Making and running agarose gels

Agarose gel electrophoresis is a simple, versatile technique for resolving DNA (*main protocol*), RNA (*Alternative A*), and large protein complexes (*Alternative B*) for analytical or preparative purposes.

Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE) buffers provide reliable separation of DNA fragments, with TBE favoring small fragments and TAE better suited for larger fragments and downstream enzymatic recovery. However, Tris-based buffers gradually lose buffering capacity during electrophoresis, causing heating and potential gel distortion at high voltages. Alternative electrolytes, such as sodium borate (borax) or lithium acetate, allow faster electrophoresis at higher voltages with lower heat buildup.

Risk assessment

- – Agarose solutions can become superheated in the microwave and may boil over suddenly when agitated, causing burns or splash injuries
- – Many DNA intercalating dyes are potent mutagens
- – Boric acid may damage fertility or the unborn child
- – Work with high voltage power sources
- ▷ Use large flasks of two- to four-times the gel volume; cover loosely and heat in short bursts, swirling gently between intervals
- ▷ Wear heat-resistant gloves or mitts when handling hot solutions
- ▷ Wear gloves, safety glasses, lab coat
- □ Collect staining solutions as HAZARDOUS WASTE

Reviewed: Nov 26, 2023

Procedures

›› ***Choosing an electrophoresis buffer for DNA separation***

(1.) Select running buffer, agarose concentration, and voltage based on the DNA fragment size and goals:

Purpose	DNA size range	Agarose content	Running buffer	Voltage	Staining
Analytical separation	<1 kbp	1.0–1.8%	0.5 × TBE	10–15 V/cm	Post-run staining
	1–12 kbp	0.7–1.0%	0.5 × TBE	5–10 V/cm	Post-run staining
	>12 kbp	0.6–0.8%	1 × TAE	5 V/cm	Post-run staining
Preparative recovery	<1 kbp	1.2–2.0%	1 × TAE	10–15 V/cm	In-gel
	1–12 kbp	0.8–1.2%	1 × TAE	5–10 V/cm	In-gel
	>12 kbp	0.6%	1 × TAE	5 V/cm	In-gel
Speed/Throughput	<1 kbp	1.0%	0.5 × Tris-borate	20–25 V/cm	In-gel
		1.0%	1 × Borax	30 V/cm	In-gel
	1–12 kbp	1.0%	1 × Lithium-acetate	30 V/cm	In-gel

Hint: For routine analytical gels, 1% agarose in 0.5 × TBE (or 1 × TAE) is suitable for most applications. For DNA recovery, TAE is preferred because borate can inhibit enzymatic reactions.

(2.) Choose a DNA stain according to sensitivity needs:

Stain	Detection limit	Wavelength	Working concentration
Ethidium bromide	1–5 ng per band	UV light	0.2–0.5 mg/L
SYBR® Safe	100 pg per band	UV *or* Blue light	1 : 10, 000
Thiazole Orange (TO)	50–100 pg per band	Blue light	1 : 10, 000
GelRed®	50 pg per band	UV *or* Blue light	1 : 10, 000
SYBR® Gold	10–25 pg per band	Blue light	1 : 10, 000

Hint: Ethidium bromide and GelRed® can be incorporated directly into the gel and running buffer. SYBR® Gold and SYBR® Green are best used as post-run stains for optimal resolution and lowest background.

[SAL+14]

›› *Casting an agarose gel*

- ☐ Erlenmeyer flask *or* Screw-cap bottle
- ☐ Stir bar and plate
- ☐ Microwave oven
- ☐ Agarose, low EEO (LE)
- ☐ Running buffer

(1.) Choose a heat-resistant container two- to four-times the intended gel volume. Add the electrophoresis buffer and a magnetic stir bar.

> ***Hint:*** Use a large Erlenmeyer flask or screw-cap bottle with a narrow neck to reduce spillage during swirling and handling. Make sure they are easy to grab.

(2.) Slowly sprinkle the agarose powder into the stirred buffer to prevent clumping.

> ***Critical:*** Use low electroendosmosis (EEO) agarose for better mobility, sharper separation, and minimized electro-osmotic backflow during electrophoresis.

(3.) *Optional:* Soak the agarose for 15 min in running buffer to reduce foaming during heating, especially for concentrations above 3%. ⏲ 15 min

(4.) Weigh the container and record the weight to monitor evaporation losses after heating.

(5.) Cover the container loosely: seal with pierced plastic wrap or leave the screw cap loosened by one turn.

> ***Safety:*** Allowing some ventilation prevents pressure buildup while minimizing excessive evaporation during microwaving.

