## 2016 H2 Biology Prelim Paper 3 ANSWERS

## **Question 1**

(a) The bacterial plasmid, pBR322, was used as a vector for Gene X as shown in Fig. 1.1 below.



Fig. 1.1

Gene X was inserted in the *Bam*HI restriction site. pBR322 also contain the *Eco*RI and *Hpa*I restriction sites. The target sites for these restriction enzymes are shown in the table below. The lines drawn in each sequence show where the enzyme cuts the DNA molecule.

restriction enzyme	specific target base sequence of DNA				
EcoRI	G A A T T C C T T A A G				
BamHI	G G A T C C C C T A G G				
HpaI	G T T A A C C A A T T G				

(i) With reference to Fig. 1.1, explain how two properties of plasmid pBR322 allow it to be used as a vector. [2]

- Presence of origin of replication; so inserted gene can be replicated;
- Has ampicillin and tetracycline resistance genes; as selection markers / allow identification of host cells that have successfully taken up the recombinant plasmid

(ii) Outline the steps taken to produce the recombinant plasmid shown in Fig. 1.1. [2]

- Cut plasmid and gene X with BamHI restriction enzyme;
- "Sticky ends" generated;
- Allow plasmid and gene to anneal with ref. complementary base pairing;

1

• Add ligase to seal the nicks in the sugar-phosphate backbone / form phosphodiester bonds between the ends of the cut plasmid and gene;

(iii) Explain the disadvantage that would arise if gene X was to be inserted into the *Hpa*l restriction site instead of the *Bam*HI site. [2]

- Hpal generates "blunt ends";
- No hydrogen bonds to hold the cut plasmid and gene together;
- Ref. extra step that will require linker;
- To generate "sticky ends"
- (b) Calcium chloride heat shock treatment was then used to introduce the recombinant plasmid into *Escherichia coli*. However, the process of creating recombinant plasmids is typically not 100% efficient. Often, a mixture of re-annealed plasmid and re-annealed DNA is produced along with the recombinant plasmid. These may be taken up by the bacteria as well. This necessitates the process of selecting for the bacteria that have successfully taken up the recombinant plasmid. As such, the bacteria was first plated onto a nutrient agar plate containing ampicillin. Replica plating was subsequently carried out onto a nutrient agar plate containing tetracycline. Bacterial growth on both plates is shown in **Fig. 1.2**.



Fig. 1.2

With reference to Fig 1.2,

- (i) Circle the colonies that were successfully transformed with the recombinant plasmid. [1]
- (ii) Account for the difference in colony numbers in Plate A and B. [3]
- Plate A selects for all successfully transformed cells/ taken up plasmid with ampicillin gene; (maybe recombinant plasmid or re-annealed plasmid);
- Selected against / killed off cells which took up re-annealed DNA;
- Plate B contain 3 fewer colonies compared to Plate A;
- Missing colonies had successfully taken up the recombinant plasmid;
- where gene X had been inserted into tetracycline gene and disrupted it (ref. to insertional inactivation) / colonies lost tetracycline resistance and died;

- Therefore comparing the difference in colony numbers between Plate A and B will allow us to identify recombinant cells/ colonies;
- (c) In a separate cloning experiment, *E.coli* cells were transformed with another type of plasmid carrying a different selectable marker. Fig. 1.3 shows the results of plating the transformed *E.coli* cells onto an agar plate with the appropriate substances.



- (i) Explain why some colonies appeared white while others appeared blue. [4]
- The cells must have been transformed by plasmids containing intact lacZ gene as the selectable marker;
- The agar plate must have contained X-gal and IPTG;
- lacZ gene codes for β-galactosidase enzyme;
- β-galactosidase enzyme catalyses the conversion of X-gal in the agar from colourless to blue;
- Hence, colonies that contain the reannealed / non-recombinant plasmids appeared blue.
- Should the lacZ gene in the plasmid be disrupted as a result of insertion of foreign DNA / gene, ref. to insertional inactivation of lacZ gene;
- no functional β-galactosidase enzyme will be produced and thus no blue product is formed;
- Hence, colonies that contain the recombinant plasmids appeared white.

