La-Net

BIO 408

APPLIED BIOTECHNOLOGY

Downoad on the App Store

GET IT ON Google Play

Course video is available on our app

lanetonline.com

Course code: BIO 408

Course title: Applied Biotechnology

Course credit load: 2 Units

Series 1: DNA and Protein Extraction

- **Part 1.1: Principles of Cell Disruption and Tissue Preparation**
  - Sub Part 1.1.1: Overview of extraction: aims, challenges, and tissue types
  - Sub Part 1.1.2: Mechanical and chemical cell disruption methods
  - Sub Part 1.1.3: Buffers, reagents, and safety considerations
- **Part 1.2: DNA Extraction**
  - Sub Part 1.2.1: Principles of DNA extraction
  - Sub Part 1.2.2: CTAB method for plant DNA extraction
  - Sub Part 1.2.3: Extraction from animal tissues, blood, and microorganisms
- **Part 1.3: Protein Extraction**
  - Sub Part 1.3.1: Principles of protein extraction and solubilisation
  - Sub Part 1.3.2: Extraction from plant and animal tissues
  - Sub Part 1.3.3: Subcellular fractionation
- **Part 1.4: Quality Assessment of Extracted Nucleic Acids and Proteins**
  - Sub Part 1.4.1: Visual and spectrophotometric assessment of DNA quality
  - Sub Part 1.4.2: Assessment of protein yield and integrity
  - Sub Part 1.4.3: Storage and handling of extracted biomolecules

lanetonline.com

Introduction

Extraction of nucleic acids and proteins from biological tissues is the starting point for virtually all molecular biotechnology applications, from PCR amplification and DNA sequencing to enzyme characterisation and recombinant protein production. The quality and yield of the extracted molecules directly determine the success of downstream analytical and preparative procedures. A good extraction must release sufficient quantities of the target molecule, remove contaminants (proteins, polysaccharides, pigments, lipids, and inhibitors), and preserve the structural integrity of the molecule.

This Series introduces the principles and practice of DNA and protein extraction from diverse biological tissues encountered in Nigerian research contexts: crop plants (cassava, maize, cowpea), livestock tissues (muscle, blood, liver), and microorganisms (bacteria, fungi). It covers cell disruption methods, extraction reagents and buffers, standard protocols (including the CTAB method for plant DNA), subcellular fractionation for protein extraction, and quality assessment of the extracted biomolecules.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 1.1** describe the aims of biomolecule extraction and compare mechanical and chemical cell disruption methods (Linked to Part 1.1),
- **SLO 1.2** explain the roles of buffers, detergents, and other key reagents used in DNA and protein extraction (Linked to Part 1.1),
- **SLO 1.3** describe the principles of DNA extraction and outline the steps of the CTAB method for plant tissues (Linked to Part 1.2),
- **SLO 1.4** describe methods for extracting DNA from animal tissues, blood, and microorganisms (Linked to Part 1.2),
- **SLO 1.5** explain the principles of protein extraction and subcellular fractionation (Linked to Part 1.3), and

lanetonline.com

- **SLO 1.6** assess the quality and integrity of extracted DNA and protein using spectrophotometric and gel-based methods (Linked to Part 1.4).

1.1 Principles of Cell Disruption and Tissue Preparation

1.1.1 Overview of extraction: aims, challenges, and tissue types

The aim of biomolecule extraction is to release the target molecule from the cellular environment in sufficient quantity and purity for the intended downstream application. The principal challenges vary with tissue type: plant tissues present a tough cell wall (cellulose and pectin), large vacuoles containing phenolic compounds and polysaccharides that co-precipitate with DNA and inhibit downstream enzymes, and plastids containing chloroplast DNA that may contaminate nuclear DNA preparations. Animal tissues are generally easier to disrupt but contain proteases and nucleases that rapidly degrade the target molecules if not inhibited promptly. Microbial cells may have rigid peptidoglycan cell walls (bacteria) or thick glucan-chitin walls (fungi) requiring more aggressive disruption.

