UNIVERSITY
OF SKÖVDE

School of Bioscience

WRITTEN EXAMINATION

Course: Molecular genetics

Course code: BV317G

Credits for written examination: 4 hp

Date: 231026

Examination time: 8.15-12.30

Examination responsible: Anna-Karin Pernestig

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: 100p

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

Note! In order to achieve a passed grade (A-E) both Part I and Part II must be passed (≥ 50%)

Examination results should be made public within 18 working days

Good luck!

Total number of pages 13 (excluding this page)

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Part I – Learning objective: describe how genes are functioning and how their expression is regulated in prokaryotic and eukaryotic organisms. (20 p)

Question 1.

Below is a figure of the tryptophan operon. There is also a table (Table 1) with different terms. I want you to combine the numbers in the figures with the correct name and letter found in Table 1. Also, briefly describe the function of each structure. Answer in your answering sheet. (8p)

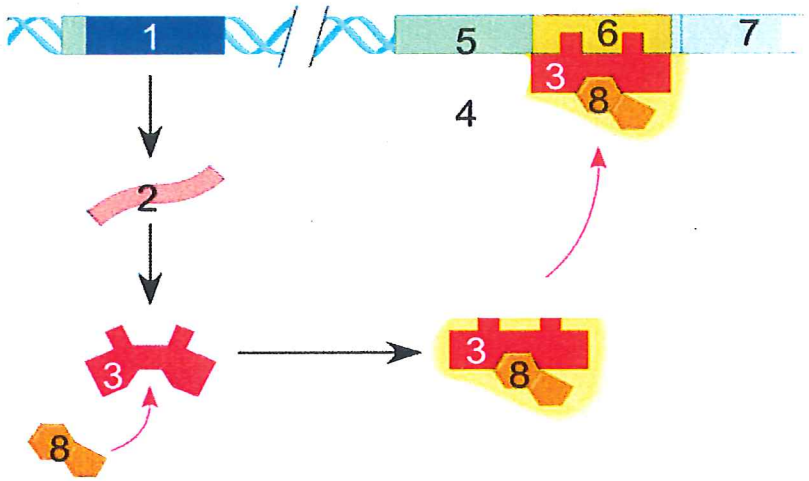


Figure 1. The tryptophan-operon, belonging to Question 1

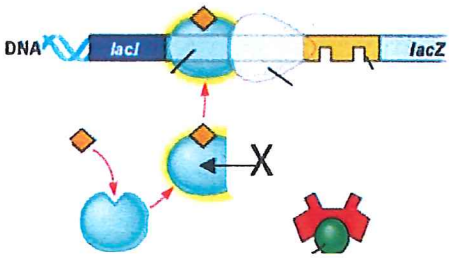
Table 1 Terms belonging to question 1

A	Operon gene, structural gene
B	Inducer
C	Corepressor
D	Primer
E	RNA polymerase
F	mRNA
G	Start codon
H	Promoter
I	tRNA
K	Regulatory gene
J	Major protein
L	Operator
M	Repressor protein

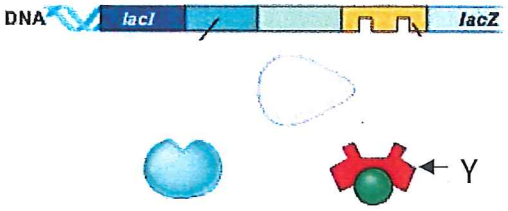
Question 2.

Below is a figure of the lactose operon in two different metabolic environments a) and b).

a) Tell me if the lactose transcription activity is on, off or has little activity? (1p)
Do also write down what the complex X consist of. (0,5p)



b) Tell me if the lactose transcription activity is on, off or has little activity? (1p)
Do also write down what the complex Y consist of. (0,5 p)



Question 3.

Multiple-choice questions (a-d). Only one alternative is correct (0,5p for each correct answer).

a) When lactose in an *E. coli* culture, allolactose bind to the _____.
   A. Repressor protein
   B. Operator
   C. Promoter

b) Which of the following statements is false?
   A. In an inducible operon, free repressor binds to the operator.
   B. In an inducible operon, free repressor cannot bind to the operator.
   C. In a repressible operon, free repressor binds to the operator.

c) What happens when a repressor is bound to the operator?
   A. RNA polymerase is prevented from transcribing the structural genes in the operon.
   B. RNA polymerase increases the rate of transcription.
   C. RNA polymerase decreases the rate of transcription.

d) In the lac operon, what type of control is exhibited by the CAP/cAMP complex?

