RIVER VALLEY HIGH SCHOOL
YEAR 6
PRELIMINARY EXAMINATION II

CANDIDATE NAME									
CENTRE NUMBER	S					INDEX NUMBER				

H2 BIOLOGY **9648/03**

Paper 3 Application Paper **20 Sep 2016**

2 hours

Additional Materials: Answer Paper

READ THESE INSTRUCTIONS FIRST

Write your index number and name on all the work you hand in.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.
DO **NOT** WRITE IN ANY BARCODES.

Section A
Answer **all** questions in the spaces provided on the question paper.

Section B
Answer **all** question on the answer paper provided.

The use of an approved scientific calculator is expected, where appropriate. You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together. The number of marks is given in brackets [ ] at the end of each question or part question.

For Examiner's Use	
Section A	
1	**/ 13**
2	**/ 13**
3	**/ 14**
4	**/ 12**
Section B	
5	**/ 20**
Total	**/ 72**

This Question Paper consists of **16** printed pages.

Section A (80 marks)

Answer **all** the questions in this section.

1 The coat colour of Norwegian cattle is mainly determined by the distribution of two pigments: red and black. Both pigments are produced by the action of the enzyme tyrosinase, in cells called melanocytes. A low level of activity of the enzyme leads to the production of red pigment, while a high activity of the enzyme produces black pigment.

The activity of the enzyme is increased by the melanocyte stimulating hormone (MSH), which binds to the MSH receptor. The receptor is encoded by the gene at **E** locus, which has three alleles, $\mathbf{E^D}$, $\mathbf{E^A}$ and **e**. $\mathbf{E^D}$ and $\mathbf{E^A}$ each encodes a receptor with different activity. No receptor is produced by the recessive allele, **e**.

Alleles $\mathbf{E^D}$ and $\mathbf{E^A}$ differs by a single base substitution; while alleles **e** and $\mathbf{E^A}$ differs by a single base deletion.

(a) Explain how a mutation at $\mathbf{E^A}$ allele may result in **e** allele. [3]

1. **This results in a nonsense mutation;**
2. **by changing reading frame of codons on mRNA;**

Accept: frameshift mutation

3. **This give rise to a stop codon;**
4. **thus premature termination of translation;**
5. **Resulting in a truncated polypeptide;**
6. **thus no protein produced;**

DNA was extracted from the frozen semen of six bulls with different genotypes at the E locus. The DNA from each animal was separately digested with two different restriction enzymes, **P** and **Q**.

The products of each digestion were separated on a gel and the banding pattern shown in **Fig. 1.1**.

