Applied Biology and Bioengineering (BT520)

EXPERIMENT- 6

ISOLATION OF PLASMID DNA

Purpose: Isolation of Plasmid DNA from a microbial source

Principle: Plasmids are extra chromosomal DNA that replicate independently of the bacterial chromosome. They are normally covalently closed, circular, super-coiled molecules. They carry genes encoding functions (such as antibiotic resistance) which may be useful to the cell but are not essential for normal cellular activities. In the recombinant DNA technology plasmid DNA are used as vectors for carrying any foreign DNA. They can replicate with in host cell and possess phenotypic traits by which they can be detected. Genetic engineering makes use of recombinant DNA technology to fuse genes with plasmid vectors and clone them in the host cells. This way large number of isolated genes and their products can be synthesized and used for industrial, therapeutic and agricultural purposes.

Requirements:

1. *E. coli* cells (DH5α) (provided)
2. Plasmid DNA Isolation Kit (Sigma) (provided)
3. Ethidium Bromide
4. Luria Bertani medium
5. Autoclaved Sigma water
6. Agarose and Buffers for Electrophoresis

Luria Bertani medium (provided)

Media Composition: All the Ingredients are in (g/L)

Bactotryptone : 10g
Bacto Yeast extract : 5g
Sodium Chloride : 10g
pH : 7.2

Electrophoresis buffers (provided)

TBE Electrophoresis buffer (10X stock)

108 g Tris Base
55 g Boric acid
40 ml of 0.5 M (pH 8.0) EDTA
Adjust volume to 1 L (Use 1X for electrophoresis)

Nucleic acid sample Buffer (5X)

50mM Tris HCl
25%Glycerol
5 mM EDTA
0.2 % Bromophenol Blue
0.2% Xylene Cyanol (pH 8.0)

Ethidium Bromide (provided)

Prepare a stock solution of 5 mg/ml. For staining, use final concentration of 0.5 µg/ml.

Equipment/glass and other wares

1. Autoclave
2. Incubator shaker
3. Micro-centrifuge
4. Horizontal electrophoresis apparatus
5. Power supply unit
6. Gel documentation / Trans-illuminator
7. Eppendorf tubes (1.5 ml)
8. Micropipettes (P1000, P200, P20).

Day 0

1. Inoculate 50 µl of bacterial cells (*E. coli*, DH5α) from frozen glycerol stock (or pick up one colony from Petri plate using sterile tooth pick) to 5ml LB medium. Allow the cells to grow overnight at 37℃ at 200 rpm.

Day 1

A. Isolation of Plasmid DNA

1. **Harvest and Lysis of bacteria:** Transfer 1.5 ml of broth containing cells into 1.5 ml centrifuge tubes and centrifuge the cells at 13000 rpm for 1 min. Discard supernatant.
2. Resuspend the cells in 200 µl of Resuspension solution. Pipette up and down or vortex for mixing.
3. Add 200 µl of Lysis Solution. Invert **gently** to mix. **Do not vortex.** Allow to clear for about 5 min.
4. **Preparation of clear lysate:** Add 350 µl of Neutralization solution (S3). Invert 4-6 times to mix.
5. Centrifuge at 13,000 rpm for 10 mins.
6. **Preparation of binding column:** Add 500 µl Column Preparation Solution to binding column in a collection tube.
7. Spin at 13,000rpm for 1 min. Discard flow through.
8. **Binding plasmid DNA to column:** Transfer the cleared lysate to the binding column
9. Centrifuge at 13,000 rpm for 1 min. Discard the flow through.
10. **Wash to remove contaminants:** Add 750 µl Wash solution to column. Spin for 1 min. Discard flow through.
11. Spin for 1 min to dry the column (dry by speed vacuum). Allow at room temperature for 2-5 min.
12. **Elution of purified Plasmid DNA:** Transfer the column to a fresh collection tube. Add 70 µl of Elution Solution (or sterile sigma water). Spin for 1 min at 13,000 rpm. The eluate contains plasmid DNA. Store the plasmid DNA at -20°C or -80°C.

Day 2:

A. Preparation of Agarose Gel for Electrophoresis of the isolated plasmid DNA:

1. Prepare 0.8% Agarose solution in 1X TBE.
2. Pour the agarose into Gel casting trays pre set along with combs. Allow the agarose gel to polymerize and then remove combs without breaking the wells. Submerge the gel into the horizontal electrophoresis tank containing 1X TBE buffer.

B. Electrophoresis of the isolated plasmid DNA:

1. Take about 5 µl of the (eluate) plasmid DNA and add 2 µl of Sample buffer.
2. Load about 7 µl into each well along with a marker DNA or Ladder.
3. Run electrophoresis at constant voltage and allow the DNA to run in 1x TBE running buffer, keeping track of the dye front.
4. Remove the gel and place in a solution of Ethidium Bromide for staining DNA for 30min. Remove the gel with gloves, rinse with water and place in the Gel Documentation system / Transilluminator and visualize the DNA bands in the UV light.
5. Quantify the DNA concentration from the standard (ladder) used.

Observation:

Result: