Laboratory Work

First day

A- Slide preparation:

- Fully frosted slides are layered twice with 100 µL 1% GP-42 agarose (normal agarose). Leave to solidify at least 10 min.

B- Sample preparation:

1- Samples collection: fresh tissue samples should be used within an hour to avoid extra DNA damage. Samples should be kept in ice container.

2- Homogenization: Tissue sample (liver, kidney, brain, testis….) homogenize in a chilled homogenizing buffer (0.075 M NaCl, 0.024 M Na_2EDTA, pH 7.5) using automatic homogenizer.

3- Centrifugation: 15 min., 1500 rpm, at 0 °C.

4- Mix 75 µL of nuclear suspension (supernatant) with 75 µL of 2% LGT agarose (low melting agarose). Cover the slide with another slide and leave to solidify.

5- Finally 100 µl of normal agarose 1% was quickly layered on the surface and covered with another slide and allowed to gel.

C- Lysis:

Immerse slides in chilled lysing solution (2.5 M NaCl, 100mM Na4EDTA, 10mM Tris base, 0.1% SDS, 10% dimethyl sulfoxide, and triton X-100) and keep at 4°C in the dark for 1-24 hours.

Second day

D- Unwinding:

The slides are placed on a horizontal gel electrophoresis platform and covered with chilled alkaline solution (300 mM NaOH and 1mM Na_2 EDTA, pH 13) in the dark at 0°C for 20 min, (OFF)

E- Electrophoresis:

Electrophoresis is conducted (25 V, 300mA) (ON) at 0°C in the dark for 20 min.

F- Neutralization:

Immerse slides in neutralizing solution (400 mM Tris buffer pH 7.5) for 7 minutes.

G- Dehydration:

Dehydrate slides in ethanol for 5 minutes and Allow slides to dry at room temperature.

H- Staining and microscopical examination:

Ethidium bromide stain, 50µl and examine with fluorescent microscope with green filter.