Tissue preparation before extraction includes: freshness (tissues processed immediately or snap-frozen in liquid nitrogen to prevent nuclease and protease degradation); homogenisation (reducing the tissue to a fine powder using liquid nitrogen and a mortar and pestle, or a bead mill for small volumes); removal of non-target material (de-fatting, removal of connective tissue, washing of blood cells); and young, actively growing tissue selection (for plant DNA extraction, young leaves have lower polysaccharide and phenolic content than mature or senescing leaves). For Nigerian crops, the optimal tissue for DNA extraction is young, fully expanded leaf material collected in the morning before significant photosynthetic activity has loaded leaves with starch.

Fill in the blank: For plant DNA extraction, young _____ tissue is preferred because it has lower polysaccharide, phenolic, and starch content than mature or senescing tissue, reducing co-precipitation of inhibitors with the extracted DNA.

lanetonline.com

1.1.2 Mechanical and chemical cell disruption methods

Mechanical disruption physically breaks open cells by applying force. Methods include: mortar and pestle with liquid nitrogen (freezing the tissue in liquid nitrogen makes it brittle; grinding produces a fine powder; standard for plant and tough animal tissues); bead milling (tissue and small glass or ceramic beads are agitated at high speed; suitable for microorganisms, fungi, and small volumes of plant material; instruments include TissueLyser and FastPrep); sonication (high-frequency ultrasound generates cavitation bubbles that lyse cells; used for bacterial and mammalian cell suspensions; generates heat that must be controlled by cooling); and French press (suspension forced through a narrow valve at high pressure; used for bacteria and yeast).

Chemical disruption uses reagents to solubilise or weaken cell walls and membranes. Detergents (sodium dodecyl sulphate, SDS; cetyltrimethylammonium bromide, CTAB; Triton X-100; NP-40) solubilise lipid bilayers and denature proteins, releasing cell contents. Chaotropic agents (guanidinium thiocyanate; guanidinium hydrochloride) disrupt hydrogen bonds and hydrophobic interactions, denaturing proteins and releasing nucleic acids; widely used in commercial DNA extraction kits and spin-column protocols. Alkaline lysis (NaOH + SDS) is used for plasmid DNA extraction from bacteria: SDS disrupts the membrane; NaOH denatures chromosomal DNA and proteins; neutralisation with potassium acetate selectively precipitates denatured chromosomal DNA and proteins while the smaller covalently closed circular plasmid DNA remains in solution.

Fill in the blank: In alkaline lysis for plasmid extraction, _____ (NaOH + SDS) disrupts bacterial membranes and denatures chromosomal DNA; neutralisation with potassium acetate selectively precipitates chromosomal DNA and proteins, leaving plasmid DNA in the supernatant.

1.1.3 Buffers, reagents, and safety considerations

Extraction buffers serve three main functions: maintaining pH to prevent acid or alkaline degradation of the target molecule; providing ionic conditions that stabilise macromolecular structure; and containing chelating agents (EDTA; ethylene diamine tetraacetic acid) that sequester divalent cations (Mg^{2+}, Ca^{2+}) required by nucleases and proteases, inhibiting their

lanetonline.com

activity. A typical DNA extraction buffer for plant tissue contains: Tris-HCl (pH 8.0; buffering); NaCl (0.1 to 0.8 M; ionic stabilisation); EDTA (20 to 25 mM; DNase inhibition); CTAB or SDS (detergent; cell membrane disruption); and beta-mercaptoethanol or dithiothreitol (DTT; reducing agents that prevent oxidation of phenolic compounds to quinones, which otherwise bind irreversibly to DNA). Beta-mercaptoethanol is highly toxic and volatile; handling must be in a fume hood.

