



MACHAKOS UNIVERSITY

University Examinations for 2018/2019 Academic Year

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF AGRICULTURAL EDUCATION AND EXTENSION

FOURTH YEAR SECOND SEMESTER EXAMINATION FOR THE DEGREE OF
BACHELOR OF SCIENCE IN AGRICULTURAL EDUCATION AND EXTENSION

SBT 420: AGRICULTURAL BIOTECHNOLOGY

TIME:

DATE:

INSTRUCTION TO CANDIDATES: Answer ALL questions from Section A and any other TWO from Section B:

SECTION A: COMPULSORY: (30 MARKS)

QUESTION ONE

- a. Differentiate between the structures of DNA and RNA (2 Marks)

Dna contains contain adenine, guanine and cytosine bases and thymine, while. RNA nucleotides contain adenine, guanine and cytosine bases, but instead of thymine they have uracil (U).

Dna is double stranded while RNA is a single strand

DNA nucleotide: lacks a hydroxyl group on the 2' carbon of the sugar (i.e., sugar is deoxyribose).

RNA nucleotide: has a hydroxyl group on the 2' carbon of the sugar (i.e., sugar is ribose).

- b. Describe the components of DNA molecule (3 Marks)

5-carbon sugar,- The deoxyribose

Phosphate group

Nitrogenous base ATGC

- c. Explain the functions of chromosomes (2 Marks)

Chromosome, the microscopic threadlike part of the cell that carries hereditary information in the form of genes.

The chromosome holds not only the genetic code, but many of the proteins responsible for helping express it

- d. i. Explain the Six requirements of polymerase chain reaction (PCR) (3 Marks)

A PCR reaction contains **the target double-stranded DNA, two primers** that Hybridize to flanking sequences on opposing strands of the target, **all four deoxyribonucleoside triphosphates** and a **DNA polymerase** along with **buffer,co-factors of enzyme** and **water**.

- ii. Describe the steps involved in polymerase chain reactions (3 Marks)

1. **Denaturation** (96°C): Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.
2. **Annealing** (55 - 65°C): Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.
3. **Extension** (72°C): Raise the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA.

- e. Describe the following techniques, indicating the applications of each:

- i. Gel electrophoresis (3 Marks)

Gel electrophoresis is a technique used to separate DNA fragments (or other Macromolecules, such as RNA and proteins) based on their size and charge.

Electrophoresis involves **running a current through a gel containing the DNA molecules** of interest. **Based on their size, the molecules will travel through the gel at different speeds, allowing them to be separated from one another.** Then **visualization under UV:**

- ii. Recombinant DNA technology/gene cloning (5 Marks)

1. Isolation of DNA [gene of interest] fragments to be clone and Insertion of isolated DNA into a suitable vector to form recombinant DNA.
2. Introduction of recombinant DNA into a suitable organism known as host.
3. Selection of transformed host cells and identification of the clone containing the gene of interest.
- 4 Multiplication/Expression of the introduced Gene in the host.
5. Isolation of multiple gene copies/Protein expressed by the gene and Purification of the isolated gene copy/protein

Applications of Gene Cloning/rDNA

- A particular gene can be isolated and its nucleotide sequence determined
- Protein/enzyme/RNA function can be investigated
- Mutations can be identified, e.g. gene defects related to specific diseases, Organisms can be 'engineered' for specific purposes, e.g. insulin production, insect resistance, etc.
- Genetic engineering of plants and animals

- f. State the important biological tools used in recombinant DNA technology (2 Marks)

Requirements (biological tools) for Gene Cloning/rDNA (Cell-based)

1. **DNA fragment** containing the desired genes to be cloned.
2. **Restriction enzymes** and **ligase enzymes**.
3. **Vectors** – to carry, maintain and replicate cloned gene in host cell.
4. **Host cell**– in which recombinant DNA can replicate.

g. i) Describe the Three techniques used in protoplast fusion (3 Marks)

1. Electrofusion
2. Polyethylene glycol - induced fusion (PEG)
3. High Ca^{2+} , high pH

ii) Explain four applications of protoplast in agriculture (4marks)

- In the production of Cybrid
- For Somatic Hybridization to overcome sexually incompatible species
- Ingesting "Foreign" material into cytoplasm
- For DNA transformation
- Used to study wall synthesis and decomposition
- Studied as Single Cell System

SECTION B: ANSWER ANY TWO QUESTIONS (40 MARKS)

QUESTION TWO (20 MARKS)

a. Describe the central Dogma in molecular biology (10. Marks)

Def 1mk, replication, transcription, translation@3mks

The 'Central Dogma' is the process by which the instructions in DNA are converted into a functional product. The central dogma of molecular biology explains the flow of genetic information, from **DNA to RNA**, to make a functional product, **a protein**.