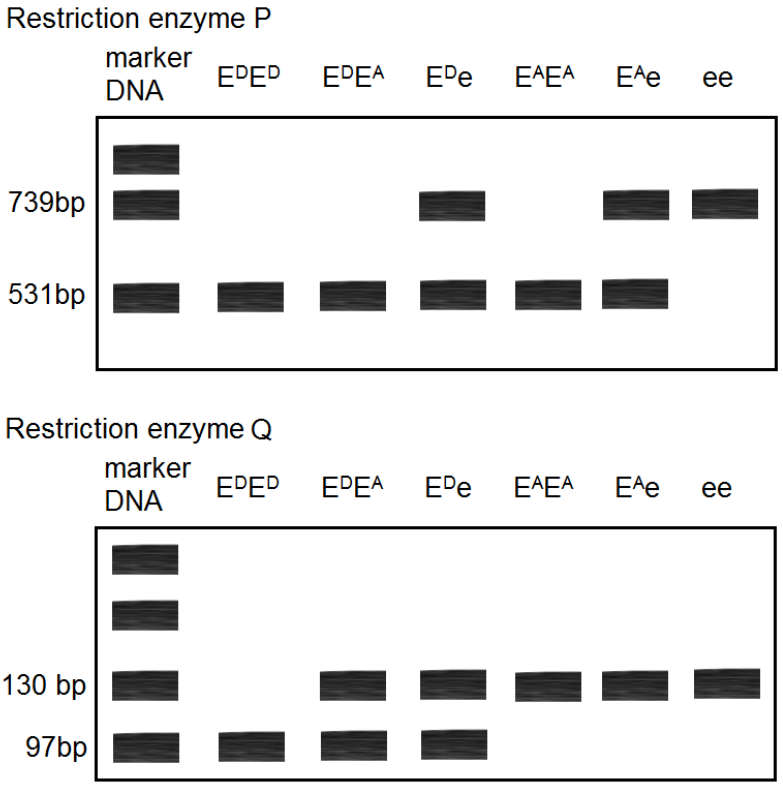

Fig. 1.1

(b) **(i)** Explain why the banding pattern from the same genotype are different when a different restriction enzyme is used. [2]

1. **Different restriction enzymes recognizes different <u>restriction sites</u>;**
2. **thus cuts the DNA at different positions;**
3. **resulting in different number;**
4. **and length of restriction fragments;**

(ii) Explain the role of marker DNA. [2]

1. **Comprises a collection of DNA fragments;**
2. **<u>Compare</u> marker DNA with DNA fragements;;**
3. **to measure length / size / base pair of DNA fragment;**

(iii) State which genotype(s) can be identified by using each of the two restriction enzymes. [2]

P: **ee**;;

Reject: multiple listing

Q: **E^DE^D**;;

Reject: multiple listing

Bovine somatotropin (BST) is an animal hormone used to increase milk production in dairy cows. BST is naturally produced in the cow pituitary gland in small quantity and is used to regulate metabolic processes. With the advent of biotechnology, *BST* gene can be cloned in *Escherichia coli*. The bacteria are grown in bioreactors to produce BST, which is purified to produce hormone for injection.

Fig. 1.2 shows the plasmid map of the plasmid BGH1, used for transformation of *BST* gene into *E.coli*. Sal 1 restriction enzyme was used to cut the pBGH1 plasmid for insertion of *BST* gene.

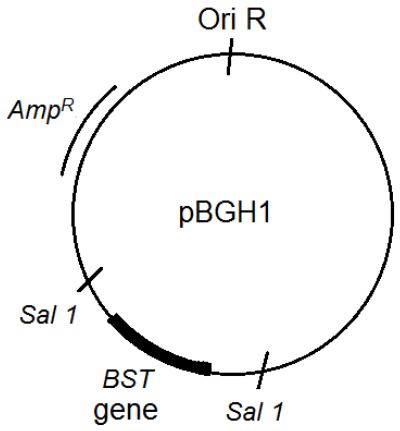

Fig. 1.2

(c) **(i)** Describe the natural role of Sal 1 restriction enzyme. [2]

1. **To protect <u>bacteria</u>;**
2. **by cutting <u>foreign</u> <u>DNA</u>;;**
3. **Such as that of invading viruses;**

(ii) Explain how *Amp^R* gene facilitates *BST* gene cloning. [2]

1. **Functions as a selectable marker;**
2. **to identify <u>transformed</u> bacteria / bacteria that took up <u>pBGH1 plasmid</u>;**
3. **Amp^R gene codes for (β-lactamase) <u>enzyme</u>;**
4. **that inactivate / breakdown ampicillin / allow bacteria to survive (in ampicillin);**

[Total: 13]

2 Omega-3 fatty acids are polyunsaturated fats that are often marketed as health supplements to prevent cholesterol deposits in arterial walls.

In the wild, omega-3 fatty acids are produced by marine algae and accumulate in small fishes that feed on them. These small fishes are then fed to farmed fishes to increase their omega-3 fatty acid content. Farmed fishes high in omega-3 fatty acids serve as a good source for omega-3 fatty acid extraction for the health supplement industry.