Protease inhibitors added to protein extraction buffers include: phenylmethylsulfonyl fluoride (PMSF; serine protease inhibitor; unstable in aqueous solution; added just before use); EDTA (metalloprotease inhibitor); pepstatin A (aspartic protease inhibitor); leupeptin (cysteine and serine protease inhibitor); and complete protease inhibitor cocktail tablets (Roche; convenient combination product). Safety considerations for common extraction reagents: phenol-chloroform (used in classical DNA extraction; highly toxic; causes chemical burns; volatile; must be handled in fume hood with nitrile gloves and eye protection; waste disposed as halogenated organic solvent); chloroform (toxic; anaesthetic; handle in fume hood); liquid nitrogen (extreme cold; risk of asphyxiation in enclosed spaces; use with cryogenic gloves and face shield); SDS (irritant; produces dust; avoid inhalation).

Fill in the blank: _____ (EDTA) is added to DNA extraction buffers to chelate divalent cations such as Mg^{2+} and Ca^{2+}; this inhibits nucleases, which require these metal ions as cofactors, thereby protecting extracted DNA from degradation during the extraction procedure.

Pilot questions 1.1

1. State three challenges specific to DNA extraction from plant tissues and explain how each is addressed.
2. Distinguish between mechanical and chemical cell disruption and give two examples of each.
3. State the function of each component in a typical plant DNA extraction buffer: Tris-HCl, NaCl, EDTA, CTAB, and beta-mercaptoethanol.
4. Explain the principle of alkaline lysis and why it selectively recovers plasmid DNA rather than chromosomal DNA.

lanetonline.com

5. State four safety precautions that must be observed when working with phenol-chloroform extraction reagents.

1.2 DNA Extraction

1.2.1 Principles of DNA extraction

DNA extraction aims to release genomic or plasmid DNA from cells, separate it from proteins, RNA, polysaccharides, and other contaminants, and recover it in a form pure and concentrated enough for downstream applications (PCR, restriction digestion, sequencing, southern blotting). The fundamental steps common to all DNA extraction methods are: (1) cell disruption (described in Part 1.1); (2) protein denaturation and removal (by heat, detergent, proteinase K digestion, or organic solvent extraction); (3) RNA removal (by RNase A treatment); (4) DNA precipitation (by addition of cold absolute ethanol or isopropanol in the presence of monovalent cations; DNA is insoluble in alcohol and precipitates as a visible pellet or thread); (5) washing (with 70 percent ethanol to remove salt and residual organic solvent); and (6) re-suspension in low-salt buffer (TE: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or nuclease-free water.

DNA precipitation with alcohol is explained by the reduction in the dielectric constant of the solution, which destabilises the electrostatic repulsion between phosphate groups and allows cations (Na+, K+, NH4+) to neutralise the negative charges, facilitating DNA aggregation and precipitation. Isopropanol requires less volume than ethanol (0.6 to 0.7 volumes vs 2 to 2.5 volumes) and is more efficient at room temperature, but co-precipitates more salt; ethanol at -20 degrees C gives cleaner DNA. Proteinase K (a broad-spectrum serine protease active in SDS and at 55 to 65 degrees C) is widely used to digest proteins during DNA extraction from animal tissues and blood.

Fill in the blank: In DNA extraction, the target DNA is precipitated from aqueous solution by addition of cold absolute ethanol or isopropanol in the presence of _____ cations (Na+, K+) which neutralise the negative charges on the phosphate backbone, allowing DNA strands to aggregate and precipitate.

