CHAPTER 10 - RECIPES

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ABC Solution (Avidin-Biotin HRP Complex):

3ml 1% DMSO, 0.1% Triton, PBS
30µl Vectastain solution A
30µl Vectastain solution B

Agar/Sucrose:

1.5% agar
5% M sucrose
Boil into solution and store as 1.5 ml aliquots at 4°C.

Atabrine Stock Solution:

10 mg/ml dH2O. Store in a light tight bottle.

Blocking Solution for Western Blots:

3% dried milk in TBS

BSA/PBS/DMSO:

1% bovine serum albumin, 1% dimethyl
sulfoxide in PBS.

BT Fix:

10 ml 10% para-formaldehyde
15 ml 1.25X fix buffer
Adjust pH to 7.3 if necessary. Add dH2O to a final volume of 25.0 ml. Final concentration is 4% para-formaldehyde, 0.15 mM CaCl2, 4%sucrose in 0.1 M PO4 buffer.

Chorion Removal:

Drain eggs add 5 ml of 0.5 mg/ml pronase for 3.5 min.
Dilute eggs with 200 ml of 8x water. Rinse 3x more with 200 ml washes of 8x water.
8x water = 12 ml stock salts per liter dH2O

Stock salts = 40 g Instant Ocean per liter dH2O

DAB (Diaminobenzidine):

DAB is prepared by dissolving the contents of 1 g bottle of DAB in 25 ml dH2O and filtering with a disposable unit attached to a disposable syringe. Aliquots containing 1 mg DAB per 25 µl of solution are stored in 1.5 ml microfuge tubes in a -20°C freezer. Thawed aliquots may be refrozen. To minimize risk in handling this carcinogen, use disposable plastic containers and pipettes wherever possible. Treat waste with bleach.

DAB Heavy Metal Stain:

Presoak solution

4 mg DAB
5 ml 0.6% Ni(NH4)2(SO4)2
5 ml 0.2 M Tris buffer, pH 7.4
50 µl DMSO

After presoak interval add

10 µl 3% H2O2

Final concentrations are 0.04% DAB, 0.1 M Tris buffer, 0.3% Ni(NH4)2(SO4)2 and 0.003% H2O2.

DAB Presoak Solution:

25 µl (=1 mg) DAB stock solution
1 ml 0.1 M PO4 Buffer, pH7.3
1 ml dH2O
20 µl DMSO
After presoak interval add
5 µl 3% H2O2
Final concentrations are 0.05% DAB, 0.05 M PO4, 1% DMSO and 0.004% H2O2.

DAB Solution:

0.05% diaminobenzidine
1% dimethyl sulfoxide in 0.05 M PO4 buffer, pH 7.3.

DNA Extraction Buffer:

10 mM Tris pH 8.2
10 mM EDTA
200 mM NaCl
0.5% SDS
200 µg/ml proteinase K

EDTA:

Stock - 10 mM EDTA, pH 7.0. Add 1 ml of stock/10 ml Ringer's (final conc. 1 mM EDTA)

Egg Water:

1.5 ml stock salts added to 1 L distilled water = 60µg/ml final concentration.

Embryo Extract:

1. Chill 200 3 d embryos after removing from chorions.
2. Rinse in 0.5% chilled bleach for 2 min and then in zero calcium Ringer for 2 min.
3. Transfer to a Dounce homogenizer with a minimum of liquid and homogenize well.
4. Resuspend in 1 ml L-15 supplemented with 0.3 mg/ml glutamine, 50 U/ml penicillin, 0.05 mg/ml streptomycin and 0.8 mM CaCl2.
5. Store at -20°C.

Embryo Medium:

1.0 ml Hank's Stock #1
0.1 ml Hank's Stock #2
1.0 ml Hank's Stock #4
95.9 ml dd H2O
1.0 ml Hank's Stock #5
1.0 ml fresh Hank's Stock #6
Use about 10 drops 1 M NaOH to pH 7.2

Epon:

12 g Epon 812
24.7 g Dodecenyl succinic anhydride
Weigh components into a 50 ml plastic beaker.
Cover with parafilm and stir on a magnetic stirrer for
15 min. Add 0.5 ml DMP-30

Continue stirring for another 15 min. Store in syringes in -20°C freezer.