A. Negative control
B. Positive control

Question 4.

Match one question to one explanation below. In your answering sheet it is enough to have number and letter. (7p)

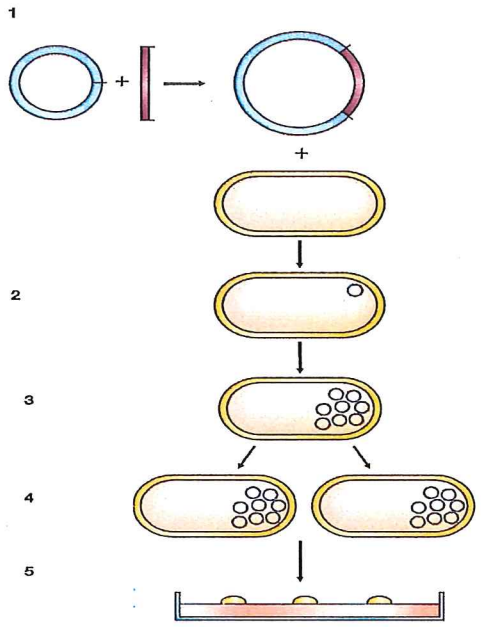
1) What are the two types of operons?
2) What is cell differentiation?
3) What is control element?
4) What is an anabolic pathway?
5) What is histone modification?
6) What is DNA methylation?
7) What are transcription factors?

A. Process of cells becoming specialized in structure and function.
B. Repressible - can be turned off and inducible - can be turned on
C. A segment of eukaryotic DNA containing multiple control elements, usually located far from the gene whose transcription it regulates.
D. RNA polymerase requires these for initiation of transcription
E. This process loosens chromatin structure, thereby promoting the initiation of transcription.
F. Pathway synthesizes molecules and requires energy.
G. This process can cause long-term inactivation of genes and cellular differentiation
H. Pathway cutting molecules and release energy.

Part II – Learning objective: describe the theories behind some molecular biological techniques where DNA, RNA and proteins are studied. (80 p)

Question 5.

a) Figure below describe the basics of gene cloning. Describe step 1-5 so the reader understands the basics of gene cloning. I want you to write in your **answering sheet** not below in the figure. Your answer should include each step 1-5 and include the following keywords at the correct step. Colony, Gene, Vector, Transformation, Multiply, Recombinant DNA molecule, Identical copies, Cell division, Clone, Host cell (5p).

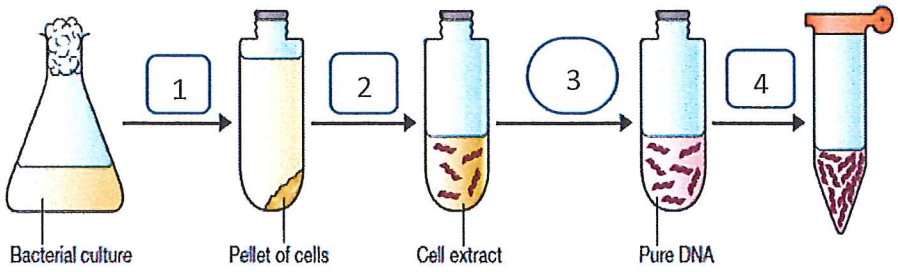


b) Plasmids are used as the cloning vector above. Do mention another cloning vector that can be used in cloning. (1p)

Question 6.

To be able to study molecular genetics you need to step into the lab and do some experiments.

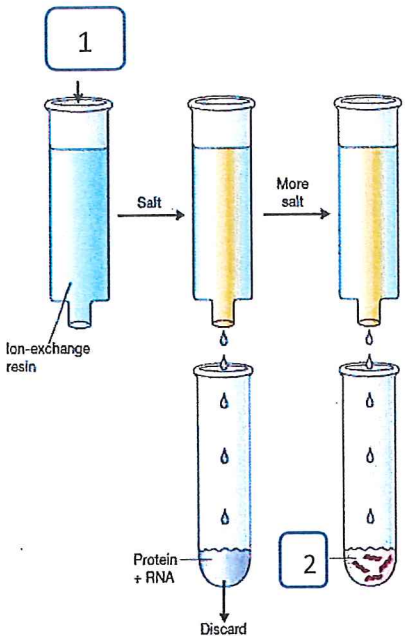
a) Your supervisor has told you isolate bacterial DNA from *E. coli.* What machines / methods / kit are you going to use at step 1, 2, 3 and 4 to get good enough pure bacterial DNA. Just write ONE example at each step. I will only correct the first example written down at each step (1 x 4 p)