lanetonline.com

1.2.2 CTAB method for plant DNA extraction

The CTAB (cetyltrimethylammonium bromide) method is the standard protocol for DNA extraction from plant tissues, particularly those with high polysaccharide and phenolic content such as cassava, yam, cowpea, and maize leaves. CTAB is a cationic detergent that forms insoluble complexes with polysaccharides and cell wall material at high ionic strength (0.7 M NaCl or higher) but releases DNA into solution. The procedure: (1) grind 100 to 200 mg young leaf tissue to fine powder in liquid nitrogen using mortar and pestle; (2) transfer to 2 ml microcentrifuge tube containing prewarmed (65 degrees C) CTAB extraction buffer (2 percent CTAB; 1.4 M NaCl; 100 mM Tris-HCl pH 8.0; 20 mM EDTA; 0.2 percent beta-mercaptoethanol; added fresh); (3) mix thoroughly and incubate at 65 degrees C for 30 to 60 minutes with occasional mixing; (4) add equal volume of chloroform:isoamyl alcohol (24:1) and mix by inversion; centrifuge at 12,000 rpm for 10 minutes; transfer aqueous upper phase to fresh tube.

(5) Repeat chloroform:isoamyl alcohol extraction if the aqueous phase is still turbid or coloured; (6) add 0.1 volumes 10 percent CTAB solution and 0.7 volumes cold isopropanol; mix gently; leave at -20 degrees C for 30 to 60 minutes or at room temperature for 1 hour; (7) centrifuge at 12,000 rpm for 10 minutes; discard supernatant; wash pellet with 70 percent ethanol; air-dry briefly; (8) dissolve pellet in TE buffer or nuclease-free water; treat with RNase A (100 micrograms per ml; 37 degrees C; 30 minutes) to remove RNA; (9) assess yield and quality by nanodrop spectrophotometry and agarose gel electrophoresis. The CTAB method was developed by Doyle and Doyle (1987) and remains the most widely used protocol for tropical crop species in Nigerian research laboratories.

Fill in the blank: In the CTAB extraction method, the chloroform:isoamyl alcohol (_____ :1) step removes proteins and lipids from the aqueous DNA solution; the DNA partitions into the upper aqueous phase while proteins and lipids partition into the organic lower phase or interphase.

1.2.3 Extraction from animal tissues, blood, and microorganisms

lanetonline.com

DNA extraction from animal muscle, liver, or heart tissue follows a proteinase K digestion protocol: tissue is homogenised in lysis buffer (50 mM Tris-HCl pH 8.0; 100 mM NaCl; 10 mM EDTA; 1 percent SDS); proteinase K (100 to 200 micrograms per ml) is added and digestion proceeds at 55 degrees C for 3 hours to overnight; protein is removed by phenol-chloroform extraction; DNA is precipitated with ethanol and re-suspended in TE. Blood is a convenient source of genomic DNA: 200 to 500 microlitres whole blood or buffy coat (white blood cell layer after centrifugation) is digested with proteinase K in SDS lysis buffer; commercial spin-column kits (QIAamp DNA Blood Mini Kit; Qiagen) yield high-quality DNA in 30 minutes and are widely used for clinical and livestock DNA banking applications.

Bacterial DNA extraction requires disruption of the peptidoglycan cell wall, achieved by incubation with lysozyme (an enzyme that cleaves the beta-1,4-glycosidic bonds in peptidoglycan) before SDS and proteinase K lysis; Gram-positive bacteria (thicker peptidoglycan) require longer lysozyme treatment or more vigorous mechanical disruption than Gram-negative bacteria. Fungal cell walls (beta-glucan and chitin) require bead milling or liquid nitrogen grinding before chemical lysis. Commercial kits (DNeasy Plant Mini Kit for plants; DNeasy Blood and Tissue Kit for animals and microorganisms; Qiagen) use silica membrane spin columns that bind DNA at high chaotropic salt concentration and release it in low-salt elution buffer; they provide fast, clean extractions without organic solvents but at higher reagent cost.