A new method to produce omega-3 fatty acids involves insertion of seven genes of the marine algae into oilseed plant, *Camelina sativa.* The resulting seeds contain as much as 200 milligrams of omega-3 fatty acids in a single tablespoon of 'fish oil' extracted. Currently, this 'fish oil' is fed to farmed fishes, in replacement of small fishes.

The effect of human consumption of 'fish oil' from genetically modified *C. sativa* seed pods is still under investigation.

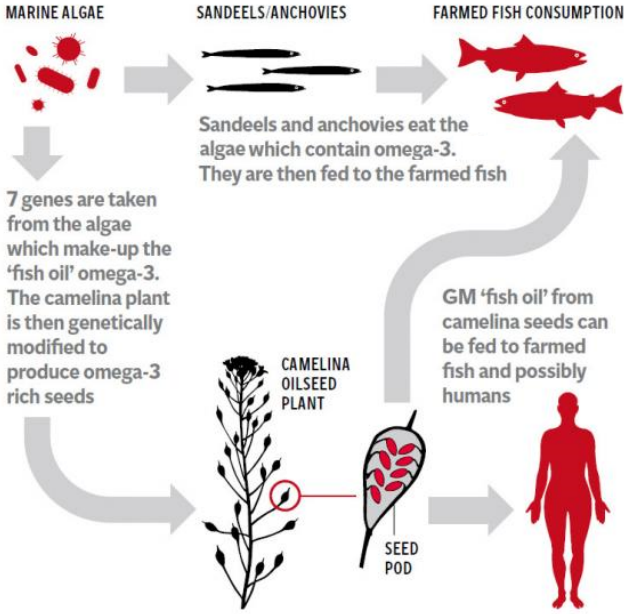

Fig. 2.1

Source: http://www.independent.co.uk/news/science/new-gm-cereal-crop-produces-fish-oil-in-its-seeds-10372772.html

(a) A Ti plasmid containing the seven genes from marine algae is constructed and reintroduced into *A. tumefaciens* before infecting *C. sativa* plant cells. Describe the properties of Ti plasmids that make them suitable as vectors in this process. [2]

1. **Ti plasmids contain T-DNA region which can be integrated into plant chromosome;;**
2. **Ti plasmids are small allowing it to be taken up by *C. sativa* cell;;**
3. **Ti plasmids contain origin of replication, allows independent replication of plasmid and the inserted genes;;**
4. **Ti plasmids contain selectable marker allows for identification of transformed *C. sativa* cells;;**
5. **Ti plasmids have multiple restriction sites, to allow introduction of genes;;**

Any 2

(b) Suggest why crop plants like *C. sativa* are better candidates for genetic modification than fishes. [2]

1. ***C. sativa* cells are totipotent / can give rise to a whole plant;;**
2. **Genetic manipulation can be performed on any somatic/plant cell (to generate crop plants with new traits);;**
3. **To create GM fishes, genes need to be injected into nucleus of fertilised egg;;**

(c) **(i)** With reference to **Fig 2.1**, suggest two benefits of using genetically modified *C. sativa* as an omega-3 fatty acid source for farmed fishes. [2]

1. **Increase the quality of farmed fishes as fishes will be rich in omega-3 oil;;**
2. **Possibility of mass production of genetically modified *C. sativa*, reduces need for fishing, less disturbance to marine food chain;;**
3. **More sustainable source of omega-3 fatty acids, seeds produced can be used to plant new genetically modified *C. sativa*;;**
4. **Easy storage of seed pods as compared to live feed;;**
5. **Reduces possibility of farmed fish contracting disease from the wild fishes;;**

(c) **(ii)** Describe an ethical and a social implication associated with genetically modified *C. sativa* 'fish oil'. [2]

Social:

1. **Healthcare threat due to possible transfer of antibiotic resistance gene to *E. coli* found in the gut;;**
2. **Possible transfer of allergens to human when consuming genetically modified *C. sativa* fish oil;;**

[Max 1 mark]

Ethical:

3. **Violation of organisms intrinsic values through mixing of genes from different species;;**
4. **Lack of consumer awareness due to lack of labelling laws;;**

[Max 1 mark]

The effectiveness of genetically modified *C. sativa* seed pods in preventing cholesterol deposition in arteries was investigated using rodents, to evaluate if they can be used to substitute fish oil from farmed fishes. Effectiveness is evaluated by analysing the levels of total omega-3 fatty acids available in blood and analysis of diameter of rodents' arteries.

