minutes.

DNA Elution

Resuspend the pellet in 50 µl of Elution Buffer. Centrifuge at 13,000 rpm for 5 minutes to remove insoluble material and transfer the supernatant containing pure plasmid DNA into a new collection tube.

Storage of the eluate with purified DNA: The eluate contains pure plasmid 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 40 mlof 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 1XTBE

buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate or burner 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 to about 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 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.

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. The concentration of DNA is calculated by the following formula:

Concentration of DNA sample (µg/ml) = 50 x A260 x dilution factor

Flowchart:

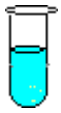
Inoculation and Harvesting

- Harvest Cells according to the O.D.
- Inoculate *E.coli* cells into 10 ml LB broth containing ampicillin.

Resuspension

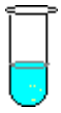
- Take 1.5 ml of bacterial culture and centrifuge at 13,000 rpmfor 3min.
- Resuspend cells by adding 250µl of Resuspension solution and10µl RNase A. Vortex gently.

Cell Lysis and Neutralization



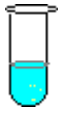
- Add 250µl lysis solution. Mix gently by inverting 4-6 times
- Add 300µl neutralization solution. Mix gently by inverting 4-6 times

Precipitation of Plasmid DNA.



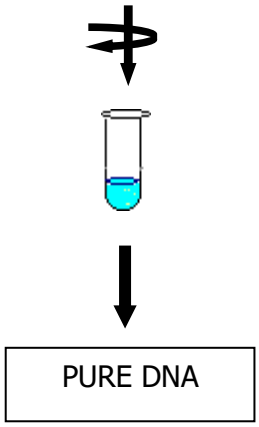
- Centrifuge for 10 minutes at 13,000 rpm.
- Transfer supernatant to new collection tube
- Add 1 ml of isopropanol, mix by gentle inversion for 5 minutes

Wash to remove contaminants



- Centrifuge for 10 minutes at 10,000 rpm, discard supernatant
- Resuspend pellet in Add 100 µl of Wash Solution (400 µl ethanol+100ul stock wash solution)

- centrifuge for 3 minutes at13,000 rpm, discard supernatant
- Add 500 µl of Wash Solution (400 µl ethanol+100ul stock wash solution)
- Centrifuge for 3 minutes at 13,000 rpm, discard supernatant
- Air dry pellet for 10 minutes at 55˚C for 10 min.



DNA Elution

- Resuspend the pellet in 50 µl of Elution Buffer
- Incubate at 55˚C for 5 minutes
- Centrifuge at 10,000 rpm for 3 minutes, transfer eluted DNA tonew tube.

Observation and Result:

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

Lanes 1 2 3 4 5

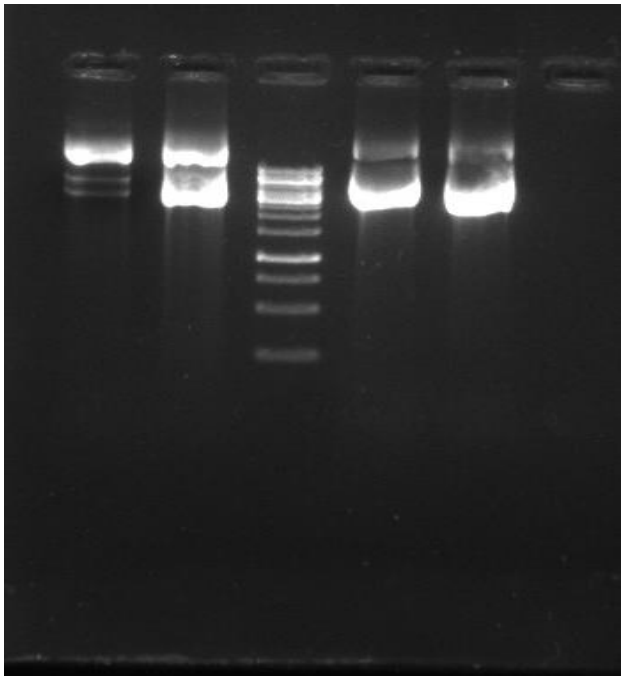


Fig 2: Gel picture of isolated plasmid DNA

Lane 1: Control DNA

Lane 2, 4 & 5: Extracted plasmid DNA

Lane 3: DNA ladder

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:

On analyzing plasmid DNA after electrophoresis, two bands were observed.

- Nicked DNA
- Super coiled DNA

The super coiled being more compact runs faster than the nicked form. The nicked form runs slowlysince their open structure experiences more resistance while passing through the gel matrix. These are seen as bands above the super coiled form.

The data in lanes 2, 4 and 5 demonstrate that highly purified plasmid 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 DN. 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 1.6-1.9, then the isolated DNA sample is considered to be pure. If higher A260/A280 ratio isobserved it indicates the possibility of RNA contamination.

Troubleshooting Guide:

Sl. No.	Problem	Possible Cause	Solution
1	Poor or low plasmid DNArecovery	Antibiotic activity is insufficient	Use a fresh antibiotic solution for growth of overnight cultures. Most antibiotic solutions are light sensitive and degrade during long term storage at 2-8˚C.
		Residual supernatant From cell media	Remove the supernatant after the initial centrifugation; the remaining supernatant canbe removed by an additional centrifugation.
		Alkaline lysis is prolonged	The lysis time should not exceed more than 5 minutes.
2	Additional bandseen ahead of super coiled plasmid during gel electrophoresis	A portion of the plasmid DNA is permanently denatured	Do not allow the lysis reaction to exceed 5 minutes. NOTE: The nicked or covalently open double- Stranded plasmid DNA runs slower than the super coiled DNA during electrophoresis.
3	Poor performance in downstream enzymatic applications	Purification is incomplete	Salts in one or more of the solutions may have precipitated. Examine the solutions for any kind of precipitation, if any solution forms a precipitate warm at 55-65°C until the precipitate dissolves completely; allow it to cool to room temperature before use

Technical Assistance:

At SN we pride ourselves on the quality and availability of our technicalsupport. For any kind of technical assistance, mail at **sales@snlifesciences.com**

Shankaranarayana Life Sciences LLP

57/90,3rd Road, Bommasandra Industrial Area, 4th Phase, Bengaluru Urban, Karnataka-560099.

MAIL: sales@snlifesciences.com

Call:+91-7975790185