Agarose gel electorphoresis

Aim of the Experiment

This method is used as a quality control of enzymatic reactions on DNA or RNA. It also useful for the separation of DNA or RNA fragments of different length.

Materials

- nuclease-free H_2O (nf H_2O, Sigma Aldrich, Germany)
- DNA or RNA of interest
- Gel ladder (2-log ladder, NEB, Germany)
- 6x purple loading dye (NEB, Germany)
- Agarose (Agarose NEEO Ultra Qualität, Carl Roth, Germany)
- 1x TAE buffer (Carl Roth, Germany)
- Gel chamber system (Peqlab, Germany)
- DNA stain (Stain G, Serva, Germany)
- UV - illuminator box (Peqlab, Germany)

Procedure

1. Mix at least 100 ng of DNA with 1x loading dye. Fill up the volume with nf H_2O if needed.
2. Vortex sample and spin down shortly in a microcentrifuge or mix loading dye and sample by pipetting up and down.
3. In 30 ml or 100 ml (depending on chmber size), dissolve 1 % (w/v) or 1.2 % (w/v) agarose in 1x TAE buffer to separate larger and smaller DNA fragments, respectively.
4. Heat agarose solution in a microwave until it is fully dissolved.

5. Cool down the solution to approximately 50 °C before adding the stain 1:30000.
6. Cast the gel in a gel chamber, add an appropriate comb and wait at least 20 min until the gel is fully polymerized.
7. Load the gel with samples and ladder.
8. Let it run at 120 V for 30 min.
9. If the gel has not been stained before, put it in a staining box and let it incubate in staining solution for 1 h.
10. Image the gel using the UV-illuminator box .

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after an agarose gel electrophoresis:

1. Gel extraction
2. Restriction digest
3. Ligation
4. Transformation