

Agarose gel electrophoresis

Introduction

Gel electrophoresis is used for separating DNA by size (length in base pairs) for visualization and purification. This is a protocol on how to make the necessary buffers, agarose gel and how to run it. Based on Liljeruhm *et al.*, 2014.

Materials

- TEA Buffer (10X)
 - Tris-base: 48.4g
 - Acetate (Acetic acid 100%): 11.42mL
 - EDTA: 7.31g
 - H₂O up to 1L
- Agarose Gel (1%)
 - 1g Agarose
 - 100mL TEA buffer (10X)
- SYBR-Safe or Diamond gel Dye

Procedure

Preparation of the gel

1. Mix agarose gel according to materials above. Roughly 25-30mL are needed for one small gel.
2. Microwave the mixture several times until the solution is completely homogeneous
 - Caution: wear gloves (recommended oven mittens) when microwaving
3. Let the gel cool down at room temperature. If you are using SYBR-Safe, add it when it no longer burns to the touch.
4. Meanwhile prepare the gel caster: put it into the caster holder and place the comb in the gel caster
5. Pour the gel into the caster equally
6. Let the gel cool down and polymerize

Preparation of the samples

- Add Loading dye to the samples and mix well

Loading and running of the gel

1. Prepare the gel chamber: put the caster holder in the chamber, fill with TAE 1X buffer until fill line and remove the comb from the gel.
2. Stabilize your hands to prevent shaking and pipette the samples into the gel wells.

- Do not forget to load the DNA ladder!
3. Plug the chamber to the power bank
 - CAUTION: Make sure the cables are in the correct orientation: DNA travels towards the positive pole, so the wells are in the negative pole.
 - Set the voltage to max 120 V, ampere up
 4. Stop when the DNA travelled through at least half of the gel
 5. If you are using Diamond Dye, stain in the shaker for 15-30min.

Visualization

1. Place gel on UV lamp
 - Caution: Wear gloves and goggles
2. Take pictures of the gel
3. Do not forget to clean up the space!

References

Liljeruhm, J., Gullberg, E., & Forster, A. C. (2014). *Synthetic biology: a lab manual*.