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Section VII: Separation of DNA in Polyacrylamide Gels

Separating DNA in Polyacrylamide Gels

Overview

Polyacrylamide gels can separate DNA that differs by 0.2% in length, well beyond the resolving capabilities of agarose [2% difference in DNA length]. Another advantage to using polyacrylamide gels is that they can accommodate large amounts of DNA (up to 10 µg) without any loss in resolution.

Depending upon the application, TBE gels can be prepared as denaturing or nondenaturing gels.

Lonza offers PAGEr® Precast TBE Gels for DNA separation. Refer to page 17 for information.

Applications

Denaturing gels: concentrations range from 8 - 20%

- Oligonucleotide purification
- Separation of single-stranded DNA
- Isolate radiolabeled DNA probes
- S1 nuclease assay
- DNA footprinting
- RNase protection assays

Nondenaturing gels: concentrations range from 3 - 20%

- Separation of di-nucleotide repeats
- Separation of DNA ranging from 20 bp - 2000 bp in length
- Study DNA-Protein interactions (Gel Shift Assays)

Buffers for Electrophoresis

To ensure adequate buffering power during vertical electrophoresis, TBE Buffer is used for polyacrylamide gel electrophoresis at a working strength of 1X. Lower dilutions of the buffer or the use of TAE Buffer may cause gels to overheat and result in band smearing throughout the gel.

TBE is commercially available as 5X or 10X solutions (Lonza AccuGENE® TBE Buffer). Alternatively, it can be prepared as follows:

10X TBE Stock Solution

(890 mM Tris base, 890 mM Boric acid, 20 mM EDTA)

1X = 89 mM Tris base, 89 mM Boric acid, 2 mM EDTA)

108.0 g Tris base
55.0 g Boric acid
7.44 g Na₂EDTA • 2H₂O

Adjust volume to 1 liter with distilled water
Filter through a 0.45 µm filter
pH adjustment is not necessary

Suggested Polyacrylamide Concentrations

Nondenaturing polyacrylamide gels	
Gel Percentage	Fragment Sizes
3.5%	1,000 bp - 2,000 bp
5.0%	80 bp - 500 bp
6.0%	75 bp - 2,000 bp
8.0%	60 bp - 400 bp
10.0%	30 bp - 1,000 bp
12.0%	40 bp - 200 bp
15.0%	25 bp - 150 bp
20.0%	5 bp - 100 bp
4 - 20%	10 bp - 2,000 bp

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Detecting DNA in Polyacrylamide Gels

Detecting DNA in Polyacrylamide Gels with GelStar® or SYBR® Green Nucleic Acid Gel Stains

GelStar® and SYBR® Green Nucleic Acid Gel Stains are highly sensitive fluorescent stains for detecting nucleic acids. These stains provide high sensitivity detection of double-stranded or single-stranded DNA. See table below for a comparison of staining sensitivities and limits of detection.

Stain	ssDNA	dsDNA	Native RNA
GelStar® Stain	25 pg	20 pg	10 ng
SYBR® Green I Stain	–	20-30 pg	–
SYBR® Green II Stain	60 pg	–	15 ng
Ethidium bromide	350 pg	100 pg	30 ng

Tips for Staining Gels with GelStar® and SYBR® Green Nucleic Acid Gel Stains

- The powder used on some laboratory gloves may contribute to background fluorescence. We recommend using powder-free gloves and rinsing gloves prior to handling gels
- Fibers shed from clothing or lab coats may be fluorescent; be cautious when handling gels
- Staining of nucleic acids with these dyes has minimal impact on blotting efficiency. To ensure efficient hybridization, use of prehybridization and hybridization solutions containing 0.1 - 0.3% SDS is important to remove stain retained during transfer
- These stains can be removed from nucleic acids by ethanol precipitation. Isopropyl alcohol precipitation is less effective at removing the dye; butyl alcohol extraction, chloroform extraction and phenol do not remove the dye efficiently
- Allow time for the stock solution to thaw completely. Removal of stain from partially thawed solutions will result in depletion of stain over time. These stains may be diluted in most common electrophoresis buffers with a pH range from 7.0 - 8.5 or in TE Buffer. Staining solutions prepared in water or in buffer with a pH below 7.0 or above 8.5 are less stable and show reduced staining efficiency

- Prepare and store the stain in polypropylene containers such as Rubbermaid® containers or pipette-tip box lids. The stain may adsorb to glass surfaces and some plastic surfaces, particularly if the surfaces carry residues of anionic detergents or reagents
- As an alternative to the protocol presented for staining gels on the cassette plate, smaller gels such as mini gels may be removed from both plates then stained using the protocol for post-staining agarose gels found in Section IV.
- Treatment of one plate with a “release” agent, such as Gel Slick® Solution, increases the ease of separating the glass plates while keeping the gel in place on the other plate for staining
- Handling or compression of gels (particularly polyacrylamide-type gels) can lead to regions of high background after staining. If possible, gels should not be handled directly; use a spatula (or other tool) and a squirt bottle to slide the gel off the plates and into the stain or onto the light box

Procedure for Staining

Incorporating these dyes into the gel or prestaining the nucleic acid in a vertical format is not recommended. The dye binds to glass or plastic plates and DNA may show little to no signal. Gels should be post-stained as described below.

