UNIVERSITY
OF SKÖVDE

School of Bioscience

WRITTEN EXAMINATION

Course: Molecular Biotechnology

Course code: BV703A

Credits for written examination: 4 hp

Date: 2025-01-16

Examination time: 08.15-12.30

Examination responsible: Sanja Jurcevic

Aid at the exam/appendices

Instructions	☒	Take a new sheet of paper for each teacher.
	☐	Write your answer in the exam sheet.
	☒	Write only on one side of the paper.
	☒	Write your name and personal ID No. on all pages you hand in.
	☒	Use page numbering.
	☒	Don´t use a red pen.
	☒	Mark answered questions with a cross on the cover sheet.

Grade points

Maximum score: 70p

Grades: A ≥ 90%, B ≥ 80%, C ≥ 70%, D ≥ 60%, E ≥ 50% of the total points.

Examination results should be made public within 18 working days

Good luck!

Total number of pages 6 (excluding this page)

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Question 1

a) Green biotechnology is the application of biotechnology processes in agriculture and food production. One example is the development of Golden Rice. What was the purpose of Golden Rice? **(2p)**

b) What is the primary application of Grey Biotechnology? **(1p)**

Question 2

True or false **(3p)**

a) Traditional plant breeding involves crossing two plants to create offspring with combined traits of both parents.

b) Genetic engineering requires plants to be able to sexually mate with each other to introduce new traits.

c) Traditional plant breeding typically takes 12-15 years to produce a new crop variety.

d) Genetic engineering allows traits from any living organism to be transferred into a plant.

e) Both traditional plant breeding and genetic engineering transfer many unwanted traits along with the desired trait.

f) Traditional plant breeding is more precise than genetic engineering because it can add a single desired trait without transferring unwanted characteristics.

Question 3

Compare transient and stable transfection in plants by describing their key differences and providing one example of their respective applications. **(2p)**

Question 4

Co-integrate vector systems for plant transformation employ two different plasmids. In detail explain the structure and function of the two types of plasmids in Agrobacterium-mediated transformation. **(8p)**

Question 5

Explain the process of creating knockout cells or knockout mice using positive and negative selection markers. **(8p)**

In your answer, include:

- The function of positive selection markers and an example of one used in knockout experiments.
- The function of negative selection markers and an example of one used in knockout experiments.
- How positive and negative selection markers work together to select for homologous recombination and eliminate cells with random integration.
- How PCR can be used to confirm the successful creation of a knockout construct

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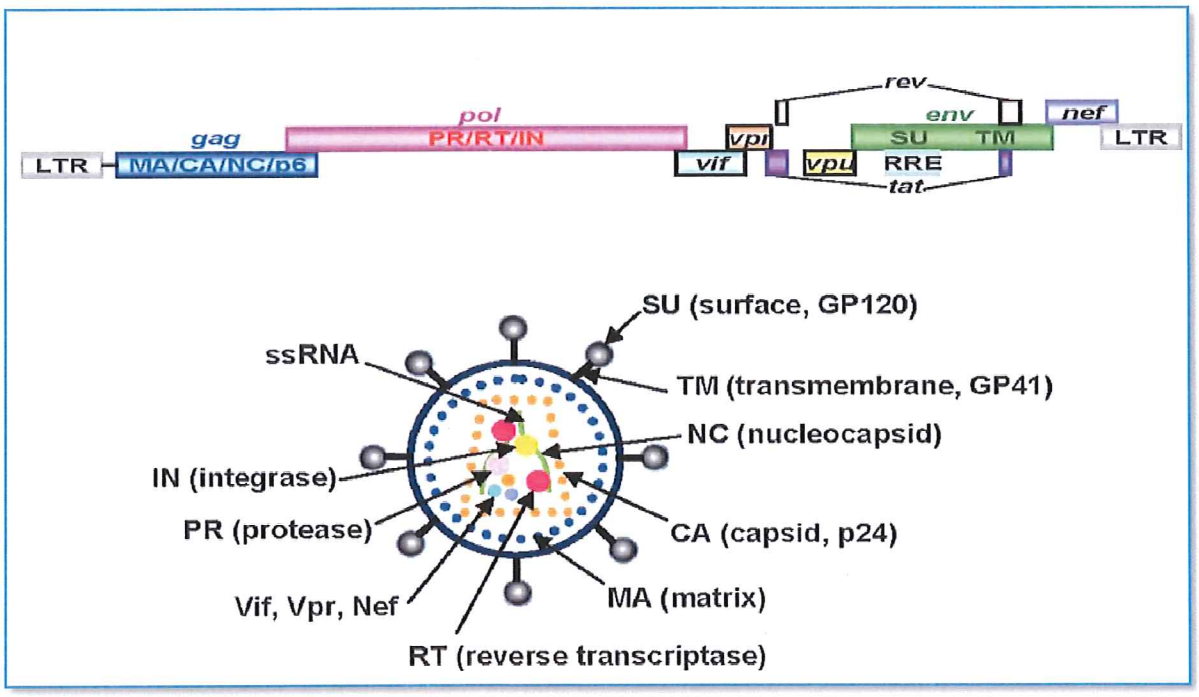
Question 6

Here is the diagram of a lentiviral genome.



