Video Article

Denaturing Urea Polyacrylamide Gel Electrophoresis (Urea PAGE)

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Abstract

Urea PAGE or denaturing urea polyacrylamide gel electrophoresis employs 6-8 M urea, which denatures secondary DNA or RNA structures and is used for their separation in a polyacrylamide gel matrix based on the molecular weight. Fragments between 2 to 500 bases, with length differences as small as a single nucleotide, can be separated using this method[1]. The migration of the sample is dependent on the chosen acrylamide concentration. A higher percentage of polyacrylamide resolves lower molecular weight fragments. The combination of urea and temperatures of 45-55 °C during the gel run allows for the separation of unstructured DNA or RNA molecules.

In general this method is required to analyze or purify single stranded DNA or RNA fragments, such as synthesized or labeled oligonucleotides or products from enzymatic cleavage reactions.

In this video article we show how to prepare and run the denaturing urea polyacrylamide gels. Technical tips are included, in addition to the original protocol [1,2].

Video Link

The video component of this article can be found at http://www.jove.com/video/1485/

Protocol

The complete and detailed text protocol for this experimental procedure is available in Current Protocols in Molecular Biology. Detailed step-by-step instructions for the assembly of the gel sandwich and for gel apparatus can be found on the BioRad website [3].

Required equipment:

Glass plates (inner and outer)

10 cm cell: 10.1 x 7.3 cm (inner plate), 10.1 x 8.2 cm (outer plate), BioRad

20 cm cell: 20 x 20 cm (inner plate), 20 x 22.3 cm (outer plate), BioRad

0.5-1.5 mm gel comb and spacers

Gel casting stand

Gel apparatus with lid and cables

High voltage power supply

Heating block or water bath

Serological pipettes and Pipette aid

Pipette and Pipette tips

Gel dryer or scanner

Reagents and Solutions:

Urea (ultrapure)

40% polyacrylamide solution (29:1)

10 x TBE solution (Tris-Borate, EDTA buffer)

Deionized, distilled water

TEMED

30% (w/v) ammonium persulfate solution

0.5 x TBE solution

Formamide

EDTA

Xylene cyanol

Bromphenol blue

Methanol

Ethanol

Volume	**50 ml**			**60 ml**			**10 ml**		
Acrylamide concentration	**10%**	**12.5%**	**15%**	**10%**	**12.5%**	**15%**	**10%**	**12.5%**	**15%**
g UREA	24	24	24	28.8	28.8	28.8	4.8	4.8	4.8
ml 40% Acryl (29:1)	12.5	15.625	18.75	15	18.75	22.5	2.5	3.125	3.75
ul 30% APS	166	166	166	199.2	199.2	199.2	33	33	33
ul TEMED	20	20	20	24	24	24	4	4	4
10 x TBE	5	5	5	6	6	6	1	1	1
Fill up the volume with deionized, distilled water									

Table 1: Reagents and solutions needed for various acrylamide concentrations and volumes for resolving single stranded oligonucleotides

Gel sandwich assembly and gel preparation

1. Assemble the gel according to manufacturers description and fix the gel in the gel-casting chamber [3]. Use 0.5-1.5 mm thick spacers.
2. Prepare the appropriate polyacrylamide solution according to current protocols in molecular biology or as listed in Table 1. For a denaturing acrylamide gel of 20 cm x 22 cm x 1.5 mm, 60 ml of gel solution and for a 10.1 x 8.2 cm x 1 mm gel 5 ml gel solution is sufficient. Larger gels are used when the expected products/bands are within the range of a few bases, then a longer gel will resolve bands with a difference of a single nucleotide. If the expected bands differ in more than 10 nucleotides, smaller gels will be suitable to resolve the fragments. Choose the percentage of polyacrylamide that suits your separation requirements, a higher polyacrylamide concentrations will resolve lower molecular weight fragments. Use ultrapure urea and mix with the desired amount of acrylamide. Add TBE buffer to the gel mix to get a final concentration of 0.5-1 x TBE and fill up the volume with deionized, distilled water. Heat the solution for 20 seconds in the microwave and mix it gently. For larger gel volumes repeat this step until the solution is hand warm.
3. Pour the gel immediately using a serological pipette and an automatic pipette aid between the two glass plates. Avoid introducing air bubbles. Insert the comb and let the gel polymerize for 30-60 minutes.

