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Protocol for preparing agarose gels for Agarose Gel Electrophoresis

Materials needed but not provided
Agarose, TAE (or TBE) buffer, 6x sample loading buffer, DNA ladder standard, electrophoresis chamber, power supply, gel casting tray and combs, staining tray, gloves, pipette and pipette tips

Pouring a standard agarose 1% agarose gel*

1. Weigh 1g of your agarose powder and add this 1g to 100mL 1X TAE or 1X TBE buffer into a glass bottle of at least 250mL.
2. Melt the agarose in a microwave for 1-3 minutes until the agarose is completely dissolved and the solution becomes clear (the solution is completely clear).
   NOTE: Caution HOT solution! Be careful!
   NOTE: Solution may boil out of the flask/bottle if the solution is boiled for too long periods!
3. **Optional for pre-stained gels (not recommended by Genaxxon bioscience)****
   Let agarose solution cool down to 55°C to 60°C, add 10µL SafeGel per 100mL gel.
   Gently stir the gel solution to mix SafeGel red stain.
4. Seal horizontal gel casting tray at both ends.
   Pour molten agarose (55°C to 60°C) onto the gel plate to a depth of 4-6mm.
   Insert comb (until its base is 1mm from the base of the gel).
   Allow cooling down to ambient temperature. This will take about 30 minutes at ambient temperature. Cooling can be speed up by placing the gel cast tray with the agarose in a fridge for 15 minutes. ***
   NOTE: Pour slowly to avoid bubbles, which will disrupt the gel.
   NOTE: Any bubbles can be pushed away from the well comb and the gel towards the sides/edges of the gel with a pipette tip.

* normally, agarose concentrations of 0.7% up to 2% were used, depending on the application and the size of the DNA to be separated.

** Genaxxon recommends „in-slot application" of a 20X SafeGel red stain-solution together with the DNA and, if needed the loading dye.

*** low melting agaroses need cooling down to +2°C to +8°C for at least 45 minutes to enable solidification.

Loading samples and running an agarose gel electrophoresis

1. Once solidified, remove the seals from the end of casting tray.
2. Place the casting tray into the electrophoresis apparatus.
3. Add 1/5 volume 6X Loading buffer to each sample and DNA ladder standard.
   NOTE: Loading buffer serves 2 purposes:
   a. It provides a visible dye that helps with gel loading and will also allow you to gauge how far the gel has already run.
   b. it contains a high percentage glycerol. So after adding it to your sample your sample will be heavier than water/buffer and will settle to the bottom of the slot and not diffusing into the buffer.
4. Fill electrophoresis apparatus with 1X TAE (or 1X TBE) buffer until the complete gel is covered by the buffer.
5. Carefully pipette 5µL of the DNA ladder standard into the first lane of the gel.
   NOTE: Place the very top of the pipette tip into the buffer just above the well. Very slowly and steadily, push the sample out and watch if the DNA ladder / your sample fills the slot.
6. Carefully pipette 5µL of each sample (already mixed with loading buffer) into other slots of the gel.
   NOTE: Take care that you mark which sample was loaded into which slot.
7. Run the gel at 80 – 150V until Bromophenol blue is near the end of the gel. Bromophenol blue runs at about 800bp.
   NOTE: The black cable is the negative pole. The red cable is the positive pole.
   NOTE: DNA is negatively charged and will run to towards the positive pole (electrode).
   NOTE: A typical run time is about 0.5 to 1 hour, depending on the gel size, the gel concentration, the buffer and the voltage applied.
8. Turn OFF power.
   Disconnect the electrodes/cables from the power source, and then carefully remove the gel from the casting tray.
9. Visualize or image the stained gel (or stained DNA bands) with a standard transilluminator (302 or 312nm), and photograph the gel using an appropriate filter (e.g., Ethidium bromide filter).