(6.) Heat the agarose suspension in the microwave at Medium power for 1–2 min. Remove, swirl gently, and repeat as needed until almost dissolved.

> ***Safety:*** Agarose solutions can become superheated. Swirl gently to prevent sudden boiling over. Point the opening of the flask away from you and others while swirling! If in doubt, let the solution rest for a couple of minutes.

(7.) Heat briefly at High power until the solution comes to a rolling boil. Hold at boiling for 30–60 s to fully dissolve any remaining particles. Swirl gently after heating.

(8.) Re-weigh the container. If weight loss exceeds 5%, add hot distilled water to compensate for evaporation. Mix thoroughly.

> ***This is why:*** This correction ensures consistent agarose concentration and gel strength across experiments.

(9.) Cool the molten agarose to 45–60 °C before casting.

> ***Hint:*** To cool quickly, swirl the container under running cold water. Avoid splashing water into the flask.

(10.) *Optional:* Add DNA staining dye to the gel as appropriate.

> ***Critical:*** Do not include SYBR® Green Stains in the agarose solution: the bands will start to smear (overloading) and alter the electrophoretic separation.

(11.) Prepare the casting tray. Position the comb so that the teeth are suspended 1–2 mm above the tray base.

> ***Hint:*** Thin combs (1 mm) give sharper bands. Use thicker or wider combs for preparative gels needing higher DNA loads.

(12.) Pour the molten agarose smoothly into the tray in one continuous motion to a thickness of 3–4 mm.

> ***Quality assurance:*** Avoid overfilling beyond the comb teeth. Thin gels improve resolution, reduce heating artifacts, and allow faster post-staining penetration.

(13.) Allow the gel to solidify undisturbed for 15–20 min at room temperature. ⏲ 15–20 min

(14.) Once solidified, place the gel with the tray into the gel tank. Cover 1–2 mm deep in running buffer. Carefully remove the comb by pulling straight upward without tilting.

[BK04]

›› *Loading samples*

- ☐ 6 × Gel loading buffer, 40 µL ⟨*R*⟩
- ☐ 20 × Gel loading dye, 12 µL
- ☐ 25 mg/L DNA ladder, 4–8 µL ⟨*R*⟩

(1.) *Optional:* To supplement the 6 × loading buffer with a loading dye, add 0.3 vol 20 × loading dye for a 5 × loading buffer with dye. The dye front will migrate at:

	Agarose content				
DNA dye	**0.7%**	**1.0%**	**1.5%**	**2.0%**	**3.0%**
Xylene cyanol	8 000 bp	4 000 bp	2 000 bp	900 bp	400 bp
Cresol red	3 000 bp	1 500 bp	900 bp	300 bp	100 bp
Bromophenol blue	600 bp	400 bp	240 bp	120 bp	25 bp
Orange G	100 bp	50 bp	20 bp	10 bp	5 bp

(2.) Choose the appropriate molecular weight marker.

> ***Note:*** For DNA fragments smaller than 1 kbp, use New England Biolab 100 bp Ladder. For fragments between 1–10 kbp, use the 1 kbp Plus Ladder. For fragments larger than 10 kbp, use a Lamda *Hin*dIII Marker.

(3.) Mix each DNA sample with loading buffer:

- Add 2.0 µL of 6 × loading buffer per 10 µL sample, or
- Add 2.5 µL of 5 × loading buffer (pre-mixed with tracking dye) per 10 µL sample.

> ***Hint:*** Alternatively, spot a small drop of loading buffer on Parafilm® and mix with the sample just before loading.

> ***Quality assurance:*** Aim to load between 10–100 ng DNA per band for ethidium bromide staining. For more sensitive dyes like SYBR® Gold, adjust accordingly. If you are unsure about how much DNA is in your sample, load varying amounts per lane.

(4.) Load the gel by gently lowering the pipette tip just into the well opening. Dispense the sample slowly without puncturing the gel bottom. Steady the pipette with one finger of your non-pipetting hand if needed. Change pipette tips between different samples.

›› *Running the gel*

(1.) Connect the electrophoresis unit: the negative electrode (cathode, black) must be closest to the wells; the positive electrode (anode, red) opposite the wells. Remember: "Run towards red!"

> ***Hint:*** Check that the power supply shows current flowing. Look for gas bubbles forming at the electrodes.

(2.) Apply voltage according to the task:

- For analytical gels: 5–10 V/cm between electrodes.
- For preparative gels: 5 V/cm for better resolution and minimal heating.

> ***Hint:*** For a typical small- or medium-sized horizontal gel (10–15 cm length), power supplies are set to between 100–150 V and gels are run for 15–90 min. Excess voltage causes asymmetric heating, leading to band smearing, slanting, or compression.