Rodents were divided into three groups of 10 and were subjected to treatment for a period of six months as shown below.

Group A: High fat diet enriched with 'fish oil' from genetically modified *C. sativa* seed pods

Group B: High fat diet enriched with fish oil from farmed fishes fed with small fishes

Control: High fat diet without fish oil

The results are summarised in the **Table 2.1**.

Table 2.1

	Group A	Group B	Control
Total omega-3 fatty acids level/ $mmolL^{-1}$	33.7 ± 0.3	27.1 ± 0.1	20.0 ± 0.3
Cross section of artery after 6 months			

(d) **(i)** With reference to **Table 2.1**, comment on whether genetically modified *C. sativa* is a more effective substitute. [3]

1. **Genetically modified *C. sativa* is a more effective substitute;;**
2. **Total omega-3 fatty acid levels in group A is 33.7 $mmolL^{-1}$ while in group B is 27.1 ± 0.1 $mmolL^{-1}$;**
3. **indicating that higher availability/absorption of omega 3- fatty acid from *C. sativa* fish oil compared to natural fish oil;**

4. **Genetically modified *C. sativa* is not a more effective substitute;;**
5. **There is no obstruction/buildup of cholesterol in both group A and B as compared to control group where cholesterol is deposited on arterial walls/ similar diameter of arterial lumen in group A and control group;**

(e) Describe how genetic engineering is used to increase quality of another named crop plant. [2]

1. **Golden rice;**
2. **Introduced genes encoding <u>enzymes</u>;**
3. **which convert a natural compound in rice to beta-carotene;**
4. **a precursor of vitamin A;**

[Total: 13]

3 Severe combined immunodeficiency disease (SCID) is caused by a severe genetic defect often found in newborns. The condition must be diagnosed and treated quickly to prevent serious complications. However, doctors continue to struggle with often ineffective treatment options.

In recent studies, researchers found that blood stem cells may effectively treat SCID caused by a deficiency in the adenosine deaminase (ADA) gene. The ADA gene is critical for the proper functioning of the immune system.

(a) Explain how an individual with SCID caused by ADA deficiency inherited this condition. [2]

1. Inherited a recessive (ADA) allele from father;

2. and a recessive (ADA) allele from mother;

3. Thus has a homozygous recessive genotype for ADA gene;;

Accept: 2 copies of recessive alleles for ADA gene

(b) **(i)** Describe the normal functions of adult stem cells obtained from the bone marrow and stem cells obtained from the zygote. [2]

Adult stem cells from bone marrow serves to

1. Maintain / replenish population;

2. of blood cells in the organism;

Stem cells from zygote responsible for

3. growth / development;

4. of embryo;

(ii) Explain how the properties of blood stem cells allow for them to effectively fight SCID. [3]

1. Blood stem cells are relatively unspecialised cells;

2. thus can differentiate into T cells;

3. under appropriate conditions;

4. through selective changes in genetic activity;

Accept: some genes turned on and some genes turned off

5. Blood stem cells can grow and divide indefinitely;

6. thus replenish (T) cells population indefinitely (via mitosis);

Accept: grow more (T) cells

A trial was carried out on an ADA-SCID patient of age 7 months. Blood stem cells were collected from the patient's bone marrow, transduced with a retroviral vector containing the therapeutic ADA allele, and injected back to the patient. The patient received 8.8 x 10^6 blood stem cells per kg body weight, containing 25% successfully transduced cells in culture.

Fig. 3.1 shows the total T cell count in the blood sample of this patient.

