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, USA)
- DNA of interest
- Gel ladder (2log ladder, NEB, USA)
- 6x gel loading dye (NEB, USA)
- Agarose (Agarose NEEO Ultra Qualität, Carl Roth, Germany)
- TAE buffer (Carl Roth, Germany)
- Gel chamber system ()
- DNA stain (Stain G, Serva, Germany)
- Electrophoresis machine ()
- UV detector ()

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. Dilute 1 % (w/v) or 1.2 % (w/v) agarose in TAE buffer to separate larger and smaller DNA fragments, respectively.
4. Heat agarose solution until it is fully dissolved.

5. Cool down the solution before adding the stain 1:30000.
6. Cast gel in gel chamber and add an appropriate comb.
7. Load the gel with samples and ladder after it is fully polymerized.
8. Let it run at 120 V for 30 min.
9. If gel was not stained before, put it in a staining box and let it incubate in staining solution for 1 h.
10. Image gel by excitation through UV-light source.

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