(3.) Stop the run when the band of interest has migrated 40–60% of the gel length or when desired separation is achieved.

Optional: *Post-run staining of DNA*

- ☐ 10 g/L Ethidium bromide
- ☐ 10 000 × Thiazole orange, 2 µL ⟨*R*⟩

- If accurate sizing is critical, stain gels post-run

(1.) Immerse the gel for 15–20 min in 1 × staining solution with gentle agitation if available. 15 min

> ***Hint:*** The 10 g/L ethidium bromide stock is 1 000 ×. Dilute ethidium bromide, thiazole orange or SYBR® dyes according to the manufacturer's instructions in water or buffer.

> ***Safety:*** Always handle ethidium bromide and DMSO-based dyes with gloved hands in a designated area. Collect staining waste separately for proper disposal.

(2.) *Optional:* Rinse the gel three times with deionized water to reduce background fluorescence.

> ***Hint:*** Short rinses (2–5 min each) are usually sufficient. Rinsing is particularly important for SYBR® dyes, which otherwise produce higher background signal.

A › *Separation of RNA in agarose gels*

- ☐ Water, 100 mL ⟨*R*⟩
- ☐ 1.3 × RNA sample buffer ⟨*R*⟩
- ☐ 5 × MOPS-acetate running buffer, 0.5 L ⟨*R*⟩

(1.) For RNA separation, use a 1–1.5% agarose gel in 1 × MOPS-acetate buffer containing 2.2 M (6.5%, w/v) formaldehyde.

> ***Critical:*** RNA is highly sensitive to degradation. Use RNase-free reagents and equipment, including DEPC-treated water. Wear gloves at all times.

> ***Safety:*** Formaldehyde is a known carcinogen. Cast the gel in a chemical fume hood, and allow to set for at least 30 min at room temperature. Melt agarose in DEPC-treated water, before combining with 5 × MOPS-acetate buffer and formaldehyde.

(2.) Combine 1.0 vol of RNA sample containing up to 30 µg per lane with 3 vol 1.3 × RNA sample buffer. Incubat for 15 min at 65 °C and chill on ice.

(3.) Mix with 0.1 vol RNA loading dye.

(4.) Pre-run the gel for 5 min at 5 V/cm. Immediately load the sample.

(5.) Run at 3–4 V/cm in 1 × MOPS-acetate running buffer until appropriate separation is achieved.

> ***Quality assurance:*** Collect the running buffer every 1–2 h from the reservoir, mix, and return to the gel apparatus.

(6.) Stain RNA with ethidium bromide or SYBR® Green II.

> ***Hint:*** RNA bands may appear less sharp than DNA due to size and secondary structure differences.

(7.) *Optional:* Proceed with Northern blotting.

[LDW+77]

B › *Separation of proteins in agarose gels*

(1.) Prepare a low electroendosmosis (EEO) agarose gel. Adjust concentration depending on target size:

Protein size range	Agarose content	Agarose type
20–200 kDa	5%	MetaPhor®, NuSieve®
150–300 kDa	3%	MetaPhor®, NuSieve®
300–600 kDa	2%	MetaPhor®, NuSieve®
>600 kDa	1–1.5%	SeaKem®, SeaPlaque®

(2.) Use 1 × Tris-borate (or Tris-Gly running buffer 0007 to cast a horizontal agarose gel.

> ***Critical:*** Do not add SDS to the gel buffer to avoid excess foaming during agarose dissolution. Only add SDS to the running and sample buffer for denaturing electrophoresis.

(3.) Load proteins with loading buffer containing bromophenol blue or xylene cyanol FF as tracking dye.

(4.) Run at 10–20 V/cm for 3–4 h until the tracking dye travels to the bottom of the gel. ⏲ 3–4 h

> ***Hint:*** Supplement 0.1% SDS if desired. Monitor carefully to avoid overheating and reduce voltage if needed.

(5.) Stain proteins with Coomassie Brilliant Blue 0010, Silver stain 0011, Amido black, Violet 17, or commercial protein stains such as SERVA Blue after electrophoresis.

> ***Hint:*** Coomassie detects 1 µg of protein per band; silver staining detects down to 10–50 ng protein.

(6.) *Optional:* To recover proteins, use low-melting agarose and extract bands by freeze-thaw or gentle buffer diffusion. ⊞

🔗 [KYK00]

Analyses

- ○ Visualize the DNA bands in a UV transilluminator (302 nm or 365 nm) for ethidium bromide detection, or blue-light imaging for SYBR® dyes and Thiazole Orange.
- ○ Record gel images promptly to avoid photobleaching or overexposure.