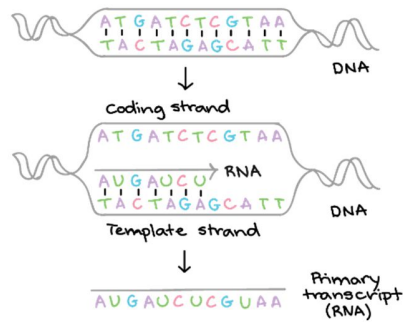
DNA replication is semi-conservative. This means that each of the two strands in double-stranded DNA acts as a template to produce two new strands.

Replication relies on complementary **base pairing** that is the principle explained by Chargaff's rules: adenine (A) always bonds with thymine (T) and cytosine (C) always bonds with guanine (G).

The replication process -4steps:

- DNA double helix.
- Hydrogen bonds break and helix opens.
- Each strand of DNA acts as a template for synthesis of a new, complementary strand.
- Replication produces two identical DNA double helices, each with one new and one old strand.

In transcription, one strand of the DNA that makes up a gene, called the **non-coding strand**, acts as a template for the synthesis of a matching (complementary) RNA strand by an enzyme called RNA polymerase. This RNA strand is the **primary transcript**.



Translation-After transcription (and, in eukaryotes, after processing), an mRNA molecule is ready to direct protein synthesis. The process of using information in an mRNA to build a polypeptide is called translation.

1. Matching tRNA binds to exposed codon in rightmost slot of ribosome.
2. Chain of amino acids is transferred from tRNA in middle slot of ribosome onto the amino acid of the tRNA in the rightmost slot. This has the effect of adding the amino acid to the end of the amino acid chain.
3. The ribosome shifts one codon over. The tRNA formerly in the middle slot moves to the leftmost slot and exits the ribosome. The tRNA formerly in the right slot moves into the middle slot and continues to hold the amino acid chain. A new codon is exposed in the rightmost slot for a new tRNA to bind to.

b. Describe the molecular markers used in biotechnology (10 Marks) any 5

Abbreviations	Molecular makers
AFLP	Amplified Fragment Length Polymorphism
CAPS	Cleaved Amplified Polymorphic Sequences
EST	Expressed Sequence Tag
IRAP	Inter-Retrotransposon Amplified Polymorphism
REMAP	Retrotransposon-Microsatellite Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
RAPD	Random Amplified Polymorphic DNA
STS	Sequence Tagged Site
SCAR	Sequence Characterized Amplified Region
SSR	Simple Sequence Repeat
PCR	Polymerase Chain Reaction
ISSR	Inter- Simple Sequence Repeat amplification

QUESTION THREE (20 MARKS)

a. Describe the restriction enzymes applied in biotechnology (10 Marks)

Restriction enzyme define, types, mode of action 5mk

DNA ligase-function, types, mode of action 5mks

b. Describe some important GMOs in Agricultures (10 Marks) any 10

Crops
Animals
Fish
Ornamentals etc

QUESTION FOUR (20 MARKS)

Describe the applications of biotechnology in agriculture (20 Marks) 20 points@1mk

Pest and disease resistance
Herbicide tolerance crops
Ornamentals.eg dwarfing plants
Secondary metabolites engineering
Saline tolerance
Drought resistance crops
Adaptation to climate change
Plants for phytoremediation
Novel hybrids
Mass propagation via TC
Conservation of endangered species
Biofuel crops
Biofertilizers
biodegradation
Improvement of taste, colour , smell in food crops etc
Bioremediation
Biomining
Biosensors
Bioreactors
Increase yields
Production of antibiotics, enzymes, secondary metabolites
Silk production
Food processing

etc

QUESTION FIVE (20 MARKS)

a. Describe the media components of plant tissue/cell culture (15 Marks)

- Inorganic nutrients; macronutrients and micronutrients 3
- vitamins, 2
- Amino acids or nitrogen supplements,2
- Source(s) of carbon, 2
- Organic supplements, 2
- Growth regulators 2
- Solidifying agents.2

a. Explain the applications of Marker assisted selection (MAS) in breeding (5 Marks)

MAS is mainly applied in situations where:-

- the selected character is expressed late in plant development,
- the expression of the target gene is recessive
- there is requirement for the presence of special conditions in order to invoke expression of the target gene(s), as in the case of breeding for disease and pest resistance
- the phenotype is affected by two or more unlinked genes (epistasis).- Gene pyramiding.
- Applications in variety distinction and identification