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Protocol for Staining agarose gels after agarose gel electrophoresis

1. **In-slot staining protocol (recommended by Genaxxon)**

   a. Prepare agarose gel as usual without adding any dye.

   b. Prepare diluted SafeGel red stain solution (final concentration of 20X).
   Add 3µL of the 10000X SafeGel red stain solution to 97µL H2O resulting in a 300X SafeGel red stain solution.
   Add 8µL of the 300X SafeGel red stain solution to 100µL 6X Loading buffer plus 12µL H2O resulting in a 20X SafeGel red stain solution.

   Add 1µL to 2µL of the 20X SafeGel red stain solution to 4µL sample and apply directly into the slot of the agarose gel!

   At Genaxxon this procedure show the best results in sharpness of bands and is the most economical way to use SafeGel red stain!

2. **Staining DNA by Post Gel Staining**

   a. Run gels according to your standard protocol.

   b. Dilute the SafeGel red stain 10000X stock solution about 3,300 fold to 10,000 fold to prepare a 3X or 1X staining solution in water with 0.1M NaCl (e.g., add 5µL up to 15µL of SafeGel red stain stock solution and 5mL NaCl to 45mL water). While SafeGel red stain 1X staining solution can be used for post gel staining, the sensitivity is generally less than with 3X staining solution (**NOTE:** inspect the 10000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial).
   NOTE: Use of NaCl in the staining solution is optional. NaCl in the solution enhances the staining, but may promote dye precipitation if the staining solution is to be used repeatedly. Any staining solution to be re-used is preferably stored at room temperature in a dark place to reduce possible dye precipitation problems.

   c. Carefully place the gel in a suitable container such as a polypropylene container. Gently add sufficient amount of the 3X staining solution to submerge the gel.

   d. Agitate gel gently at room temperature for about 30 minutes. Optimal staining time may vary somewhat depending on the thickness of the gel and the percentage of agarose. For polyacrylamide gels containing 3.5 – 10% acrylamide, typical staining time is 30 minutes up to 1 hour with gels of higher acrylamide content requiring longer staining time. The staining solution can be re-used at least 2-3 times. The unused staining solution can be stored at room temperature in a dark place.

   e. View the stained gel with a standard transilluminator (302nm o 312nm) and photograph the gel using an ethidium bromide filter. Similarly, a SYBR™ or SafeGel red stain filter may also be used for photographing with equally good results.

3. **Staining of DNA by Precasting SafeGel red stain Gels***

   a. Prepare agarose gel solution using your standard protocol.

   b. Dilute SafeGel red stain 10000X stock solution into the agarose gel solution at 1:10000 (e.g., 5µL stock solution added to 50mL of gel solution). Let agarose solution cool down to about 50-55°C before adding SafeGel red stain. Make sure that the dye is thoroughly mixed with the gel solution by swirling, stirring, or inversion.
   NOTE: inspect the 10000X vial carefully. If dye precipitation occurs, heat up or sonicate the vial).

   c. Cast the gel and allow it to solidify. To avoid mould formation, we recommend that the precast gels are stored in a refrigerator.

   d. Load samples and run the gels using your standard protocol.
   NOTE: Use only 1/3 or less of your normal DNA-marker amount to avoid overexposure (no bands of marker visibly separated).

   e. View the stained gel with a standard transilluminator (302nm o 312nm) and photograph the gel using an ethidium bromide filter. **NOTE:** If you consistently see band smearing and/or poor band separation, run a gel and post-stain by following the protocol provided (point 1) to confirm if the problem is caused by the dye or other factors unrelated to the dye. If post gel staining is normal and the problem is not caused by the dye, try any of the followings:

   - Lower the amount of nucleic acid loaded.
   - Lower running voltage
   - Lower the amount of agarose in the gel
   - Run a longer gel
   - Increase the thickness of the gel
   - Increase the solidification time to ensure sharp well formation
   - Improve your loading technique or select post gel staining

*** Precasting SafeGel red stain Gels is not suitable for acrylamide gels. Use post gel staining for acrylamide gels.**

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