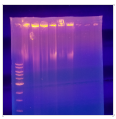

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Agarose Gel Electrophoresis

Forked from Agarose Gel Electrophoresis

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ABSTRACT

Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode. Shorter DNA fragments migrate through the gel more quickly than longer ones. Thus, you can determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).

MATERIALS

NAME	CATALOG #	VENDOR
Gel Loading Dye Purple (6X) - 4.0 ml	B7024S	New England Biolabs
100 bp DNA Ladder - 500 gel lanes	N3231L	New England Biolabs
1 kb DNA Ladder - 1,000 gel lanes	N3232L	New England Biolabs
Agarose		Sigma
Gel and PCR Clean-up kit	740609.250	Macherey and Nagel
SYBR SAFE DNA stain	S33102	Life Technologies

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Agarose	75510019	Thermo Fisher
10ml Ethidium Bromide Solution [10mg/ml]	R034	G-Biosciences
Gel Loading Dye, Purple (6X), no SDS - 4.0 ml	B7025S	New England Biolabs
100 bp DNA Ladder - 500 gel lanes	N3231L	New England Biolabs
1 kb DNA Ladder - 1,000 gel lanes	N3232L	New England Biolabs
Gel and PCR Clean-up kit	740609.250	Macherey and Nagel

SAFETY WARNINGS

This protocol uses Ethidium Bromide (EtBr), which is a known mutagen. Wear a lab coat, eye protection and gloves when working with this chemical.

Pouring a Standard 1% Agarose Gel

1 Measure 1 g of agarose

Agarose
by Thermo Fisher
Catalog #: 75510019

'Pro-Tip': Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of bands needed to be separated. Simply adjust the mass of agarose in a given volume to make gels of other agarose concentrations (e.g., 2 g of agarose in 100 mL of TAE will make a 2% gel).

2 Mix agarose powder with 100 mL 0.5x TAE in a microwavable flask.

'Pro-Tip': TBE can be used instead of TAE, labs usually use one or the other, but there is very little difference between the two.

Note: *Make sure to use the same buffer as the one in the gel box (do not mix different buffers and do not use water).*

3 Microwave for 1-3 min until the agarose is completely dissolved (but do not overboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up.).

CAUTION: *HOT! Be careful stirring, eruptive boiling can occur.*

'Pro-Tip': It is a good idea to microwave for 30-45 sec, stop and swirl, and then continue towards a boil. Keep an eye on it the solution has a tendancy to boil over. Placing saran wrap over the top of the flask can help with this, but is not necessary if you pay close attention.

4 Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins.

5 Add **0.8 µl** to a final concentration to agarose solution and swirl the solution.

10ml Ethidium Bromide Solution [10mg/ml]
by G-Biosciences
Catalog #: R034

6 Pour the agarose into the gel tray with the well comb in place.

'Pro-Tip': Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.

7 Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

'Pro-Tip': If you are in a hurry, the gel will set more quickly if you place the gel tray at 4 °C earlier so that it is already cold when the gel is poured into it.

Loading Samples and Running an Agarose Gel

8 Add loading buffer to each of your DNA samples.

Gel Loading Dye, Purple (6X), no SDS - 4.0 ml
by New England Biolabs
Catalog #: B7025S

Note: *Loading buffer serves two purposes: 1) it provides a visible dye that helps with gel loading and allows you to gauge how far the DNA has migrated; 2) it contains a high percentage of glycerol that increases the density of your DNA sample causing it settle to the bottom of the gel well, instead of diffusing in the buffer.*

9 Once solidified, place the agarose gel into the gel box (electrophoresis unit).

10 Fill gel box with 0.5x TAE (or TBE) until the gel is covered.

11 Carefully load a molecular weight ladder (according to the expected size of the sample) into the first lane of the gel.

100 bp DNA Ladder - 500 gel lanes
by New England Biolabs
Catalog #: N3231L

1 kb DNA Ladder - 1,000 gel lanes
by New England Biolabs
Catalog #: N3232L

Note: *When loading the sample in the well, maintain positive pressure on the sample to prevent bubbles or buffer from entering the tip. Place the very top of the tip of the pipette into the buffer just above the well. Very slowly and steadily, push the sample out and watch as the sample fills the well. After all of the sample is unloaded, push the pipettor to the second stop and carefully raise the pipette straight out of the buffer.*

12 Carefully load your samples into subsequent wells of the gel.

13 Run the gel at 80-150 V until the dye line has traveled approximately 75-80% down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration and used voltage.

14 Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

15 Using any device that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as 'bands' due to their appearance on the gel.

'Pro-Tip': If you will be purifying the DNA for later use, use long-wavelength UV and expose for as short time as possible to minimize damage to the DNA.

Note: *When using UV light, protect your skin by wearing safety goggles or a face shield, gloves and a lab coat.*

Analyzing Your Gel

16 Using the DNA ladder in the first lane as a guide (the manufacturer's instruction will tell you the size of each band), you can determine the size of the DNA in your sample lanes.

Purifying DNA from Your Gel

17 If you are conducting certain procedures, such as molecular cloning, you will need to purify the DNA from the agarose gel.

18 Cut the desired band out of the gel, with a sterilized razor blade.

Note: *be sure to take as little as possible of residual gel (with additional bands in) when you cut out the band. Most commercial sold gel purification kits can handle only a specific amount of gel and DNA.*

19 Place the cut out band in a microcentrifuge tube and weight additional weight of the cut out gel band for further steps.

20 Finally, isolate the DNA from cut out gel band with a commercial gel purification kit or your preferred method.

Gel and PCR Clean-up kit
by Macherey and Nagel
Catalog #: 740609.250