- (ii) Suggest why replica plating was not necessary in this experiment. [1]
- By their colours, colonies that contain the recombinant plasmids (appeared white) can be differentiated from those that do not (appeared blue);
- Replica plating is necessary only when the selection process kills off the desired colonies.
- In the case of using two antibiotic resistance genes as the selection markers, the insertion of foreign DNA / gene disrupts only one of the two antibiotic resistance genes in the plasmid.
- To identify colonies that contain the recombinant plasmids, the colonies must be treated with two types of antibiotics.
- Those that survived one antibiotic treatment but not the other would be the desired colonies.
- However, adding both antibiotics to the same agar plate would kill off the desired colonies.
- To get living cells that contain the recombinant plasmids, replica plating must be done. )

**d)** In a separate cloning experiment, another plasmid pBR33 was used to introduce Gene Z into a different strain of *E.coli* bacteria. Gene Z was inserted into one of the three genetic markers found in pBR33 – neomycin resistance gene, kanamycin resistance gene and streptomycin resistance gene.

The bacteria were then plated onto nutrient agar plate containing neomycin. Replica plating was subsequently carried out onto nutrient agar plate containing streptomycin. Bacterial growth on the two plates is shown in Fig. 1.4 below.



Account for the results obtained in Fig. 1.4. [3]

- Gene Z inserted in kanamycin-resistant gene;
- Insertional inactivation;
- 3 functional genes in re-annealed/non-recombinant plasmids vs 2 functional genes is recombinant;

- Bacteria with recombinant plasmid can survive in presence of neomycin and streptomycin
- Bacteria with re-annealed plasmid can survive in all 3 antibiotics
- Both plates have same number; and position; of colonies; QV: 10 colonies
- Both plates do not contain any non-transformed bacteria; consists of transformed cells with recombinant and non-recombinant plasmid

#### Alternative:

- Gene Z inserted neomycin-resistant gene;
- So plate N has killed off all bacteria with recombinant plasmids and nontransformed bacteria
- So when is done on from Plate N to plate S, only the bacteria that survive (i.e. the recombinant bacteria) are picked up by the nitrocellulose membrane and transported to plate S;

Question 2

[Total: 18]

Explain why two primers are used for polymerase chain reaction. [2]

- Ref. to the 2 primers as forward and reverse primers [1/2]
- Flank the targeted sequence to be amplified [1/2]
- Amplify large quantities of a specific sequence of DNA in a short period of time [1/2]
- 1 primer anneals to 1 of the separated DNA strands after denaturation [1/2]
- Provide free 3' OH for *Taq* polymerase to elongate the complementary strands of both templates to produce 2 DNA molecules [1/2]

Duchenne muscular dystrophy is a genetic disease in which there is a progressive loss of muscle mass, leading to physical weakness, difficulty in standing and walking, and eventually paralysis and death. Early symptoms of the disease can only be observed between the ages of 2 and 3 in most patients. A group of doctors and medical biologists discovered a RFLP marker, found on the same chromosome as the disease gene, which can be used in the screening of the disease during pregnancy.

To investigate the effective of the RFLP marker in disease screening, samples of DNA were obtained from a family known to have the disease. The RFLP locus was isolated and amplified using polymerase chain reaction, which was then mixed with *Bam*HI restriction enzymes. The pedigree tree of the family and results of gel electrophoresis are shown in Fig. 2.1.



(a) With reference to Fig. 2.1,

- (i) state the mode of inheritance of the Duchenne muscular dystrophy. [1]
- Sex-linked recessive [1]

(ii) explain why there are different fragment lengths after restriction digest. [3]

- Ref. to the RFLP marker having 2 alleles [1/2]
- 1 allele contains a *Bam*HI restriction site while the other does not [1/2]
  > Variation due to a mutation [1/2]
- Allele with restriction site produces 2 RFLP fragments of 360 bp and 250 bp [1/2]
  Due to digestion by *Bam*HI restriction enzyme [1/2]
- Allele without restriction site produces 1 RFLP fragment of 610 bp [1/2]
- BamHI restriction enzyme cannot recognise the mutated sequence resulting in no restriction digest [1/2]

(iii) Explain the difference in the band patterns between the father and the daughter. [3]

- The daughter has **thicker bands** than the father corresponding to the same sizes [1/2]
  - > 360 and 250 bp [1/2]
  - Twice as thick [1/2]
- The RFLP marker is found on the X chromosome [1/2]
  - Father has only 1 X chromosome thus 1 copy of the allele of the RFLP marker [1/2]
  - Daughter has 2 X chromosomes thus 2 copies of the allele of the RFLP marker [1/2]
- Ref. to thickness of the bands due to amount of RFLP fragments [1/2]

