BIOTECHNOLOGY_Labclass No. 4

DNA ELECTROPHORESIS, PCR PURIFICATION, LIGATION AND TRANSFORMATION

PROCEDURE:

a) DNA ELECTROPHORESIS:

- to get the AMPLICON (i.e. I get the necessary section of DNA, without admixtures of non-specific amplicons)
- **2% agarose gel**: - 80 ml (weight 1,6 g) or 120 ml (2.4 g agarose)
  - up to **0.5xTBE** BUFFER !!! (contains salts, leads the sample and it splits better)
  + **GELRED** (1: 1000 → 120 µl per 120 ml) or MIDORI GREEN (1:10 000 → 10-12µl per 120 ml)

b**) PREPARATION OF THE GEL (MEDIA) on a P. dish:**
- preparation of LB agar (basic nutrient medium): agar 40g / L → into Erlenmayer flask
- 1 dish (V = 25ml) → agar: 1g, H2O purif. to 25 ml
- sterilization in pressure cooker: 30 min; (foil, sterilization tape)
- when it cools to 41 ° C (it solidifies at 30 ° C!) add selection and reference markers:

Selection markes	Stock concentration	Volume used/dish
Ampicilin	10 mg/ml	25 µl
X-Gal	20 mg/ml	100 µl
IPTG	200 mg/ml	12.5 µl

- sterile petri dishes → put in the flow box, pour out here
- evaluation of colony growth (next class): - larger white → it was possible to introduce a plasmid with an insert
- blue → plasmid introduced without insert
- small white (with blue dots) → plasmid with insert out of reading frame
- bb without plasmid → they do not grow, they are sensitive to ampicilin

c) **APPLY SAMPLES to gel (electrophoresis)**
- **7 µl** PCR mix and **2 µl** dye (bromophenol blue ???), don't forget the water !!!
- ladder: **3 µl**
- electrophoresis 110-120 V, 30 min

d) when sterilization is over, pour in the flow box of the bowl (25 ml / bowl / student), when they solidify, turn upside down

e) **CUTTING bands out of gel,** weight the eppendorf in advance !!!!
- picture: amaranth: **330** kbp (bottom)
  potatoes: **540** kpb (top)
- cut a DNA fragment from the gel → put in a weighed ependorf tube

f) **COLUMN ISOLATION** – according to: (*NucleoSpin Gel Extraction Kit Protokol*)

1. Weight eppendorf tube with the gel and add 3times of NTI buffer (per weight of the gel) – e.g. the gel is 50 mg, so add 3x50 (=150) µl of NTI buffer (NTI buffer contains guanidinium salts that bind DNA)
2. Incubated for 10 minute at 50°C (to solve the gel) – vortex every 3 minutes
3. Transfer the sample into a column and centrifuge 30s/11.000*g*
4. Discard the flow-thorough and put column back to the tube
5. Add 700 µl of NT3 (wash) buffer, centrifuge again
6. Discard the flow-thorough and put column back to the tube
7. Centrifuge again for 1 minute to get rid off the buffer

8. Place the column to clean epp tube and add 15 µl of the NE (elution) buffer to the column, incubate for 1 minute at RT and centruguge again.

g) **LIGATION AND TRANSFORMATION:**

- TA cloning (Taq polymerase - at the end of amplicon A overlaps → connection with plasmid (T vector) with overlaps T → ligase (topoisomerase II) connects amplicon and vector

1. Ligation mixture:

Acceptor vector 1 µl
PCR product 2 µl
Clonable 2X Ligation Premix 2,5 µl

Incubate in fridge for 15 minutes.

2. Transformation with heat-shock:

Add 4 µl of the ligation mix to 500 µl of the cells.
Put to **ice** for **1** minute
42°C for **30** sec
Ice again **2** minutes

Add SOC medium to revive the cells (glucose and nutrients)

Pores are thought to form in the cell membrane due to heat shock; DNA passes through them. DNA is negatively charged, the bacterial wall is also → positively charged Ca2 + ions bind to the plasmid and "cover it"

Plasmid "coated" with Ca2 + ions sticks to the cell (on ice), heating to 42 ° C → passes into the cell interior (30 sec.) → ice, pores close