Jul 08, 2015 Version 1

# Gel Electrophoresis V.1

DOI
dx.doi.org/10.17504/protocols.io.dbd2i5

Alan J. Cone[1]

[1]Wright State University

Ju Lab

Alan Cone
Wright State University





**DOI: https://dx.doi.org/10.17504/protocols.io.dbd2i5**

**Protocol Citation:** Alan J. Cone 2015. Gel Electrophoresis. **protocols.io https://dx.doi.org/10.17504/protocols.io.dbd2i5**



**Protocol status:** Working

**Created:** July 08, 2015

**Last Modified:** March 08, 2018

**Protocol Integer ID:** 1093

**Keywords:** gel electrophoresi, checking dna, dna, restriction digest, molecule

## Abstract

Separates molecules based on size. Great for checking DNA after a Restriction Digest.

## Materials

MATERIALS

- Ethidium **P212121**
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #**B7025S
- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Agarose **Catalog #**A5304

STEP MATERIALS

- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Agarose **Catalog #**A5304
- Ethidium **P212121**
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #**B7025S
- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Agarose **Catalog #**A5304
- Ethidium **P212121**
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #**B7025S

## Protocol materials

- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #**B7025S
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #**B7025S
- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Agarose **Catalog #**A5304
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- Ethidium **P212121**
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Agarose **Catalog #**A5304
- Agarose **Catalog #**A5304
- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Ethidium **P212121**
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #**B7025S
- Ethidium **P212121**
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Agarose **Catalog #**A5304
- Ethidium **P212121**
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- 1 kb DNA Ladder - 1,000 gel lanes **New England Biolabs Catalog #**N3232L
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml **New England Biolabs Catalog #**B7025S

## Troubleshooting

## Safety warnings

Ethidium Bromide potentially acts as a mutagen or carcinogen.

## Before start

Have a DNA Sample ready, typically either from PCR or a recently performed Restriction Digest. Dilute down the 50X TAE Buffer to 1X.

## Prep Work

1 Pour 50 mL of 1X TAE Buffer into an Erlenmeyer Flask.

50 mL

TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49

2 Weigh out 0.5 g Agarose and add it to the Erlenmeyer Flask.

1 g

Agarose **Catalog #**A5304

3 Place Erlenmeyer Flask in a microwave on high power for two minutes or until solution is clear and agarose is completely dissolved.

00:02:00

Note

Every time it starts to boil open the microwave and swirl the flask around then place the flask back in the microwave and continue heating.

4 Remove Erlenmeyer Flask from microwave and let it sit on the lab bench to cool just until you can comfortably pick it up.

00:05:00

5 Add 5 uL Ethidium into the flask and swirl to mix, taking care not to introduce bubbles.

5 µL

Note

Ethidium intercalates with DNA and floureses orange under UV light.

Ethidium **P212121**

6 Place gel tray on clamp and clamp securely. Add well plates where you want wells and use a level to ensure it is balanced.

7 Pour contents of the Erlemeyer Flask into the gel tray and let it sit for 30 minutes, or until a blue tint appears.

00:30:00

## Loading the Gel

8 Remove the well plates carefully as to not tear the gel and remove the tray from the clamp, but ensure the gel remains in the tray.

9 Place gel tray into gel electrophoresis apparatus with the wells closer to the negative/black end.

Note

As DNA is negatively charged it will be attracted to the positive end and repelled from the negative end.

10 Pour additional TAE Buffer to fill each side of the apparatus and to create a thin layer of buffer covering the top of the gel.

10 µL

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

11 Pipette 10 uL of the 1kb DNA Ladder with Loading Dye into a well. Typically this is placed into one of the wells near an edge.

10 µL

Note

Be careful when loading not to puncture the sides or bottom of the wells as the sample may then leak out.

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

12 Pipette your DNA with Loading Dye mixture into another well. Repeat for each sample.

5 µL

Note

Make sure you pipette to mix your sample before loading it into the well. Loading Dye contains glycerol which will sink to the bottom of your sample and not appropriately stain your DNA or ensure it stays in the well.

Note

For a 25 uL PCR reaction you can add 5 uL of the Loading Dye to yield a final volume of 30 uL with 1/6 of the mixture being Loading Dye.

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

## Running the Gel

13 Place lid on apparatus and plug cables into amplifier. Set amplifier to stay at a constant voltage of 100 V.

14 Let run for 30 minutes or until the loading dye has sufficiently moved.
00:30:00

15 Remove gel from gel tray after draining excess TAE Buffer and place on plastic wrap.

## Reading the Gel

16 Place gel with plastic wrap on UV lamp to view bands, or store in the plastic wrap at +4 C for later use.