Epon/Araldite:

24.7 g Epon 812
33.25 g Dodecenyl succinic anhydride
31.05 g Araldite 506

Mix well by stirring. Use disposable syringes to add:
2.3 ml Dibutylphthalate
2.5 ml DMP-30

Continue to mix well by stirring. Try to avoid incorporating excess air. If this occurs resin may be degassed by subjecting it to a mild vacuum. Store resin in 10 ml syringes at -20°C.

Finquel (MS222):

See Tricaine. (Finquel is the trademark brand of Argent Chemical Laboratories, Inc.)

Fish Water:

60 mg "Instant Ocean" per liter dH2O.

Fix Buffer:

Dilute 1.25X fix buffer 3:2 with dH2O.

1.25X Fix Buffer:

1.0 g Sucrose
18.75 µl 0.2 M CaCl2
5 ml 0.5 M PO4 buffer, pH 7.3
Check pH. Adjust if necessary to 7.3 with 1 M NaOH or HCl.
Add H2O to a final volume of 15 ml.

Fixatives:

Fix buffer: 4% sucrose, 0.15 mM CaCl2, 0.1 M PO4 pH 7.3.
For general fixation: 1.5% glutaraldehyde, 0.5% paraformaldehyde in fix buffer.
For antibody staining: 4% paraformaldehyde in fix buffer.

Gelatin Embedding Medium:

17% gelatin in 10% Hank's saline.

Genomic DNA Extraction Buffer:

10 mM Tris pH 8
100 mM EDTA pH 8
0.5% SDS
200 µg/ml Proteinase K

GHCl Buffer:

7.5 M guanidinium hydrochloride
0.025 M NaOAc pH 7.0
5 mM dithiothreitol
0.5% N-laurylsarcosinate.

Giemsa:

4 mls Giemsa Stock (Sigma Diagnostics)
4 mls 0.5 M Na Phosphate pH7
200 mls distilled water

Ginzburg Fish Ringers:

6.5 g NaCl
0.25 g KCl
0.3 g CaCl2 (0.4 g CaCl2•2H2O)
Add ddH2O to almost 1 liter
0.2 g NaHCO3
Add ddH2O to 1 liter
Note: The order of addition is important to prevent precipitation

GIT Buffer:

4 M guanidinium isothiocyanate
0.1 M Tris-HCl pH 7.5
1% ß-mercaptoethanol.

Growth Medium:

L-15 (Sigma)
0.3 mg/ml glutamine
50 U/ml penicillin
0.05 mg/ml streptomycin
0.8 mM CaCl2
10% embryo extract
3% fetal calf serum.

Hank's (Final):

9.9 ml Hank's Premix
0.1 ml Stock #6

Hank's (Full Strength):

0.137 M NaCl
5.4 mM KCl
0.25 mM Na2H PO4
0.44 mM KH2 PO4
1.3 mM CaCl2
1.0 mM Mg SO4
4.2 mM NaH CO3

Hank's Premix:

Combine the following in order:
10.0 ml Solution #1
1.0 ml Solution #2
1.0 ml Solution #4
86.0 ml ddH2O
1.0 ml Solution #5

Store Hank's Premix in the refrigerator along with the Hank's solutions.

Hank's Stock Solutions:

Stock #1

8.0 g NaCl
0.4 g KCl
in 100 ml dd H2O

Stock #2

0.358 g Na2HPO4 Anhydrous
0.60 g KH2PO4
in 100 ml ddH2O

Stock #4

0.72 g CaCl2
in 50 ml ddH2O

Stock #5

1.23 g MgSO4x7H2O
in 50 ml dd H2O

Stock #6

0.35 g NaHCO3
10.0 mls dd H2O

Heat Shock (Diploid eggs):

T = 0 Fertilize eggs with UV-irradiated sperm
T = 5 min Transfer embryos to 28.5°C cylinder
T = 13 min Move cylinder from 28.5°C to 41.4°C
T = 15 min Move cylinder from 41.4°C to 28.5°C

In situ Hybridization Staining Buffer:

100 mM Tris pH 9.5
50 mM MgCl2
100 mM NaCl
0.1% Tween-20
1 mM Levamisol (add fresh)

Paramecia Seed Cultures:

1. Add 10-15 grains of boiled wheat to 175 mls of dH2O.
2. Inoculate with 20 ml from an excellent existing seed culture dish or with a sample from the commercial inoculant.
3. Grow for 7 to 12 days before using.