Name and describe the function of:

a) Major genes **(2p)**

b) Regulatory genes **(2p)**

c) Accessory genes **(2p)**

Question 7

Matching questions **(8p)**

(1p for correct answer, 0p for no answer, and -1p for incorrect answer)

1. What does CRISPR stand for?
2. What is CRISPR?
3. What is tracrRNA?
4. In what type of organisms can the CRISPR/Cas system be found naturally?
5. What is spacer?
6. CRISPR method is based on what?
7. What does Cas9 do after binding DNA that is complementary to its guide RNA?
8. What is a palindrome?

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A) Clustered International Societies of Photosynthesis Research

B) Clustered Regularly Interspaced Short Palindromic Repeats

C) A gene-editing tool

D) Sequences derived from human DNA fragments

E) Binds to crRNA and forms an active complex with Cas9

F) Viruses

G) miRNA

H) Prokaryotes

I) A natural system used by bacteria to protect themselves from infection of viruses

J) Unique sequence complementary to virus

K) Cuts it

L) Transcribes it

M) It is a word or number that reads the same in both directions, backward and forward

Question 8

Fill in the blanks using terms from the list below. Each blank space corresponds to one point (1p for correct answer, 0p for no answer, and -1p for incorrect answer). **(7p)**

This small RNA (_____) is processed from double-stranded RNA by the enzyme _____ and incorporated into the _____complex. This complex then binds to complementary mRNA to induce cleavage.

miRNAs and siRNAs share similarities but have distinct roles. miRNAs often cause _____ of target mRNAs through imperfect pairing, whereas siRNAs achieve gene silencing via _____. In the processing pathway, miRNAs require a nuclear cleavage step by the enzyme _____ before being further processed by the enzyme Dicer.

When _____ bind to the complementary mRNA, an RNA-RNA hybrid is formed, and the mRNA from this hybrid cannot be translated into a protein.

List of terms:

siRNA

Dicer

RISC

asRNA

miRNA

Translational inhibition

Complementary binding

Drosha

Exportin 5

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Question 9

Answer the following five multiple choice questions (1p for correct answer, 0p for no answer, and -1p for incorrect answer). **(1 point per question, totally 5p)**

a) What is a key feature of a useful diagnostic test in molecular diagnostics?

A) It requires advanced equipment and extensive training.
B) It is specific, sensitive, and technically simple.
C) It focuses exclusively on DNA analysis.
D) It detects multiple diseases simultaneously without specificity.
E) It provides results without needing any sample preparation.

b) Which of the following best describes the role of Amplification-Created Restriction Sites (ACRS) in molecular biology?

A) ACRS introduces artificial restriction sites through PCR to enable the identification of specific mutations.
B) ACRS amplifies entire genomes to detect restriction enzyme activity.
C) ACRS uses restriction enzymes to directly sequence DNA fragments.
D) ACRS eliminates natural restriction sites to simplify DNA amplification.
E) ACRS identifies tandem repeats in non-coding DNA regions for forensic analysis.

c) How does DNA microarray technology detect mutations? By sequencing DNA base-by-base.

A) By amplifying DNA using primers specific to the mutation.
B) By electrophoresis of tandem repeats.
C) By visual inspection of chromosome images under a microscope.
D) By directly editing mutated sequences to restore normal function.
E) By fluorescent signals indicating hybridization strength.

d) What advantage does whole-exome sequencing (WES) provide over whole-genome sequencing (WGS)?

A) Covers non-coding regions of the genome.
B) Requires less computational power but generates more data.
C) Detects structural variations more accurately.
D) More cost-effective and focuses on protein-coding regions.
E) Ensures a higher sequencing depth for targeted regions.

e) What is an advantage of using STR loci in DNA profiling?

A) STRs are identical in all individuals, making analysis simpler.
B) STR loci are only found on one chromosome.
C) STRs always indicate genetic diseases.
D) STR regions are only inherited maternally, making them reliable for tracing ancestry.
E) Analyzing multiple STR loci increases the test's discriminating power.

Question 10

CRISPR technology has recently expanded beyond gene editing to include diagnostics. Two notable tests developed using CRISPR for diagnostic purposes are SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing) and DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter).

a) What are the benefits of these tests, and what can they detect? **(2 p)**
b) In detail, describe how one of these tests works (either SHERLOCK or DETECTR). **(8 p)**

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Question 11

Transgenic expression of a eukaryotic protein in a bacterium or yeast cell can sometimes be challenging for several reasons. Give one example of what you can optimize in each of the four steps AND describe in detail what you do. **(4 x 2p)**

a. Gene or selection of vector
b. Selection of expression organism
c. Cell cultivation procedure
d. Purification procedure

Question 12

The protein you have purified is a dehydrogenase enzyme that catalyzes the reaction to the left in the figure. Knowing the absorbance spectrum of NADH and NAD^+ (right figure) how would you measure the enzyme activity (that it is functional)? **(2p)**