PAPER 3

QUESTION 1

(a) Outline how the isolated viral RNA can be converted into a double-stranded cDNA in step 1. [2]

1. Reverse transcriptase catalyses synthesis of the single-stranded cDNA using the isolated viral RNA template
2. DNA polymerase converts the single-stranded cDNA into a double-stranded cDNA

(b) (i) Explain how the recombinant Ti plasmid in step 2 can be formed. [3]

1. The same restriction enzyme is used to cleave both the viral gene coding for TMV coat protein from the cDNA formed in Step 1 and the Ti plasmid
2. to produce complementary sticky ends which facilitate annealing of / formation of hydrogen bonds between the viral gene and linearized Ti plasmid
3. DNA ligase is added to covalently link / form phosphodiester bonds between the viral gene and linearized Ti plasmid to produce a recombinant Ti plasmid

(ii) Explain the purpose of having a leaf-specific promoter in the recombinant Ti plasmid formed in step 2. [2]

1. The leaf-specific promoter will ensure that the viral gene coding for TMV coat protein is only expressed in the leaves of transgenic plants
2. to ensure production of TMV coat protein

(c) Suggest another important role of *Agrobacterium tumefaciens* in the process of creating transgenic plants which are resistant to TMV. [1]

1. *Agrobacterium tumefaciens* allows for *in vivo* amplification / production of numerous / multiple copies of the recombinant Ti plasmid

(d) (i) Describe the functions of two other named ingredients in the growth media in the development of plant tissues. [4]

Ingredient	Use in developing tissue
1a. Carbon source, e.g. glucose / sucrose	1b. As respiratory substrate due to lack in autotrophic ability for proliferation of cells and regeneration of shoots
2a. Plant growth regulators, auxins	2b. Stimulates root growth by promoting cell growth & differentiation
3a. Plant growth regulators, cytokinins	3b. Stimulates shoot growth by promoting cell growth & differentiation
4a. Amino acids	4b. Precursors for protein synthesis to stimulate cell growth
5a. Vitamins	5b. As catalysts / coenzymes / enzyme activators in various metabolic processes
6a. Antibiotics	6b. Prevent growth of systemic microbes / effectively control systemic infection

(ii) Explain the significance of the change of environment for the newly formed plantlets in the final stage of tissue culture. [2]

1. The change of environment represents the shift from heterotrophic / sugar-requiring to autotrophic / free-living condition
2. and the acclimatization of the plantlets to the outdoor environment before they develop into whole transgenic plants

[Total: 14 marks]

QUESTION 2

(a) State what is meant by *genetic testing*. [1]

1. identification of changes in chromosomes/ genes/ alleles/ DNA
2. to detect suspected genetic disease

(b) Using the information given in Fig. 2.1, explain why

(i) the three-day-old embryo will develop into a child affected by cystic fibrosis. [2]

1. reference to cystic fibrosis as a recessive condition
2. requiring two copies of mutant allele/ allele 2
3. Three-day-old embryo has same banding pattern as affected child

(ii) the position of allele 2 on the agarose gel indicates that it contains a deletion in comparison with allele 1. [2]

1. allele 2 is shorter / lighter / has a lower molecular mass than allele 1
2. allele 2 is able to migrate a longer distance / move faster through agarose gel

(c) Suggest why the child cannot pass on the normal *CFTR* allele to his offspring. [1]

1. tracheal epithelial cells are somatic cells / are not germ cells

(d) State one limitation of liposome-mediated gene therapy and suggest one improvement to this limitation. [2]

limitation:

1. transient expression of *CFTR* allele
2. *CFTR* allele delivered is not integrated into chromosome
3. short-lived/ transient nature/ repeated treatment needed
4. not specific in delivering *CFTR* allele into target cells

improvement:

1. Use retrovirus-mediated gene therapy
2. design liposomes carrying glycoproteins/glycolipids in their membranes that are recognised by cell receptors

(e) Describe how RFLP analysis facilitated linkage mapping of the *CFTR* gene on chromosome 7. [4]

1. restriction sites occur more than once outside the CFTR locus, i.e. *Taq* I
2. point mutations that create / destroy the restriction sites
3. restriction digestion / cutting with *Taq* I produce unique patterns of restriction fragments respectively
4. each pattern / set of restriction fragment is an RFLP marker
5. determine the COV between each RFLP marker and the CF condition
6. the longer the distance between an RFLP marker and a particular CFTR allele, the higher the frequency of crossing over
7. Application: to determine order of CFTR gene in relation to RFLP markers and relative distances between the loci

[Total: 12 marks]

QUESTION 3

(a) Describe how the expression of the miRNA gene controls the expression of the structural gene. [3]