Paramecia for Baby Fish (Traditional Method):

1. Use glass finger bowls filled two-thirds full with system water.
2. To each bowl add 8-9 grains of of boiled wheat and 8 ml from a paramecia seed culture.
3. Stack the finger bowls 6 or 8 high, cover, and store at 28.5°C on well-lit shelves.
4.After 10-14 days, the paramecia are ready to feed to the fish larvae. Cultures remain useable and healthy for a month or more.

Paramecia for Baby Fish (Streamlined Procedure):

1. Fill plastic mouse cage or equivalent with 2 liters of system water.
2. Add a large pinch (about 40-50 grams) of boiled wheat, four 250 mg tablets of brewer's yeast, and 100 ml (or half the contents of a plastic seed culture dish) of paramecia culture to each mouse cage.

3. Cover and store in a warm, well-lit place. The covered mouse cages may be stacked three layers high with the highest layer closest to a warm light. It will be ready to feed first.
4. Brewer's yeast may also be added to the seed cultures (one half of a 250 mg tablet per 200 ml of seed culture). The cultures are then ready to use in four days and are depleted after two weeks.

Paramecia Medium Stock Solution I:

Component - g/l in dH2O - Source
Calcium pantothenate - 1 - Sigma P-2250
Nicotinamide - 1 - Sigma N-3376
Riboflavin - 1 - Sigma R-9881
Pyridoxamine HCL - 1.16 - Sigma P-9158
Folic Acid - 0.5 - Sigma F-7876
Thiamine HCL - 3 - Sigma T-4625
Biotin - 0.00125 - Sigma B-4639
(make 100x stock) Store at -20°C the riboflavin is dispersed through-out the solution and will settle to the bottom. Shake well before using

Paramecia Medium Stock Solution II:

Component - g/l in ETOH - Source
Stigmasterol - 2 - Sigma S-6126
Lipoic Acid (Dl-6,8-thioctic acid) - 0.02 - Sigma T-1395
Palmitic Acid - 3 - Sigma P-5917
Stearic Acid - 2 - Sigma S-4751
Oleic Acid - 0.4 - Sigma 0-4379
Linoleic Acid - 0.2 - Sigma L-1376
Linolenic Acid - 0.06 - Sigma L-2376
(Note: Blow nitrogen gas over the solution and store at -20°C)

Paramecia Storage Medium (Long Term):

Component - Stock - Amount (ml)
NaH2PO4 - 0.01 M - 10
Na2HPO4 - 0.01 M - 10
Sodium citrate - 0.1 M - 20
CaCl2 - 0.1 M - 15
dH20 - 945

PBS:

0.8% NaCl
0.02% KCl
0.02 M PO4, pH 7.3.

2X PBS:

8.0 g NaCl
0.2 g KCl
200 ml 0.1 M PO4 Buffer, pH 7.3
300 ml dH2O

When diluted 1:1 with dH2O, final concentrations are 0.8% NaCl, 0.02% KCl and 0.02 M PO4.

PBS/BSA/DMSO:

50 ml 2X PBS, pH 7.3
1 g BSA
1 ml DMSO

Check pH with paper and adjust if necessary. Add dH2O to a total volume of 100 ml

PCR Extraction Buffer:

10 mM Tris pH 8
2 mM EDTA
0.2% Triton X-100,
200 µg/ml Proteinase K

PMSF:

Stock - 100 mM phenylmethylsulfonylfluoride in isopropanol. Immediately before use, add 30 µl of stock/10 ml Ringer's (final conc. 0.3 mM PMSF).

PO4 buffer (0.1 M, pH 7.3):

77 ml 0.1 M NaH2PO4 (13.8 g NaH2PO4xH2O/liter dH2O)
23 ml 0.1 M Na2HPO4 (14.2 g Na2HPO4/liter dH2O)

Pronase:

5 mg/ml pronase diluted to 1 mg/ml in embryo medium

Protein Extraction Buffer:

10 mM tris, pH 7.4
2% Triton-X 100
1 mM PMSF
1 mM aprotinin
1 mM leupeptin
1 mM trypsin inhibitor

PTU:

0.003% 1-phenyl-2-thiourea in 10% Hank's saline.

