

APPENDIX

Reagents and buffer

Reagents and buffer for DNA-isolation:

Cold lysis buffer (pH 7.5): 100 mM Tris-HCl, 0.32 M sucrose and 1% of Triton X-100, dissolved in distilled water. The pH is adjusted with conc.HCl. The buffer is kept in the refrigerator.

10x STE buffer (pH 7.5): 500 mM Tris-HCl, 1.0 M NaCl and 10 mM EDTA are dissolved in distilled water. The pH is adjusted with conc.HCl, and the buffer is sterilized at 112°C for 15 min.

10% (w/v) SDS solution: 100 gm of SDS dissolved in 1000 ml distilled water.

80% (w/w) phenol solution: 1.0 Kg of phenol dissolved in 250 ml of 20 mM Tris-HCl (pH 8.0).

* If not used within a short time, 0.1% 8-hydroxyquinoline and 0.2 % of 2-mercaptoethanol are added.

3.0 M Sodium Acetate (pH 6.0): The pH is adjusted with acetic acid and the solution is sterilized at 112°C for 15 min.

10 x TE buffer (pH 8.0): 100 mM Tris-HCl and 10 mM EDTA.

in distilled water. The pH is adjusted with conc.HCl and the buffer is sterilized at 112°C for 15 min.

Reagents and buffer for Restriction endonuclease digestion:

10x reaction buffer: the composition of the buffer is different for each enzyme, but all are kept at -20°C .

For Bam HI, Bgl II and Eco RI: 50 mM Tris-HCl (pH 8.0), 10 mM MgCl_2 .

For Hind III and Pst I: 50 mM Tris-HCl (pH 8.0), 10 mM MgCl_2 and 50 mM NaCl.

For Rsa I: 50 mM Tris-HCl (pH 8.0) and 10 mM MgCl_2 .

For Apa I: 20 mM Tris-HCl (pH 7.4), 5 mM MgCl_2 and 50 mM NaCl.

5 x loading buffer: 15% Ficoll (Type 400), 1% SDS, 50 mM EDTA and 0.3% Brom phenol blue.

Reagents and buffers for gel electrophoresis:

Tris-acetate buffer (pH 8.0): 40.0 mM Tris-HCl, 20.0 mM NaOAc, 18 mM NaCl and 2.0 mM EDTA. The pH is adjusted with conc.HCl.

Tris-borate buffer: 0.1 M Tris-HCl, 0.1 M Boric acid and 2.5 mM EDTA.

Ethidium bromide (stock): 10 mg ethidium bromide are dissolved in 10 ml of 1x TE buffer and kept in a dark and cool place.

20% Polyacrylamide gel: 20% acrylamide gel (Acrylamide gel : bis-acrylamide = 19:1) and 7 M Urea (BRL, Enzymatic grade) in Tris-borate buffer.

The gel solution (50 ml) is degassed in a vacuum desiccator for 5-10 min, then 80 ul of TEMED and 80 ul of Ammoniumperoxodisulfate (40% w/v in distilled water, freshly prepared) is added. The solution is mixed well for 3 min and immediately poured into the mold.

Polyacrylamide gel loading buffer: 90% Formamide and 0.05% Azorubin dye in Tris-borate buffer. Keep in the refrigerator.

Hind III lambda DNA marker: lambda DNA (40 ug) are digested with 50 units of Hind III in 160 ul. The reaction is stopped with 40 ul of 5x loading buffer. Keep in the refrigerator.

Reagents and buffer for Southern blotting:

Denaturation solution: 1.0 M NaOH and 1.5 M NaCl.

Neutralization solution (pH 5.5): 1.0 M Tris-HCl and 3.0 M NaCl. The pH is adjusted with conc.HCl.

20x SSC buffer (pH 7.0): 3.0 M NaCl and 0.3 M Sodium Citrate Trihydrate. The pH is adjusted with conc.HCl. Then the buffer is sterilized at 112°C for 15 min.

Reagents and buffer for preparation of DNA-specific probe:

Selective LB broth: Luria-Bertani media 0.5% NaCl, 1.0% Tryptone, 0.1% Glucose and 0.5% Yeast extract. After sterilization at 112°C for 15 min and cooling down to room temperature, the medium is supplemented with 1.0 mg% Thiamine and 8.0 mg% Ampicillin.

