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APPENDIX A

BUFFERS AND REAGENT

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1. Buffer P1

100mM glucose	50	ml
100mM Tris-HCl (pH 8.0)	25	ml
100mM EDTA (pH 8.0)	10	ml
Distilled water to	100	ml

Sterilize the solution by autoclaving and store at 4°C.

2. Buffer P2

10N NaOH	2	ml
10% (w/v) SDS	10	ml
Distilled water to	100	ml

Sterilize the solution by autoclaving and store at room temperature.

3. Buffer P3

5 M potassium acetate	60	ml
Glacial acetic acid	11.5	ml
Distilled water to	100	ml

The resulting solution is 3M with respect to potassium and 5M with respect to acetate. Store the solution at 4°C and transfer it to an ice bucket just before use.

4. 10% SDS solution

Sodium dodecyl sulfate	10	g
Distilled water to	100	ml

Mix the solution and store at room temperature.

5. 1.5 M Tris-HCl

Tris base	12.11	g

Dissolve in distilled water and adjusted pH to 7.5 with HCl

Distilled water to	100	ml

6. 0.5 M EDTA (pH 8.0)

Disodium ethylenediamine tetraacetate.$2H_2O$	186.6	g
Dissolve in distilled water and adjusted pH to 8.0 with NaOH		
Distilled water to	1,000	ml

Sterilize the solution by autoclaving and store at room temperature.

7. 1.0 M $MgCl_2$

Magnesium chloride.6 H_2O	20.33	g
Distilled water to	100	ml

Dispense the solution into aliquots and sterilize by autoclaving.

8. 5 M NaCl

Sodium chloride	29.25	g
Distilled water to	100	ml

Dispense the solution into aliquots and sterilize by autoclaving.

9. 10X Tris borate buffer (10X TBE)

Tris base	100	g
Boric acid	55	g
0.5 M EDTA (pH 8.0)	40	ml

Adjust volume to 1,000 ml with distilled water. The solution was mixed and stored at room temperature.

10. 7.5M Ammonium acetate

Ammonium acetate	57.81	g
Distilled water	80	ml

Adjust volume to 100 ml with distilled water and sterilize by autoclaving.

11. 6X loading dye

Bromphenol blue	0.25	g
Xylene Cyanol	0.25	g
Glycerol	50	ml
1M Tris (pH 8.0)	40	ml
Distilled water to	100	ml

Mix and stored at 4°C

11. 1% agarose gel (w/v)

Agarose	1.0	g
1x TBE	100	ml

Dissolve by heating and occasional mix until no granules of agarose gel are visible.

12. 16% polyacrylamide gel (w/v)

1.5M Tris-HCl pH 8.8	1.25	ml
10% SDS	50	µl
40% Acrylamide/Bis	2	ml
10% APS	25	µl
TEMED	2.5	µl
Distilled water	1.75	ml

13. 8% polyacrylamide gel (w/v)

1.5M Tris-HCl pH 8.8	1.25	ml
10% SDS	50	µl
40% Acrylamide/Bis	1	ml
10% APS	25	µl
TEMED	2.5	µl
Distilled water	2.75	ml

14. 6% polyacrylamide gel (w/v)

0.5M Tris-HCl pH 6.8	500	µl
10% SDS	100	µl
40% Acrylamide/Bis	750	µl
10% APS	50	µl
TEMED	25	µl
Distilled water	3.7	ml

13. Lysed buffer (for cytoplasm lysis)

5% Tween20	10	ml
50mM PIPES pH8.0 piperazine-N,N'-bis(2-ethanesulfonic acid)	10	ml
1M KCl	8.5	ml
Distilled water	71.5	ml

Mix and store at room temperature.

When using, add 100X Proteinase inhibitor to final concentration at 1X and keep on ice.

14. Lysis buffer (for nuclear lysis)

10% SDS	10	ml
1M Tris-HCl pH8.1	5	ml
100mM EDTA	10	ml
Distilled water	75	ml

Mix and store at room temperature.

When using, add 100X Proteinase inhibitor to final concentration at 1 and keep on ice.

15. Ethidium Bromide

Ethidium Bromide	10	mg
Distilled water	1	ml

Mix the solution and store at 4°C

BIBLIOGRAPHY

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