Troubleshooting

Casting an agarose gel

In Step 9:

– Agarose starts to solidify before pouring
  - ◦ Reheat gently (short microwave burst) and swirl until fully molten again before pouring.

In Step 14:

– Wells collapse or tear when comb is removed
  - ◦ Flood the gel surface with buffer before removing the comb.
  - ◦ Chill the gel and buffer at 4 °C for 30 min before comb removal to stabilize low-percentage gels.

Loading samples

In Step 1:

– U-shaped bands
  - ◦ Too much glycerol in the sample or loading buffer. Replace glycerol with sucrose or Ficoll® 400 where possible.
– Wavy or distorted bands
  - ◦ Salt concentration in the samples is too high. Dilute samples or match salt concentrations before loading. Aim for salt in final loading mix.
– Supposedly identical molecular weight bands are offset between lanes
  - ◦ Salt concentration differences between samples cause migration shifts. Normalize salt concentrations before gel loading.

In Step 4:

– Sample floats out of the well while loading
  - ◦ Ensure the sample contains enough loading buffer for density.
  - ◦ Avoid rapid dispensing or disrupting the well.

Running the gel

In Step 3:

– Band streaking or smearing
  - ◦ Reduce voltage for high molecular weight DNA. Increase voltage slightly for low MW DNA to prevent diffusion.

Post-run staining of DNA

In Step 1:

– Blurry or fuzzy bands
  - Prolonged storage of gels before imaging can cause diffusion. Image promptly after staining.

Recipes

Borax (SB), 20 ×

Amount	Ingredient	Stock	Final
38.1 g	Sodium tetraborate, decahydrate [1303-96-4]	381.37 g/mol	100 mM
To 1 L	Water, reagent-grade		

Filter through 0.45 µm filter or autoclave. Stable for 6 months at room temperature.

20 × Borax (SB)

5 mM Sodium tetraborate *[At 1 × dilution]*

DANGER

Reproductive toxicology

Expiry: Sign: R0174

Lithium-acetate (LA), 20 ×

Amount	Ingredient	Stock	Final
20.4 g	Lithium acetate, dihydrate [1303-96-4]	102.0 g/mol	200 mM
To 1 L	Water, reagent-grade		

Filter through 0.45 µm pore or autoclave. Stable for 6 months at room temperature.

20 × Lithium-acetate (LA)

10 mM Lithium acetate *[At 1 × dilution]*

Expiry: Sign: R0175

Tris-borate (TB), pH 8.3, 10 ×

Amount	Ingredient		Stock	Final
121.1 g	Tris base	[77-86-1]	121.14 g/mol	1 M
61.8 g	Boric acid	[10043-35-3]	61.83 g/mol	1 M
To 1 L	Water, reagent-grade			

Filter through 0.45 µm pore or autoclave. Stable for 6 months at room temperature.

10 × Tris-borate (TB)

100 mM Tris base, 100 mM Boric acid, pH 8.3

[At 1 × dilution]

DANGER

Reproductive toxicology

Expiry: Sign: R0176

Gel loading buffer, pH 8.0, 6 ×

Amount	Ingredient		Stock	Final
10 mL	Tris-Cl, pH 8.0	R0056	1 M	20 mM
7.5 g	Ficoll® 400	[26873-85-8]		15%
6 mL	EDTA, pH 8.0	R0017	0.5 M	60 mM
1.2 mL	SDS	☐ R0047	20%	0.48%
To 50 mL	Water, reagent-grade			

Omit SDS with SYBR® Safe or GelRed DNA staining dyes. ***Hint:*** Polysucrose (Ficoll®) in the loading buffer results in sharper bands than 10% glycerol- or 10% sucrose-based 1 × loading buffers.

6 × Gel loading buffer

3.3 mM Tris-Cl, 2.5% Ficoll® 400, 10 mM EDTA,

☐ 0.08% SDS, pH 8.0 *[At 1 × dilution]*

Date: Sign: R0177

Orange G, 20 ×

Amount	Ingredient		Stock	Final
250 mg	Orange G	[1936-15-8]	452.4 g/mol	5 g/L
To 5 mL	Water, reagent-grade			

20 × Orange G

0.025% Orange G *[At 1 × dilution]*

Date: Sign: R0178

Xylene cyanol FF, 20 ×

Amount	Ingredient		Stock	Final
50 mg	Xylene cyanol FF, sodium salt, 70–85% dye content	[4463-44-9]	554.62 g/mol	1 g/L
To 5 mL	Water, reagent-grade			

Note: This is a saturated solution.