1. Transcription of the miRNA gene gives a double-stranded miRNA precursor/ RNA folding onto itself/ forming a hairpin loop
2. Drosha cleaves/cut the free ends of the double stranded miRNA precursor and this is exported out of the nucleus

OR

Dicer then cleaves/ cut the loop and gives a double stranded miRNA

Reject: Non-specific terms (e.g processed/ modify miRNA)

3. RISC binds to one of the miRNA strands
4. and becomes complementary to the mRNA of the structural gene, preventing its translation/ downregulating the expression of structural gene

(b) Researchers believe that cancer cells may have mutations in some of the miRNA genes. With reference to proto-oncogenes, suggest how these mutations may have contributed to the progression of cancer. [2]

1. (i) Mutation of miRNA gene results in miRNA that are no longer complementary to target mRNA and thus cannot bind to mRNA to down-regulate expression

OR

(ii) Mutation of miRNA gene results in miRNA that cannot form double stranded miRNA thus Drosha/Dicer/RISC cannot bind to it

OR

(iii) Mutation of miRNA gene results in miRNA promoter of the miRNA is mutated resulting in no miRNA being produced

Reject: Vague description (inactive/ non-functional miRNA etc) or reference to nonsense mutation resulting in truncated miRNA.

2. hence leading to overexpression of the proto-oncogene products resulting in excessive proliferation

Reject: Ref to disruption of tumour-supressor gene or lack of ref to proto-oncogene.

(c) State a role of stem cells in an adult organism. [1]

1. maintain and repair the tissue in which they are found
2. replace cells that die because of injury or disease

(d) Describe how the knockout of DGCR8 affects RNA interference [2]

1. Drosha is inactivated
2. miRNA/precursor is not processed / not cleaved
3. RISC can't bind miRNA
4. RNA interference is reduced / translation is left on

(e)(i) Describe how self-renewal in stem cells allows for their proliferation and subsequent differentiation. [2]

1. By process of mitosis / symmetric divisions
2. to produce a pool of undifferentiated cells

3. Through assymmetric division
4. to generate committed precursors that then become committed to specific lineages

5. Differentiation occurs when precursor cells are exposed to different (internal and external) signals / chemicals
6. and undergo differential gene expression

Note: point 1 and 2 are required to obtain full marks

(ii) Account for the results obtained. [4]

Trend 1
T1a. In normal cells, self-renewal marker decreases from 1 unit (day 0) to 0.8 unit (day 8) to 0.5 unit (day 16)
T1b. Differentiation marker increases from 0.8 units (day 0) to 8 unit (day 8) to 16 units (day 16)

Explanation 1
E1a. This shows that self-renewal is switched off as differentiation proceeds
E1b. The inverse relationship is due to presence of miRNA which regulated the translation of mRNA of self-renewal and differentiation markers
E1c. AVP

Trend 2
T2a. In KO cells, self-renewal marker increases from 1.5 unit (day 0) to 1.8 units (day 8) before decreasing to 1 unit (day 16)
T2b. Differentiation marker increases from 1.2 unit (day 0) to 4 units (day 8) before decreasing to 2 units (day 16)
OR
T2c. AVP

Explanation 2
E2a. The interaction of self-renewal and differentiation is abnormal in knockout cells
E2b. No relationship is observed due to the absence of miRNA, thus the loss of regulation in translation of mRNA of self-renewal and differentiation markers
E2c. AVP

[Total=14 marks]

QUESTION 4

1. Cell vacuoles of salt-tolerant onions are of more negative solute potential than that of non-tolerant onions.
2. Use the scalpel to cut 1 section of 2 cm by 1 cm epidermal tissue from salt-tolerant onion.
3. Using the forceps, put the tissue into 5 cm^3 of 0.1 mol dm^{-3} NaCl solution in a glass specimen tube.
4. Incubate the tube for 15 minutes as timed using a stopwatch.
5. Remove the onion epidermal tissue and place it on a microscopic slide.
6. Add a drop of iodine in potassium iodide solution using a dropper.
7. Cover the sample with a coverslip using mounting needle and place the slide onto the microscope.
8. Count the number of cells undergoing plasmolysis in the first 30 cells seen in this field of view.
9. Repeat for the remaining salt-tolerant onion samples in other concentrations of NaCl solution and for non-tolerant onion samples.
10. Calculate percentage of plasmolysed cells at each NaCl concentration.
11. The NaCl concentration at which there is incipient plasmolysis can be used to further determine the respective solute potential.
12. Care must be taken when using the scalpel to avoid cutting oneself.
13. AVP

[Total: 12 marks]

QUESTION 5

(a) Describe the polymerase chain reaction and explain one limitation of this procedure. [8]

Stage 1
1. DNA denatures / H bonds broken, resulting in separation of DNA strands
2. by heating to 95 °C

