

APPENDIX IV. Gel Electrophoresis.

Migration of biological molecules in the presence of an electric field through a gel matrix is the heart of many biochemistry experiments. The variety of electrophoresis techniques, conditions and apparatus are almost as vast as the types of molecules that can be applied to gels. Regardless of the particular conditions reported for a given application, the fundamental physical principle remains the same: negatively charged molecules migrate through the gel matrix towards the positive electrode!

Excellent techniques, tips, history and procedures for numerous electrophoresis experiments can be found throughout Sambrook and Russell, *Molecular Cloning, A Laboratory Manual*, Third Edition, CSHL Press, 2001.

Below are four fundamental electrophoresis techniques

1. Agarose Gel Electrophoresis

Most common application - resolving larger DNA fragments

Agarose is a linear polymer of alternating residues of D and L-galactose joined by α -(1-3) and β -(1-4) glycosidic linkages. Agarose gels have a lower resolving power than polyacrylamide gels, but they have a greater range of separation (Table 1). Agarose gels are often run in a horizontal configuration in an electric field of constant strength and direction.

Table 1. Sieving properties of different percentage agarose gels

Concentration of agarose (% w/v) ^a	Range of Separation (bp)	Xylene cyanol ^b	Bromophenol Blue ^b
0.5	70-25,000	4,000	300
0.8	500-15,000	4,000	300
1.0	250-12,000	4,000	300
1.2	150-6,000	4,000	300
1.5	80-4,000	4,000	300

a standard high melting agarose

b approximate length of dsDNA that co-migrates with the dye

Agarose gels can be considered non-denaturing or native environments for biomolecules. This means DNA migrating through the gel with remain double stranded and retain alternate conformations. Super helical, nicked circular and linear DNAs migrate through agarose gels at different rates. The relative mobilities of the three forms depends primarily on the concentration and type of agarose used to make the gel, but they are also influenced the strength of the applied current, the ionic strength of the buffer and the density of the super helical twists. In most cases, the best way to distinguish between the different conformational forms of DNA is to simply load control samples of untreated circular DNA and the same DNA linearized by digestion with a restriction enzyme that cleave the DNA in only one place.

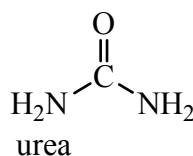
Agarose gels are typically prepared by weighing out a given amount of agarose solid, adding buffer and heating the solution until the agarose melts. The molten agarose is then poured into a horizontal apparatus. The gels are run in 0.5X Tris-Boric acid-EDTA (TBE) buffer with samples loaded in glycerol loading buffer containing xylene cyanol and bromophenol blue.

Nucleic acids are typically visualized by staining with ethidium bromide or SYBR gold.

2. Denaturing polyacrylamide gel electrophoresis

Most common application - resolving DNA/RNA samples based on molecular weight/length

Denaturing polyacrylamide gel electrophoresis is a technique that uses a gel made of polymerized acrylamide with the addition of urea, a molecule that hydrogen bonds to DNA/RNA bases and denatures the double strands into single strands.



Acrylamide, $\text{CH}_2=\text{CH}-\text{C}(=\text{O})-\text{NH}_2$, forms a linear polymer, polyacrylamide, that can be cross-linked with *N,N'*-methylene bisacrylamide, $\text{CH}_2=\text{CH}-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{NH}-\text{C}(=\text{O})-\text{CH}=\text{CH}_2$, to form a gel matrix of controlled pore size. Cross-linked polyacrylamide is shown in Fig. 1.

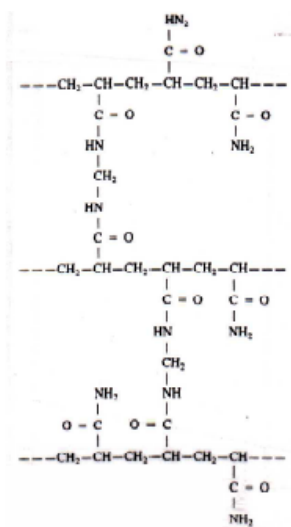


Fig. 1. Structure of cross-linked polyacrylamide

Acrylamide stock solutions are typically found with varying ratios of acrylamide to bis-acrylamide to create pores of different size. Ratios of 19:1, 29:1 and 37.5:1 are routinely used. If the pore size of the gel approximately matches the size of the molecules to be separated, smaller molecules can move freely in an applied electric field whereas larger molecules have restricted movement.

Polymerization is catalyzed by free radicals, generated by agents such as ammonium persulfate in the presence of *N,N,N',N'*-tetramethylethylenediamine (TEMED). Polymerization is inhibited by oxygen, so gel solutions should be degassed.

Denaturing-PAGE most often makes use of vertical slab gels ranging in thickness from 0.5 mm to 3.0 mm. Samples are prepared in formamide loading buffer, heated slightly (65°C, 3 minutes) and loaded while still warm. Denatured DNA migrates through these gels at a rate that is almost completely independent of sequence. Table 2 indicates some common conditions.

Table 2. Sieving properties of different percentage gels (denaturing)

Concentration of Acrylamide ^a	Range of Separation (nts)	Xylene cyanol ^b	Bromophenol Blue ^b
5.0	>250	130	35
6.0	250-60	106	29
8.0	120-40	76	26
10.0	60-20	55	12
20.0	40-5	28	8

a 29:1 acrylamide/bis-acrylamide

b approximate length of DNA that co-migrates with the dye

A common laboratory practice uses a concentrated solution of acrylamide to prepare gels of different percentage. This allows for maximum efficiency and flexibility among researchers. For example, a common solution of 40% acrylamide can be used as follows to prepare gels of the indicated percentage. Note: the amount of APS and TEMED does not change.