b) What machine will you use to be able to measure the concentration and quality of your DNA isolation? (1p)

c) Your supervisor has told you to also isolate DNA from a plant. What method are you going to use? (1p)

d) What method is described in the figure below and what is added in number 1 and what do get out at number 2? (2p)

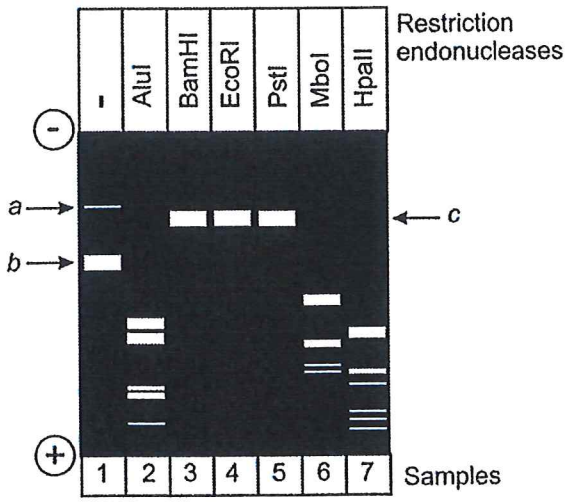


Question 7.

Look into the gel below and tell which suggestion is correct for describing

a) ___ are circular. Write the letter / letters in the answering sheet. (1p)

A: DNA molecules in band a
B: DNA molecules in band b
C: Both of them
D: Neither of them



b) Look into the gel above again and tell which suggestion is correct for describing ___ contain single-strand breaks. Write the letter / letters in the answering sheet (1p)

A: DNA molecules in band a
B: DNA molecules in band b
C: Both of them
D: Neither of them

c) Look into the gel above again and tell what is the most likely explanation for the difference between samples 2 and 3? Write the letter / letters in the answering sheet (1p)

A: The recognition site of AluI is longer than that of BamHI
B: The recognition site of AluI is shorter than that of BamHI
C: AluI has random endonuclease activity
D: AluI has exonuclease activity

Question 8.

Match one statement to one explanation below. In your answering sheet it is enough to have number and letter. If you answer is wrong I will withdraw points. (10p)

Statement
1. Nucleases
2. cDNA
3. Polymerases
4. Transformation
5. Hybridization probe
6. Microinjection
7. Competent
8. Insertional inactivation
9. Ti plasmid
10. Copy number

Explanation

A) The introduction of purified virus DNA molecules into any living cell.
B) The large plasmid found in those *Agrobacterium tumefaciens* cells able to direct crown gall formation on certain species of plants.
C) A group of manipulative enzymes, that cut, shorten or degrade nucleic acids.
D) The number of molecules of a plasmid contained in a single cell.
E) A cloning strategy whereby insertion of a new piece of DNA into a vector inactivates a gene carried by the vector.
F) A culture of bacteria that has been treated to enhance their ability to take up DNA molecules.
G) A group of manipulative enzymes, that make copies of molecules
H) A culture of eukaryotic cancerogenic cells that has been treated to decrease their ability to take up DNA molecules.
I) A method of introducing new DNA into a cell by injecting it directly into the nucleus.

J) The introduction of any DNA molecule into any living cell.
K) A method for separating molecules according to how tightly they repel the gel red present in a chromatographic matrix.
L) The number of optimal DNA copies during PCR
M) DNA synthesized from a single-stranded RNA using reverse transcription
N) A labeled nucleic acid molecule that can be used to identify complementary or homologous molecules through the formation of stable base-paired hybrids.