Fill in the blank: Bacterial DNA extraction requires prior treatment with _____, an enzyme that cleaves beta-1,4-glycosidic bonds in the peptidoglycan cell wall, before detergent lysis; Gram-positive bacteria require more extensive treatment because their peptidoglycan layer is thicker than that of Gram-negative bacteria.

lanetonline.com

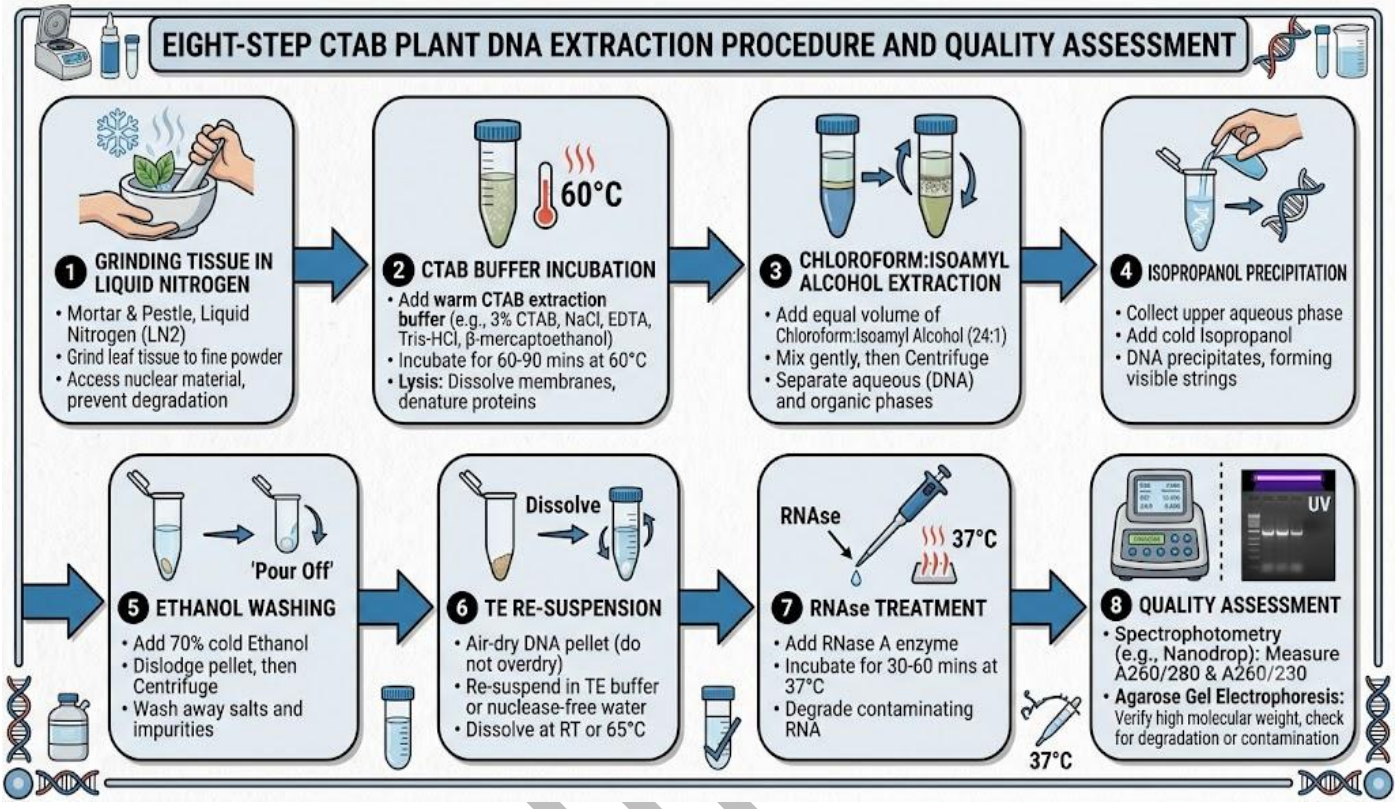


Figure 1.1: Flowchart of the CTAB method for plant DNA extraction

Pilot questions 1.2

1. State the six fundamental steps common to all DNA extraction methods.
2. Explain the role of CTAB in plant DNA extraction and why high NaCl concentration is important.
3. Describe how proteinase K digestion is used in DNA extraction from animal muscle tissue.
4. Explain why lysozyme treatment is necessary before extracting DNA from bacteria.
5. Compare phenol-chloroform extraction with commercial spin-column kits for DNA extraction, stating one advantage of each.