These cells were then isolated and analysed for presence of ADA mRNA in the cytoplasm. **Fig. 3.2** shows the percentage of cells carrying ADA mRNA.

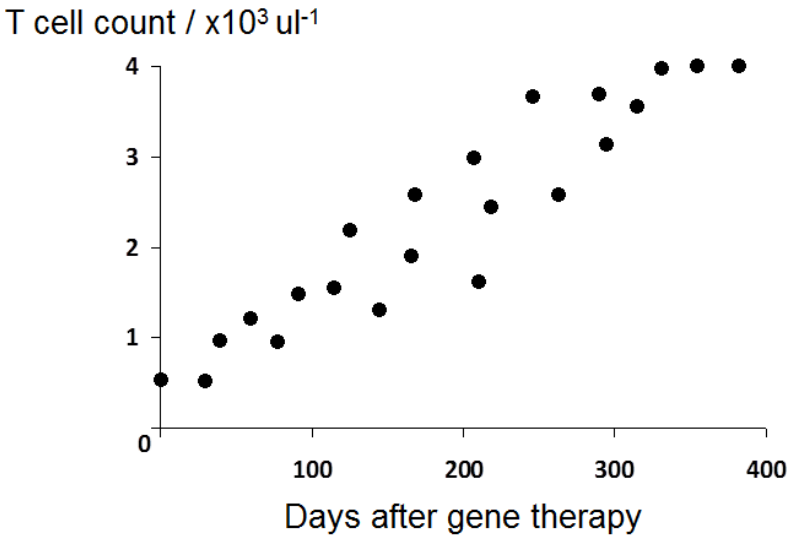

Fig. 3.1

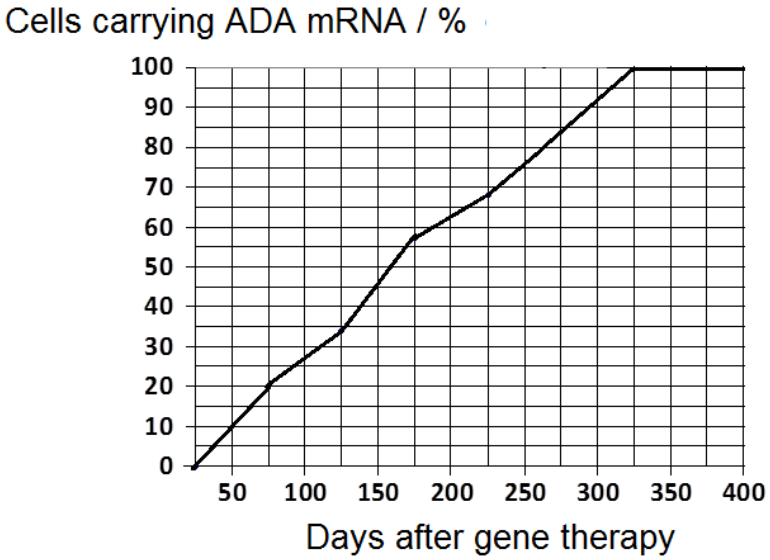

Fig. 3.2

(c) **(i)** Suggest a medical advantage of using patient's own blood stem cells rather than stem cells from a healthy donor. [1]

No cell rejection;

Reject: tissue rejection as blood cells do not form tissues

(ii) Explain why a retroviral vector was chosen for this trial. [2]

1. **Able to insert the therapeutic allele into the host chromosome;**
2. **offering opportunity for long-term stability;**
3. **Able to introduce therapeutic allele into the nucleus;**
4. **protecting it from degradation by (cytoplasmic) enzymes;**

Reject: targeted gene delivery as this is ex-vivo gene therapy / large capacity to carry foreign genes as this is not unique to retroviral vector

(d) With reference to **Fig. 3.1** and **3.2**, account for the success of this gene therapy in treatment of SCID, 350 days after the therapy. [4]

