

Appendix 1: Reagents and buffers used.

Amino linking Buffer (10x)

500mM KCl,
25mM MgCl₂,
50mM Tris/HCl pH 8.5
Made with autoclaved distilled water.

HindIII Digestion mix (for a 96 well plate)

Hind III (Boehringer 40U/ml),	55µl
Buffer B (Boehringer)	99µl
Sterilised water	286µl

Hybridisation Buffer

50% deionised formamide
2xSSC
10% dextran sulphate
0.1% SDS
10mM Tris pH 7.4
0.1% Tween 20

LB Agar

Tryptone	10g
Yeast Extract	5g
NaCl	10g
Agar	15g

Make up to 1 litre with autoclaved distilled water.

LB Broth

Tryptone	10g
Yeast Extract	5g
NaCl	10g

pH to 7.5 (using 1M NaOH)
Make up to 1 litre with autoclaved distilled water.
Autoclave at 121°C for 15 minutes.

Orange G (10mls)

Orange G	0.1g
Ficoll	1.2g

Make up to 10ml with sterilised distilled water

Polyamine isolation buffer (PAB)

80mM KCl
20mM NaCl
2mM EDTA
0.5mM EGTA
15mM Tris
3mM dithiothreitol

0.25% (vol:vol) Triton X-100
pH adjusted to 7.2

Sheath Buffer

10mM Tris-HCl pH 8.0
1mM EDTA
100mM NaCl
0.5mM Sodium Azide

SSC (1x)

0.15M NaCl
0.015M Sodium Citrate
pH 7.0

TAPS 2 Buffer (10x)

250mM TAPS pH 9.3,
166mM (NH₄)₂SO₄,
25mM MgCl₂,
0.165% w/v Bovine serum albumin (Sigma),
0.7% v/v 2-mercaptoethanol
Made with autoclaved distilled water.

TBE Buffer (10x)

Tris Base	121g
Boric Acid	61.83g
EDTA	18.612g

pH 8.0
Make up to 1 litre with autoclaved distilled water.

TY Media (2x)

Bacto-tryptone	16g
Bacto-yeast Extract	10g
NaCl	5g

Make up to 1 litre with autoclaved distilled water.
Autoclave at 121°C for 15 minutes.

Vista Green Stain (for 500ml – 1 gel)

1M Tris HCl	5ml
0.5M EDTA pH 7.4	0.5ml
Vistra Green	0.05ml

Make up to 500ml with sterilised distilled water.