- (b) Some years later, the baby boy with unknown phenotype is born and has reached 2 years of age. Clinical diagnosis reveals that he too suffers from Duchenne muscular dystrophy.
- (i) Explain why the band pattern of the baby boy is different from that of his older brother even though both are with the disease. Assume no new mutations occurred in the disease gene or RFLP locus. [2]
- Crossing over occurred during gamete formation in the mother [1/2]
- Between the disease gene and RFLP locus [1/2]
- Giving rise to gametes with a X chromosome with **different combinations of alleles** [1/2]
  - X chromosome inherited by older brother has RFLP allele producing 610 bp fragment linked to the disease allele [1/2]
  - X chromosome inherited by baby boy has RFLP allele producing 360 bp and 250 bp fragments linked to the disease allele [1/2]
- (ii) Suggest an ethical implication that may arise due to the use of this RFLP marker to screen for Duchenne muscular dystrophy. [1]

Any 1:

- Stigmatisation of the parents and child even before the onset of the disease
- Parents may want to **terminate the pregnancy** if the unborn baby is diagnosed with the disease
- Parents may have to pay a higher premium for child insurance even before the onset of the disease
- AVP

## **Question 3**

[Total: 12]

(a) Patients with severe combined immunodeficiency disorder (SCID) are vulnerable to serious infections and death. There are two main types of SCID, X-SCID and ADA-SCID. Besides the location and difference in their modes of inheritance, give two differences between the two types of SCID.

Difference	X-SCID	ADA-SCID	
1	Mutation of IL2RG (interleukin-2	Mutation of gene coding for	
(Name of gene; Idea of what the	receptor gamma) gene	adenosine deaminase (ADA)	
gene codes for / function of normal protein)	that codes for <b>gamma chain</b> on <b>lymphocyte receptor</b>	involved in <b>purine</b> metabolism / breakdown of deoxyadenosine	
<b>2</b> (Consequence)	Defective receptor unable to activate cell signal pathways leading to normal development of specific white blood	Hence, ADA deficiency leads to the accumulation of deoxyadenosine which kill developing white blood cells	

(b) Gene therapy can be used to treat SCID by introducing retrovirus containing the normal gene into hematopoietic stem cells (HSCs). The HSCs are then infused into the patient. Explain how this choice of vector and target cells may theoretically lead to long term treatment of the disease. [2]

Retroviral vectors are able to **integrate** the **normal gene into the** <u>chromosome</u> [1/2]. The HSCs are able to undergo **long term self-renewal** by **mitotic** <u>cell</u> <u>divisions</u> [1/2]

Hence, patient will have the normal gene in his/her white blood cells since the **normal gene would be replicated and passed to the daughter cells** [1/2] When the HSCs **differentiate into white blood cells** [1/2], the normal **gene can be expressed** and produce the **normal enzyme** [1/2]

(c) In one attempt to treat ADA-SCID, hematopoietic stem cells (HSCs) from the bone marrow of baby X patient were collected and treated with retrovirus containing the ADA gene before infusing them back.

Genomic DNA from the T lymphocyte cells of baby X was later extracted. The extracted DNA was digested with a restriction enzyme and subjected to Southern blot analysis using a specific probe that binds to a known RFLP marker found within the gene associated with the disease.

The RFLP allele 1 associated with the disease allele gives rise to a 0.8kb band while the RFLP allele 2 associated with the normal allele gives rise to a 2.2kb band.



(i) With the information provided, draw to indicate the position of another restriction site and the position where the probe binds to. You should also indicate the length of the restriction fragments that would be produced. [1] The RFLP band patterns from baby X's normal brothers, Tom and John, are shown in Fig. 3.1.



#### Fig 3.1: Results of Southern Blot analysis of DNA extracted from T lymphocytes

(ii) With reference to Fig 3.1, explain the difference in the band pattern of Tom and John. [2]

ADA-SCID is an **autosomal recessive condition**. [1/2] Tom has **one copy of the <u>recessive</u> and one copy of the <u>dominant</u> allele / heterozygous. [1/2] Ref to two bands of <b>2.2kb and 0.8kb** [1/2]

John has **two copies of the normal <u>dominant</u> allele / homozygous <u>dominant</u> [1/2] Ref to <b>one thicker band of 2.2kb** [1/2]

(iii) Draw the expected band pattern of baby X after gene therapy treatment. [1]

Over the next 360 days after infusion, the total number of various blood cells (e.g. red blood cells and white blood cells) of baby X were recorded at regular intervals.



