Indian Institute of Technology, Delhi

BBL231 Molecular Biology and Genetics, 2015 (II-LT2, 2:30-3:30 pm)

MINOR I (Maximum Marks: 22) (Each question carries 2 marks)

J Physiol Biochem. 2015 Feb 10. [Epub ahead of print]

Liver histone H3 methylation and acetylation may associate with type 2 diabetes development.

Tu P[1], Li X, Ma B, Duan H, Zhang Y, Wu R, Ni Z, Jiang P, Wang H, Li M, Zhu J, Li M.

⊕ Author information

Q1. Given above is the title of a paper. Why do you think that variations in histone methylation/acetylation patterns may be involved in disease development?

Q2. What has been the contribution of Dr. Paul Rothemund to the field of DNA nanotechnology?

Q3. I identify a chemical compound called "WOW" that is involved in inhibiting telomerase activity. What use this compound can be put in and why?

Q4. An organism has 2n= 12. Predict the ploidy of the cell that shows following chromosome numbers- - 6, 36, 10, 13.

Q5. A woman with red-green color-blindness has a mother with normal vision. Knowing that color-blindness is a X-linked recessive gene, can you determine what her father's phenotype is? The woman marries a man with normal vision. What is the probability they will have sons who are red-green color-blind? What is the probability they will have daughters who are red-green color-blind?

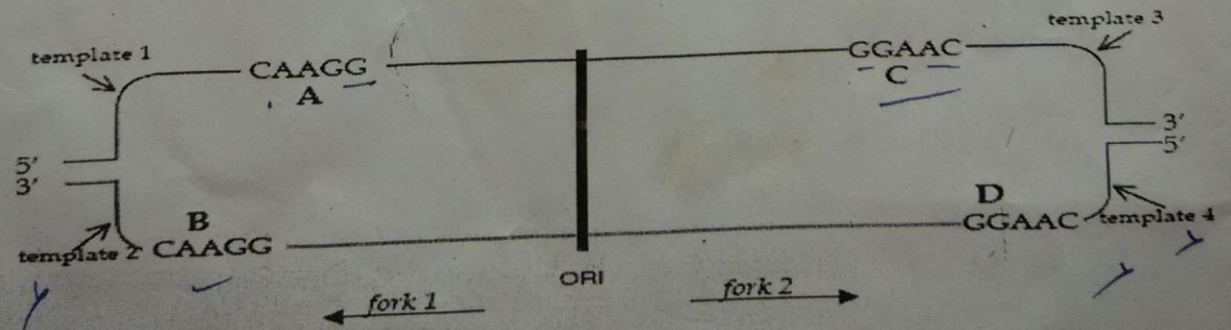
Q6.

a) Differentiate a strong and a weak promoter.
b) DNA polymerase I versus DNA polymerase III versus RNA polymerase.

Q7. Shown is a representation of an origin of replication. Synthesis if new DNA occurs on both strands and in both directions.

a) On which strand/strands will replication be continuous a) template 1, b) template 2 c) template 3 d) template 4.

b) To which site/sites (A, B, C or D) can the primer 5'GUUCC3' bind to initiate replication?



flow chart through.

Q8. Transposon insertions are flanked by a short direct repeat (usually 5-10 bp) of the target DNA sequence. Draw a flow diagram to indicate how these direct repeats are formed.

Q9. What are VNTRs, and why are they valuable for DNA fingerprinting? How do VNTRs compare for unrelated individuals versus for closely related individuals (for example, parent and child or brother and sister)?

Q10. Strain X of E. coli contains a mutated lac regulatory gene on its bacterial genome. As a result, the gene produces a nonfunctional lac repressor protein. You add a plasmid (an extra circular piece of double-stranded DNA) to these cells. The plasmid contains a normal regulatory gene and a normal lac operon.

a. Before the addition of the plasmid, would the E. coli strain X cells be able to produce the enzymes for lactose digestion? Explain.

b. After the addition of the plasmid, would the plasmid's lac operon produce the enzymes for lactose digestion constitutively (all the time) or only when lactose was the available sugar source? Explain.

c. After the addition of the plasmid, would the bacterial genome's lac operon produce the enzymes for lactose digestion constitutively or only when lactose was the available energy source? Explain.

