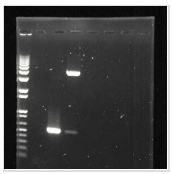

Jul 11, 2022

Agarose gel electrophoresis

Brian P Teague[1]

[1]University of Wisconsin - Stout

This protocol is published without a DOI.

Yeast ORFans CURE

Brian Teague
University of Wisconsin - Stout

ABSTRACT

We use agarose gel electrophoresis to analyze DNA samples -- is there DNA present? What size is it?

PROTOCOL CITATION

Brian P Teague 2022. Agarose gel electrophoresis. **protocols.io** https://protocols.io/view/agarose-gel-electrophoresis-ccs9swh6

KEYWORDS

dna, agarose, gel, electrophoresis

IMAGE ATTRIBUTION

Wikimedia user TransControl, https://commons.wikimedia.org/wiki/File:Agarosegelphoto.jpg

CREATED

Jul 06, 2022

LAST MODIFIED

Jul 11, 2022

PROTOCOL INTEGER ID

66113

GUIDELINES

This protocol has been optimized so it can be run in a 2-hour laboratory section.

MATERIALS TEXT

1X LAB buffer

Make LAB buffer using this protocol:

LAB Agarose Gel Electrophoresis Buffer Recipe
by Brian Teague

Agarose **Fisher Scientific Catalog #BP1356** Step 2 (any biotechnology-grade agarose is fine)

SYBR Safe **Invitrogen - Thermo Fisher Catalog #S33102** Step 2 (or another DNA stain)

Quick-Load Purple 1 kb Plus DNA Ladder - 250 gel lanes **New England Biolabs Catalog #N0550S** In 2 steps (or another DNA molecular weight standard, pre-mixed with loading dye)

Gel Loading Dye Purple (6X) - 4.0 ml **New England Biolabs Catalog #B7024S** Step 13

Parafilm™ M Laboratory Wrapping Film, 4 in. W x 125 ft. L; (10cm x 38m) **Thermo Fisher Catalog #1337410** Step 12

SAFETY WARNINGS

Lithium acetate: May cause eye and skin irritation. May cause respiratory and digestive tract irritation. The toxicological properties of this material have not been fully investigated.

Boric acid: May damage fertility. May damage the unborn child.

SYBR Safe DNA stain: Anything that binds DNA is a potential mutagen / carcinogen.

Wear appropriate personal protective equipment (PPE), including a lab coat, nitrile gloves and safety glasses.

BEFORE STARTING

Before starting, make sure you have the DNA samples you'll be analyzing (either PCR or restriction digest) thawed on ice.

Cast the gel 30m

1 Using a balance, weigh out **0.5 g agarose** in a weigh boat.

2 In a 250 Erlenmeyer flask, mix **50 mL LAB buffer** , **0.5 g** Agarose **Fisher Scientific Catalog #BP1356** , and **2 µL**

SYBR Safe **Invitrogen - Thermo Fisher Catalog #S33102** DNA stain. Swirl to mix.

Use a graduated cylinder to measure out the LAB buffer.

3 Microwave on HIGH for **00:00:30** ; the solution should begin to boil. Remove from the microwave and swirl. 30s

Be careful, it will be very hot! Use a folded-over paper towel and grab the flask at the top to avoid burning yourself.

4 Look carefully at the contents of the flask while you swirl it. If there are any flecks of undissolved agarose remaining, microwave again for **00:00:15** . Repeat until the solution is completely clear. 15s

It should look as clear as water when you swirl it.

5 Set the agarose solution on the bench to cool slightly. While it is cooling, insert the black wedges and the gel comb into the gel box.

Make sure the combs are on the side of the gel box near the black electrode.

6 When the agarose solution is cool enough that you can hold your hand against it for 10 seconds, carefully pour the solution into the gel tray.

It is particularly important that the agarose solution be cool enough, because if it isn't the gel box can be damaged.

If you would like to cool it faster, you can swirl the flask under running cold tap water. Don't let it solidify in the flask!

7 Wait **00:15:00** for your gel to solidify. 15m

The gel should be solid and uniformly translucent.

8 Carefully remove the gel combs and the black wedges.

PAUSE POINT -- You can put your gel in a sandwich baggie with a splash of buffer and store it in the refrigerator for up to 48 hours. Longer than that and the DNA stain will degrade.

Run the gel 30m

9 If you removed your gel from the gel box (or cast it outside of the gel box), put your gel in the box with the wells toward the black electrode.

10 Pour just enough 1X LAB buffer into the box to entirely submerge the gel.

11 Below, select whether you're analyzing a PCR or a restriction digest:
Step 11 includes a Step case.
PCR
Digest

step case

PCR

Follow these steps to analyze a PCR on the gel.

12 Cut a 1 cm x 4 cm (half a square) of
Parafilm™ M Laboratory Wrapping Film, 4 in. W x 125 ft. L; (10cm x 38m) **Thermo Fisher Catalog #1337410**
off of the roll.

13 For each sample (not including the molecular weight standard), carefully deposit **1 µL** of
Gel Loading Dye Purple (6X) - 4.0 ml **New England Biolabs Catalog #B7024S** onto the square of parafilm, then pipette **2 µL** of the DNA sample onto the spot. Pipette up and down to mix.

Keep careful track of which spot is which sample!

14 Carefully pipette **5 µL** of
Quick-Load Purple 1 kb Plus DNA Ladder - 250 gel lanes **New England Biolabs Catalog #N0550S** into the

left-most well. Then, pipette your samples mixed with loading dye into successive wells.

15 Assemble the top of the gel box onto the bottom. Plug the box into a power supply and run the gel at 10for 20m
00:20:00 .

After you start it, look closely at the electrodes in the gel box to make sure there are bubbles. If there aren't, the gel box may be assembled incorrectly or damaged.

The LAB buffer allows us to run the gel at a higher voltage, and for a shorter time, than we would with a traditional buffer like TAE or TBE. However, this decreases the resolution somewhat. For a "prettier" gel, you can run for 30 minutes at 100 volts.

16 After you have run your gel, image it with a transilluminator.