Briefly explain how to assess contamination of an extracted DNA sample by proteins? 4 pts

Tell three main features (and their roles) of a plasmid vector in molecular biology. 3 pts

1-

2-

3-

Briefly explain the role of maintenance methylases. 4 pts

How many DNA fragments are obtained: when a previously linearized plasmid:
is digested with a restriction enzyme that has two recognition sites? 3 pts

is mildly digested with micrococcal DNase? Justify briefly. 3 pts

- When possible, match the number with a letter (based on the <u>most specific and direct</u> logical link) (10 pts)

1- Phenol extraction	a- DNA removal from an extracted RNA sample
2- EDTA	b- RNA removal from an extracted DNA solution
3- SDS	c- inactivation of DNases by chelating magnesium ion
4- RNase	d- deproteinization
5- ligase	e- concentration of a nucleic acid sample
6- dideoxynucleoside triphosphate	f- protein denaturation and solubilization of the lipid bilayers.
7- Reverse transcriptase	g- insertion of a cloned DNA into a vector
8- specific restriction enzyme	h- cDNA preparation
9- DNase	i- preparation of the vector prior to insertion
10- precipitation with ethanol and salt	j- hybridization of the probe

a	b	c	d	e	f	G	h	i	j

Using complete sentences, list in the logical chronological order the steps of Northern blot experiment starting from cells till bands visualization. 4 pts

1-

2-

3-

4-

5-

6-

7-

8- . . . . . .
9- . . . . . .

Rank the steps (numbered 1 to 8) in the logical order during cDNA cloning through library preparation and screening. 3 pts

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Where **1**- total RNA extraction, **2**- transformation of bacteria with recombinant vectors and incubation at 37^0C, **3**- hybridization with a probe specific to the wanted cDNA and revelation of the clone of interest, **4**- mRNA extraction, **5**- ligation of the cDNA with the vector which have cohesive extremities. **6**- reverse transcription of mRNA into cDNA, **7**- replica of colonies of the library on nitrocellulose membrane, and fixation and denaturation, **8**- addition of poly dC to cDNA ends and poly dG to linearized vector ends using TdT.

Briefly explain the mechanism of transferrin receptor mRNA stabilization triggered by aconitase during iron starvation (when iron concentration is low inside the cell). 3 pts

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Briefly explain two different modes that control gene regulatory proteins activity in eukaryotes. 3 pts

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2-. . . . . .
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Histone tails deacetylation is associated with inhibition of gene expression. Justify without giving any example 3 pts

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What is the intrinsic protein feature that is necessary for its import to the nucleus after its translation is done? 2 pts

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Give two main structural features of antisense RNA. 3 pts

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Nucleosome position in the proximal promoter may be changed by a nucleosome remodeling complex. Explain this fact without considering any specific example. 3 pts

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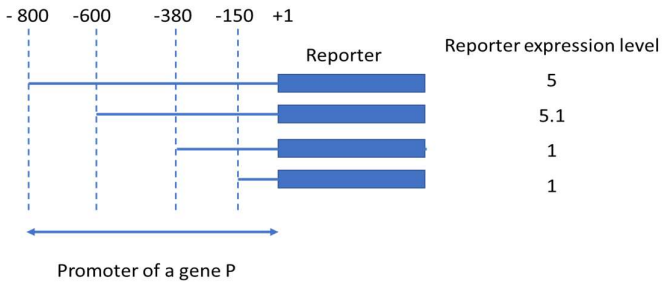
-What are the two types of trans-factors that control gene expression? 4 pts
1- . . . . . .
2- . . . . . .

The promoter of gene P was sequentially deleted and inserted into a vector in front of a reporter. After transfection of the suitable cells, reporter expression was quantified (see figure). Give two justified conclusions (briefly) regarding importance of the regulatory sequences in the promoter. 4 pts



1st . . . . . .
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. . . . . .
. . . . . .
. . . . . .
2nd . . . . . .
. . . . . .
. . . . . .
. . . . . .

The abbreviation DNA stands for . . . . . and the abbreviation RNA stands for . . . . . 1 point.

Encircle the "#" next to one correct answer: 1 point/question:

Which of the followings may be used to achieve radioactive high-density labeling?
α^{32}P-dATP. # T7 DNA polymerase. # β^{32}P-dATP. # γ^{32}P-ATP.