1.3 Protein Extraction

1.3.1 Principles of protein extraction and solubilisation

Protein extraction aims to solubilise the target protein(s) from the cellular environment while preserving their structural integrity, biological activity (for enzyme and functional studies), or

lanetonli

antigenic properties (for immunological studies). The choice of extraction conditions depends on the intended use: native extraction (mild conditions; non-denaturing detergents; cold temperatures; protease inhibitors) preserves protein activity; denaturing extraction (SDS; boiling; reducing agents) is used when only protein size and identity are needed (e.g. for SDS-PAGE analysis). The key determinants of extraction success are: the solubility of the target protein (hydrophilic vs hydrophobic; cytoplasmic vs membrane-bound); the presence of disulphide bonds (reduced by DTT or beta-mercaptoethanol); and the pH, ionic strength, and temperature of the extraction buffer.

Solubilisation buffers for native protein extraction typically contain: phosphate-buffered saline (PBS; pH 7.0 to 7.4) or HEPES buffer (pH 7.0 to 7.5) for pH maintenance; NaCl (0.1 to 0.5 M) for ionic stabilisation; glycerol (10 to 20 percent; stabilises proteins against denaturation during freeze-thaw cycles); DTT or beta-mercaptoethanol (1 to 5 mM; reduces disulphide bonds and maintains thiol groups in reduced state); and protease inhibitor cocktail (prevents proteolytic degradation). For membrane proteins, non-ionic detergents (Triton X-100; Tween-20; digitonin; dodecyl maltoside) are added at low concentrations (0.1 to 1 percent) to solubilise membrane lipids without fully denaturing the protein. For total protein extraction for SDS-PAGE, RIPA buffer (150 mM NaCl; 1 percent NP-40; 0.5 percent SDS; 50 mM Tris-HCl pH 8.0; protease inhibitors) is widely used.

Fill in the blank: For native protein extraction, _____ buffer (containing pH buffer, NaCl, glycerol, DTT, and protease inhibitors) is used to solubilise proteins while preserving their biological activity; denaturing conditions (SDS; boiling) are used only when protein activity is not required.

1.3.2 Extraction from plant and animal tissues

Plant protein extraction is complicated by phenolic compounds (which oxidise and bind to proteins, causing brown discolouration and loss of activity), polysaccharides (which increase viscosity and interfere with protein assays), and proteases in vacuoles. To address these: polyvinylpolypyrrolidone (PVPP) is added to the grinding buffer to adsorb phenolic compounds before they oxidise; reducing agents (DTT, ascorbic acid) prevent phenolic oxidation; protease inhibitor cocktails are added; and cold conditions (ice; cold centrifuge) limit protease activity.

lanetonline.com

For tissues with high lipid content (seeds, oil palm mesocarp), an initial defatting step with cold acetone is performed: tissue is ground in cold acetone and centrifuged to remove lipids; the acetone powder is then extracted with buffer.

Animal tissue protein extraction: 0.1 to 0.5 g tissue is homogenised with a motorised homogeniser or dounce homogeniser in ice-cold lysis buffer; insoluble debris is removed by centrifugation (600 to 1,000 x g; 10 minutes); the resulting post-nuclear supernatant (PNS) contains total cellular protein. For blood plasma or serum protein extraction, whole blood is collected in an anticoagulant tube (EDTA; sodium citrate) and centrifuged to separate plasma; serum is obtained by allowing blood to clot and removing the clot. For intracellular red blood cell proteins (haemoglobin extraction), red blood cells are washed, lysed by hypotonic shock or freeze-thaw, and centrifuged to remove membranes (ghost membranes), yielding haemolysate containing predominantly haemoglobin.