20 × Xylene cyanol FF

0.005% Xylene cyanol FF *[At 1 × dilution]*

Date: Sign: R0179

Bromophenol blue, 20 ×

Amount	Ingredient		Stock	Final
0.5 mL	Sodium hydroxide (NaOH), pH 14.0	R0048	1 M	0.1 M
50 mg	Bromophenol blue	[115-39-9]	669.96 g/mol	1 g/L
To 5 mL	Water, reagent-grade			

Note: This is a saturated solution.

20 × Bromophenol blue

0.005% Bromophenol blue *[At 1 × dilution]*

Date: Sign: R0180

Cresol red, 20 ×

Amount	Ingredient		Stock	Final
50 mg	Cresol red, sodium salt	[62625-29-0]	404.4 g/mol	1 g/L
To 5 mL	Water, reagent-grade			

Note: This is a saturated solution.

20 × Cresol red

0.005% Cresol red *[At 1 × dilution]*

Date: Sign: R0181

DNA ladder, 25 mg/L

Amount	Ingredient	Stock	Final
125 µL	DNA ladder	1 g/L	25 mg/L
1 000 µL	Loading buffer	6 ×	1 ×
300 µL	Gel loading dye	20 ×	1 ×
To 6 mL	Water, reagent-grade		

Store at room temperature. ***Hint:*** Load 1–2 µg (4–8 µL) for a 5 mm wide gel lane; scale accordingly.

25 mg/L DNA ladder

25 mg/L DNA ladder, 1 × Loading buffer, 1 × Gel loading dye

Date: Sign: R0182

Thiazole orange (TO), 10 000 ×

Amount	Ingredient	Stock	Final
150 mg	Thiazole orange [107091-89-4]	476.6 g/mol	30 mM
To 10 mL	Dimethyl sulfoxide (DMSO), reagent-grade		

Stable for 2 years at 4 °C.

10 000 × Thiazole orange (TO)

WARNING

Serious eye irritation; Skin irritation

Expiry: Sign: R0183

Water, DEPC-treated

Amount	Ingredient	Stock	Final
0.1 mL	Diethyl pyrocarbonate (DEPC) [1609-47-8]	162.14 g/mol	0.1%
To 100 mL	Water, reagent-grade		

Water

DEPC-treated

Date: Sign: R0172

MOPS-acetate running buffer, pH 7.0, 5 ×

Amount	Ingredient	Stock	Final
10.5 g	4-Morpholinepropanesulfonic acid (MOPS) [1132-61-2]	209.27 g/mol	100 mM
8.3 mL	Sodium acetate (NaOAc), pH 5.2 R0045	3 M	50 mM
5 mL	EDTA, pH 8.0 R0017	0.5 M	5 mM
To 0.5 L	Water, DEPC-treated		

Adjust the pH with 2 M sodium hydroxide before adding EDTA. Stable for 6 months at room temperature.

5 × MOPS-acetate running buffer

20 mM MOPS, 10 mM NaOAc, 1 mM EDTA, pH 7.0 *[At 1 × dilution]*

Expiry: Sign: R0184

RNA sample buffer, 1.3 ×

Amount	Ingredient	Stock	Final
200 µL	MOPS-acetate running buffer, pH 7.0 R0184	5 ×	0.65 ×
350 µL	Formaldehyde [50-00-0]	27%	8.45 ×
1 000 µL	Formamide [75-12-7]	45.04 g/mol	65%

Note: Deionized formamide can be stored in small aliquots under nitrogen at −80 °C. ***Hint:*** If any yellow color is present, the formamide should be deionized by adding Dowex XG8 mixed-bed resin and stirring for 1 h; filter twice through Whatman® No. 1 paper.

1.3 × RNA sample buffer

0.5 × MOPS-acetate running buffer, 6.5% Formaldehyde, 50% Formamide *[At 1 × dilution]*

DANGER

Carcinogenicity; Serious eye irritation; Skin irritation

☐ Collect as HAZARDOUS WASTE

Date: Sign:

List of references

J.R. Brody and S.E. Kern, *Anal. Biochem.* **333**(1), 1—13 (2004).

R. Kim, H. Yokota, and S. Kim, *Anal. Biochem.* **282**(1), 147—149 (2000).

H. Lehrach, D. Diamond, J. Wozney, and H. Boedtker, *Biochemistry* **16**(21), 4743—4751 (1977).

B.A. Sanderson, N. Araki, J.L. Lilley, G. Guerrero, and L.K. Lewis, *Anal. Biochem.* **454** 44—52 (2014).

Change log

2021-04-01	Nick Coleman	Origianl protocol.
2023-11-26	Benjamin C. Buchmuller	Adaptation as SOP.