To label a cloned DNA radioactively and use it for DNA foot printing we use:
phosphatase-kinase. c- γ^{32}P-CTP. # DNase. # α^{32}P-dCTP.

A polytene chromosome:
results from 10 DNA replication cycles without cytokinesis. # is a tetrad chromosome present in oocytes.
contains domains that are differently condensed. # has the standard size of a mitotic chromosome.

During splicing, an exon may be considered as intron, and therefore removed along with the upstream and downstream introns, if:
the aim is to produce two mRNA versions that differ by the 5'UTR.
a protein hides the donor site of the upstream intron
a protein hides the acceptor site of the upstream intron.
no correct answer.

A leucine zipper domain:
contains 4 helices rich in leucine. # consists of 3 helices
contains an α helix and a β sheet. # contains two α helices belonging to two distinct protein subunits.

DNA degradation is best assessed by:
Southern blot. # optical density measurement at 260 nm.
optical density measurement at 280 nm. # gel electrophoresis.

Which of the followings is correct regarding PCR amplification technique?
It is useful to quantify the exact amount of a nucleic acid template when it is followed by electrophoresis.
It uses a couple of primers that are complementary to each other.

The DNA polymerase is added at each cycle.
It may help investigating DNA fingerprinting.

Among the following processes, which one is relevant to epigenetic regulation?
splicing of the mRNA. # Phosphorylation of transcription factors.
HDAC activity that is associated with repressors of gene expression. # Regulation of mRNA stability.

In eukaryotes, examples of non-coding moderately repeated DNA are:
Histone genes, SINES and LINES. # rRNA encoding genes, SINES and LINES..
Satellite DNA, SINES and LINES. # no correct answer.

Classes of repeated DNA in eukaryotes:
are all tandemly arranged. # may differ in terms of density.
have the same repeated sequence pattern. # represent an extremely minor fraction of the genome.

Which of the following is true regarding regulation of gene expression?
gene regulatory proteins always act individually to control gene expression.
The half-life time of mRNA may depend on cis elements in the 5'UTR or 3'UTR.
The half-life time of mRNAs is always proportional with the length of the poly A tail.
TATA box sequence is responsible for controlling mRNA stability.

- A transgene:
is inserted in the Ti plasmid outside the T-DNA when preparing a transgenic plant.
must be present in all somatic and germ cells of a transgenic mouse.
is accompanied by a gene for resistance to antibiotics when preparing transgenic drosophila.
no correct answer.

In the DNA finger printing technique, :
restriction fragments of variable sizes are revealed. # the used probe is complementary to a specific gene.
position of a cis-element is determined. # DNase-1 is used.

Transcriptional activators in eukaryotes:
increase stability of the transcribed mRNA. # are associated with acetylase activity.
contain DNA binding domain that interacts with RNA polymerase. # no correct answer.

DNA labeling by random multi-priming involves the following logical order of the steps:
1-2-3-4 # 3-2-1-4 # 2-4-1-3 # 4-3-1-2
1-annealing of the random hexaprimers. 2- Addition of DNA polymerase and dNTP. 3-Addition of random hexaprimers. 4-Denaturation of the DNA molecule.

Insulators that are involved in the control of gene expression:
are gene regulatory proteins. # are non-coding RNA. # no correct answer.
are cis elements that prevent chromatin condensation from reaching the active domains.

Concerning real-time PCR:
it requires use of restriction enzyme.
it enables the study of transcription rate.
it helps investigating the differences in the amount of a specific nucleic acid in different samples.
size of the transcript is directly revealed.

In eukaryotes, localization of mRNA in the cytoplasm:
is random. # is determined by cis elements in the UTR.
is the same for all transcripts. # is not relevant to cell differentiation.

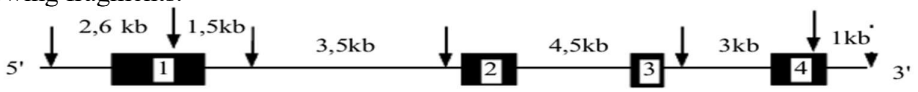
Dideoxynucleoside triphosphates (ddNTPs) are used in DNA sequencing since they:
are fluorescent. # form phosphodiester bonds.
cannot be incorporated into DNA. # no correct answer

- Two versions of the same protein (encoded by the same gene) that differ by the presence of a signal peptide sequence at the N-terminus. The process that generates them is probably:
a missing 5'UTR. # the use of different termination and cleavage sites.
alternative splicing. # the use of two different transcription initiation sites.

