Agarose gel electrophoresis

Adapted from https://www.addgene.org/protocols/gel-electrophoresis/

Basic principle

Gel electrophoresis is a standard laboratory procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electric field to move negatively charged DNA through an agarose gel toward the 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 molecular ladder (a collection of DNA fragments of known lengths).

Required reagents

1. 1X TBE or TAE buffer
2. Agarose
3. Loading buffer (check protocol)
4. Molecular ladder

Required consumables and equipment

1. Electrophoresis unit including a gel tray
2. Microwave
3. Transilluminator
4. Power supply
5. Well combs
6. 96-well PCA tray
7. Parafilm tape
8. Pipets and tips

Procedure

Pouring a standard 1% agarose gel:

1. Measure 2 g of agarose.
9. Take the gel tray of the electrophoresis unit and put the well comb in place
2. Mix agarose powder with 200 mL 1x TBE in a microwavable flask.
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.
4. Let the agarose solution cool down to about 50°C (when you can comfortably keep your hand on the flask).
5. Pour the agarose into the gel tray with the well comb in place.
6. Place newly poured gel at 4°C for 10-15 mins or let it sit at room temperature for 20-30 mins, until it has completely gelified, especially the area where the comb is located.
7. Once the agarose is completely gelified, remove the comb carefully.

Loading samples and running an agarose gel:

1. Place the agarose gel into the electrophoresis unit.
2. Fill gel box with 1x TBE buffer (or TAE) until the gel is covered.
3. Connect the electrodes from the power supply to the electrophoresis unit.
4. Place a piece of parafilm tape over a 96-well PCR tray and adjust the tape by pressing it to form shallow wells.
5. Add 4 ml of the loading buffer, including the same number of samples. Add an extra drop of loading buffer on the tape (one lane containing the DNA molecular ladder will be included in the gel).
6. Carefully add and mix 10 ml molecular weight ladder (depending on brand) to the first loading buffer drop placed over the parafilm tape, mix with the buffer drop using the pipette, and put the mixture into the first lane of the gel.
7. Carefully add and mix the first sample to the next loading buffer drop over the parafilm tape. Place the first mixture into the next lane of the agarose gel. Do the same for each sample to be loaded. By the end, the DNA molecular ladder and all your samples should have been mixed with loading buffer and loaded into separate wells of the agarose gel.
8. Run the gel at 90 V until the blue dye line in the gel is approximately 75-80% of the way down the gel. A typical run time is about 120 minutes, depending on the gel concentration, DNA size, and voltage.
9. Turn off the power supply, disconnect the electrodes from the power supply, and then carefully remove the gel from the gel box.
10. Using the Transilluminator, visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.

Gel analysis:

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

Additional tips:

A few simple ways to increase the resolution (crispness) of your DNA bands include:

- Running the gel at a lower voltage for a more extended period
- Using a wider/thinner gel comb
- Loading less DNA into the well.

Another method for visualizing very short DNA fragments is polyacrylamide gel electrophoresis (PAGE), which is typically used to separate 5-500 bp fragments.

If you have similarly sized bands running too close together, you can adjust the agarose concentration of the gel to improve separation. A higher percentage of agarose gel will help resolve smaller bands, while a lower percentage of agarose will help separate larger bands.

This protocol is a 1% agarose gel (2 g of agarose in 200 mL of buffer). If you need to increase the agarose concentration, you only need to adjust the amount of agarose to be included in the mixture in the following way:

- 2% agarose gel: 4 g of agarose in 200 mL of buffer
- 4% agarose gel: 8 g of agarose in 200 mL of buffer

Precautions and recommendations:

1. Make sure to use the same buffer as the one in the gel box (do not mix different buffers and do not use water).
2. 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 tends to boil over.
3. Pour slowly to avoid bubbles, which will disrupt the gel. Bubbles can be pushed away from the well comb or toward the sides/edges of the gel using a pipette tip.
4. 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.
5. When loading the sample in the well, maintain positive pressure on the sample to prevent bubbles or buffer from entering the tip. Place the tip of the pipette into the buffer just above the well. Push the sample out slowly and steadily, and watch as it fills the well. After all the sample is unloaded, push the pipettor to the second stop and carefully raise the pipette straight out of the buffer.
6. Black is negative, red is positive. The DNA is negatively charged and will run towards the positive electrode. Always Run to Red. The wells inside the gel must be closer to the black electrode.