Question 9.
Match each enzyme below with its function. In your answering sheet you can write number – letter (**8 p**)

Enzyme	Function
1) DNA polymerase	A) Removes the phosphate group at the 5′-end
2) Proteinase K	B) A modified enzyme that lacks 5' → 3' polymerase activity but contains 5' → 3' exonuclease activity
3) Alkaline phosphatase	C) Adds phosphate groups onto free 5′-end
4) Exonuclease	D) Adds deoxyribonucleotides to the 3′-end
5) Ribonuclease (RNase)	E) Degrades RNA
6) Reverse transcriptase	F) Breaks down X-gal (colorless) to a product that is colored deep blue.
7) Type II restriction endonuclease (Restriction enzymes)	G) Remove nucleotides one at a time from the end of a DNA molecule
8) Deoxyribonuclease (DNase)	H) Cutting DNA backbone at a specific sequence (recognition sequence)
	I) Degrades protein (break polypeptides)
	J) Degrades DNA
	K) Join nucleic acid molecules together
	L) Use RNA as a template to build up a copy of DNA

Question 10.
Some experiments have taken place and the DNA has been loaded on an agarose gel. Look into the gel below and tell which suggestion is correct for each statement. Write the letter / letters in the answering sheet. (2p x 3)

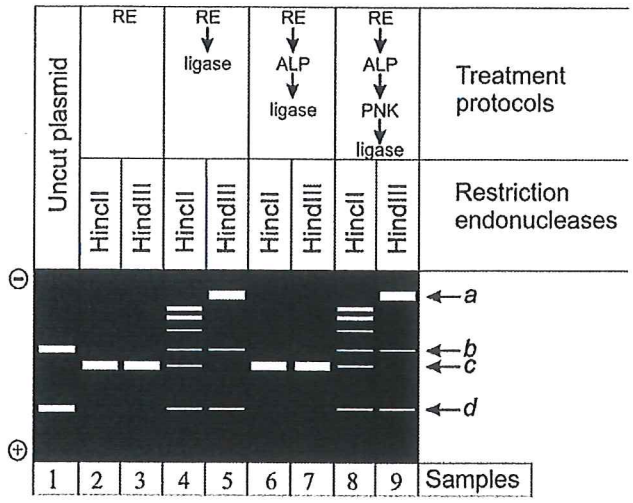


Figure X

I. The plasmid not treated with restriction enzyme migrates as two bands (b and d) during electrophoresis (see Sample 1 in Fig. X). What is the reason for the higher mobility of molecules in band d? (2p)

A. Molecules of band b consist of more nucleotides
B. Molecules of band d have more negative charges
C. Molecules of band d are superhelical, whereas molecules in band b are open circular
D. A and B
E. A, B, and C

II. What is the difference between the DNA of Sample 2 and sample 6 in Figure X? (2p)

A. The DNA in Sample 2 is linear, whereas the DNA in Sample 6 is circular
B. The DNA in Sample 2 is double-stranded, whereas the DNA in Sample 6 is single-stranded
C. DNA strands in Sample 2 have 5′-phosphate and 3′-OH ends, whereas DNA strands in sample 6 have -OH groups at both ends
D. The DNA in Sample 2 has blunt ends, whereas the DNA in Sample 6 has sticky ends
E. There is no difference between them

III. Joining of blunt ends by DNA ligase is less efficient than ligating cohesive ends in Figure X. (2p)

A. the statement is supported by the information given in Figure X
B. the statement is contradicted by the information given in Figure X
C. the statement is neither supported nor contradicted by the information given in Figure X

Question 11.

Describe how PCR works by including the following keywords and put them in the right order Denaturation, temperature is lowered, primers, Taq-polymerase, annealing, extension, template DNA, temperature is increased to above 80° C (8p).

Question 12.
Match each statement/question below to the correct description. In your answering sheet you can write number – letter. (10p)