Fill in the blank: _____ (PVPP) is added to plant protein extraction buffers to adsorb phenolic compounds before they can oxidise and bind irreversibly to proteins, preventing brown discolouration and loss of enzyme activity during extraction.

1.3.3 Subcellular fractionation

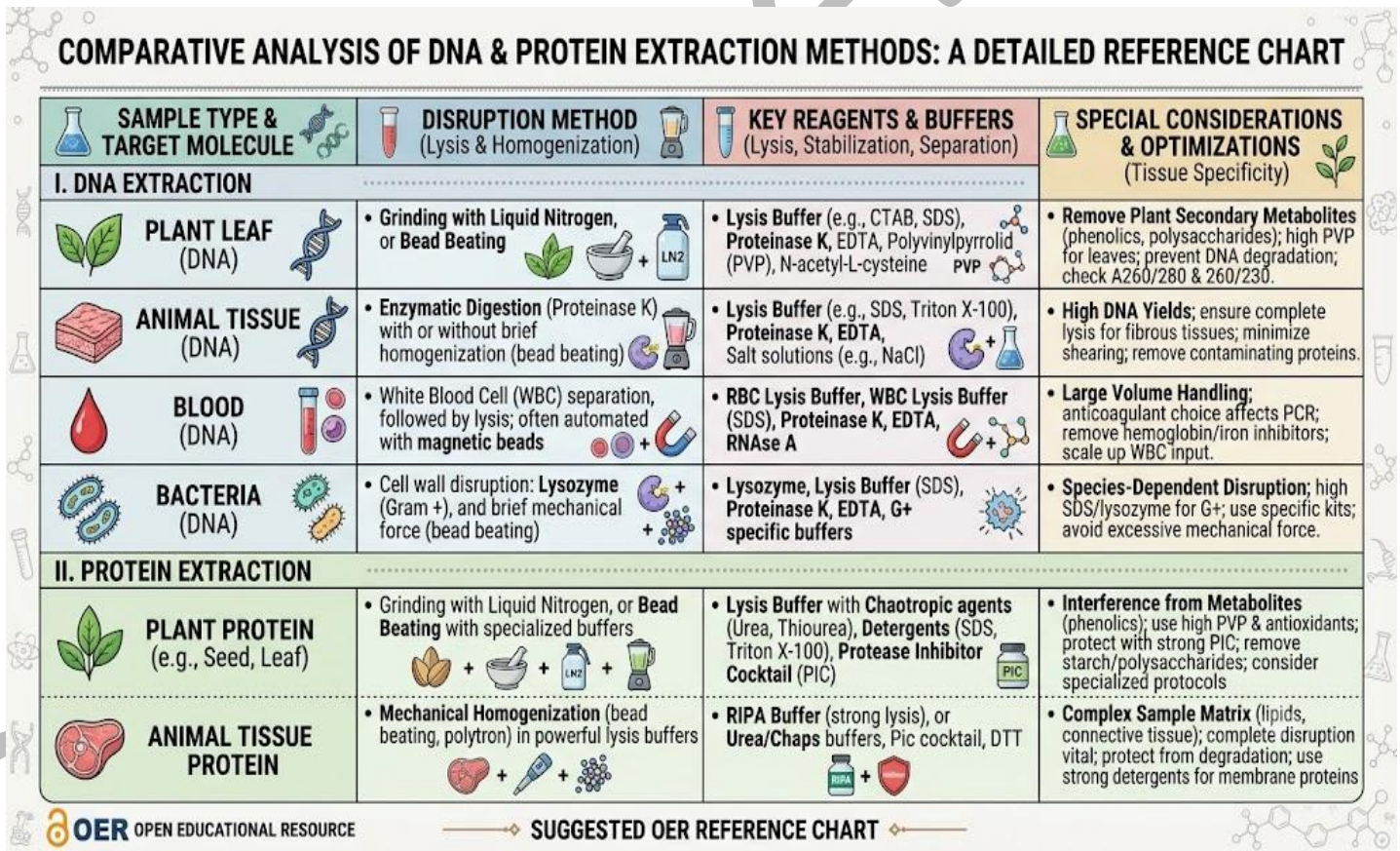
Subcellular fractionation separates cell components into distinct fractions enriched for specific organelles or subcellular compartments, allowing study of proteins associated with particular organelles. The technique exploits differences in the size, density, and sedimentation rate of organelles during centrifugation. Differential centrifugation is the sequential application of increasing centrifugal forces to separate progressively smaller and denser particles: (1) 300 to 600 x g for 5 to 10 minutes: pellets intact cells and cell debris; supernatant is post-nuclear fraction; (2) 1,000 to 3,000 x g for 10 to 20 minutes: pellets nuclei; (3) 10,000 to 20,000 x g for 20 to 30 minutes: pellets mitochondria and lysosomes; (4) 100,000 x g for 60 to 90 minutes (ultracentrifugation): pellets ribosomes and microsomes (endoplasmic reticulum vesicles); supernatant is cytosol.