Stage 2
3. Primers / oligonucleotides anneal / hybridize to single-stranded DNA template
4. by cooling to 54 °C

Stage 3
5. new strands (of DNA) are synthesized from the position of the primers / amplification of target region flanked by primers
6. by TAQ DNA polymerase
7. by heating to 72 °C

General
8. entire 3 step cycle repeat 20 – 30 times in a thermocycler
9. PCR products serve as templates for further amplification

Any one pair:

L1a. Highly sensitive to contamination
L1b. hence non-target sequences can be amplified

L2a. *Taq* polymerase used in PCR lack proof-reading / 3' to 5' exonuclease activity
L2b. resulting in infidelity / inaccuracy of DNA replication *in vitro* / possible errors in DNA replication
L3a. Loss of *Taq* polymerase activity resulting in accumulation of inaccuracies in longer PCR products
L3b. Therefore, PCR products are shorter / are synthesised in smaller / limited amount

L4a. Specific primers required to selectively amplify target sequence
L4b. Hence, some prior sequence information is needed

L5. It can only be applied to amplify nucleic acids but not proteins

(b) Explain the problems associated with the expression of eukaryotic genes in prokaryotes and how these problems are overcome. [6]

A. Presence of introns
PA1. Eukaryotic genes contain introns OR prokaryotic genes do not contain introns
PA2. There is no RNA splicing machinery OR introns are not removed in prokaryotes
PA3. pre-mRNA not converted into mature mRNA OR additional amino acid incorporated
SA1. cDNA for the gene of interest is synthesised using mRNA as a template via reverse transcriptase

B. Transcriptional initiation
PB1. Bacterial transcriptional machinery does not recognise eukaryotic promoter OR lack enhancers and activators
PB2. hence eukaryotic gene is not expressed OR expressed at very low rates when it is placed in a prokaryote host
SB1. Eukaryotic gene is placed under the control of a strong bacterial promoter to allow for expression of the eukaryotic gene

C. Translational initiation
PC1. Eukaryotic mRNA lack a Shine-Dalgarno sequence
PC2. hence prokaryotic ribosomes / translation machinery cannot recognise the start site
SC1. Insert the DNA form of the Shine-Dalgarno sequence OR leader sequence
OR
different 5' UTR sequence in the 5'UTR of the eukaryotic gene / upstream of gene of interest

D. Post-translational modifications
PD1. Prokaryotes do not have membrane-bound organelles
OR
have no / different post-translational modifications

PD2. hence proteins are not folded into their native / specific 3D conformations/ glycosylation
SD1. Recombinant proteins have to be further purified and modified manually

(c) Discuss the benefits and difficult ethical concerns of the human genome project for humans. [6]

B1a. allows for early detection of mutant genes that causes disease / risk assessment for disease
B1b. screening allows for diagnosis of inherited mutations that predisposes them to specific diseases

B2a. provides insight into the types of protein encoded by genes
B2b. able to treat genetic and acquired diseases by gene therapy

B3a. allows genetic profile of an individual to be determined
B3b. customised drugs / molecular medicine / OWTTE can be designed

B4a. associated techniques eg. genetic testing / RFLP / DNA fingerprinting / DNA sequencing techniques can be used
B4bi. in forensic science eg. analysis of DNA at crime scenes
OR
B4bii. to establish family relationships e.g. paternity testing
OR
B4biii. to assess risk of mutagenesis / health damage posed by exposure to radiation or carcinogens

B5a. Comparative genomics where the genes in the human genome project of unknown functions can be used as comparison against known sequences of other organisms
B5b. for identification of gene functions in HGP

B6a. To elucidate evolutionary history
B6b to seek out relationship between species and to check for degree of relatedness eg: between the 3 domains of life

B7. AVP

E1a. Ownership of personal genetic information
E1b. Difficult to determine who owns and controls the genetic information or who should have access to it
OR
E1c. Legislation needed to ensure that there is no discrimination on the basis of genetic information; e.g. at work or for health insurance

E2a. Commercialisation of products
E2b. Difficult to determine who own genes and other pieces of DNA
OR
E2c. To ascertain if patenting DNA will limit the accessibility and development into useful products

E3a. Use of genetic information in reproductive decision making and reproductive rights
E3b. Concerns that prenatal genetic testing could lead to genetic manipulation or a decision to abort based on undesirable traits disclosed by the tests

E4a. Physiological impacts and stigmatisation
E4b. due to an individual's genetic differences, may affect the society's perceptions and treatment of an individual

E5a. Clinical issues including the professional education of doctors and other health service providers and patients; as well as public education
E5b. in genetic capabilities, scientific limitations, and social risks and implementation of standards and quality-control measures in testing procedures

E6a. Treatment versus enhancement of humans
E6b. No clear distinction between medical treatment and enhancement

E7. AVP

[Total: 20 marks]