

UNIVERSITY OF OSLO

Faculty of Mathematics and Natural Sciences

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

Day of exam: **June 10, 2008**

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 45.

1. What were the following compounds used for in the MBV2020 course? (10)
(answer in 1-2 sentences)
- a) Ampicillin
antibiotic that was used in growth medium for *E. coli* (page 98-102)
 - b) Xylene cyanol FF
dye in gel loading buffer (page 84)
 - c) RNase A
added in digestion of miniprep DNA in order to degrade RNA (page 104)
 - d) Ammonium persulfate
initiated polymerization of the polyacrylamide gels (pages 117, 120)
 - e) Methanol
used in staining and destaining solutions of the protein gels (page 125)
 - f) Imidazole
used for elution of histidine-tagged proteins from the Ni-sepharose column (page 115)
 - g) IPTG (Isopropyl- β -D-thiogalactopyranoside)
inducer of *lac* promoter, used in blue-white color selection (page 99-100)
 - h) Ammonium acetate
used as salt in precipitation of DNA after isolation from an agarose gel (page 111-112)
 - i) Ni-sepharose
affinity column material that bound the histidine-tagged RB38 protein (pages 114-115)
 - j) dNTPs (deoxynucleotides)
in PCR (page 108)

2. Which of the following statements are **false**?
- a) Low melting point agarose melts at approximately 65°C.
true
 - b) Large DNA fragments (>10 kb) can best be separated in an 1.5% agarose gel.
false (1.5% agarose gels separate best small DNA fragments)
 - c) Ethidium bromide binds to DNA and RNA.
true (RNA could be seen on the gels when separating miniprep plasmid DNA)
 - d) Cloning vectors are usually larger than 10,000 bp.
false (they are designed as small as possible, most are 3-5 kb in size)
 - e) The gene for the green fluorescent protein (GFP) is smaller than 1,000 bp.
true
 - f) The *lacZ* gene codes for β -glucuronidase.
false (it codes for β -galactosidase)
 - g) A multiple cloning site (MCS) is also called a polylinker.
true
 - h) SDS denatures proteins.
true
 - i) Bromphenolblue stains proteins.
false (it is used as a dye in gel loading buffers)
 - j) Acrylamide is a neurotoxin.
true
- (10)

3. a) Describe the steps involved in analyzing plasmid DNA by a restriction digestion. (5)
- mix plasmid DNA (normally 0.5 – 100 μ g) with 1X restriction enzyme buffer (normally in 10-50 μ l)
 - add restriction enzyme (normally 1-5 units per μ g of DNA)
 - mix and incubate at optimum temperature for restriction enzyme (37°C for most enzymes; some have an optimum of 25°C; some work best at 65°C)
 - incubate at least 30 min for complete digestion (preferably 1-2 hours), several hours or overnight is also possible
 - mix sample with gel loading buffer and check results of digestion on an agarose gel (agarose concentration depends on size of expected fragments)
- b) What has to be considered when setting up a ligation reaction? (5)
- sizes of DNA fragments to be ligated (should normally not be more than 20 kb total)
 - ratio of DNA fragments to be ligated (e.g. 1:1.3 for vector and insert sizes of 3000 bp and 1000 bp, respectively)
 - amount of DNA ligase used (blunt-end ligations need 10 times higher ligase concentrations than sticky end ligations)
 - ligation temperature and time of ligation (16°C overnight has been optimum for T4 DNA ligase but depending on the type of ligation (blunt or sticky end, sizes of DNA fragments) much shorter incubation times and room temperature can be used.

4. Six plasmids that have been isolated by minipreps were digested with *Bam*HI and fragments separated on an agarose gel (Figure 1 below). Try to answer the following questions:

a) What is the size of the plasmid? (5)

can be deduced from lanes 2, 4, 6, and 7 which all show completely cut plasmids.

The size is around 5000 bp (5 kb-6 kb as answer is fine)

b) Explain the fragment patterns in each of the seven lanes on the gel. (10)

Lane 1: 1kb plus DNA ladder

Lanes 2, 4, and 7: plasmids cut once by *Bam*HI (size around 5000 bp, the bottom of the bands usually gives the correct size of the fragments).

Lane 6: plasmid cut twice by *Bam*HI. Fragment sizes of approximately 2000 bp and 3000 bp which adds up to 5000 bp.

Lanes 3 and 5: Uncut (lane 5) and partially cut (lane 3) plasmid. Supercoiled DNA is seen. The fragment in lane 3 that has the same size as the fragments in lanes 2, 4, and 7 shows that *Bam*HI has cut this miniprep DNA partially (perhaps 10%).

The diffuse bands at around 100 bp on the bottom of the gel are RNAs that have not been digested by RNase A.

