OBJECTIVE: Restriction enzyme digestion

Chemicals required: pUC19 DNA, BamH1 enzyme, 10X buffer, 1Kb Ladder, Sterile water, Agarose, 6X loading dye, Ethidium Bromide, 1X TAE buffer

Plastic ware/glassware: autoclaved micro centrifuge tubes, 0.02ml-0.2 ml tips, enzyme tips

Equipments required: Dry bath

PROTOCOL

1. Take 1.5 µg of pUC19 DNA (10 µl) in a fresh autoclaved microcentrifuge tube.
2. To this, add 10.5 µl of sterile water followed by 2.5 µl of 10X buffer.
3. Add 2 µl of BamH1 enzyme (2 units) and incubate the mixture at 37°C for 2 hrs.
4. Mix 10 µl of the sample and 2 µl of the loading dye
5. Prepare 1 % agarose gel and load the samples including 1 Kb DNA ladder, undigested pUC19 DNA and BamH1 digested pUC19 DNA.
6. Run the gel at 70 V for 1 hr.
7. Visualize the gel under UV transilluminator.

Reaction Protocol:
pUC19 DNA : 10 µl (1.5µg)
Sterile water : 10.5 µl
10X buffer : 2.5 µl
BamH1 : 2 µl

Total : 25 µl (Incubate at 37° C for 2 hrs)