For plating: add 1.5 % Bacto agar.

Glucose buffer (pH 8.0): 50 mM Glucose, 25 mM Tris-HCl and 10 mM EDTA.

The pH is adjusted with conc.HCl. Keep in the refrigerator.

1% SDS solution: 1 gm% of SDS in 0.2 M NaOH (fresh preparation).

5.0M Potassium acetate (pH 4.8): 98.15gm of KOAc dissolved in distilled water, pH adjusted with acetic acid.

Reagents and buffer for radiolabelling:

Multiprimer DNA labelling kit: the system is composed of
 solution 1: dATP, dGTP, dTTP in a concentrated solution containing Tris-HCl pH 7.8, $MgCl_2$ and 2-mercaptoethanol.
 solution 2: primer solution: random hexanucleotide in an aqueous solution containing nuclease free BSA.
 solution 3: enzyme solution 1 unit/ul DNA polymerase I "Klenow fragment" in 50 mM Potassium phosphate pH 6.5, 10 mM 2-mercaptoethanol and 50% glycerol.

Alpha ^{32}P -dCTP: 3,000 Ci/mole from Amersham company.

Gamma ^{32}P -ATP : 5,000 Ci/mole from Amersham company.

Sephadex G-75 column chromatography (1 ml): 1.0 ml of gel Sephadex G-75 gel is packed in a Pasteur pipette and equilibrated with 1x TE buffer.

Single stranded Salmon sperm DNA: DNA is boiled in distilled water in the concentration of 1.0 mg/ml or 10.0 mg/ml. Store at $-20^{\circ}C$.

10x reaction buffer for T4-kinase enzyme (pH 7.8): 600 mM Tris-HCl, 150 mM Dithioerythritol and 10 mM $MgCl_2$. Keep at $-20^{\circ}C$.

DE-cellulose 52 column chromatography: 300 ul of DE-cellulose 52 gel is packed in an autopipette tip (volume 1.0 ml) and equilibrated with 1x TE buffer.

Eluant: 0.2 M NaCl and 0.5 M NaCl in 1x TE buffer.

Reagents and buffer for hybridization:

Formamide solution: formamide (Merck, Germany) is deionized with 5% of Dowex mixed bed resin for 1 hr by inverting at 4°C. Remove Dowex by filtration with filter paper (to be prepared freshly before use).

50x Denhardt' solution: 1% Ficoll type 400, 1% Polyvinyl-pyrrolidone -40, and 1% BSA fraction V (Sigma USA) (fresh preparation kept in the refrigerator).

Prehybridization solution: 6x SSC, 6.25x Denhardt' solution, 50% Formamide and 1 mg of single stranded Salmon sperm DNA. (Prepared in a waterbath at 60°C).

Hybridization solution: 6x SSC, 1x Denhardt' solution, 50% Formamide, 10% Dextran sulfate (sodium salt-Pharmacia) and 50 pg/ml of sonicated Salmon sperm DNA. (Prepared in a waterbath at 60°C).

Gel denaturation solution: 0.5 M NaOH and 0.15 M NaCl.

Gel neutralization solution (pH 8.0): 0.5 M Tris-HCl and 0.15 M NaCl, pH adjusted with conc.HCl.

Sodiumphosphate buffer (pH 7.0): 0.2 M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 0.2 M $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, adjust pH 7.0 by mixing the solution in a ratio of about 1:2 by volume. Sterilized at 112°C for 15 min.

Gel hybridization solution: 5x SSC, 20 mM Sodiumphosphate pH 7.0, 7% SDS and 10% Dextran sulfate. Keep at -20°C.

Reagents and buffer for washing:

For nitrocellulose membrane hybridization:

- washing solution 1: 2 x SSC and 0.1% SDS
- 2: 2 x SSC and 0.1% SDS
- 3: 0.1 x SSC and 0.1% SDS.

For gel hybridization:

- washing solution 1: 2 x SSC
- 2: 3 x SSC, 10 mM Sodiumphosphate pH 7.0
- 3: 1 x SSC and 1% SDS.

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