Science Education Collection

DNA Gel Electrophoresis

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Abstract

DNA gel electrophoresis is a technique used for the detection and separation of DNA molecules. An electric field is applied to a gel matrix comprised of agarose, and within the gel, charge particles will migrate and separate based on size. The negatively charged phosphates of the DNA backbone cause DNA fragments to move toward the anode - a positively charged electrode.

The video explains the mechanism by which DNA fragments are resolved on an agarose gel, and it provides a step-by-step generalized procedure for how to prepare agarose gels, load DNA samples, run a DNA gel, visualize DNA fragments, and properly dispose of the gel and running buffer after the experiment is concluded.

Transcript

DNA Gel Electrophoresis is a technique used to separate and identify DNA fragments based on size.

DNA fragments of various sizes are loaded into a porous gel made from agarose – a carbohydrate found in red algae.

When an electric field is applied, the fragments will migrate through the gel, thanks to the negatively charged phosphate groups in DNA nucleotides.

Smaller pieces of DNA will more easily migrate through the gel than larger fragments, which have a more difficult time moving through the gel matrix.

When the gel run is complete the location of your DNA samples can be compared to a series of fragments or bands of known sizes, called a DNA ladder.

The presence of your fragment of interest can then be confirmed based on its size, which is determined by comparing the relative location of your test sample to the fragments of the ladder.

Agarose gels are prepared using a weight over volume percentage solution. So 1 gram of agarose in 100 ml of buffer will make a 1% gel. Lower percent gels will better resolve larger fragments and higher percent gels will make smaller fragments easier to identify. To start the gel-making procedure, weigh out the appropriate mass of agarose into an Erlenmeyer flask.

Add running buffer to the flask, so that the volume of buffer is no greater than 1/3 of the capacity of the flask. Then swirl to mix.

Melt the agarose/buffer mixture by heating in a microwave at maximum power. Every thirty seconds, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.

Next add ethidium bromide to a concentration of 0.5 mg/ml. Ethidium Bromide is an aromatic compound that fits in between individual base pairs of DNA, or intercalates, and causes DNA to emit intense orange fluorescence under UV light. It is important to note that ethidium bromide is a carcinogen so gloves should always be worn when handling gels containing this compound.

To prevent the gel tray from warping, let the agarose cool by placing it in a 65ºC water bath.

As the agarose is cooling, prepare the gel mold by placing the gel tray into the casting apparatus. As an alternative, you can use tape to seal the open edges of the gel tray to create the mold. Placing a comb into the gel creates the wells where DNA is loaded. Make sure the comb will create a well that is the appropriate size for your DNA sample.

Pour the molten agarose into the gel mold and allow it to harden at room temperature.

After the agarose has hardened, take out the comb. If the gel is not going to be used immediately, wrap it in plastic wrap and store at 4ºC until use.

If the gel is going to be used immediately, place it in the gel box.

To begin this procedure, add gel-loading dye to the DNA samples to be separated. Loading dye is typically made at a 6X concentration. Loading dye helps to visualize and load samples into the wells and helps determine how far the samples have migrated during the run.

Set the power supply to the desired voltage.

Now add enough running buffer into the gel box to cover the surface of the gel. Make sure to use the same running buffer as the one used to prepare the gel.

Connect the leads of the gel box to the power supply and turn it on. Remember that DNA is negatively charged and will move toward the anode, which is positive, and generally red in color. Make sure not to connect the black lead, or cathode, to the bottom of the gel box. So you don't forget, keep in mind that black cats are bad luck, or negative, and the black CAThode is therefore negative. Run your gel to red, or the anode. To verify that both the gel box and the power supply are working; the appearance of bubbles at the electrodes indicates that current is passing through.

Remove the lid of the gel box. Slowly and carefully load the DNA samples into the gel. Again, the loading dye in the sample allows the sample to sink into the gel and will help to track how far the sample has traveled. A DNA size marker, or ladder, should always be loaded along with the experimental samples.

Replace the lid. Double check that the electrodes are plugged into the correct slots in the power supply.

Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

When the electrophoresis run is complete, turn off the power supply and remove the lid of the gel box.

Remove the gel from the gel box and drain off excess buffer on the surface of the gel. Place the gel tray on paper towels to absorb any remaining running buffer.

To visualize the DNA fragments, remove the gel from the gel tray and expose the gel to ultra violet light.

DNA fragment should show up as orange fluorescent bands. Take a picture of the gel.

At the end of the experiment, properly dispose of the gel and running buffer per institution regulations. Again, remember to always handle the gel and running buffers with gloves to avoid ethidium bromide exposure.

Now that you've seen how to perform DNA gel electrophoresis. Let's have a look at some downstream applications and variations of this highly useful method.

Here you see an agarose gel electrophoresis result after separating PCR products. The DNA fragments loaded into the gel are visible as clearly defined bands. The DNA standard or ladder should be separated to a degree that allows for the useful determination of the sizes of sample bands. In this example, DNA fragments of 765 base pairs, 880 base pairs and 1022 base pairs are separated on a 1.5% agarose gel with a 2-log DNA ladder.

In addition to confirming the presence of a DNA fragment of interest, DNA gel electrophoresis can be combined with gel purification procedures. Typically a razor blade is used to cut out the DNA fragment of interest, so that it can be collected and the DNA sample within it recovered.

Agarose gel electrophesis can also be combined with transfer blotting, which involves allowing DNA, or RNA, to transfer to a cellulose membrane where radioactive probes can be used to identify specific DNA or RNA sequences in your electrophoretically-separated sample.

Standard DNA gel electrophoresis is not ideal for the separation of high molecular weight DNA greater than 15-20 kb in size, like genomic DNA. To separate large DNA samples, pulse field gel electrophoresis is used, which involves subjecting the gel to a changing, or pulsing, electric field in different directions. This technique involves a specialized gel running apparatus, which has pairs of electrodes arrayed in different orientations around the gel. This can be used to detect differences in genome sizes between populations of organisms, like the pooled DNA samples from different microbial communities, you see here which are taken from different lake environments.

You've just seen an introduction to DNA gel electrophoresis. We showed you the concept behind the method, how to prepare the agarose gel, how to load your samples, how to run the gel, and analyze it and some common applications of agarose gel electrophoresis. Thanks for watching and good luck with running your gel.