Fig 3.2

(iv) With reference to Fig 3.2, describe the effectiveness of the treatment in Baby X. [1]

The treatment was **ineffective / effective only for a short term** [1/2] **Decline of the white blood cells** / QV: from about **0.5** x10<sup>3</sup>/µL in day 60 to 0 by day 180 [1/2]

(v) Suggest a reason for your answer. [1]

Any one:

The gene inserted into the chromosome **could not be expressed** The inserted gene was **mutated** and so **could not produce a functional enzyme** 

[Total: 10]

(4) An orange plantation owner wants to find out the amount of ascorbic acid (vitamin C) that his breed of oranges produces. He believes that his oranges produce the most vitamin c compared to the standard orange breeds which typically contain 0.8 to  $1.6 \text{ mmolL}^{-1}$ .

The amount of ascorbic acid present in a sample can be determined using a bioassay method. At pH 7 and above, ascorbic acid reduces solutions of the dye dichlorophenol indophenol (DCPIP) from blue to colourless. For the bioassay to work, the pH of the samples must be adjusted to pH7 - 9. Ascorbic acid does not chemically change when neutralised by sodium hydroxide or when boiled.

Using this information and your own knowledge, design an experiment to determine the validity of the plantation owner's claim that orange juice from his plantation contains higher concentrations of ascorbic acid.

Your planning must be based on the assumption that you have been provided with the following equipment and materials which you must use:

- 100 cm<sup>3</sup> of 5.0 mmolL<sup>-1</sup> stock solution of ascorbic acid, adjusted to pH 7
- 100 cm<sup>3</sup> distilled water
- 100 cm<sup>3</sup> molten agar containing DCPIP
- Sterile petri dishes
- 1ml syringe
- plastic straw to create wells in the agar plate
- Ruler / 2mm graph paper
- Labels
- Timer , e.g. stopwatch
- Forceps
- Bunsen burner
- Normal laboratory glassware e.g. test tubes, beakers, graduated pipettes, droppers, glass rods etc
- 10 cm<sup>3</sup> orange juice, supplied by the plantation owner
- 10% sodium hydroxide solution
- pH indicator paper to indicate alkaline pH

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 variable,
- describe the method with the scientific reasoning used to decide the method so that results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of tables and graphs,
- use correct technical and scientific terms,
- include references to safety measures to minimize any risk associated with the proposed experiment.

[Total: 12]

#### **Proposed answer**

#### Introduction

- •Ascorbic acid reduces blue DCPIP to colourless. .
- Increase in concentration of ascorbic acid will increase the rate of decolourisation of DCPIP
- •Different concentrations of ascorbic acid can be created from the stock solution. A standard curve of the amount of decolourisation of DCPIP by the different concentrations of ascorbic acid can be created. The amount of ascorbic acid in orange can be determined by reading off the standard curve

#### Procedure

1. Obtain 10cm<sup>3</sup> of different concentrations of ascorbic acid solution by dilution.

Concentration of	Volume of 5.0	Volume of distilled	
ascorbic acid solution /	mmolL-1 ascorbic	water / cm <sup>3</sup>	
mmolL-1	acid solution / cm <sup>3</sup>		
5	10	0	
4	8	2	
3	6	4	
2	4	6	
1	2	8	
0	0	10	

- Pour the molten agar containing DCPIP into the petri dishes and allow the agar to cool.
- 2. Once the agar is cooled, use the plastic straw to make eight equal sized wells in the agar gel plate. Ensure that the wells are well-spaced.
- 3. Prepare a control experiment using boiled and cooled orange juice, following the same experimental procedures and conditions, to show that the decolourisation of DCPIP is due to the action of ascorbic acid and not due to the action of any enzymes in the juice.
- 4. Add 10% sodium hydroxide solution to the boiled and cooled orange juice, drop by drop with a dropper, until the pH is between 7 to 9. Check the pH by removing a drop of solution with a clean glass rod and placing it on indicator paper.
- 5. Neutralise the fresh orange juice in the same manner as described in step 4.
- 6. Using the 1 ml syringe, place 0.2 ml of each of the ascorbic acid solutions prepared according to the dilution table, 0.2 ml of orange juice and 0.2 ml of boiled and neutralised orange juice into one well each. Label the wells.

