

## **Appendix 6: medium recipes**

### Buffers:

Nuclear Extraction Buffer (NEB) 1 L: 100 mM Tris, pH 8.0, 1 M KCl, 100 mM EDTA, adjust to 1 L, store at 4°C.

Sucrose Extraction Buffer with  $\beta$ -mercaptoethanol (SEB-M) 1 L: 10% NEB, 550 mM Sucrose, 4 mM Spermidine, 1 mM Spermine, 0.13% w/v Carbamic acid, 1.20% w/v PEG 8000, 0.2%  $\beta$ -mercaptoethanol, adjust to 1 L, store at 4°C or on ice. Prepare on day of extraction.

SEB-MT (Sucrose Extraction Buffer with  $\beta$ -mercaptoethanol and Triton) 1 L: 45 mL SEB-M, 5 mL Triton x100, Store at 4°C.

TE 4 L: 10 mM Tris, 1 mM EDTA, Adjust to 4L, store at 4°C.

### **BNM**

This media is made by making a series of stock solutions

Formula for 1 litre of de-ionised water: 200x NOD Major Salts: 24.4 g  $\text{MgSO}_4$ , 13.6 g  $\text{KH}_2\text{PO}_4$ ; 200x NOD Minor Salts: 920 mg  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 620 mg  $\text{H}_3\text{BO}_3$ , 580 mg  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ; 200x NOD Minor Salts 2: 50 mg  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ , 5 mg  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 5 mg  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ; 200x Iron-EDTA: 3.73 g  $\text{Na}_2\text{EDTA}$ , 2.78 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ;

To make 1 litre of BNM: 344 mg  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ; 390 mg MES Buffer; 5 mL 200x NOD MAJOR SALTS; 5 mL 200x NOD MINOR SALTS; 5 mL 200x NOD MINOR SALTS 2; 5 mL 200x IRON EDTA; PH to 6 with KOH; For solid media add 11.5 g agar.

### **DWA**

Formula per 1 litre of de-ionised water: 15.0 g Bacto agar.

## **MFP (Modified FP)**

This medium is made with 7 stock solutions per 100mL: 13.23 g Calcium Chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ); 12.32 g Magnesium Sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ); 9.53 g Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ); 11.36 g Di-sodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ); 0.49 g Ferric Citrate; 4 g Ammonium Nitrate ( $\text{NH}_4\text{NO}_3$ );

The following 5 components are put into one bottle of 100 mL all together: 10 mg Manganese Chloride ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ); 10 mg Copper Sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ); 10 mg Zinc Chloride ( $\text{ZnCl}_2$ ); 10 mg Boric Acid ( $\text{H}_3\text{BO}_4$ ); 10 mg Sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ).

To make MOD FP add 1 mL of all seven stock solutions per 1 litre. pH to 7.5 with NaOH. For solid media add 8 g Formedium Agar a litre.

## **TY**

Formula per 1 litre of de-ionised water 5.0 g Tryptone; 3.0 g Yeast Extract; 1.32 g  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ . If solid medium is required add per litre 10.0 g Lab M No.1 agar.