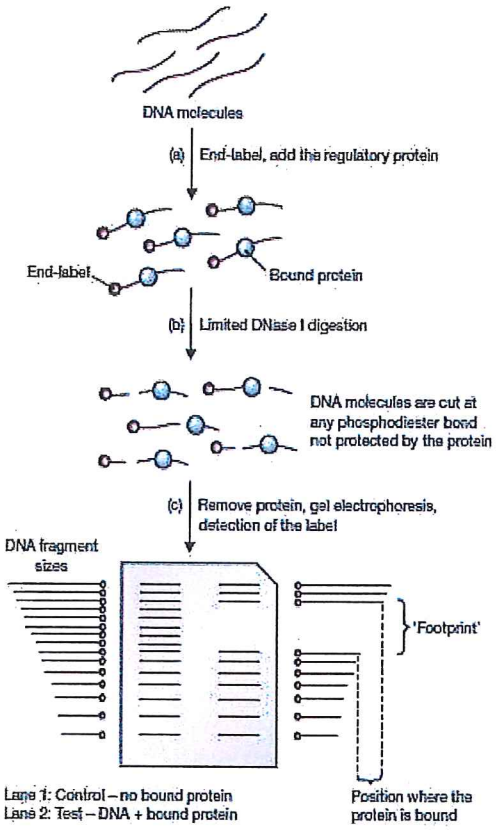
Statement/Question	Description
1) What does qPCR stand for?	A. 3' end of probe, low energy dye
2) What is the goal of qPCR?	B. Reflects the cycle number at which the fluorescence generated within a reaction crosses the threshold.
3) What is a quencher?	C. Quantitative Pyro cycling enzyme reaction
4) Higher Cq means?	D. NGS termination, amplifying the cDNA and analyze the data.
5)The steps included in construction of a library for Next generation sequencing	E. To detect the number of copies of a template within a sample
6) What do all next generation sequencing methods have in common when it comes to terms of steps?	F. Quantitative (Real-Time) Polymerase Chain Reaction
7) What are Dideoxynucleotides missing?	G. Construct a library, clonal amplification, sequence the library and analyze the data.
8) What is baseline?	H. 3´-OH group
9) What is the Sanger method	I. Sugar molecule
10) What is the challenge when using SYBR green in qPCR	J. The initial cycles of PCR during which there is little change in fluorescence signal. Usually cycles 3 to 15.
	K. It is not very specific, since it intercalates in DNA and can give signals from primer dimer
	L. Chain termination method. By using ddNTP´s and dNTP's you will find different length of DNA that correspond to each nucleotide.
	M. Less starting material
	N. Breakage of the starting DNA into fragments. Immobilization of the fragments on to the solid support and amplification of the immobilized fragments.

Question 13.

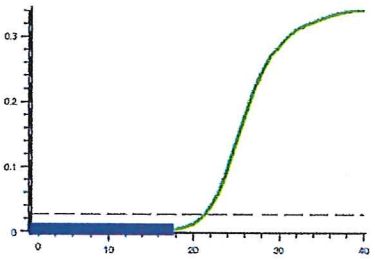
Combine the method below with one figure. Combine number with letter and write in your answering sheet. (6 x 0,5p)

1. Gel electrophoresis
2. Quantitative (real-time) PCR
3. Sanger sequencing
4. Next generation sequencing
5. DNA foot printing
6. Rapid Amplification of cDNA Ends (RACE)