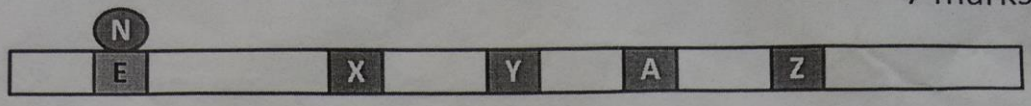
d. If equal amounts of lactose and glucose were present in the cell, would the lac operon in the bacterial DNA be off or on? Would the lac operon on the introduced plasmid be off or on? Explain.

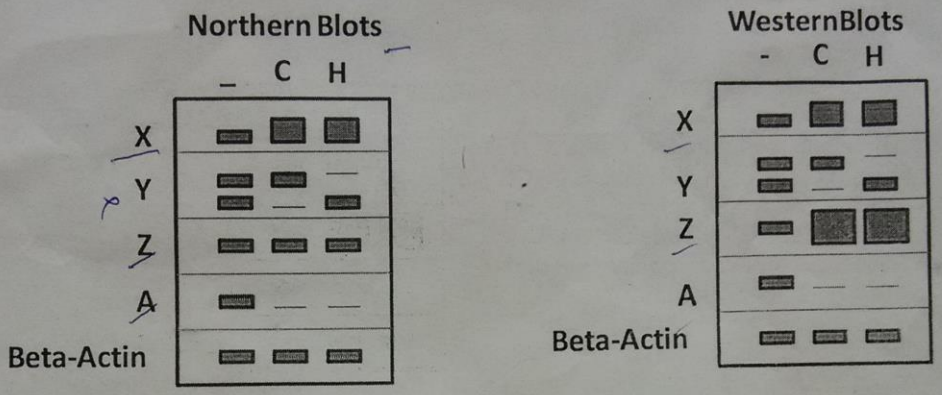
Q11. In a hybridization assay what is meant by a probe, and what is the point of a hybridization assay?

Minor - II II - LT I (2:30 - 3:30pm)

7 marks

Q1.





X, Y, Z and A are different genes. E is the enhancer bound by activator protein N. Shown in the picture is a northern blot showing expression levels of transcripts of various genes and western blot showing protein levels of various genes. – (no treatment), C (Cold Shock) and H (heat shock).
E is the enhancer bound by activator protein N and is present 2 kb upstream of X. N can activate genes X and Y but not A and Z

1. How N protein sitting on E can activate genes present so far ?
2. What could be the reasons that it is not able to activate A and Z?
3. What if the E is transferred downstream of X and upstream of Y. Will it still be able to activate X and Y?
4. What if I replace the promoter region of A with a promoter enriched with CpG islands. How will it affect the expression of A?
5. For gene Y I see two bands in a Northern blot. Why? Also I notice that when given cold shock, the upper band is more while on heat shock the lower band. Why is that?
6. Which of X, Z or A genes is transcriptionally regulated and which is post-transcriptionally regulated? What mechanisms might be involved may please be mentioned.
7. When I am studying gene regulation of A, X,Y, Z then why have I included Beta-actin gene in my study? What does it tell?

Q2. What is the role of XIST RNA in X-chromosome inactivation? 2 marks

Q3. What is the significance of pause terminator and antitermination sequences in tryptophan leader region? 2 marks

8 marks

Q4. Now that the complete genetic code has been determined, you can use the strand of DNA shown here and the codon chart (given) to answer the next questions.

Original template strand of DNA: 3' TAC GCA AGC AAT ACC GAC GAA 5'

a. If this DNA strand produces an mRNA, what does the sequence of the mRNA read from 5' to 3'?

b. For what sequence of amino acids does this mRNA code? (Assume it does not contain introns.)

c. Below are listed five point mutations that may occur in the original strand of DNA. What happens to the amino acid sequence or protein produced as a result of each mutation? (*Note:* The last base in the DNA strand, at the 5' end, is at position 21.)