1. **T cell count increases from 500μl^{-1} on day of treatment to 4000μl^{-1} at 350 days after treatment;;**
2. **for immune response;**
3. **Percentage of cells carrying ADA mRNA increases from 0% on day of treatment to 100% at 350 days after treatment;;**
4. **All cells are actively transcribing the ADA gene;**
5. **to produce ADA enzyme;**
6. **thus reduces dATP buildup;**

[Total: 14]

4 Planning question

The use of β-galactosidase for the hydrolysis of lactose in milk is a promising enzyme application in the food processing industry. A large fraction of the human population is lactose intolerant, due to the low levels of β-galactosidase present in the intestine. This causes difficulty in digesting milk products. Therefore lactose hydrolysis, which lowers lactose concentration in milk, allows the lactose intolerant population to consume milk.

Milk products containing lactose may have different pH. In order for β-galactosidase to work, its optimal pH for catalytic activity must coincide with the pH of the milk product. Hence, β-galactosidase is extracted from different sources due to their difference in optimal pH. Sources of β-galactosidase include mould species such as *Aspergillus niger*, and yeast species such as *Saccharomyces cerevisiae*.

ONPG, can be used to study the catalytic function of β-galactosidase as shown in the following reaction:

ONPG is a colourless solution while ONP produced from the reaction is a yellow solution. The reaction can be stopped by adding sodium carbonate solution in 1:1 ratio. By comparing the colour intensity of the resulting product to a colour standard, the concentration of ONP at the end of the reaction can be determined.

Using this information and your own knowledge, design an experiment to determine the respective optimal pH at which β-galactosidase of *Aspergillus niger* and *Saccharomyces cerevisiae* work.

You must use:

- 10% ONPG solution
- *A. niger* β-galactosidase
- *S. cerevisiae* β-galactosidase
- pH buffers for different pH
- 10% ONP solution
- 10% sodium carbonate solution
- Distilled water
- Stopwatch

You may select from the following apparatus:

- Normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, graduated pipette, glass rods, etc.
- Droppers
- Eye protection
- Gloves

Your plan should:

- Have a clear and helpful structure such that the method you use is able to be

repeated by anyone reading it,

- Be illustrated by relevant diagrams, if necessary,
- Identify the independent and dependent variables,
- Describe the method with scientific reasoning used to decide the method so that the results are as accurate and reliable as possible
- Show how you will record your results and the proposed layout of results tables and graphs,
- Use the correct technical and scientific terms,
- Include reference to safety measures to minimize any risks associated with the proposed experiment.

Mark scheme

Theoretical consideration or rationale of the plan to justify the practical procedure, including the effect of pH on rate of enzyme-catalysed reaction. [1]

1. **β-galactosidase speeds up rate of reaction/lower activation energy;**
2. **β-galactosidase has specific active site;**
3. **ONPG fits into active site to form an E-S complex;**

Describe expected results [1]

4. **At optimum pH, rate of reaction/concentration of ONP produced in a fixed time is the highest;**
5. **Beyond optimal pH/ above or below optimum pH, rate of reaction decreases/concentration of ONP produced in a fixed time decreases.;**

Explanation of results [2]

6. **At optimum pH, shape of β-galactosidase active site intact;**
7. **Highest number of enzyme-substrate complex form per unit time;**
8. **Above and below optimal pH, ionic and hydrogen bonds between R group of amino acid residues at active site disrupted;**
9. **Number of enzyme-substrate complex formed per unit time decreases;**

Variables [2]

10. **IV – 5 pH of equal intervals e.g. pH4, pH6, pH8, pH10, pH12);**
11. **DV – concentration of ONP produced;**
12. **Controlled variable – CV w quantity; CV w quantity;**

Procedure [4.5]