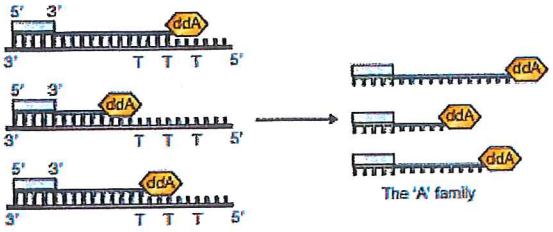
A



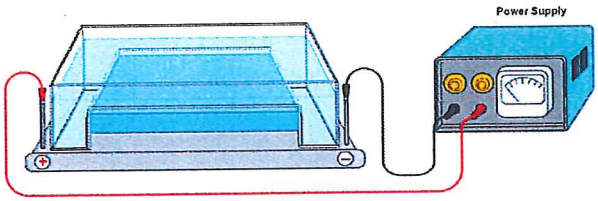
B



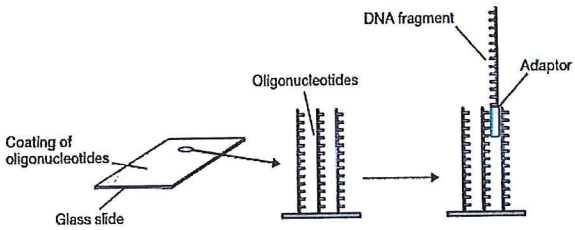
C


5' 3'
ddA
3'
T T T 5'
The 'A' family

D


Power Supply

E


DNA fragment
Adaptor
Oligonucleotides
Coating of oligonucleotides
Glass slide

F

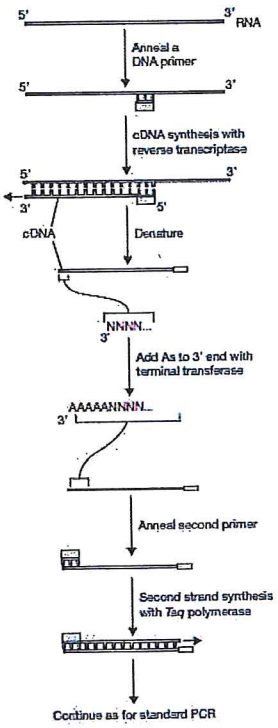
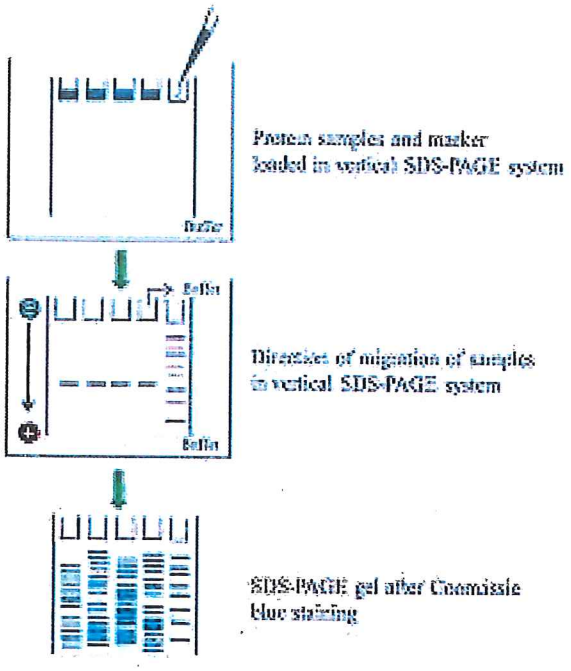

5'
3'
RNA
Anneal a DNA primer
cDNA synthesis with reverse transcriptase
cDNA
Denature
NNNN...
Add As to 3' end with terminal transferase
AAAAANNNN...
Anneal second primer
Second strand synthesis with Taq polymerase
Continue as for standard PCR

Fig
One

G.



Question 14.

Match one method below to one of the statements. In your answering sheet it is enough to have number and letter. You can just use one number one time. (8 p)

Statements A-L

A. This method is used to study the RNA transcript of a gene.

B. Introns, which are present in DNA but not in RNA, will "loop out" as additional single-stranded regions.

C. detect interaction between protein and DNA by reducing mobility of a small DNA bound to a protein.

D. This method requires a cell extract is obtained by lysing the cell of interest and centrifuging out the cell walls, DNA genome, and other debris. The remains are the necessary cell machinery including ribosomes, aminoacyl-tRNA synthetases, translation initiation and elongation factors, nucleases, etc.

E. Computer-based methods to identify the genes, control sequences, and other interesting features of a genomic sequence, as well as experimental techniques to determine the functions of unknown genes that are discovered.

F. Amino acids and their ordered are recognized by green fluorescent probes

G. The template DNA molecule is amplified by two PCRs. In each of these, one primer is normal and is 100% complementary to template DNA, but the other primer is a mutant primer because it contains a single base pair mismatch corresponding to the mutation we wish to introduce to the DNA sequence.

H. Searching genome sequence for ORFs is the first step in gene location. In order to find or locate a gene, the computer must investigate both DNA strings.

I. The sample is embedded in an excess of matrix, which is a solution of an ultraviolet compound. When the liquid evaporates, the sample consists of dry crystals of sample mixed with matrix. When this mixture is irradiated with a laser, the matrix assists in the volatilization and ionization of the analyte.
J. Protein-protein interactions can be investigated by using this method.
K. Determining the order of the nucleotide bases adenine, guanine, cytosine and thymine in DNA

Method
1. ORF scanning
2. Gel Mobility Shift
3. Hybrid release translation
4. oligonucleotide-directed mutagenesis
5. Genome annotation
6. Maldi TOF analysis
7. Primer extension assay
8. Yeast two-hybrid system

Question 15.

a) How does miRNA affect gene expression in mammals? (1p)
b) What is siRNA? (1p)
c) Name three major differences between miRNA and siRNA? (3p)

Question 16.

a) What is Cas9? (1p)
b) What is spacer DNA? (1p)
c) CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. What is a palindromic repeat? (1p)
d) What is CRISPR? (1p)
e) What is tracrRNA? (1p)