Set up the electrophoresis apparatus and prerun the gel

1. Dismount the gel from the casting chamber and assembly it to the gel apparatus according to manufactures instructions [3].
2. Fill the lower buffer chamber wit running buffer (0.5-1 x TBE) that the glass plates will be submerged 2-3 cm with buffer. Fill the upper buffer chamber up to the top of the gel with running buffer.
3. Carefully remove the comb and rinse the wells with running buffer by using a pipette and gel loading tips.
4. Attach the lid of the gel system and plug in the cables to a high voltage power supply. Before you can load your samples you have to prerun the gel for at least 30 minutes to heat the gel up and to remove remaining urea from the gel. The optimal temperature should be between 45-55 °C. Avoid temperatures higher than 60 °C as bands could smear or the glass plates could crack. Choose constant watts for the prerun (15-25 W per gel).

Sample preparation

1. In the meantime prepare your samples. Therefore, add the appropriate amount of 2 x gel loading mix to your sample. The loading mix usually contains 90% formamide, 0.5% EDTA, 0.1% xylene cyanol and 0.1% bromphenol blue.
2. Before the samples can be loaded on the gel, samples must be heat denatured by heating the samples between 70-90 °C for a few minutes.

Load and run the gel

1. When the prerun is finished, remove the lid and rinse the pockets thoroughly as described before as urea has leached into the wells.
2. Apply the samples carefully from the bottom of the pocket. Avoid introducing air bubbles. Loading buffer has to be applied to empty pockets to maintain equal conditions during the run.
3. Assemble the lid and run the gel at constant watts to maintain a gel temperature of 55 °C similar to the prerun. Observe the migration of the marker dyes until the dye front reached the lower end of the gel. The run duration is dependent on the percentage of used acrylamide, ionic strength of the buffer and gel thickness. A run can last between 2-4 hours.

   Check on the temperature of the gel. A gel thermometer can help to monitor the right temperature during the run.

Process the gel

1. When the dye front has reached the end of the gel put the gel out of the lower buffer chamber. Remove the gel from the chamber and loosen the clamps. Pull away the spacers and carefully dissemble the glass plates. If necessary cut away the upper well containing gel part.
2. Carefully transfer the gel into a dish with gel fixation solution (same concentration of TBE plus 5-10% Methanol and Ethanol). Leave the gel into the solution for 5-10 minutes and change the buffer twice.
3. The gel is ready for further processing using a vacuum gel dryer or direct scanning of the gel.

Discussion

1. The band sharpness depends on several factors. The volume of sample loaded for sharp bands should be as small as possible, i.e. ideally between 1-5 µl. However, up to 15 µl can be loaded which still ensures acceptable resolution. Less sample volume results in sharper bands.
2. The band quality is also dependent on the gel thickness. Thinner gels, such as 0.75 mm show better results than 1.5 mm thick gels.
3. The shape of bands can also be influenced during gel loading, if the sample is not applied equally into the gel pocket. Including 10% glycerol in the loading buffer helps during the gel loading and the sample sinks into the well more easily.
4. Another potential problem that is connected with the sample loading buffer can occur, if the expected product(s) runs at the same level as one of the loading dyes. Additionally, if fluorescent labels are used, some dyes show a fluorescent signal at a similar wavelength. If this is the case removing one dye or all dyes can solve this problem. Then it is advised to run the dye-containing sample in an empty well as a size standard.
5. Low voltage at the beginning of the run also helps to get sharper bands, as the sample enters the gel front smoothly and it avoids that gel pockets collapse due to high voltage. A short low percentage acrylamide stacking gel (4%) can have the same band-sharpening effect.
6. An often-encountered problem is gel “smiling”, which is due to uneven heat distribution through the gel during electrophoresis. If the gel is not surrounded by buffer, depending which gel system is employed, attaching of a metal plate to the glass plate supports an equal heat distribution and can substantially increase the gel quality.
7. Silanizing of glass plates for larger gels is advised, since it greatly improves the handling of gels after the run, as gels tend to stick to the glass plates.

Denaturing urea polyacrylamide gel electrophoresis (Urea-PAGE) is useful to analyze or separate single-stranded DNA or RNA fragments as well as radionucleotide- or fluorescent-labeled samples.

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