Original template strand: 3' TAC GCA AGC AAT ACC GAC GAA 5'

Mutation Effect on amino acid sequence

i. Substitution of T for G at position 8.

ii. Addition of T between positions 8 and 9.

iii. Deletion of C at position 15.

iv. Substitution of T for C at position 18.

v. Deletion of C at position 18.

vi. Which of the mutations produces the greatest change in the amino acid sequence of the polypeptide coded for by this 21-base-pair gene?

Second Letter

1st letter	U	C	A	G	3rd letter
U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G
A	AUU AUC Ile AUA AUG Met	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G

Q5. What are guide RNAs and why are they important? 1 Mark

30 Marks

BBL231 Major Exam (6/5/2015, 8-10am, II-LT1)

Q1. MicroRNAs are referred to as post-transcriptional gene regulators. Explain. 2

Q2. Consider conjugation in Escherichia coli. In which of the following matings would chromosomal genes be transferred most frequently and why? 2

A) F+ x F–
B) F– x F–
C) Hfr x F–
D) Hfr x F+

Q3. Which of the following features are common to transformation, transduction and conjugation? 1

(1) Unidirectional transfer of genes
(2) Incomplete gene transfer
(3)Homologous recombination
(4) Meiosis occurring in the recipient

Q4. You carry out the following experiment. You mix large populations of two mutant strains of Escherichia coli, each requiring a different, single amino acid. After plating them out on minimal medium, you note that 45 colonies have grown. Which of the following may explain this result?

A) The colonies may be due to back mutation.
B) The colonies may be due to recombination.
C) Either A or B is possible.
D) Neither A nor B is possible.

Q5. You have performed the following mating experiment using Hfr and F– strains of Escherichia coli: Hfr (thr+ leu+ gal+ strs) × F– (thr– leu– gal– strr). If you intended to map genes from the donor appearing in the recipient, which of the following selective media would you use to score recombinant colonies? 1

A) Minimal medium
B) Minimal medium + streptomycin
C) Minimal medium + appropriate nutrient
D) Minimal medium + streptomycin + appropriate nutrient

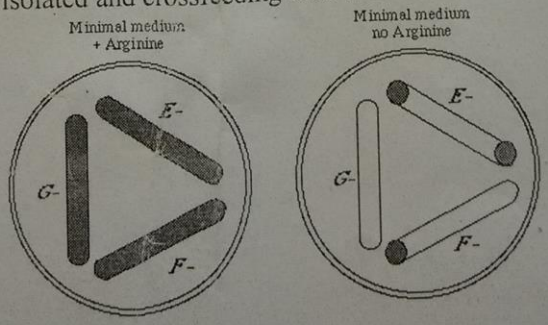
2

Q6. Match the following

Caenorhabditis elegans	Plant development
Drosophila	Stem Cell research
Arabidopsis thaliana	Second site screening
Planaria	Aging and RNAi research

Q 7. Under what conditions lysogenic termination takes place for lambda phage and how? 2

Q8. Three Arg- mutants were isolated and crossfeeding tests were done as shown below. 1



Based upon these crossfeeding results, indicate the order of the G, E, and F genes in the arginine biosynthesis pathway.

1

Q 9. Differentiate gene knock-down and gene knock-out.

Q 10. Provide any three advantages of mutagenesis. 2

Q 11. Describe briefly any two naturally occurring base damages. Diagrammatically represent steps of nucleotide excision repair in E.coli. 4

Q12. Differentiate generalized and specialized transduction. 1

Q13. Match the following (more than one can match) 2

DNA polymerase I	5'-3' polymerization
RNA polymerase	5'-3' proofreading
DNA polymerase III	3'-5' proofreading
Reverse transcriptase	RNA to cDNA
	DNA to RNA

Q14. Fill in the blanks/mark the correct answer/true or false 8

i. The process of _____ removes introns from a pre-mRNA molecule and joins the exons into a mature mRNA.

ii. A(n) _____ binds and cleaves a specific double-stranded DNA sequence.

iii. A DNA sequence that is homologous to a functional gene but does not produce a functional polypeptide because of deficiencies in transcription or translation of the gene is called a(n) _____.

iv. Protein modification such as _____ is associated with protein degradation.

v. Deacetylated histones are usually associated with a _____ chromatin state.

vi. Which of the following statements about introns is true?