Explain how to determine concentration of ascorbic acid in oranges using the standard curve [1m]

Describe how to obtain different concentrations of ascorbic acid [1m]

Describe the settling up of the DCPIP agar plates and wells in the plates[1m]

Describe control to prove reaction is due to ascorbic acid in the orange juice and is not enzyme catalysed [1m]

Describe neutralisation of fresh orange juice.[1m]

State appropriate volumes scorbic acid [1m]

13

- 7. Replace the lid of the petri dish and leave the plates on the table for one hour.
- 8. After one hour, place the dish on the graph paper and measure the diameter of each of the rings where the blue DCPIP has been decolourised
- 9. Repeat step 1 to 7 three times.



Describe the measurement of ring of decolourisation [1m] Describe repeats [1m]

Draw a labelled diagram [1m]

10. Record the results in the table below and calculate the area of decolorisation

Concentration of	Diam	Area decolourised/ mm <sup>2</sup>			
	Experiment 1	Experiment 2	Experiment 3	Average	
5					
4					
3					
2					
1					
0					
Sample of					
neutralised					
orange juice					
Sample of boiled					
and cooled					
orange juice					
which has been					
neutralised					

[1m]



From the standard curve drawn, calculate the concentration of ascorbic acid found in the sample of orange juice from the area of decolorisation obtained from the experiment with the sample of orange juice. If the concentration is higher than 0.8 to 1.6 mmolL<sup>-1</sup>, the plantation owner's claim of his breed producing a higher concentration of Vitamin C than standard orange breeds is valid. [1m]

## Safety

Sodium hydroxide and ascorbic acid may cause irritation when in contact with skin. Wear gloves when handling these reagents. [1m]

## Question 1

- (a) Outline the procedure for the production of human growth hormone by genetic engineering techniques. [8]
- (b) Describe the benefits of the Human Genome Project. [8]
- (c) Discuss the ethical concerns that have arisen from genetically modified organisms. [4]

## Question 5

(a) Outline the procedure for the production of human growth hormone by genetic engineering techniques. [8]

## Isolation of human growth hormone - max 2

- Human growth hormone mRNA (1/2)
- extracted from anterior pituitary gland is used (1/2)
- as template to synthesize **complementary DNA** (**cDNA**) (1/2)
- Using the enzyme **reverse transcriptase** (1/2)
- Reason: *E.coli*, being prokaryotes → lack mRNA processing machinery (1/2)
- Introns are not excised and exons spliced (1/2)
- Therefore, **no mature mRNA** can be formed from the **eukaryotic gene** (1/2)
- Ref to use of **DNA polymerase**  $\rightarrow$  double stranded DNA (1/2)

#### Formation of recombinant DNA – max 2

- Both plasmid vector and cDNA are cut with restriction enzyme that produces blunt ends [1]
- (In separate reactions) terminal transferase (1/2)
- is used to add extra guanines to vector and extra cytosines to the cDNA (or vice-versa) [1]
- To create complementary sticky ends (1/2)

#### Or

- Both plasmid vector and cDNA are cut with same restriction enzyme (1/2)
- Any e.g. of appropriate restriction enzyme (HindIII, BamHI, etc) (1/2)
- To create **complementary sticky ends** (1/2)

- DNA ligase is used to facilitate the joining of cDNA to vector to form recombinant DNA (1/2)
- By forming **phosphodiester bond** between the sugar and the phosphate group / nucleotides (1/2)

## Transfer of recombinant DNA to bacteria host followed by screening – max 3

- Bacteria (e.g. *E.coli*) is transformed with recombinant DNA (1/2)
- By CaCl<sub>2</sub> heat-shock method (1/2)
- Transformed bacteria with the recombinant DNA are selected (1/2)
- In the presence selection markers E.g. **antibiotics resistance genes** transformed cells survive in the presence of antibiotics (1/2)
- Identification of **correct transformed colonies** with **recombinant plasmid** from transformed colonies with re-annealed vector only by *(either one)* **blue-white screening** or **replica plating** (1/2)
- Elaboration of either method:
  - 1. <u>Replica plating</u>
  - Bacterial cells are plated on a nutrient plate with **one antibiotics** and then replica plated on **another plate with another antibiotics** or idea of 2 plates (each with an antibiotics) are used (1/2)
  - Colonies with re-annealed vector only are **resistant to both antibiotics** because both antibiotic resistant genes are intact. (1/2)
  - Colonies with correct recombinant DNA are resistant to one antibiotics but susceptible to another because the corresponding antibiotic resistant gene is disrupted during insertion of the gene (or ref to insertional inactivation) (1/2)
  - Hence **comparing the position of the colonies on both plates** help to identify correct colonies (1/2)