1. Remove the concentrated stock solution from the freezer and allow the solution to thaw at room temperature
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube
3. Dilute the 10,000X concentrate to a 1X working solution for DNA and a 2X working solution for RNA, in a pH 7.5 - 8.5 buffer, in a clear plastic polypropylene container
4. Open the cassette, and leave the gel in place on one plate
5. Place the plate, gel side up, in a staining container
6. Gently pour the stain over the surface of the gel; a disposable pipette may be used to help distribute the stain evenly over the gel surface
7. Stain the gel for 5 - 15 minutes. No destaining is required
8. Remove the gel from the staining solution and view with a 300 nm UV transilluminator, Dark Reader® Transilluminator or CCD imaging system.

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Detecting DNA in Polyacrylamide Gels — continued

For highest sensitivity, the gel should be carefully removed from the plate and placed directly on the transilluminator or scanning stage. Alternatively, if a relatively low fluorescence plate is used, the results may be visualized by placing the gel and plate gel side down on the transilluminator and photographing or by scanning the gel directly on the plate.

NOTE: More detailed information on photographing gels and decontaminating staining solutions is described in Detecting DNA with GelStar® or SYBR® Green I Stains (see Section IV).

Detecting DNA in Polyacrylamide Gels with Ethidium Bromide

The procedure for post-staining DNA in polyacrylamide gels with ethidium bromide is identical to the procedures used for post-staining agarose gels. Follow the procedures described in Detecting DNA with Ethidium bromide (see Section IV).

■ Materials

- Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)
- Microcentrifuge
- UV transilluminator
- Photographic Filter for GelStar® Stain or SYBR® Green Stain (Wratten® #15 or Wratten® #9 Filter respectively)

■ Reagents

- GelStar® Nucleic Acid Gel Stain or SYBR® Green I or II Nucleic Acid Gel Stain
- Buffer between pH 7.5-8.5 (TBE or TE)

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

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Recovery of DNA in Polyacrylamide Gels

The two primary methods for recovering DNA from polyacrylamide gels are the “Crush and Soak” method or electroelution. Described below is the “Crush and Soak” procedure. The procedure for electroeluting DNA from polyacrylamide gels is similar to the procedures used for agarose gels with one exception; 0.5X TBE Buffer should be used rather than TAE Buffer. Follow the procedures described in Electroelution of DNA from Agarose Gels (see Section VI).

Tips for increasing DNA recovery from polyacrylamide gels

When recovering DNA from polyacrylamide gels, we recommend the following:

- Stain the gel for no more than 15 minutes
- If ethidium bromide staining, destain the gel in distilled water for two, 20-minute washes
- Do not expose the DNA to UV light for any longer than 1 minute. Longer exposures may result in DNA nicking
- Cut the smallest gel slice possible
- If recovering small amounts of DNA, the addition of 10 µg of carrier RNA prior to ethanol precipitation may improve recovery yields

Crush and soak procedure

1. While the gel is on the transilluminator, cut out the band of interest using a razor blade or scalpel. Cut the smallest size gel slice possible.

NOTE: If the gel has been covered with plastic wrap, do not remove the plastic wrap before cutting.

2. Peel the small piece of gel containing the DNA from the plastic wrap.
3. Transfer the gel slice to a microfuge tube.
4. Crush the gel slice against the wall of the microfuge tube with the disposable pipette tip.
5. Add 1 - 2 volumes of elution buffer to the gel slice (e.g., if the estimated gel slice volume is 200 µl add 200 µl - 400 µl of elution buffer).
6. Incubate the tube at 37°C on a rotating wheel or rotary platform. For fragments less than 500 bp incubate for 3 - 4 hours. For fragments greater than 500 bp incubate for 12 - 16 hours.
7. Centrifuge the samples at 12,000 rpm for 1 minute at 4°C.
8. Transfer the supernatant to a fresh microfuge tube, being careful to avoid transferring fragments of polyacrylamide.
9. Add 0.5 volumes of elution buffer to the pellet of polyacrylamide.
10. Vortex briefly.
11. Centrifuge at 12,000 rpm for 1 minute at 4°C.
12. Combine the two supernatants.
13. Remove any remaining polyacrylamide by passing the supernatant through a disposable plastic column or a syringe barrel containing a Whatman® GF/C Filter or packed siliconized glass wool.
14. Add 2 volumes of cold ethanol.
15. Store the solution on ice for 30 minutes.
16. Recover the DNA by centrifugation at 12,000 rpm for 10 minutes at 4°C.
17. Decant the supernatant.
18. Redissolve the DNA in 200 µl of AccuGENE® 1X TE Buffer, pH 7.6.
19. Add 25 µl of AccuGENE® 3 M Sodium Acetate, pH 5.2.
20. Repeat steps 14 - 17.
21. Rinse pellet once with 70% ethanol.
22. Add 10 µl of AccuGENE® 1X TE, pH 7.6 to pellet and dissolve.

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Recovery of DNA in Polyacrylamide Gels — continued

■ Materials

- Scalpel or razor blade
- Microfuge tube
- Disposable pipette tip
- Rotary wheel or platform
- 37°C oven
- Microcentrifuge at 4°C
- Vortex
- Disposable plastic column or a syringe barrel containing a Whatman® GF/C Filter or packed siliconized glass wool
- Ice

■ Reagents

- Elution buffer
 - (3.85 g ammonium acetate, 0.215 g magnesium acetate, 0.2 ml AccuGENE® 0.5 M EDTA Solution, 1.0 ml AccuGENE® 10% SDS Solution, fill to 100 ml with distilled water)
- 100% and 70% ethanol
- AccuGENE® 1X TE, pH 7.6
- AccuGENE® 3M Sodium Acetate, pH 5.2

References

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