A. Most eukaryotic genes have about the same size of introns.
B. Some classes of genes generally do not have introns.
C. Introns often contain protein coding information.
D. Some types of organisms generally do not have introns.
E. Most eukaryotic genes have about the same number of introns.

vii. In comparing homologous genes from different species, which statement is true?

A. Intron sequences vary more than intron positions and exon sequences.
B. Exon sequences vary more than intron sequences.
C. Intron positions vary more than intron sequences.
D. Exon length varies more than intron length.
E. Gene length variation can be attributed primarily to exon length variation.

viii. Which of the following is *not* a way in which a DNA sequence can code for more than one polypeptide?

A. alternative splicing of the mRNA of a gene that sometimes includes all exons but sometimes excludes some exons
B. overlapping homologous genes in the same reading frame
C. tRNAs with the same anticodon that carry different amino acids
D. overlapping nonhomologous genes in different reading frames
E. alternative splicing of the mRNA of a gene that selects among alternative exons that are never expressed together

ix. Eukaryotes have several origin of replication but only one is active at a time. T/F

x. Transcription requires _____ and translation requires _____ as energy source. (ATP/GTP).

xi. The eukaryotic transcript is protected from degradation by presence of a _____ and _____.

xii Telomerase enzyme is composed of two parts _____ and _____.

xiii Human cells with two barr bodies will have _____ genotype.

xiv Maternally imprinted gene means_____

xv Kozak sequence refers to _____.

xvi The lac repressor protein (made by lacI) has 2 states: it can either bind to _____ or it can bind to the _____.

BBL231

~~BEE204~~ Practical Quiz (15 marks)

28/04/2015 (10:00-10:40am)

Q1. What would be the consequences if following mistakes are made during molecular biology experiments: **(0.5mark each)**

1. Long incubation in Solution II during plasmid DNA isolation.
2. Magnesium chloride is not added in PCR reaction mix.

Q2. What is the significance of heat shock during transformation? What will happen if it is carried for more than 90 seconds? **(1 mark)**

Q3. What are the advantages (**two**) of using *Pfu* DNA polymerase over *Taq* DNA polymerase during PCR reaction? **(1 mark)**

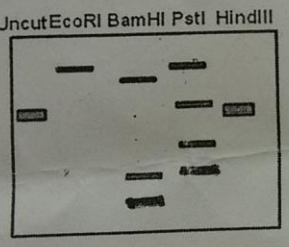
Q4. a) Design a PCR reaction cycle (temperature and time) to amplify a 3 kb long fragment from genomic DNA using *Taq* DNA polymerase. Average melting temperature of forward and reverse primers is 58°C. **(2 marks)**

b) How will the PCR reaction cycle change if *Pfu* DNA polymerase is used instead of *Taq* DNA polymerase? **(0.5 mark)**

Q5. What is the significance of following in RNA isolation? **(0.5 mark each)**

a) Chloroform →
b) TRIzol reagent →
c) 70% Ethanol →

Q6. See the figure of an agarose gel below and predict the number of site of each restriction enzyme (EcoRI, BamHI, PstI, HindIII) in the original circular plasmid. **(2 marks)**



Q7. Mention **four** considerations that should be taken into account while designing primers. **(2 marks)**

Q8. Elaborate the following: **(1 mark each)**

a) Transformation efficiency
b) Ct value

Q9. Amp sensitive competent cells were transformed using Amp resistant plasmid and both competent cells and transformed competent cells were plated on Ampicillin plates 1 and 2, respectively. The next day following was seen: **(2 marks)**

a. No growth has taken place in 1 and 2.
b. Several colonies in both 1 and 2.

What would have been the ideal result and what has gone wrong in conditions a and b.