13. **Correct dilution of ONP;**
14. **Use diluted ONP to construct colour standards;**
15. **Investigation carried out using 5 different pH buffers;**
16. **Description of correct sequence of adding reagents to test tube (i.e. enzyme/substrate added just before start time;**
17. **Description of quenching reaction using equivolume of sodium carbonate;**
18. **Description of concentration determination using colour standard;;**

19. **Reaction using *A. niger* β-galactosidase;**
20. **Reaction using *S. cerevisiae* β-galactosidase;**

Reliability of results [0.5]

21. **Three replicates/repeat experiment two more times/statistical test;**

Data recording and manipulation [2]

22. **Data recording for *A. niger* β-galactosidase and *S. cerevisiae* β-galactosidase;**
23. **Correct headings and units for Table showing effect of pH on concentration of ONP produced/rate of reaction;**
24. **Replicates and average;**
25. **Correct labelling of axis (x-axis - pH, y-axis - concentration of ONP/rate of reaction);**
26. **Correct shape of graph;**

Risk assessment [1]

27. **Description of 2 risk and precaution;;**

[Total: 12]

Section B (20 marks)

Answer **all** question.

Write your answers on the separate answer paper provided.
Your answers should be illustrated by large, clearly labelled diagrams, where appropriate.
Your answers must be in continuous prose, where appropriate.
Your answers must be set out in sections **(a)**, **(b)** etc., as indicated in the question.

A **NIL** return is necessary if you have not attempted this section.

5. (a) Explain how restriction fragment length polymorphism (RFLP) analysis facilitates the construction of a genomic linkage map. [6]

1. **A linkage map portrays the order of genes along a chromosomes;**
2. **and their relative distance;**
3. **RFLPs serves as a genetic marker for a locus in the genome;**
4. **Linkage map can be constructed based on recombination frequencies;**
5. **between two RFLPs;**
6. **and the map units calculated;**
7. **using the formula;**

$$\text{Recombinant frequency} = \frac{\text{number of recombinant offspring}}{\text{total number of offspring}} \times 100\%$$

8. **Two homozygous individuals with different RFLPs at both loci are crossed;**
9. **to produce a heterozygote;**
10. **The heterozygote is then crossed with a homozygous individual;**
11. **The resulting offspring will have more parental RFLP combinations than recombinant RFLP combinations;;**

Reject: offspring phenotypic ratio is not 1:1:1:1 ratio, without reference to expected phenotypic outcome

12. **The genotype is detected using two probes;**

Accept: nucleic acid hybridisation

13. **One for each RFLP;**
14. **and analysed using Southern Blot;**

Accept: gel electrophoresis followed by autoradiography

(b) Plant tissue culture techniques allows for aseptic growth of excised plant parts *in-vitro*. Describe how this is carried out. [9]

1. **Cells from meristematic regions of a plant are excised;;**
2. **and sterilized;;**
3. **before transferring to culture vessels containing essential minerals / vitamins / amino acids / fixed carbon (any 2);;**
4. **Cells grown in culture to form callus tissue;;**
5. **Number of calli is increased via subculturing;;**
6. **Callus can be induced to differentiate by varying the ratio of plant growth regulators;;**
7. **Low level of cytokinin and high level of auxin triggers root growth;;**
8. **High level of cytokinin and low level of auxin triggers shoot growth;;**
9. **Organised callus tissues form a plantlet;;**
10. **which is acclimatised in enriched soil;;**
11. **before transferring to a greenhouse to grow into a whole plant;;**

(b) *"The Human Genome Project was one of the great feats of exploration in history… (giving) us the ability, for the first time, to read nature's genomic blueprint for building a human being." – National Human Genome Research Institute.* [5]

Discuss the main objectives of this project.

1. **To map all human genetic markers by identification of their chromosomal location;;**
2. **To construct a detailed physical map of the entire human genome;;**
3. **To determine the base sequence of all 24 human chromosomes;;**
4. **To develop a technology for the management of human genomic information;;**
5. **To serve as an umbrella for similar mapping / sequencing of the genomes of other organisms;;**

[Total: 20]