WCSB, Wageningen University	IBISBA-SOP-WU10
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Agarose gel electrophoresis of DNA fragments

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Instruction

Agarose gel electrophoresis of DNA fragments

1. Introduction / Purpose

In this protocol, the procedure of agarose gel electrophoresis of DNA fragments is described.

Keywords: Agarose – gel - electrophoresis - DNA

2. Equipment and chemicals

2.1. Equipment

- Electrophoresis machine
- Microwave
- Machine to take pictures of the agarose gel after electrophoresis

2.2. Chemicals

- Agarose (SigmaAldrich)
- SYBR Safe DNA Gel Stain (Thermo Fisher Scientific)

2.3 Bacterial strains

- Strain of interest

2.4 Other materials

- The DNA that needs to be analyzed

3. Media and Buffers

- TAE buffer (Tris-Acetate-EDTA buffer): take the store-bought 50x stock solution of TAE buffer (Fisher Scientific) and dilute it 100 times with demi water before use

4. Procedures

For detailed instruction regarding the use of the electrophoresis machines, making the agarose solution, and pouring the gel, ask your supervisor or a technician. Never perform agarose gel electrophoresis without receiving instructions first.

In general:

- 0.8%-1.0% agarose gels are routinely used for analysis of DNA fragments

- Dissolving agarose is done by placing the erlenmeyer with agarose and 0.5x TAE buffer in the microwave. The agarose is completely dissolved when no colourless particles are visible any more.
- After dissolving agarose in TAE buffer 0.01% SybrSafe is added, mix well by shaking.
- Pour the solution in a tray of suitable size
- Let solidify for 20-30 minutes
- Before loading your samples DNA, load two ladders, one to the left and one to the right of your samples
- Electrophoresis is routinely done for 40 minutes at 100 V
- Taking a picture of the gel and how to analyse and store the picture:

  Use the dedicated machinery for taking pictures that is available in your lab. Ask your supervisor or a technician about how to operate it. Ask how to properly analyse and store the picture as well.

5. Remarks / troubleshooting

- Cooling down the hot dissolved agarose solution under cold tap water will speed up the solidifying of the gel
- SYBR Safe is generally added to the hot dissolved agarose solution. It can however also be added before boiling the solution. In this case, it is better to add a bit more of it, since it will lose part of its activity during boiling.

6. Biosafety

- Agarose is dissolved by boiling. Be careful not to burn yourself.
- SYBR Safe is a chemical that binds to DNA. It can therefor cause cancer. Only use it and solutions containing it while wearing gloves, lab coat, and safety goggles.
- All equipment that comes into contact with SYBR Safe also only can be used while wearing gloves, lab coat, and safety goggles.

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