DNA Gels

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Abstract

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Guidelines

FIGURE OUT WHAT YOU NEED:

A. Type of gel: regular agarose or low melting point (LMP) agarose

B. Concentration of gel
1. Mini gels (50 mL gels):
   0.7% = 0.35 gm per 50 mL
   0.8% = 0.40 gm per 50 mL
   1.2% = 0.60 gm per 50 mL
2. Small gels (100 mL gels):
   0.7% = 0.70 gm per 100 mL
   0.8% = 0.80 gm per 100 mL
   1.2% = 1.20 gm per 100 mL
3. Large gels (250 mL gels):
   0.7% = 1.75 gm per 250 mL
   0.8% = 2.00 gm per 250 mL
   1.2% = 3.00 gm per 250 mL

The concentration of the gel will depend on the size fragments you are working with.
Low concentration gels are used for large DNA fragment and high concentration gels are used for small DNA fragments.

C. Type of buffer
1. TPE (Tris/Phosphate/EDTA), 10X stock
2. TBE (Tris/Borate/EDTA), 10X stock
3. TAE (Tris/Acetate/EDTA), 10X stock

TPE buffer is a general use buffer. TBE and TAE buffers are generally used when electroeluting fragments from gels.

D. Size of gel
1. Mini - 50 ml gel + 250 ml running buffer
2. Small - 100 ml gel + 900 ml running buffer
3. Large - 250 ml gel + 2000 ml running buffer (slope end gel boxes) or 2500 ml running buffer

(square end gel boxes)

E. Gel combs
1. Mini - 8 lane combs (to 25 µl each well)
2. Small - 14 lane or 20 lane combs (to 25 µl each well)
3. Large - 20 lane or 30 lane combs (to 20 µl each well)

GEL BUFFERS AND ELECTROPHORESIS MATERIALS:

1) TPE buffer, 2 liters, 10X
216.0 gm of Tris base
30.2 mL of 85% Phosphoric acid
80.0 mL of 500 mM EDTA, pH 8.0

2) TBE buffer, 2 liters, 10X
216.0 gm of Tris base
110.0 gm of Boric acid
100.0 mL of 500 mM EDTA, pH 8.0

Filter the 10X buffer through Whatman #1 filter paper before placing the buffer in the bottle.

3) TAE buffer, 2 liters, 10X
193.76 gm of Tris base
108.86 gm of Sodium acetate
26.9 gm of disodium EDTA

Adjust the pH to 8.0 with glacial acetic acid.

Protocol

Gel Preparation and Electrophoresis

Step 1.
Place the agarose in an appropriately sized flask (usually a flask that holds at least twice the volume of the gel you are preparing) with a stir bar, buffer, and d-H_2O.

Gel Preparation and Electrophoresis

Step 2.
Melt the agarose in a microwave oven, autoclave, or on a hot plate/stirrer.

Gel Preparation and Electrophoresis

Step 3.
When the agarose has been melted, place the flask on a stir plate and gently stir the contents until the agarose cools to 50-60°C.

NOTES
Irina Agarkova 25 Mar 2016
The flask should be warm, but not hot, when holding the flask.

Gel Preparation and Electrophoresis

Step 4.

Pour the gel at room temperature for regular agarose or in the cold room for low melting point agarose.

Gel Preparation and Electrophoresis

Step 5.
Tape the ends of the gel tray.

Gel Preparation and Electrophoresis

Step 6.
Place the comb in the tray.

Gel Preparation and Electrophoresis

Step 7.
Level the gel tray so that the gel will solidify evenly.

Gel Preparation and Electrophoresis

Step 8.
Let the gel solidify (30 min).

DURATION
00:30:00

Gel Preparation and Electrophoresis

Step 9.
Remove the tape.

Gel Preparation and Electrophoresis

Step 10.
Pour the running buffer into the gel apparatus.

Gel Preparation and Electrophoresis

Step 11.
Place the gel in the apparatus.

Gel Preparation and Electrophoresis

Step 12.
Remove the comb from the gel.

NOTES
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The comb is easier removed from the gel when the gel is submerged in buffer; it is possible to tear the wells trying to remove the comb before the gel is submerged in buffer.

Gel Preparation and Electrophoresis

Step 13.
Load the gel.

Gel Preparation and Electrophoresis

Step 14.
Connect the leads from the gel apparatus to the power supply.

Gel Preparation and Electrophoresis

Step 15.
Electrophorese the samples toward the positive (red) electrode.

NOTES
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Mini gels are generally run for 100-150 volt-hours, small gels are generally run for 300-350 volt-

hours, and the large gels are generally run for 900-1000 volt-hours. These are general conditions and may be changed for particular experiments.

Gel Preparation and Electrophoresis

Step 16.

Stain the gels with 0.5 µg/mL of ethidium bromide for 30 min.

DURATION

00:30:00

Gel Preparation and Electrophoresis

Step 17.

Stain the regular gels at room temperature and the low melting point gels at 4°C.

Gel Preparation and Electrophoresis

Step 18.

Destain the gels with d-H_2O and view.