GENERAL REAGENTS- MOLECULAR PROTOCOLS

POLYMERASE CHAIN REACION (PCR):

Standard Buffer:

500 mM KCl

100 mM Tris. Cl

15 mM MgCl

Volumes of Components

Sterile Water	30 uL
10 X amplification buffer	10 uL
Mixture of 4 dNTPs (conc. 1.25 mM)	16 uL
primer 1 (in 5 uL of water)	100 pmoles
primer 2 (in 5 uL of water)	100 pmoles
template DNA (up to 2 ug)	
Water to a final volume of 100 uL	

MINIPREP OF E. COLI:

Buffer P1:

50 mM Tris, pH 8.3
10 mM EDTA, pH 8
100 ug/mL RNAse A
---store at 4ºC---

Buffer P2:

200 mM NaOH
1% SDS
---only good for a few weeks, store at RT---

Buffer P3:

3 M KAc
pH to 5.5
---store at RT but chill before using---

TRANSFORMATION OF E. COLI:

SOC:

2% Tryptone

0.5% Yeast Extract

10mM NaCl

10mM $MgSO_4$

10mM $MgCl_2$

SEQUENCING:

Sequencing Reaction Mixture:

~50 ng Template

Primer 2 uM- 1 uL

5X BFF- 1.5 uL

*Big Dye- 1 uL

HPLC H_2O- up to 10 uL

*B.D. is light sensitive

SOUTHERN BLOTTING:

Buffer	Working Solution	Conc. Stock (per liter)
Tris acetate (TAE)	1 x : 0.04 M Tris acetate 0.001 M EDTA	50 x :242 g Tris base 57.1 mL glacial acetic acid 100 mL 0.5 M EDTA (pH 8)
Tris phosphate (TPE)	1 x :0.09 M Tris phosphate 0.002 M EDTA	10 x :108 g Tris base 15.5 mL 85% phosphoric acid(1.679 g/mL) 40 mL 0.5 M EDTA (p H 8.0)
Tris borate (TBE)	0.5 x :0.045 M Tris borate 0.001 M EDTA	5 x :.54 g Tris base 27.5 g boric acid 20 mL 0.5 M EDTA (pH 8.0)
Alkaline	1 x :50 MN NaOH 1 mM EDTA	1 x :.5 mL 10 N NaOH 2 mL 0.5 M EDTA (pH 8.0)

Gel Loading Buffers

Buffer Type	6X Buffer	Storage Temp
1	0.25% bromophenol blue 0.25% xylene cyanol FF 40% (w/v) sucrose in water	4°C
2	0.25% bromophenol blue 0.25% xylene cyanol FF 15% Ficoll (Type 400) in water	Room temp.
3	0.25% bromophenol blue 0.25% xylene cyanol FF 30% glycerol in water	4°C
4	0.25% bromophenol blue 40% (w/v) sucrose in water	4°C
5	*Alkaline Loading Buffer* 300 mN NaOH 6 mM EDTA 18% Ficoll in water 0.15% bromocresol green 0.25% xylene cyanol FF	4°C

NORTHERN BLOTTING:

SSC 20X:

Dissolve the following in 800ml of distilled H2O.

175.3g of NaCl

88.2g of sodium citrate 0.3 M

Adjust the pH to 7.0 with a few drops of 1M HCl.

Adjust the volume to 1L with additional distilled H2O.

Sterilize by autoclaving.

DIG EASY HIB:

BLOCKING SOLUTION: for 50 mL

1 g powdered nonfat milk (1%)
50 mL Washing Solution (components below)

ANTIBODY SOLUTION:
Centrifuge Anti-Digoxygenin-AP for 5 min at 10krpm in its original vial prior to each use. Pipet the necessary amount carefully from the top surface.Dilute Anti-Digoxygenin-AP 1:10 000 (75mU/ml) in Blocking Solution. Prepare fresh (2hrs, 40C)

WASHING BUFFER:
500 uL Tween 20 (0.05%)
Make to 1 L with 1X PBS

10X Wash Buffer (for protein samples):
0.1 mM MES pH 6.8
2.5 mM EGTA
1 mM $MgCl_2$

DETECTION BUFFER:
0.1 M Tris-HCl
0.1 M NaCl
pH to 9.5 (200C)
Store at room temp

COLOR SUBSTRATE SOLUTION:
200 uL of BCIP vial 5 into 40 of detection buffer

RNA ISOLATION:
TRIZOL:
A reagent and mono-phasic solution of phenol and guanidine isothiocyanate from invitrogen .

RNA-AGAROSE GEL:

10X MOPS ELECTROPHORESIS BUFFER:

41.8 g MOPS

in 700 ml of sterile DEPC-treated H2O.

Adjust the pH to 7.0 with 2 N NaOH.

Add 20 ml of DEPC-treated 1 M sodium acetate

20 ml of DEPC-treated 0.5 M EDTA (pH 8.0).

Adjust the volume to 1 liter with DEPC-treated H2O.

SAMPLE BUFFER (Makes 14.5 uL):

5 uL 10XMOPS

17.5 uL formaldehyde

50 uL formamide

GENOMIC DNA ISOLATION:

STARVATION BUFFER:

20 mM MES pH 6.8

0.2 mM $CaCl_2$

2 mM $MgSO_4$

Autoclave

TE:

10 mM Tris, pH 8

1 mM EDTA