Applied Biology and Bioengineering (BT520)

EXPERIMENT- 7

RESTRICTION ANALYSIS of PLASMID DNA

Purpose Restriction digestion of plasmid DNA for confirmation of clones.

Principle Recombinant Plasmids containing the target gene are digested by restriction enzymes to confirm the ligation of the inserted gene and hence the positive clones. The digested fragment is run on agarose gel and its size can be determined by comparing the size obtained with the DNA ladder, the recombinant clones are confirmed.

Materials requirement

1. Plasmid DNA (isolated)
2. Restriction enzymes
3. Agarose and Buffers for Electrophoresis
4. Sterile nuclease free water, sterile tips

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)

50 mM Tris HCl
25%Glycerol

Equipment

Water bath, Agarose gel electrophoresis Kit, Gel documentation System, Transilluminator, Micropipettes

Procedure

Day 1

A. Restriction digestion of Plasmid DNA

Take 10 µl of plasmid DNA and add prescribed reaction buffer and then 1 µl of restriction enzyme (NheI, 1 U/mg) and make volume to 25 µl and incubate at 37°C in a water bath for 2h. Then add 1 µl of second restriction enzyme (XhoI, 1 U/mg) and the required reaction buffer and further incubate for 2 h at 37°C in a water bath.

Day 2

B. 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.

C. 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