Ringer's Solutions:

Normal

116 mM NaCl
2.9 mM KCl
1.8 mM CaCl2
5 mM HEPES, pH 7.2.

High calcium

116 mM NaCl
2.9 mM KCl
10 mM CaCl2
5 mM HEPES, pH 7.2.

Calcium free

116 mM NaCl
2.9 mM KCl
5 mM HEPES, pH 7.2.

Salt Stock:

20 tablespoons (280 g) Instant Ocean Sea Salts (Aquarium Systems, Inc.) dissolved in 2 liters distilled water

SDS Sample Buffer:

0.63 ml 1M Tris-HCl, pH 6.8
1.0 ml glycerol
0.5 ml ß-mercaptoethanol
1.75 ml 20% SDS
6.12 ml H2O
(10 ml total)

Store at -20°C in aliquots.

Schönefeld's Medium for Growing Paramecia:

Component - Concentration - Source
Powdered Skim Milk - 8.5 g/l - Any grocery store
Ribonucleic Acid - 1.0 g/l - Sigma R-6625
MgSO4 - 0.5 g/l - Sigma M-2643
Phosphatidylcholine - 250 mg/l - Sigma P-5638

Stock Solution I: 5 ml/l
Stock Solution II: 2.5 ml/l

Sodium Bacarbonate

0.35 g NaHCO3
10.0 ml ddH2O

Sperm Freezing Medium:

9 ml Ginzburg Fish Ringers
1 ml Methanol
1.5 g Carnation Powdered Skim Milk
Note: This order is important to prevent precipitation of milk

Sperm Thawing Medium:

a. Measure the mm of sperm + freezing medium (sm) in the capillary.
b. Convert to volume:

$$\frac{10\mu l \text{ (cap. vol.)} \times \text{mm sm}}{90 \text{ mm (cap. length)}} = \text{vol } (\mu l)$$

c. Multiply the calculated volume x 10 and use that much 10% Hank's saline for thawing.

Stock Salts

40 g "Instant Ocean" Sea Salts added to 1 L distilled water

Subbing Solution for Slides:

0.5% gelatin
0.05% CrK(SO4)x12H2O.

TBS:

20 mM Tris, pH 7.5
500 mM NaCl

TES buffer:

1% sodium dodecyl sulfate
5 mM EDTA
10 mM Tris-HCl pH 7.4.

Tricaine:

Tricaine (3-amino benzoic acid ethy lester also called ethyl m-aminoboenzoate) comes in a powdered form from Sigma (Cat.# A-5040). It is also available as Finquel (Part No. C-FINQ-UE) from Argent Chemical Laboratories, Inc. Make tricaine solution for anesthetizing fish by combining the following in a glass bottle with a screw cap:

400 mg tricaine powder
97.9 ml DD water
~2.1 ml 1 M Tris (pH 9).

Adjust pH to ~7. Store this solution in the freezer. (Buy the smallest amount possible because tricaine gets old.)
To use tricaine as an anesthetic combine the following in a 250 ml beaker:

4.2 ml tricaine solution
~100 ml clean tank water.

TTBS:

20 mM Tris, pH 7.5
500 mM NaCl
0.05% Tween-20

Western Blot color development buffer:

102 mg MgCl2
4.2 g NaHCO3
500 ml dH2O

Western Blot color development solution:

66 µl nitroblue tetrazolium (NBT) stock
33 µl 5-bromo-4-chloro-3-indolyl galactopyranoside
(BCIP) stock
10 ml color development buffer
Both NBT and BCIP stocks are 50 mg/ml in dimethylformamide. NBT stock is made by suspending 50 mg NBT in 700 ml dimethylformamide. Vortex. Add 300 µl distilled water to dissolve. Store both stocks at 4°C in dark.

Whole-Mount in situ Hybridization Solutions:

PBST - PBS plus 0.1% Tween
SSCT - SSC plus 0.1% Tween
HYB* - 50% formamide, 5xSSC, 0.1% Tween-20
HYB+ - HYB* with 5mg/ml torula (yeast) RNA, 50 µg/ml heparin