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Agarose gel electrophoresis V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

Agarose gel for DNA electrophoresis
This protocol is useful to check the approximate size of a mixture of DNA fragments.
For example, after a HMW gDNA extraction, it is good to check that the DNA we extracted is actually high-molecular weight. Another use is for separating PCR amplicons when checking for a mutation. Primers can be designed in such a way that PCR will yield two fragments of different sizes depending on whether a sequence is present or not. If a deletion is present, then the amplified fragment will be smaller than for the DNA where there is no deletion.

Materials

MATERIALS

- Gel Loading Dye Orange (6X) - 4.0 ml **New England Biolabs Catalog #**B7022S
- TAE Buffer (Tris-acetate-EDTA) **Catalog #**B49
- Hyperladder 4 (100bp) **Bioline Catalog #**BIO-33056
- Agarose **Merck MilliporeSigma (Sigma-Aldrich)**
- Image Lab Software **Bio-Rad Laboratories**
- Autoclave Indicator Tapes **Bio Basic Inc. Catalog #**AT011.SIZE.1ROLLS
- Ethidium bromide 10 mg/ml **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E1510
- PowerPac™ HC Power Supply **Bio-Rad Laboratories Catalog #**1645052
- Hyperladder 1kb **Bioline Catalog #**BIO33025
- agarose gel container
- Bio-Rad electrophoresis machine
- Bio-Rad Gel Doc XR with Image Lab Software
- Microwave oen

Safety warnings

Ethidium bromide is a carcinogen and you should be trained on how to handle it before use. EtBr also needs to be diposed of in a special way.

1 Get a 250 ml flask and a 250 ml measuring cylinder.

part1.mp4

2 Add 1 gram agarose to the flask. Add 130 ml TAE in measuring cylinder. Taper container. Put comb in container.

3 Microwave flask with agarose + TAE for 1 minute. After one minute, check that the solution is homogeneous. If not, microwave in steps of 15 seconds until it is homogeneous.

4 Move taped and combed container to the cold room along with the quasi-boiling agarose solution. Add 1 microlitre ethidium bromide to the agarose solution. Pour the agarose solution into the container. Make sure there is no spillage. If there is spillage, stop pouring, clean the mess and start again.

5 Add a small amount of water to the flask, rinse it and dispose of the contaminated water in the liquid EtBr waste through the white material.

6 Wash flask with water and leave it in the dirty dishes trolley.

7 Leave the gel to solidify in the cold room. If you want to use the gel the following day, wrap it in a plastic sheet to protect it from desiccation. 2h

8 Remove tape, put gel in electrophoresis tank. Add 5 microlitres ladder.

9 Add 2 microlitres loading buffer to 5 microlitres of DNA sample (~ 100 nanograms) and load into gel.

10 Attach the cables from the tank to the Bio-Rad power machine. Set the programme to 150 V, 400 mA and 30 minutes. Start run. 30m

11 Check gel every 10 minutes. Put gel in Imager. Open Image Lab. New Protocol, Position gel, zoom in/out. Start protocol. Image is ready on computer. Invert contrast, change contrast, print image. Don't touch computer without gloves. It is contaminated.

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