SOP

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Agarose Gel Electrophoresis

Agarose Gel Electrophoresis is a technique used to separate DNA fragments (or other macromolecules, such as RNA and proteins) based on their size and charge. Electrophoresis involves running a current through a gel containing the molecules of interest. Based on their size and charge, the molecules will travel through the gel in different directions or at different speeds, allowing them to be separated from one another.

Workflow:

Preparation of the Gel → Loading Samples and Running → Observing Separated DNA fragments

Equipment and materials:

Agarose

Electrophoresis buffer, usually Tris-acetate EDTA (TAE) or Tris-borate-EDTA (TBE).

Loading dye

SYBER Green

DNA ladder/marker

DNA samples

Sample combs, around which molten medium is poured to form sample wells in the geTAE/TBE buffer

Gel casting trays

, or gel docsGel documentations systems

An electrophoresis chamber and power supply.

pipettes, pipette tips

Safety:

See the Material Safety Data Sheet (MSDS) regarding safety precautions for this method.

Procedure:

1.Preparation of Agarose gel:

-Weigh out the agarose and add it to the flask/beaker containing running buffer. For example, for a 1% agarose gel, add 1 g agarose to 100 ml running buffer. Allow the agarose to sit in solution for a few minutes before swirling the flask/beaker and suspending it in the solution. Higher percentage gels (> 1.5%) should hydrate for longer than lower percentage gels. Use a stir bar and stirring plate to rapidly mix the solution. (The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).

Note: Remember to remove the stir bar before microwaving.

-Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well.

Note: If solid agarose or gel pieces remain, return the flask to the microwave and continue heating in 30-second intervals until all product is in solution. This may take a few minutes, depending on the gel concentration you are making and the power of the microwave.

-Add 1 µL of the SYBER Green per each 10 mL of gel solution and swirl to mix well. SYBER Green is used to visualize DNA.

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

-Pour the agarose into the gel tray, with combs in place. Remove any air bubbles.

-once gel is completely solidified, gently pull out the combs and put the gel into the electrophoresis tank, (it will solidify around the combs last).

2.Setting up of Gel Apparatus and Separation of DNA Fragments

-Add loading dye to the DNA samples to be separated. Gel loading dye is typically made at 6X

concentration (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol). Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel. -Load 5 µl DNA ladder and controls into their own wells.

3. Electrophoresis

-Program the power supply to desired voltage (1-5V/cm between electrodes).

-Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.

-Attach the leads of the gel box to the power supply.

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

4. Observing Separated DNA fragments

-When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.

-Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.

-Expose the gel to UV light. This is most commonly done using a gel documentation system. DNA bands should show up as orange fluorescent bands.

-Take a picture of the gel.

References:

1.https://www.addgene.org/protocols/gel-electrophoresis/

2.Agarose Gel Electrophoresis, Daniel Voytas: June 1992.

3. https://doi.org/10.1002/0471142735.im1004s02.

4. Agarose Gel Electrophoresis for the Separation of DNA Fragments. J Vis Exp. 2012; (62): 3923.

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5.Cell Viability and Proliferation, Sigma Aldrich.

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