Reaction Mixtures, Media and Solutions:

These are used by me in my work;

5′ End labeling:

Use T4 Polynucleotide kinase.

10 x buffers:

0.5M Tris-Cl pH 8,
0.1 M MgCl2,
0.1M DTT,

3′ Recessive end labeling:

One can use Klenow fragment, T4 DNA-pol or T7 DNA-pol,
Use 10x Klenow Buffer: containing 10x Hepes buffer pH 6.6.
0.5 M Tris-Cl pH 7.6,
0.1 M Mg Cl2,

RNA labeling:

10x Transcription buffer:

0.4 M Tris-Cl pH 8,
0.08 M Mg Cl2,
0.5 M NaCl,
0.02 M Spermidine-HCl.

5′ end labeling:

10x buffer:
0.5 Tris-Cl pH 8,
0.1 M Mgcl2,
0.1 M DTT (Dithiothretol).
Use T4 polynucleotide kinase.

Labeling 3′ overhangs:

10x buffer:
1M Potassium cocodylate pH 7.2,
20mM cacl2,

2mM DTT.
Use Terminal Transferase.

TBE buffer:
10x buffer:
108gms Tris base,
55gms Borate,
40ml 0.5M EDTA (pH 8.0).

TAE buffer: 50x:
242 gm Tris base,
57.1 ml Glacial acetic acid,
100ml 0.5M EDTA ph 8.0.

Strength of the Agarose gel for DNA fragments:
0.3% 5-50KB,
0.5% 1-25KBP,
0.8% 700bp- 10KBP,
1% 500bp – 10KBP,
1.5% 200bp- 5KBP,
2% 100bp-2KBP,
2.5% 20bp-1KBP.

20x buffer SSC (NaCl-Na citrate):
174.3 gms NaCl,
88.2 Na citrate,
Make it to 1000ml, pH 8.0.

Hybridization buffer 10X:
0.5M Sodium phosphate (pH 7.2),
7% SDFS,
1mM EDTA,
50% Farmamide (ultrapure0,
1x Denhardt sol.
100ug/ml Calf thymus sheared DNA.

Wash buffer:
0. X ssc+0.1%SDS at room Tm,
0.2x SSC +0.1 % SDS at room Tm,
0.2x SSC + 0.1% SDS at 62^o C.

Northern Blotting:

Denaturation buffer:
DMSO4- 20ul,
0.1M Na H2 PO4 pH7- 4ul,
Deionized glyoxal- 5.9ul,
RNA -10ug.

Mix and incubate at 50^0C for 60 minutes,
Then mix with glyoxal loading buffer;
50% glycerol,
10mM Na H2Po4 pH 7.0
0.25% Xylene cyanol,
0.25% Bromophenol blue.

Glyoxal removal buffer:
Submerge the membrane in 20mM Tris-Cl pH 8.0 at 65 ^oC for few minutes.

Preparation of Gel for northern blotting:
RNase is the most dreaded enzyme in all labs. Take precautions to avoid the contamination with RNase.
10mM NaH2PO4 ph 7.01 gm of Agarose,
All RNase free.
Running buffer is the same.

Gel loading buffer:
0.25% Bromophenol blue,
0.25% Xylene cyanol,
40%sucrose or
15% Ficol in water.

Denhardt solution 100 x:

10gm Ficol 400,
10gm PVP,
10gm BSA'
Water to 500ml.

Phage 6M buffer:
50mM Tris-Cl pH 7.8,
8mM Mgcl2,
100mM NaCl,
0.1% Gelatin.

Phage diluting buffer:
10mM Tris-Cl pH 7.5,
10mM MgCl2.

Stock EtBr:
10mg/ml.

Depurination solution:
0.25M HCl.

Denaturation buffer:
1.5M NaCl,
0.5M NaOH.

Neutralization buffer:
1M Ammonium acetate,
20mM NaOH,
or
Tris Cl and NaCl.

SDS PAGE buffers:
Stock: 29.2 gms acrylamide,
0.8 gms Bis acrylamide,
Make it to 100ml, store it in cold room.

Separation Gel:
Tris-Cl 10ml (1.5M Tris),
SDS 0.4ml (10% SDS),
Ammonium per sulfate 0.170ml,
TEMED 0.032ml.
Add 16ml of Acrylamide stock solution,
Make it to 40ml.
This is for 10 % gel.

Stacking gel:
Stock acrylamide 1.7ml,
Triscl-6.8 1.25ml (1 M),
SDS 0.1ml,
APS 0.075 ml (10%stck),
TEMED 0.016ml.
Make it to 10ml.

4x sample buffer 10ml:
SDS 2ml,
Tris pH 6.8, 1.2ml (1M Tris),
Glycerol 4ml,
Bromophenol blue 2.5mg,
Coomassie Brilliant Blue 2.5 mg.
B-Mercaptoethanol 2ul/20ul.

