SOUTHERN BLOTTING

Depurination solution

11 ml HCL

989 ml distilled wáter

mix. store at room temperatura for up to 1 month.

Denaturation buffer

87.66 g NaCl

20 g NaOH

Add approximately 800 ml of distilled wáter. Mix to dissolve.

Make up to a final volumen of 1000 ml. Store at room temperatura for up to 3 months.

Neutralization buffer

87.66 g NaCl

60.5 g Trizma base

Add approximately 800 ml of distilled wáter. Mix dissolve. Adjust to pH 7.5 with concentrated hydrochloric acid.

Make up to a final volumen of 1000 ml. Store at room temperatura for up to 3 months.

Nucleic acid transfer buffer (20x SSC)

88.23 g Tri-sodium citrate

175.32 g NaCl

Add approximately 800 ml of distilled wáter. Mix dissolve. Check the pH is 7-8.

Make up to a final volumen of 1000 ml. Store at room temperatura for up to 3 months.

PROTOCOL

1.- Separate the DNA samples on a suitable neutral agarose gel.

2.- following electrophoresis visualize the DNA samples in the gel with UV light.

3.- Process the gel for blotting, between each step rinse the gel in distilled wáter.

4.-Depurination

Place in 0.125 M HCl so that the gel is completely covered in the solution. Agitate gently for approximately 10 minutes. During this time the bromophenol blue dye present in the samples will change color.

Note:

 Depurination is not required for DNA fragments ≤ 10 kb in size. Do noto ver depurinate, 10 minutes (or until the bromophenol blue turns yellow) is usually sufficient for most samples.

5.-Denaturation

Submerge the gel in sufficient denaturation buffer. Incubate for 30 minutes with gentle agitation.During this time the bromophenol blue dye will return to its original color.

6.-Neutralization

Place the gel in sufficient neutralization buffer to submerge the gel. Incubate for 30 minutes with gentle agitation.

7.- set up the capillary blot.

