IMPERIAL COLLEGE LONDON

B.Sc. Examination 2017

This paper is also taken for the relevant examination for the Associateship of the Royal College of Science

MOLECULAR BIOLOGY

Thursday 22 June 2017 10.00 - 13.00

FOR FIRST YEAR STUDENTS IN BIOCHEMISTRY AND BIOTECHNOLOGY

Answer ALL questions in SECTION A using the answer sheet provided. Answer THREE questions from SECTION B, C & D using a SEPARATE answer book for each answer. You must choose ONE from SECTION B and ONE from SECTION C and ONE from SECTION D. Each question has equal weight to section A (i.e. 25 marks).

SECTION A

This section consists of 25 compulsory multiple choice questions. Using the answer sheet provided, mark the box or boxes to indicate your answer. Some questions in this section have more than one correct answer. Credit will be given for all correct answers but you will be penalised with a negative mark for incorrect choices. You will not be penalised if you do not select an answer.

1. Which of the following statements concerning genes and genome sizes is/are TRUE?

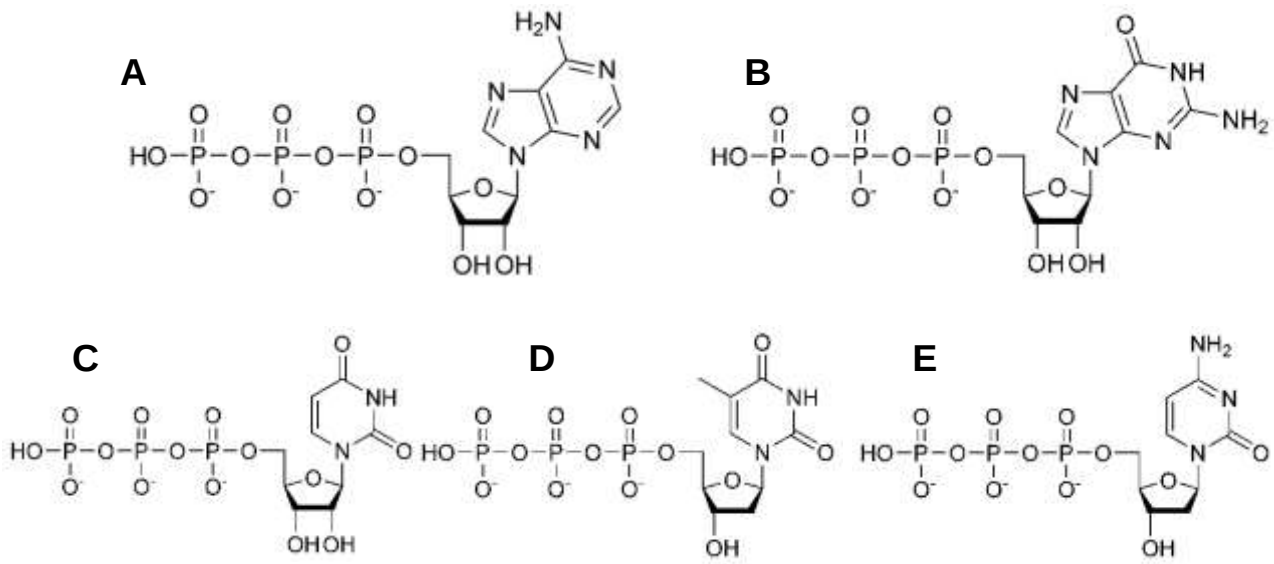
A Bacterial genomes are around 2 - 6 x 10^6 base pairs in size, and contain around 2000 - 6000 genes.
B The average size of a bacterial gene is about 1 kbp.
C Bacterial genomes are around 2 - 6 x 10^3 base pairs in size, and contain around 200 - 600 genes.
D 50 % of an average bacterial genome is composed of repetitive DNA.
E At least 10,000 bp are necessary to encode each bacterial gene.

2. Which of the following factors affects the migration of DNA in agarose gels?

A The amount of voltage applied.
B The size of the DNA molecule.
C The concentration of agarose used in the gel.
D The amount of cross-linker used during polymerization.
E Whether the DNA is of prokaryotic or eukaryotic origin.

