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Agarose Gel Electrophoresis

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We use this protocol and it's working

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Abstract

Agarose gel electrophoresis is a very common method used to analyze the results of a PCR or any DNA sample in general. It can also be used to purify DNA by gel extraction methods.

The underlying principle of gel electrophoresis is that DNA strands of different lengths travel with different speeds through a gel material, when a electrical current is applied. Based on this, it becomes possible to separate DNA in a sample based on the length of the strands and thus access which DNA fragments are present in a sample.

The gel material commonly used for dsDNA analysis is agarose powder, dissolved in TAE buffer. The percentage of agarose for optimal spearation depends on the expected length of the samples: it is lower for larger fragments and higher for shorter ones.

This protocol describes the fabrication of a X% agarose gel and the run of an agarose gel electrophoresis on a DNA sample.

Materials

Material required for X% agarose gel electrophoresis

- 42 µl of TAE 1x buffer
- X% times 42 g of agarose powder
- 3.5 - 4.0 µl Midori Green gel stain
- 5 µl DNA Ladder
- Loading dye
- DNA sample
- Thermo Scientific™ Owl™ EasyCast™ B1A Mini Gel Electrophoresis Systems

1 Melt X% times 42 mg of agarose in 42 ml of 1X TAE buffer in microwave oven until the liquid is fully transparent.

2 Add 3.5 to 4 µl Midori green gel stain in the melted agarose.

3 Pour the melted agarose in the gel cast with the comb set.

4 Wait 30 minutes until the gel solidifies (faster in the 4°C fridge).

5 Cover the gel with 1X TAE buffer and remove the combs carefully.

6 Load the samples in the wells:
- 5 µl of 1 kb DNA ladder
- Mix 5 µl of DNA with 1 µl of 6X times loading buffer

7 Run the gel at 100 volts for 30 minutes or 110 volts without letting the bands run out of the gel.

8 Remove the gel from the chamber.

9 Visualize the DNA fragments using a UV transilluminator.