Or

## 2. Blue-white screening

- Bacteria cells are plated on a nutrient plate containing an **antibiotics** and the substrate **X-gal** (1/2)
- Colonies with re-annealed vector only are resistant to antibiotics / contain antibiotics resistance gene and intact / functional β-galactosidase / Lac Z gene. (1/2)
- β-galactosidase enzyme that act on X-gal resulting in blue colonies (1/2)
- Colonies with correct recombinant DNA will appear white because the βgalactosidase / Lac Z gene is disrupted during insertion of the gene (or ref to insertional inactivation) (1/2)

# Culture of correct transformed cells and extraction and purification of insulin - max 1

- And cultured in a nutrient / growth medium / fermenter (1/2)
- Ref to **prokaryotic promoter** inserted next to eukaryotic gene (1/2)
- The eukaryotic **gene** is **expressed** in the bacteria (1/2)
- The protein is **extracted** and **purified** for use (1/2)

(b) Discuss the benefits of the Human Genome Project. [8]

## A. Molecular medicine (no marks for heading; max 2 mks, @ 1 mk)

1 Earlier diagnosis/detection of genetic diseases;

2 Gene therapy;

3 Rational drug design/control systems for drugs/rational drug design/pharmacogenomics & custom drugs;

#### B. Energy and Environmental Applications (max 1 mk, @ 1 mk)

4 Use microbial genomics research to create new energy sources (biofuels); 5 Use microbial genomics research to develop environmental monitoring techniques to detect pollutants ;

6 Use microbial genomics research for safe, efficient environmental remediation;

#### C. DNA Forensics (max 3 mk, @ 1 mk)

7 Identify potential suspects whose DNA may match evidence left at crime scenes;

8 Exonerate persons wrongly accused of crimes;

9 Identify crime and catastrophe victims;

10 Establish paternity and other family relationships;

11 Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers);

12 Detect bacteria and other organisms that may pollute air, water, soil, and food;

13 Match organ donors with recipients in transplant programs;

14 Determine pedigree for seed or livestock breeds;

15 Authenticate consumables such as caviar and wine;

## D. Agriculture, Livestock Breeding, and Bioprocessing (max 1 mk, @ 1 mk)

16 Healthier, more productive, disease-resistant crops/ farm animals / higher yield;

17 More nutritious produce ;

18 Edible vaccines incorporated into food products;

19 New environmental cleanup uses for plants like tobacco;

## E. Bioarchaeology, anthropology, evolution and human migration (max 1 mk, @ 1 mk)

20 Study human evolution (through germline mutations in lineages);

21 Study of migration of diff pop groups based on female genetic inheritance/lineage and migration of males via Y chromosomes;

22 Compare breakpoints in the evolution of mutations with ages of populations and historical events;

## F. Risk assessment (@ 1 mk)

23 Assess health damage and risks caused by radiation exposure/mutagenic chemicals/ cancer-causing toxins;

Total max: 8 mk

(c) Discuss the ethical concerns that have arisen from genetically modified organisms. [4]

## Ethical (deals with right or wrong; equity; fairness)

- Genetic manipulation of plants may not be acceptable by some as it involves altering the genetic makeup of the plants which can be seen as tampering with nature;;
- Religious groups with strong dietary restrictions may not be informed about the genetic content of the food they are eating and may unknowingly consumed GM food with genes from unacceptable sources;; (example to illustrate)
- Patenting of the transgenic crops is viewed as unethical as it promotes the treatment of living things as mere objects or commodities to be owned and redesigned at will;
- Patenting of the transgenic crops by companies in order to ensure they profit from the technique may end up causing farmers to be dependent on them;; (Use example of the patent for the genetically engineered seeds)
- Companies with the patents become very rich at the expense of the farmers or consumers who have to pay for the high cost of the seeds or plants;;
- World food production may be controlled/ dominated by a small number of large biotechnology companies with the technical know-how;;