Gel Running Buffer:
Tris 15gm,
SDS 5gms,
Glycine 90gms,
Make it to 1liter.

Gel staining soln.:
Methanol 400ml,
Acetic acid 100ml,
Water 500ml,
Coomassie Blue 200 mg/200ml of the above solution.

Destaining solution: same as the above with out the stain.

Sequencing Gel:
TBE buffer (5x):
Tris base 54gms,
Boric acid 27.5 gms,
EDTA 20ml (0.5 M pH 8.0).

Gel loading buffer:
Bromophenol blue 0.25%,
Xylene cyanol 0.25%,
Sucrose 40% or Ficol 15%

Prepare 5% Polyacrylamide-Urea Gel;
Acrylamide stock 30%,
Urea (7M stock),
TBE (10x) - 5ml,
Acrylamide 30%- 25ml,
Urea -21.gms,
APS- 200ul,
TEMED- 25ul

Western Blot Buffers:
Transfer buffer:
Tris-Glycine SDS containing 20% methanol.

Membrane treatment Buffer:
TBS (50mM Tris *.0, 150mM NaCl) or PBS,
TBST or PBST,
PB=phosphate buffer, (10mM NaPHO4 (7.0) + 150mM NaCl)
TB=Tris buffer,
T= Tween (.03%),

Blocking buffer: TBST + Skimmed milk 0.5 to 3%; or 0.1%BSA
Washing 2 to 3 times.

Incubation with IgG in PBS (T) or TBS (T) buffer;
Incubation with anti-antibody conjugated with AP in PBS/TBS,
Washing twice to three times;

Alkaline phosphate buffer (pH9.5); 5mM Mgcl2, 50mM Na2CO3 (or 100mM NaCl, 100mM Tris-Cl pH 9.5, 5mM MgCl2.
Then add Nitro BlueTetrazolium (NBT) (100ug/ml, and 5, Bromo 5-chloro 3-Indolyl phosphate (BCIP) 50ug/ml.

Culture media:

Bacterial culture media: LB medium (Luria Broth):
10 gms Bacto Tryptone,
5 gms Bacto-yeast extract,
10 gms NaCl,
Make it to 1 liter.

Phage buffer:
200mM Tris-Cl 7.4,
100mM NaCl,
10mM MgSO4.

Supplement medium:
For Y1090 cells:
10gm Bacto-Tryptone,
5gm Bacto-yeast extract,
5gm NaCl,
pH 7.5;
50ml LB
0.5 ml 20% maltose,
0.5ml of 1M MgSO4.

TEP buffer:
100mM Tris-Cl 7.4,
10mM EDTA,
1mM PMSF (10mM PMSF in EtOH),

SM buffer:
50mM Tris-Cl 7.5,
100mM NaCl,
8mM MgSO4,
0.01% Gelatin.

PCR buffer:
670mM Tris-Cl (.0,
67mM MgCl2,
1.7mg/ml BSA, 160mM (NH) 4 SO4.

Yeast culture media:
YP:
10gm Yeast extract,
20gms Bacto-peptone,
Make it to 1 liter.

YPD:
YP + 0.1 volumes of 20% Glucose.
LB for Bacterial transformation or electroporation:
Bacto-Tryptone 2%,
Bacto-yeast extract 0.5%,
NaCl 10mM,
KCl 2.5mM;
Mgcl2+MgSO4- 22mM,
PH 6.5;
Mg and Mg So4 -20mM each,

TFB:
K MES 10mM,
KCl 100mM,
MnCl2.4h2O- 45mM,
CaCl2-2H2O- 10mM,
H4COCl3 (Cobalt chloride)-3mM,
pH- 6.2.

FSB:
K.Acetate 10mM,
Glycerol 10%,
KCl 100mM,
MnCl2 45mM,
CaCl2 10mM,
HACoCl3 3mM. Adjust the pH to 6.2.

Random primining:

10X Hepes pH 6.6
Klenow
3 dNTPs
ααα32P CTP (high Ci/mM).

RNA Labeling:
Stock:
10x Transcription buffer,
0.4 Tris-Cl pH 8,
0.8M MgCl2,
0.5M NaCl,
0.2M Spermidine,
RNA polymerase,
α32P-UTP

Purify with G-50 column

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Purify with G-50 column

Meanwhile one has to have freshly grown E.coli strains such as Y1089 (lysogenic) or Y1090 (lytic). These have to be grown in LB medium containing MgSO4 (20mM) and maltose 2%. The density of the cells to be at 0.5 to 0.7 OD.

LB medium:
10 gm Bacto Tryptone,
5 gm Bacto yeast extract,
2% maltose,
20mM MgSO4,
Adjust the pH to 7.5.

Phage buffer:
200 mM Tris-Cl 7.4,
100 mM NaCl,
10 mM MgSO4

A list of Solutions and Buffers is unending- the above are some basic solutions used by me.