III. Buffers and Gels for Electrophoresis

Running buffers for non-denaturing gel electrophoresis

(Adapted from Brown, 1991)

Buffer	Components (1 x buffer)	Recipe for 1 liter of 10 x buffer
TBE[a,b] (Tris-borate)	89 mM Tris-borate (pH 8.3) 2 mM Na_2EDTA	108.0 g Tris-base 55.0 g boric acid 9.3 g Na_2EDTA
TAE (Tris-acetate)	40 mM Tris-acetate (pH 7.6) 1 mM Na_2EDTA	48.4 Tris-base 11.4 ml glacial acetic acid 20.0 ml 0.5 M Na_2EDTA (pH 8.0)
TPE (Tris-phosphate)	89 mM Tris-phosphate 2 mM Na_2EDTA	108.0 g Tris-base 15.5 ml phosphoric acid (85%) 40.0 ml 0.5 M Na_2EDTA (pH 8.0)

a) 0.5 x TBE can be used for agarose gels
1 x TBE is the standard running buffer for DNA separation in polyacrylamide gels.

b) Concentrated stock solutions of TBE tend to develop a precipitate when stored for long periods of time. Store at room temperature and discard any solution that develops a precipitate.

Recommended agarose gel concentration for resolving linear DNA

% Agarose	DNA size range (bp)
0.40	2000 – 30,000
0.75	1000 – 15,000
1.00	500 – 10,000
1.25	300 – 5000
1.50	200 – 4000
2.00	100 – 2500

Gel loading buffers for non-denaturing agarose gel electrophoresis

(Adaped from Brown, 1991)

Loading buffer	Recipe for 6 x buffer
SBX (store at 15 – 25°C)	40% (w/v) Sucrose 0.25% (w/v) Bromphenol blue 0.25% (w/v) Xylene cyanol
FBX (store at 2 – 8°C)	15% (w/v) Ficoll 400 0.25% (w/v) Bromphenol blue 0.25% (w/v) Xylene cyanol
GBX (store at 2 – 8°C)	30% (v/v) Glycerol 0.25% (w/v) Bromphenol blue 0.25% (w/v) Xylene cyanol

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Denaturing agarose gel electrophoresis

	Alkaline gels (McDonell et al., 1977)	**Glyoxal gels** (Bailey and Davidson, 1976)	**Formaldehyde gels** (Rueger et al., 1996)
Nucleic acid	DNA only	DNA, RNA	RNA (DNA)
Principle	Gels run in alkaline buffer which denatures DNA	Nucleic acids are denatured with glyoxal prior to electrophoresis	Gels are run in the presence of formaldehyde to denature nucleic acids
Gel buffer	50 mM NaCl 1 mM Na_2EDTA (neutral pH, because at high temperatures alkaline pH hydrolyzes agarose)	10 mM sodium phosphate, pH 7.0 (neutral pH, because glyoxal dissociates from nucleic acids at pH >8.0)	1 x MOPS* 2% (v/v)Formaldehyde
Running buffer	30 mM NaOH, 1 mM Na_2EDTA (Soak gel in running buffer 30 min before loading.)	Same as gel buffer (The running buffer must be circulated during electrophoresis.)	1 x MOPS*
Loading buffer	Resuspend DNA in: 50 mM NaOH 1 mM Na_2EDTA 3% (w/v) Ficoll 400 0.025% (w/v) Bromocresol green 0.025% (w/v) Xylene cyanol	Resuspend DNA/RNA in: 8 µl 1 M glyoxal 50% (v/v) DMSO 10 mM sodium phosphate, pH 7.0 Incubate at 50°C for 1 h; cool, then add: 2 µl 10 mM sodium phosphate, pH 7.0 50% (v/v) glycerol 0.4% (w/v) Bromphenol blue	**Prepare fresh!!!** 250 µl formamide (deionized) 83 µl formaldehyde, 37% (w/v) 50 µl 10 x MOPS-buffer* 0.01% (w/v) Bromphenol blue Add RNA and incubate for 10 min at 65°C; then immediately cool on ice.

* 10 x MOPS:
200 mM Morpholinopropanesulfonic acid, pH 7.0
50 mM sodium acetate
10 mM Na_2EDTA

Nondenaturing polyacrylamide gels: Recommended polyacrylamide gel concentration for resolving dsDNA and behavior of marker dyes

(Adapted from Sambrook et al., 1989)

% Acrylamide	DNA size range (bp)	Bromphenol Blue*	Xylene Cyanol*
3.5	100 – 1000	100	460
5.0	75 – 500	65	260
8.0	50 – 400	45	160
12.0	35 – 250	20	70
15.0	20 – 150	15	60
20.0	5 – 100	12	45

* Approximate size of DNA fragments (in base pairs) with which the dyes would migrate.

Denaturing polyacrylamide gels: Migration of marker dyes

(Adapted from Sambrook et al., 1989)

% Acrylamide	Bromphenol Blue*	Xylene Cyanol*
5.0	35	130
6.0	26	106
8.0	19	76
10.0	12	55

* Approximate size of DNA fragments (in nucleotides) with which the dyes would migrate.
(Acrylamide: Bisacrylamide (w/w) should be 29:1.)

Agaroses and molecular weight markers

see inside back cover page.

Stains and tracker dyes

Compound	MW	Application	Concentration
Acridine Orange	301.8	DNA, RNA stain in gel electrophoresis	1 – 10 µg/ml in staining solution
Bromphenol Blue	670.0	Gel electrophoresis marker dye	0.01 – 0.05% in gel loading buffers
Ethidium bromide	394.3	Fluorescent dye for DNA/ RNA in density gradients and electrophoresis gels	1 µg/ml in running buffer or staining solution
Xylene Cyanol	554.6	Gel electrophoresis marker dye	0.01 – 0.05% in gel loading buffers

IV. Other Useful Information

Length of rRNAs from various species

(Lewin, 1987)

Species	RNA	Length (bases)
E. coli	16 S rRNA 23 S rRNA	1542 2904
Yeast (*S. cerevisiae)*	18 S rRNA 28 S rRNA	2000 3750
Drosophila *(D. melanoglaster)*	18 S rRNA 28 S rRNA	2000 4100
Mammal	18 S rRNA 28 S rRNA	1874 4718