3. Which of the following statements regarding Mendelian dihybrid ratios is/are TRUE?

A A 9:3:3:1 ratio will be seen in the F2 even when one gene has codominant alleles.
B 3/16 of the F2 progeny would be expected to exhibit doubly dominant phenotypes.
C A 9:4:3 ratio would imply that one gene is recessively epistatic to the other.
D Linkage would disrupt the classical Mendelian phenotypic ratio.
E 1/16 of the F2 progeny would be expected to exhibit the dominant phenotype of one of the two genes.



Which of the above molecules:

4. Is required by RNA Polymerase in *E. coli*?
5. Is used by T4 DNA ligase?
6. Is required in chain termination di-deoxy DNA sequencing?
7. Is required for translocation of the ribosome?

8. Which of the following positions of DNA can be methylated by DNA methylase?

A C5 position of the cytosine base.
B N4 position of the cytosine base.
C C6 position of the guanine base.
D N3 position of the thymine base.
E N6 position of the adenine base.

9. Which of the following statements regarding classical Mendelian inheritance is/are TRUE?

A Both parents contribute an equal number of autosomes to their offspring.
B In mammals and *Drosophila* both sexes contribute an equal number of genes to their offspring.
C Classical Mendelian monohybrid phenotypic ratios are disrupted if one of the alleles of the gene is lethal.
D A test or back cross involves inter-breeding F1 progeny.
E Pure breeding strains can be heterozygous for some genes.

10. Which of the following statements regarding DNA supercoiling is/are TRUE?

A DNA supercoils are introduced into a molecule by Topoisomerase I.
B Negative supercoils twist the DNA in the same direction as the turns of the right handed DNA double helix.
C Relaxed DNA molecules completely lack supercoils.
D Supercoiling does not occur in bacterial DNA which lack histones.
E Negatively supercoiled DNA is underwound.

11. Which of the following statements concerning eukaryotic transcription is/are TRUE?

A Eukaryotic mRNA is produced in the nucleus and transported to the cytosol for translation.
B Eukaryotic mRNA is made by RNA polymerase II.
C Eukaryotic mRNA has polyA added to the 5' end.
D There are 4 types of eukaryotic RNA polymerase.
E Eukaryotic mRNA primary transcripts include introns.

12. Which of the following statements regarding the mRNA translation initiation complex in bacteria is/are TRUE?

A IF-3 enables the 30S and 50S subunits to combine.
B fMet-tRNA^{fMet} can only bind at the P site.
C The initiating AUG is correctly positioned by proximity to the TATA box sequence.
D IF-1 binds at the A site to prevent tRNA binding during initiation.
E During elongation the uncharged tRNAs leave from the A site.

13. Which of the following statements regarding the properties of DNA Polymerase I is/are TRUE?

A DNA Polymerase I is essential for *E. coli*.
B DNA Polymerase I has dual activities.
C The polymerisation rate of DNA polymerase I is 5-fold lower than that of DNA polymerase II.
D DNA Polymerase I has a 5'-3' exonuclease activity.
E DNA polymerase I is composed of more than 4 subunits.

14. Which of the following statements concerning horizontal gene transfer in bacteria is/are TRUE?

A During transformation, uptake of DNA is initiated by the recipient.
B Transformation requires the donor to synthesize a competence pilus.
C In conjugation, the donor initiates DNA transfer.
D Transduction requires cell-cell contact.
E Transduction is mediated by bacteriophage.

15. Which of the following statements regarding Restriction Fragment Length Polymorphism (RFLP) detection is/are TRUE?

A DNA from different individuals is digested with DNaseI.
B Digested DNA is electrophoresed on agarose gels.
C DNA bands are transferred to a nitrocellulose filter.
D Hybridization of filters to single copy DNA probes allows different individuals to be genotyped.
E RFLP loci tend to occur near the ends of human chromosomes.

16. Which of the following statements regarding prokaryotic tRNA is/are TRUE?

A One tRNA molecule can recognise and bind to more than one codon due to wobble base pairing.
B An activated amino acid attaches to the 3' end.
C The 5' end is always methylated.
D tRNA charging does not rely on Watson-Crick base pairing.
E The site where an activated amino acid attaches has invariant nucleotides: GGA.

17. Which of the following statements regarding CRISPR-Cas9 is/are TRUE?

A CRISPR - Cas arrays are found in bacteria and contain multiple palindromic DNA repeats, spacer DNA and associated genes.
B The spacer DNA sequences are composed of restriction enzyme recognition sequences.
C The spacer DNA sequences are derived from previous encounters of the bacterium with foreign DNA, e.g. phage DNA.
D The associated Cas genes encode restriction enzymes.
E The associated Cas genes encode nucleases that target invading foreign DNA.

