

# UNIVERSITY OF OSLO

Faculty of Mathematics and Natural Sciences

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

Day of exam: **June 9, 2005**

Exam hours: **(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.*

1. Which of the compounds listed below have been used in the MBV2020 course in the processes a)-g)?:

Compounds: BSA (bovine serum albumin), PEG (polyethylene glycol), gel loading buffer, RNase A, dNTPs (deoxynucleotides), competent cells, ampicillin, SDS (sodium dodecyl sulfate), 1 kb DNA ladder.

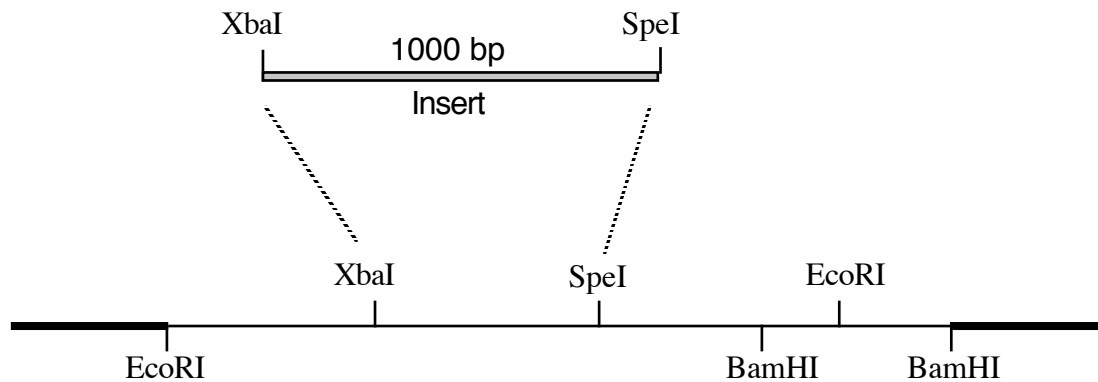
Processes:

- a) agarose gel electrophoresis
- b) plasmid isolation
- c) ligation
- d) transformation
- e) polymerase chain reaction
- f) restriction enzyme analysis
- g) growth of *E. coli* cells

2. You receive a batch of *E. coli* cells that harbor a plasmid carrying the gene for the green fluorescent protein (GFP) in the polylinker.

- a) How can you find out whether the GFP gene is expressed in the bacterial cells?
- b) Outline the major steps to isolate the plasmid from the bacterial cells (mini prep).
- c) Outline how you can verify the presence of the GFP gene in the isolated plasmid.  
[Note: You know all the restriction sites in the polylinker and the complete sequence of the GFP gene.]
- d) Which methods can be used to produce large amounts ( $\mu\text{g}$  amounts) of the GFP gene sequence from the plasmid?

3. You tried to clone a XbaI-SpeI DNA fragment of 1000 bp into the polylinker of a plasmid as shown below.



Note: not drawn to scale. distance between the restriction sites is just a few base pairs.

You isolate the plasmid from transformants in order to check the cloning. Try to answer the following questions:

- What would be the easiest way to verify that the fragment is in the plasmid? Explain.
- What would be the easiest way to determine the orientation of the insert in the polylinker? Explain. [Note: XbaI and SpeI produce ends that are compatible. This means that the fragment can have ligated into the polylinker in both orientations].

4. You have isolated genomic DNA from cells and digested 4 samples with a restriction enzyme. An agarose gel with the separated fragments is shown below. Explain what you see on the gel.

