

# UNIVERSITY OF OSLO

Faculty of Mathematics and Natural Sciences

Exam in: **MBV2020 Laboratory course in biochemistry and molecular biology**

Day of exam: **June 5, 2015**

Exam hours: **14:30-16:30 (2 hours)**

This examination paper consists of **2** pages.

Appendices: **None**

Permitted materials: **None**

*Make sure that your copy of this examination paper is complete before answering.*

Numbers in brackets indicate the maximum number of points for each question. The maximum number of points for the entire exam is 50.

1. What were the following compounds used for in the MBV2020 course?:  
(answer in 1-2 sentences)

- a) dNTPs (deoxyribonucleotides) used in PCR
- b) Coomassie blue used in staining of protein gels
- c) TAE buffer used in agarose gel electrophoresis
- d) Ethidium bromide used to visualize DNA after electrophoresis
- e) Polyethylene glycol used in ligation (reduces the water space in the sample) (5)

2. Which **5** of the following **10** statements are **false**? **False statements are marked red.**

- a) Cloning can be used to amplify DNA.
- b) **The molecular weight of the green fluorescent protein is above 37 kD.**
- c) DNA polymerase adds nucleotides to the 3' end of primers in PCR.
- d) **Bromphenol blue stains proteins.**
- e) **The *lacZ* gene codes for the lac repressor.**
- f) **X-gal has a blue color.**
- g) A histidine tag can be used to purify proteins from cell extracts.
- h) T4 DNA ligase is an enzyme from a virus.
- i) **Gel loading buffers have a lower specific weight than water.**
- j) ColE1 is an origin of replication. (10)

3. Try to answer in a few sentences the following questions related to the work in the MBV2020 course:

a) What is denaturing polyacrylamide electrophoresis and what is it used for? (5)  
Electrophoresis carried out in a polyacrylamide gel under protein-denaturing conditions (usually SDS is the denaturing agent), i.e. proteins are separated by size, not by shape or charge. SDS (denaturing) PAGE is used to determine the size of proteins.

b) What is ampicillin and what is it used for? (5)  
Ampicillin is an antibiotic. Similar to penicillin, it inhibits synthesis of bacterial cell walls, thereby inhibiting growth of bacteria. It is used as a selective marker to identify or grow cell lines that harbor a plasmid carrying an ampicillin resistance gene.

c) What is IPTG and what is it used for? (5)  
IPTG is an inducer of transcription. It binds to and inactivates the lac repressor. In the MBV2020 course IPTG has been used to induce transcription of the lacZ gene in the pBluescript vector and for inducing transcription of the GFP gene for expression of the his-tagged protein.

4. DNA has been cut with *Pst*I, *Eco*RI, and *Bam*HI, together and in separate reactions. DNA fragments of 5 samples were separated on an agarose gel (see photo below).

Try to answer the following questions:

a) What was approximately the size of the original DNA molecule? (5)  
 $\approx 4000$  bp. This can be concluded quite accurately by adding the sizes of fragments in lane 4 (2500 bp + 1000 bp + 500 bp).

b) Was the original DNA a linear or a circular molecule? (5)  
Linear.  
If it were circular, four fragments would be expected in lane 4 because the DNA in lanes 2 and 3 would have been cut twice by *Eco*RI and *Bam*HI, respectively.  
If it were linear, *Eco*RI and *Bam*HI would have cut once (lanes 2 and 3), and twice in lane 4, releasing 3 fragments.

c) Draw a map indicating the approximate positions of the restriction sites. (10)

