OBJECTIVE: To determine the DNA damage induced by UV

Chemicals required: T4 endonuclease V, 1X T4 Reaction Buffer, 100X BSA, dry bath, agarose, ethidium bromide, supercoiled pUC19 plasmid DNA

Plastic ware/glassware: autoclaved micro centrifuge tubes, 1.0 ml tips, 0.02ml-0.2 ml tips, enzyme tips

Equipments required: Dry bath, horizontal gel electrophoresis assembly, UV source

PROTOCOL

1. Plasmid pUC19 (0.5 µg) is irradiated with 40, 80, 160 or 240 J/m2 of UV.

2. The DNA damage is determined by treating the irradiated samples with T4 endonuclease V (TEV).

Reaction Conditions

Plasmid DNA= 5 µl (0.5 µg)
1X Reaction Buffer= 2 µl
100X BSA= 0.2 µl
MilliQ water= 11.8 µl
T4 Endonuclease V= 1 µl (1 unit)
Reaction volume = 20 µl

Incubate at 37°C for 30 minutes.

3. Load the sample along with control DNA (not exposed to UV) in a 1% agarose gel. Following incision by TEV, supercoiled plasmids containing UV lesions are converted to a relaxed open circular conformation that can be monitored in an agarose gel stained with ethidium bromide.