Protocol

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Agarose Gel Preparation Protocol

Introduction

Agarose gels are a standard component of gel electrophoresis, an effective technique used in the separation and analysis of deoxyribonucleic acid (DNA) fragments. Agarose is a polysaccharide composed of agar (agarobiose), a natural product of seaweed. During gelation, agarose forms a porous matrix that allows diffusion of nucleic acids. In gel electrophoresis, when DNA is loaded into the agarose gel and current is applied, the negatively charged phosphate backbone of DNA migrates through the agarose and towards the positive electrode. This process allows the separation of DNA fragments according to size. Here, we describe a procedure that will help identify and prepare the correct buffer and agarose combination for your experiment, allowing for the most efficient use of your agarose.

Materials

- GoldBio Agarose LE Powder (GoldBio Catalog # A-201) or
  GoldBio High Resolution Agarose (GoldBio Catalog # A-202) or
  GoldBio Low Melt Agarose (GoldBio Catalog # A-204)
- Microwave safe beaker or flask
- dH_2O
- Microwave oven
- 1x or 0.5x TAE/TBE Buffer
- A balance
- Plastic wrap
- Thick gloves or potholders
- Gel casting device

Method

First, determine the best agarose for your application. Agarose LE works best for analysis of nucleic acids from 50 bp to 25 kbp and is recommended for cloning experiments. High resolution agarose is ideal for analysis of nucleotides below 1 kb. Low melting (LM) agarose facilitates separation and purification of large intact DNA fragments; it is recommended for applications requiring enzymatic processing or cloning.

Preparation of buffer

1. Tris-Acetate-EDTA (TAE) or Tris-Borate-EDTA (TBE) buffer may be used in the preparation and running of the gel, depending on the application. TAE buffer allows for faster electrophoretic migration of linear DNA and better resolution of genomic and

supercoiled DNA. Alternatively, TBE buffers have a stronger buffering capacity, ideal for longer or higher voltage electrophoresis. They are preferred when separating < 2 kb fragments. However, borate is an enzyme inhibitor; thus, it is not recommended for applications requiring enzymatic activity. Buffers can be first prepared in a concentrated solution (50x or 10x) and diluted (1x or 0.5x) when ready to add agarose and cast the gel. Prepare the concentrated buffers as follows:

a. Instructions for preparing a 50x TAE buffer
b. Instructions for preparing a 10x TBE buffer

2. Dilute the buffer to a 1x or 0.5x concentration. Refer to Table 1 in the Calculations section to determine the best buffer concentration for you DNA fragment analysis.

3. Place the diluted buffer in a beaker or flask that can hold 2-4 times the volume of agarose to be prepared.

Preparation of Agarose gel

Agarose gels are usually prepared using a weight/volume solution in the 0.5-2% range, which should be optimized for the size of DNA fragments being analyzed. Optimal percentage of agarose gels will result in best separation and resolution of bands (DNA fragments).

1. Weigh out the agarose and add it to the flask/beaker containing the buffer. For example, for a 1% agarose gel, add 1 g agarose to 100 ml buffer. Allow the agarose to sit in solution for a few minutes before swirling the flask/beaker and suspending it in the solution. Higher percentage gels (> 1.5%) should hydrate for longer than lower percentage gels. Use a stir bar and stirring plate to rapidly mix the solution.

Note: Remember to remove the stir bar *before* microwaving.

2. Cover the mouth of the flask/beaker with plastic wrap and make a small hole in the top to allow the solution to vent.

3. Heat the flask/beaker in the microwave. After 30 seconds, remove and swirl to mix well. Repeat every 30 seconds until all the agarose has dissolved.

Note: If solid agarose or gel pieces remain, return the flask to the microwave and continue heating in 30-second intervals until all product is in solution. This may take a few minutes, depending on the gel concentration you are making and the power of the microwave.

4. Remove the flask/beaker from the microwave and very gently swirl.

WARNING: The microwaved solution can become superheated and foam over quickly when agitated. Wear appropriate protection and use caution.

5. Ethidium bromide (EtBr) is used to visualize DNA. Add 0.5 µg/ml EtBr to the melted agarose and swirl to mix well.

Note: EtBr is a potential carcinogen and should be handled appropriately.

6. Cool the solution to 55-60°C.

7. Cast the gel following the instructions provided for your casting apparatus.

Calculations

Table 1. Optimal buffer concentrations for best nucleic acid fragment separation.

TAE/TBE	DNA Size Resolution (bp)
0.5	1,000 – 25,000
0.75	800 – 12,000
1.0	**500 – 10,000**
1.2	400 – 7,500
1.5	200 – 3,000
2.0	50 – 1,500

Associated Products

- GoldBio Agarose LE Powder (GoldBio Catalog # A-201)
- GoldBio High Resolution Agarose (GoldBio Catalog # A-202)
- GoldBio Low Melt Agarose (GoldBio Catalog # A-204)
- Tris (GoldBio Catalog # T-400)
- Boric Acid, ACS Grade (GoldBio Catalog # B-030)
- EDTA Disodium (GoldBio Catalog # E-210)

References

Hickson, T. and Polson, A. (1968). Some physical characteristics of the agarose molecule. *Biochimica Et Biophysica Acta (BBA) - General Subjects,* 165(1), 43-58. Doi:10.1016/0304-4165(68)90186-4.

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Smith, S., Aldridge, P., and Callis, J. (1989). Observation of individual DNA molecules undergoing gel electrophoresis. *Science,* 243(4888), 203-206. Doi:10.1126/science.2911733.

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