Regarding RNA editing, it is correct that it:
is a proofreading function performed by RNA polymerase.
is a deletion of U from the mRNA with the help of guide RNA.
is found in prokaryotes but not in eukaryotes.
requires enzymes and, for certain editing types, RNA molecules.

Which of the following techniques investigates protein binding to an oligonucleotide?
Northern blot # Real time PCR # Southern blot # EMSA

The following map shows gene "G" exons (black boxes) and restriction map for a restriction enzyme "E" (arrows). Numbers refer to fragments sizes in kilobases (kb) (distance between two restriction sites). Genomic DNA was extracted, and Southern blot performed using as probe the cDNA of the gene "G". The used probe will reveal the following fragments:



2,6 kb - 1,5 kb - 3,5 kb - 4,5 kb - 3 kb - 1kb # 2,6 kb - 4,5 kb - 3 kb - 1kb
2,6 kb - 1,5 kb - 4,5 kb - 3 kb - 1kb # no correct answer.

A helix-turn-helix motif:
has always the same amino acid consensus sequence.
is found in diverse gene regulatory protein types that act as dimers.
is found in diverse gene regulatory protein types that act as monomers.
no correct answer.

Electrophoresis of nucleic acids:
is always performed in a denaturing gel. # sorts nucleic acid molecules depending on their charges.
is performed on an agarose gel during a sequencing reaction. # no correct answer.

- A chimera gene between gene P and the GFP coding sequence:
helps tracking the product of gene P inside the cell.
must respect the reading frame of both.
produces a chimeric protein made up of two covalently linked polypeptides.
all of the above.

- Which of the followings does not involve (in general) a cis-element in the 3'UTR?
mRNA localization in the cytosol. # polyadenylation. # mRNA stability. # no correct answer.

- Antisense RNA:
may be produced by replicases. # are unfolded single stranded molecules.
act only at the translation initiation level. # no correct answer.

- Transcription termination by RNA polymerase II and transcript cleavage:
may be variable leading to a difference in the 3'UTR. # occur at a specific site in all eukaryotic genes.
occur on the stop codon. # no correct answer.

- The yeast mating type:
is determined by repeated genes. # switch requires an active HO gene.
switch is possible in the haploid and diploid states. # no correct answer.

- Euchromatin and heterochromatin differ from each other by:
their sensitivity to DNase. # their methylation states. # their acetylation. # all answers are correct.

- Combinatorial control by gene regulatory proteins:

occurs only in drosophila larvae.
achieves diverse regulation patterns by few GRP.
is just a dimerization of two GRP types.
no correct answer.

- Bicoid:
is a GRP that is uniformly distributed in the drosophila egg.
is a GRP encoded by eve gene.
combines with three other GRP to determine 7 stripes of eve gene expression.
no correct answer.

- A DNA molecule with 3' overhang may be converted into a blunt ended molecule by:
Nuclease S1.
DNA polymerase + dNTP
phosphatase / kinase
no correct answer.

- An enhancer sequence is:
a single cis-element.
a complex of overlapping cis-elements.
a trans factor that activates transcription.
always found in the proximal promoter.

- The DNA binding motif of gene regulatory proteins:
reads the sequence of its specific cis-element by chemical fit between amino acid side chains and chemical groups exposed by AT and CG in the major groove.
reads the sequence of its specific cis-element by unwinding the double helix.
is the sane as the RNA binding motif.
is always the same in all GRP types.

- The gene regulatory protein that binds to a certain cis-element:
enables DNase 1 digesting in a DNA foot print experiment.
is attached by means of covalent bonds.
may be identified by EMSA using specific oligonucleotide competitors.
no correct answer.

- Separation of two proteins that have the same size but different charges is achieved by:
denaturing gel electrophoresis.
two dimensional gel electrophoresis.
non denaturing gel electrophoresis.
no correct answer.

- A knock-out multicellular organism:
preparation is started on the fertilized egg.
has a missing gene in the germ cells only.
is prepared using antisense RNA.
no correct answer.

- Yeast colonies may become red because:
ADE2 gene is deleted.
inversion of a chromosome segment that moved ADE2 gene close to telomere which caused its inactivation due to heterochromatin spreading.
an insulator sequence position was altered.
second and third answer are both correct.

- Degradation of a mRNA may be triggered:
by deadenylation or decapping.
by non coding RNA.
by a short poly A tail.
all answers are correct.