Labclass No.2 – Cleavage of plasmid DNA using restriction nendonucleases

Workflow:

3 party**: A) Restriction cleavage of plasmid DNA**
B) Gel preparation (0.8% agarose gel)
C) Plasmid DNA electrophoresis

A) Restriction digestion of plasmid DNA

Method:
1) Incubate for **30 minutes**
2) **thermal inactivation** of the enzyme - 65 ° C, 5 minutes
3) the so-called **L-form** of the plasmid is formed (linearized)

Reaction mixture:
• plasmid DNA **15 µL** (recalculate the actual amount of DNA according to the determined concentration)
• NEB4 10X **2 µL** buffer
• BSA 10X **2 µL**
=> centrifugation (short spin, 6000g)
• **Xmn I** **1 µL**
TOTAL: 20 µL

B) Gel preparation (0.8% agarose gel)

- we prepare 0.8% agarose gel in TBE buffer.

Method:
1) - first it is necessary to glue the pads (into which we pour the gel) and insert combs into them
2) - weigh agarose and pour 100 ml of 0.5% TBE buffer
3) - boil agarose in the microwave (note: the solution must be transparent, 3-4 times boiling, no bubbles)
4) - then the boiled agarose is cooled to 50 ° C under running cold water
5) - add MIDORI GREEN 1:10 000 (4 µl per 100 ml gel)
6) - pour the gel into the pad and let it solidify for 30 minutes.
Pour 0.5% TBE buffer into the electrophoresis baths.

C) Plasmid DNA electrophoresis

- is performed to control restriction cleavage
- agarose, after solidification, forms a gel = molecular sieve, in which DNA moves (has a negative charge), i.e. it moves from a negatively charged pole (electrode)
Method:
1) use 0.8% gel prepared in block B
2) remove the comb => this will create holes, remove the adhesive TAPE !!
3) insert the gel into the tub so that the wells are above the red stripe
4) pour buffer to the MAX line

5) sample pipetting: ladder 3 µl (1kbp); plasmid DNA: 7 µl and 2 µl bromophenol blue - make drops on parafilm, mix
6) each student applies his undigested and digested plasmid to the gel, first always a sample of undigested DNA, then digested; (students - write down the hole numbers with your sample !!!)
7) 150 V, 30 min
After electrophoresis, the gel is transferred to a transluminator and photographed (under UV light).