Table 3. Denaturing gel conditions

Gel Composition	6%	8%	10%	12%
Water (mL)	6.4 ^a	5.9 ^a	5.4 ^a	4.9 ^a
5x TBE (mL)	2.0	2.0	2.0	2.0
Urea (g)	4.8	4.8	4.8	4.8
40% acrylamide (mL)	1.5	2.0	2.5	3.0
10% APS (mcL)	100	100	100	100
TEMED (mcL)	10	10	10	10
Total Volume (mL)	10	10	10	10

^a the best practice would be to dissolve the urea in a small volume of water, add the buffer and acrylamide then adjust the volume to 10 mL

Polyacrylamide gels have the following advantages over agarose gels: 1) their resolving power is so great that they can separate molecules of DNA whose lengths differ by as little as 0.1% (i.e. 1 bp in 1000). 2) They can accommodate much larger quantities of DNA, 3) DNA recovered is extremely pure.

Nucleic acids are typically visualized by staining with ethidium bromide or SYBR gold.

3. Non-denaturing or Native polyacrylamide gel electrophoresis

Most common application - resolving DNA/RNA samples while retaining structure

Native-PAGE most often makes use of vertical slab gels ranging in thickness from 0.5 mm to 3.0 mm. Samples are prepared in glycerol loading buffer and loaded attempting to retain intimate molecular interactions whether it is hydrogen bonding between nucleotides or the preservation of RNA-protein interactions. Native DNA migrates through these gels at a rate that is dependent on structure. Table 4 indicates some common conditions.

Table 4. Sieving properties of different percentage gels (native)

Concentration of Acrylamide ^a	Range of Separation (bp)	Xylene cyanol ^b	Bromophenol Blue ^b
3.5	1000-2000	460	100
5.0	80-500	260	65
8.0	60-400	160	45
12.0	40-200	70	20
15.0	25-150	60	15
20.0	6-100	45	12

^a 29:1 acrylamide/bis-acrylamide

^b approximate length of dsDNA that co-migrates with the dye

Table 5. Native gel conditions

Gel Composition	6%	8%	10%	12%
Water (mL)	6.4	5.9	5.4	4.9
5x TBE (mL)	2.0	2.0	2.0	2.0
40% acrylamide (mL)	1.5	2.0	2.5	3.0
10% APS (mcL)	100	100	100	100
TEMED (mcL)	10	10	10	10
Total Volume (mL)	10	10	10	10

Nucleic acids are typically visualized by staining with ethidium bromide or SYBR gold.

4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Most common application - resolving protein samples based on molecular weight

SDS-PAGE most often makes use of vertical slab gels, although gels can also be formed in cylinders. A discontinuous gel system is also used frequently, consisting of a stacking gel and a separating gel. The stacking gel makes up only approx. 10% of the volume of the total gel and is of a lower percentage acrylamide (usually 2.5-4.5%) and a lower pH (6.8). In the stacking gel, charged molecules move freely in an applied electric field and proteins in a sample should accumulate in *stacks* of closely spaced bands. A stacking gel is used to concentrate larger volumes of sample before encountering the separating gel. In the separating gel, containing a higher percentage acrylamide (usually 7-15%) and at a higher pH (8.8), proteins separate into discrete bands based on molecular size.

Table 6. Sieving properties of different percentage gels (SDS-PAGE)

Acrylamide ^a (%)	Linear Range of Separation (kD)
5.0	57-212
7.5	36-94
10	20-80
12	12-60
15	10-43

^a 29:1 acrylamide/bis-acrylamide

Separation is not on the basis of shape or intrinsic charge of the protein, because proteins are treated with sodium dodecyl sulfate (SDS), a detergent with both polar and nonpolar properties. The long hydrocarbon chain of SDS is buried in nonpolar regions of proteins and the (-)-charged sulfate is exposed on the surface of proteins. SDS coats proteins with an approximately uniform charge-to-mass ratio of (-) charge (saturation occurs at approx. 1.4 g SDS/g protein). In addition, proteins are approximately uniformly shaped into spheres when coated with SDS, because the detergent disrupts native ionic and hydrophobic interactions. Thus, proteins separated by SDS-PAGE are denatured. Disulfides in proteins are broken by addition to the gel sample buffer of β -mercaptoethanol or 1,4-dithiothreitol, both of which reduce disulfide bonds.

A common laboratory practice uses a concentrated solution of acrylamide to prepare gels of different percentage. This allows for maximum efficiency and flexibility among researchers. For example, a common solution of 40% acrylamide can be used as follows to prepare gels of the indicated percentage. Note: the amount of APS and TEMED does not change.

Table 7. Conditions for SDS-PAGE

Gel Composition	6%	8%	10%	12%
Water (mL)	5.7	5.2	4.7	4.2
1.5M Tris (mL)	2.6	2.6	2.6	2.6
10% SDS (mcL)	100	100	100	100
40% acrylamide (mL)	1.5	2.0	2.5	3.0
10% APS (mcL)	100	100	100	100
TEMED (mcL)	10	10	10	10
Total Volume (mL)	10	10	10	10

Proteins in the gel are typically visualized by staining with coomassie brilliant blue.

The molecular weights of proteins in a sample can be estimated on a gel by measuring the mobility of protein standards of known molecular weight on the same gel. A calibration curve can be generated from the mobilities of the standards by plotting the distances migrated from the top of the resolving gel versus the log of molecular weight. A linear regression can be calculated and used to estimate unknown molecular weights. The estimation of molecular weight by this method is accurate to approximately 5-10% of the actual value.