18. Which of the following statements regarding Type II restriction enzymes is/are TRUE?

A They recognize symmetric sequences.
B They cleave polypeptide bonds.
C They form homodimers.
D They cleave DNA on both sides of their recognition sequence.
E They require ATP for cleavage.

19. Which of the following statements regarding bacteriophage is/are TRUE?

A Bacterial cells infected with bacteriophages are lysed and form colonies.
B Bacteriophage cannot be replicated once they are in bacteria cells.
C Bacteriophage can be replicated without host cells.
D Bacteriophage can be replicated without causing cell lysis.
E Bacteriophage cleave DNA in host cells during cell lysis.

20. Which of the following statements regarding the properties of the Single-Stranded DNA binding (SSB) proteins is/are TRUE?

A SSB proteins are unique to bacteria.
B SSB proteins do not interact with other proteins at the replication forks.
C SSB proteins help to align strands.
D SSB coating is intrinsic to each SSB and unrelated to the former, previously-bound protein.
E SSB proteins must be stripped off for replication to occur.

21. Which of the following statements regarding mtDNA is/are TRUE?

A Yeast with defective mitochondria which are unable to carry out oxidative phosphorylation caused by large deletions in their mtDNA cannot grow.
B Liver cells have thousands of mtDNA genomes per mitochondrion.
C mtDNA genomes cannot recombine during meiosis.
D The D loop or control region of human mitochondrial DNA is hypervariable.
E Mutations in mtDNA accumulate more slowly than in nuclear DNA.

22. Which of the following reagents could normally be found in a typical DNA purification experiment?

A Sodium Dodecyl Sulphate (SDS).
B DNases.
C Hydrochloric Acid (HCl).
D Ethylene Diamine Tetra-Acetic Acid (EDTA).
E Magnesium Chloride ($MgCl_2$).

23. Which of the following statements regarding Quantitative Trait Loci (QTLs) is/are TRUE?

A The *CFTR* gene of humans is an example of a QTL.
B Height in humans is an example of a continuously variable complex trait.
C QTL mapping in plant inbred lines is performed by selfing for six generations individual F2 that derive from a F1 hybrid.
D The DeCODE genetics company made use of very large Icelandic pedigrees to identify new disease loci.
E QTLs are easily identified by traditional linkage analysis.

24. Which of the following statements about DNA sequence variation is/are TRUE?

A The most common type of DNA sequence variation in the human genome are VNTRs.
B The most common VNTR allele frequencies are less than 0.001 in human populations.
C The SNP mutation rate in humans is of the order of $3x10^{-8}$ per base pair.
D Indels are always caused by insertion of new bases into a chromosome.
E The length of a branch in a haplotype network corresponds to the number of DNA sequence differences between two haplotypes.

25. Which of the following statements is/are TRUE?

A Erwin Chargaff stated that the amount of guanine in DNA is equal to thymine and the amount of adenine is equal to cytosine.
B Albrecht Kossel developed dideoxy DNA sequencing.
C Phoebus Levene formulated the tetranucleotide hypothesis.
D Walter Sutton isolated DNA polymerase I.
E Hamilton Smith isolated the first type II restriction enzyme.

SECTION B

Answer ONE question from this section, in a SEPARATE answer book

26. You have been asked to work with a DNA fragment, called fragment A. Fragment A has an *Eco*RI site at one end and a *Hind*III site at the other end, and another *Eco*RI site within. Fragment A was digested with *Eco*RI and *Hind*III, and the digested fragments were cloned into plasmid B which was pre-digested with either *Eco*RI only or *Eco*RI + *Hind*III. The resultant two plasmids were named plasmid C and plasmid D. There is only one site for *Eco*RI, *Hind*III, *Kpn*I, and *Pst*I in plasmid B. The fragment sizes from the restriction enzyme digest patterns of plasmid B, C, D are shown in Tables 1-3.

