Appendix 2

Buffers, Solution, Media Composition and Suppliers

Buffers

Electrophoresis 10X TAE (Tris-acetate-EDTA)

48.4g of Tris-base was added to 11.42ml of glacial acetic acid and 20ml of 0.5M EDTA, adjusted to pH 8.0 and made up to 1L with H_2O

Loading Buffer

0.25% [w/v] of bromophenol blue or 0.25% [w/v] xylene cyanol, 30% [v/v] glycerol were dissolved in H_2O and stored at 4°C

Cracking Buffer (to test bacterial cells after ligation)

2ml of 5M NaOH, 2.5ml of 10% [w/v] SDS, 10g of sucrose were added to a sterile bottle and the total volume was made up to 50ml with sterile dH_2O. Bromocresol green was added until a blue colour developed

Lysis Buffer

50mM glucose and 25mM Tris-HCl adjusted to pH 8.0 were added to 10mM EDTA

Trapping Buffer

0.6M sorbitol was added to 100mM Tris-HCl at pH 7.0

20X SSC

175.3g of NaCl, 88.2g of Sodium Citrate were dissolved in 1L of dH_2O and adjusted to pH 7.0

Denaturing Solution

0.5M NaOH and 1.5M NaCl dissolved in 1L of dH20

Neutralizing Solution

0.5M Tris brought to pH7.4 with HCl and 3M NaCl made up to 1L with dH20

20X SSPE

175.3g NaCl, 27.6g NaH_2PO_4-H_2O and 7.4 g of EDTA was dissolved in 1L of dH_2O, adjusted to pH 7.4 with 10N NaOH

Transfer Buffer

288g glycine, 60.4 g Tris base were dissolved in 200ml of methanol and

1.8L of dH_2O

5 X Running Buffer

15g Tris, 72g Glycine and 5g of SDS

10 X TBS

9 % NaCl, 100 mM Tris, pH 7.4

TBST

1 X TBS + 0.1 % Tween 20

Gel Stain

1g Coomassie Blue dye R-250 was dissolved in 450ml Methanol, 450ml dH_2O and 100 ml AcCOOH

PAGE Destain

900 ml Ethanol, 900 ml DW, 200 ml AcCOOH

APS

10% Ammonium Persulfate was dissolved in sterile dH_2O and left at -4°C for a fortnight

Solutions

Proline Stock

11.51g of proline powder was added to 100ml of dH_2O and filter filtered

Luria and glycerol

20ml of glycerol was added to 100ml of Luria Broth and autoclaved

$NaNO_2$ Stock

34.5g of NO_2 powder was added to 500ml of dH_2O and filter filtered

$NaNO_3$ Stock

89.44g of NO_3 powder was added to 500ml of dH_2O and filter filtered

NH_4 Tartrate Stock

92.07g of NH_4 powder (FW 184.15) was added to 500ml of dH_2O. Filter filtered

p-aminobenzoic acid (paba)

0.1mg of paba was dissolved in 1ml of dH_2O and filter filtered

Vitamins

400mg of paba was added to 50mg aneurin, 100mg inositol, 100mg nicotinic acid, 200mg ca. pantothenate, 50mg pyridixine (Pyro), 100mg riboflavin, 1.4g choline chloride, 10ml biotin stock and made up to 1L of dH_2O and kept in a dark bottle at -4°C.

1X STC (Sorbitol Tris Calcium)

1.2M sorbitol and 10mM Tris-HCl at pH 7.5 was added to 10mM $CaCl_2$ and sent to autoclave

2X STC

2.4M sorbitol and 20mM Tris-HCl at pH 7.5 was added to 20mM $CaCl_2$ and sent to autoclave

Osmotic Medium (OSMO)

1.2M $MgSO_4$ was added to 1ml of 1M NaOP at pH 7.0. The pH was adjusted to 5.8 with 0.2M Na_2HPO_4 and filter filtered

Trace Element

400mg of $MnCl_2 4H_2O$ was added to 1g $ZnSO_4$, 0.5g of $CuSO_4$, 1.1g of $Na_2MoO_4 2H_2O$, 0.5g of $CoCl_2 6 H_2O$, 0.5g of $FeSO_4 7 H_2O$, 1g of HBO_3, 3.72g of citric acid

Media

All media used were sterilised by autoclaving at 121°C for 20 minutes.

***Aspergillus* Minimal (Cove, 1966)**

10g of glucose was added to 1ml of trace element, 1.3g of KCl, 1.3g of $MgSO_4.7H_2O$, 3.8g of KH_2PO_4, made up to 1L of H_2O and adjusted to pH 6.5 with 5M KOH

***Aspergillus* Complete (Cove, 1966)**

10g of glucose was added to 1g yeast extract, 2g of peptone, 0.075g of adenine, 10ml of vitamin solution, 14g of agar, 10ml of casamino acids (added after melting the medium), made up to 1L of dH_2O and adjusted to pH 6.5 with 5M NaOH

Luria

10g of tryptone was added to 5g yeast extract, 10g of NaCl, made up to 1L of dH_2O and adjusted to pH 7.0 with 5M NaOH. For agar plates 15g/L of agar was added and for liquid media 15.5g of luria was dissolved into 1L of dH_2O

SOB (Super Optimal Broth)

20g of tryptone was added to 5g yeast extract, 0.5g of NaCl, 10ml of 250mM KCl, made up to 1L of dH_2O and adjusted to pH 7.0 with 5M NaOH. After autoclaving 5ml of sterile solution of 2M $MgCl_2$ was added before use

SOC (Super Optimal Broth with Catabolite repression)

20g of tryptone was added to 5g yeast extract, 0.5g of NaCl, 10ml of 250mM KCl, made up to 1L of dH_2O and adjusted to pH 7.0 with 5M NaOH. After autoclaving 20ml of sterile solution of 20mM glucose was added before use

Name	**Address**
Biogene	Kimbolton, UK
BioSpec	Bartlesville, USA
BDH	Poole, UK
Fermentas	York, UK
Fisher Scientific	Loughborough, UK
Fisson	Loughborough, UK
Fluka Biochemika	Buchs, Switzerland
GE Healthcare	Amersham, UK
GFS Chemicals	Powell, USA
ICN Biomedicals	San Fransico, USA
Invitrogen	Gibco Paisley, UK
Marligen	Ijamsville, USA
Melford	Ipswich, UK
Merck Chemicals	Nottingham, UK
Millipore	Watford, UK
New England Biolabs	Hitchin, UK
Novagen	Madison, USA
NovoBilabs, Novoindustries	
Novo Nordisk	Dihingen, Switzerland
Promega	Southampton, UK
Qiagen	Crawley, UK
Roche	East Sussex, UK
Santa Cruz	Calne, UK
Severn Biotech	Kidderminster, UK
Sigma Aldrich	Dorset, UK
Thistle	Glasgow, UK
VWR International	Lutterworth, UK