

## **APPENDIX 1: REAGENTS AND SOLUTIONS**

### 0.5% Acetic anhydride

5ml 1M Tris

45ml DEPC water

0.25ml acetic anhydride

### Agar plates

14g agar

500ml distilled water

Boil to dissolve and autoclave.

Dissolve 0.1g ampicillin in 1ml sterile distilled water.

Add 50 $\mu$ l to slightly cooled agar and swirl to mix.

Pour agar in petri dishes, wrap in foil and store at 4°C.

### Running an agarose gel

1g agarose

100ml 1x TBE.

Melt in microwave. When slightly cooled add 3 $\mu$ l ethidium bromide, swirl to mix.

Pour into tray set with comb. Allow to set.

Remove comb, load 5 $\mu$ l of DNA ladder with 3 $\mu$ l of loading dye, and 15 $\mu$ l of sample with 5 $\mu$ l of loading dye.

6x Agarose gel loading dye

0.63g bromophenol blue

150ml distilled water

500ml glass beaker. Stir for 60 minutes.

Add 25ml 100% anhydrous glycerol using a syringe.

Stir for 30 minutes till mixed. Add distilled water till 250ml.

Stir for 30 minutes. Store at 4°C in 50ml tubes.

1 x Anti-Dig

1µl 10 x anti-Dig

1ml 10x blocking buffer

1ml 10x malic acid

8ml sterile distilled water

1 x Blocking buffer

5ml 10x malic acid

5ml 10x blocking buffer

40ml sterile distilled water

DAB chromogen mix

25µl DAB in 475µl sterile distilled water

25µl 20x DAB Chromogen

25µl 20x Hydrogen peroxide

Cover in foil, keep on ice till use – prepare just before use.

1x Detection buffer

5ml 10x detection buffer

45ml sterile distilled water

DEPC water

Autoclave a 1l flask and 1l bottle.

To flask add 1l water and 1ml DEPC. Shake at 37°C overnight.

Autoclave in bottle again.

40% Dextran

40g dextran sulphate sodium salt in 70ml DEPC water.

Heat for 3-4 hours in 68°C waterbath.

Make up to 100ml and store at 4°C.

0.5M EDTA

18.61g EDTA

400ml DEPC H<sub>2</sub>O

Adjust pH to 8 using NaOH

Make up to 500ml, store at room temperature.

0.1M HCl

2.5ml 9M (32%) HCl

247.5ml DEPC water

Hybridization buffer

1ml 20x SSC

2.5ml 40% Dextran

20µl 10% SDS

5ml formamide

1.4ml DEPC water

Store at -20°C in 1ml aliquots.

4M LiCl

8.478g LiCl

50ml DEPC water

Store at room temperature.

Luria broth (LB)

50g Bacto-tryptone

25g yeast extract

50g NaCl

Bring to 1 litre with distilled water and autoclave.

10x MOPS

41.86g MOPS

4.1g NaAc

3.72g EDTA

Make up to 1l with distilled water, wrap bottle in foil.

3M NaAc

24.6g NaAc in 100ml distilled water.

Autoclave.

0.85% NaCl

4.25g NaCl in 500ml distilled water.

Autoclave.

5M NaCl

73.05g NaCl in 250ml distilled water

Autoclave.

0.1M Na-citrate

12g Na-citrate in 400ml distilled water.

Adjust pH to 6, autoclave.

1x NBT/BCIP

200µl 10x NBT/BCIP

9.8ml 1x detection buffer

PBS 1% BSA

5 PBS tablets in 1l distilled water

Autoclave.

10g Bovine Serum Albumin

4% PFA (prepared fresh daily)

2.5g paraformaldehyde

25ml 200mM phosphate buffer

25ml distilled water

Heat at 65°C under laminar flow to dissolve.

Store at 4°C.

200mM Phosphate buffer

4.3g Na<sub>2</sub>HPO<sub>4</sub>

3.1g NaH<sub>2</sub>PO<sub>4</sub>

250ml DEPC water

Autoclave.

10mg/ml Proteinase K

2µl proteinase K

998µl TBS

10% SDS

1g SDS in 10ml DEPC water.

20x SSC

175.3g NaCl

88.2g Trisodium citrate

800ml distilled water

Adjust pH to 7, make up to 1l, autoclave.

0.1M Sodium citrate

29.4g in 1l distilled water.

Adjust pH to 6 and autoclave.

10 x TBE

108g Tris

55g Boric acid

9.3g EDTA

Dissolve and make final volume to 1l, adjust pH to 8.3

TBS

50ml 1M Tris (pH 7.5)

30ml 5M NaCl

Make up to 1l with DEPC water. Autoclave and keep at 4°C.

TBS-Tween

1ml of Tween per 1l TBS

10 x TE

12.11g Tris-Cl (Sigma, Steinheim, Germany)

5.72g EDTA (Saarchem Merck, Gauteng, South Africa)

700ml distilled water

Adjust pH to 7.5, make final volume to 1l, autoclave.

TNB

2ml malic acid

2.5ml blocking buffer

45ml TBS

1M Tris, pH 7.5

6.1g Tris in 50ml distilled water

Adjust pH to 7.5 and autoclave.

Wizard Plus SV Cell Resuspension Buffer

50mM Tris-HCl (pH 7.5)

10mM EDTA

100µg/ml RNase A

Wizard Plus SV Cell Lysis Solution

0.2M NaOH

1% SDS

Wizard Plus SV Neutralization Solution

4.09M guanidine hydrochloride

0.759M potassium acetate

2.12M glacial acetic acid