Table 1: The digest patterns of plasmid B

*Eco*RI + *Hind*III	3.4 kbp, 0.4 kbp
*Eco*RI + *Kpn*I	2.9 kbp, 0.9 kbp
*Eco*RI + *Pst*I	2.7 kbp, 1.1 kbp
*Hind*III + *Kpn*I	2.5 kbp, 1.3 kbp
*Hind*III + *Pst*I	3.1 kbp, 0.7 kbp

Table 2: The digest patterns of plasmid C

*Eco*RI + *Hind*III	3.4 kbp, 0.8 kbp, 0.4 kbp
*Eco*RI + *Kpn*I	2.9 kbp, 0.9 kbp, 0.7 kbp, 0.1 kbp
*Eco*RI + *Pst*I	2.7 kbp, 1.1 kbp, 0.6 kbp, 0.2 kbp
*Hind*III + *Kpn*I	2.5 kbp, 1.6 kbp, 0.5 kbp
*Hind*III + *Pst*I	2.9 kbp, 1.0 kbp, 0.7 kbp
*Kpn*I + *Bam*HI	3.0 kbp, 1.6 kbp

Table 3: The digest patterns of plasmid D

*Eco*RI + *Hind*III	3.4 kbp, 1.4 kbp
*Eco*RI + *Kpn*I	3.9 kbp, 0.9 kbp
*Eco*RI + *Pst*I	2.7 kbp, 1.2 kbp, 0.9 kbp
*Hind*III + *Pst*I	3.9 kbp, 0.7 kbp, 0.2 kbp
*Bam*HI	4.6 kbp, 0.2 kbp
*Bam*HI + *Eco*RI	4.3 kbp, 0.3 kbp, 0.2 kbp
*Bam*HI + *Hind*III	3.7 kbp, 0.9 kbp, 0.2 kbp

(a) Construct maps of plasmid B, C, and D. The distances (in kbp) between each restriction sites should be indicated. (30%)

(b) What are the fragment sizes for a *Hind*III + *Eco*RI + *Bam*HI digest of plasmid C? (10%)

(c) Construct a restriction map of fragment A. The distances (in kb) between each restriction sites should be indicated. (30%)

(d) Describe strategies that might be used to construct a plasmid harbouring fragment A using plasmid C and D. (30%)

Explain your reasoning throughout.

27. Female *Drosophila* from a stock that is pure-breeding for the character *claret* eye colour (symbol: *ca*) are crossed to males from a stock that is pure-breeding for *sable* body colour (symbol: *s*). The wild-type alleles (ca^+, s^+) are dominant to the mutant alleles (*ca*, *s*). The wild type eye colour is brick red and the wild type body colour is brown.

(a) From the information given so far, there are 2 possible alternative phenotypes for the F1 males. For each of these alternatives give the phenotype, the possible genotypes, and a brief statement of the linkage relationship(s) that would give that phenotype. (40%)

(b) The F1 from the above parental cross are crossed to each other to produce the F2. The F2 are:

phenotype	Number of females	Number of males
wild-type	297	151
claret	103	50
sable	0	153
claret, sable	0	46

i. From this additional information, what are the parental genotypes? (20%)
ii. What are the F1 genotypes and phenotypes? (20%)
iii. What are the expected numbers of each of the F2 phenotypic classes? (20%)
N.B. It is not necessary to use the Chi – square test

Explain your reasoning throughout.

SECTION C

Answer ONE question from this section, in a SEPARATE answer book

28. Describe in detail proof-reading mechanisms that occur during DNA replication.

29. Discuss the roles of RNA polymerase and DNA in the process of transcription initiation and elongation in *E.coli*.

30. Describe how supercoiling allows compaction of DNA into chromosomes, both prokaryotic and eukaryotic. Your answer should include explanations of the terms **unwinding**, **supercoiling** and **torsional stress**.

31. Explain how restriction enzymes provide bacteria with a primitive form of immune system.

SECTION D

Answer ONE question from this section, in a SEPARATE answer book

32. A new genome has been sequenced using Illumina next-generation sequencing. Discuss how the reads produced are assembled. Your answer should include the potential problems caused by sequencing errors and repeats and the methods used to address these.

33. With reference to specific types of DNA sequence variation, describe how this variation can be detected using molecular biological techniques.

34. Transduction and transformation are two methods where DNA can be introduced into bacteria. Compare and contrast these methods (75%) and outline how you would distinguish between these processes if you observed gene transfer in an experimental situation that was known not to be due to conjugation (25%).

35. Describe how recombination mapping can be performed to map genes in terms of relative chromosomal position and distance apart. Indicate why these types of experiments could not be done in mapping of the human genome and briefly explain how alternative methods were used.

End of paper