Density gradient centrifugation (sucrose gradient or iodixanol/OptiPrep gradient) provides finer separation: organelles migrate to their isopycnic (buoyant density) position in a pre-formed

lanetonline.com

gradient. Sucrose gradients (10 to 50 percent) are used to separate mitochondria, lysosomes, peroxisomes, and plasma membrane vesicles by their different buoyant densities. In plant cells, chloroplast isolation uses a Percoll (colloidal silica) gradient: ground plant material is layered on 40/80 percent Percoll cushion; intact chloroplasts band at the 40/80 percent interface while broken chloroplasts and other debris remain at the 40 percent layer. Subcellular fractionation is essential for studying organelle-specific metabolic pathways and for producing highly purified organelle preparations for proteomics.

Fill in the blank: In differential centrifugation, mitochondria are sedimented at approximately 10,000 to 20,000 x g after nuclei have been removed at _____ x g; ribosomes and microsomes require ultracentrifugation at 100,000 x g for pelleting.



Box 1.1: Comparison of DNA and protein extraction methods for different tissue types

Pilot questions 1.3

1. Explain the difference between native and denaturing protein extraction conditions and state when each is used.
2. State three problems specific to plant protein extraction and describe how each is addressed.
3. Describe the steps of differential centrifugation and state which organelle is pelleted at each centrifugal force.
4. Explain how chloroplasts are isolated from plant tissue using a Percoll gradient.
5. Describe how haemoglobin is extracted from red blood cells.

1.4 Quality Assessment of Extracted Nucleic Acids and Proteins

1.4.1 Visual and spectrophotometric assessment of DNA quality

Before using extracted DNA in downstream applications, its yield, purity, and integrity must be assessed. Visual assessment: placing 3 to 5 microlitres of extracted DNA on a 0.8 percent agarose gel stained with ethidium bromide (EtBr) or safe alternative stains (SYBR Safe; GelRed) and running at 80 to 100 V for 30 to 45 minutes allows visual detection of: high-molecular-weight genomic DNA (migrates as a tight, high-MW band well above a 10 kb ladder); RNA contamination (a smear or bands below 2 kb if RNase treatment was incomplete); and degraded DNA (a smear of small fragments throughout the gel, indicating nuclease activity or mechanical shearing during extraction). A single high-MW band is the hallmark of good quality genomic DNA.

Spectrophotometric assessment using a nanodrop or UV spectrophotometer provides quantitative purity data. DNA absorbs UV light maximally at 260 nm; proteins absorb maximally at 280 nm; residual phenol and other aromatic compounds absorb at 270 to 280 nm. The A260/A280 ratio indicates protein contamination: a ratio of 1.8 to 2.0 indicates pure DNA; ratios below 1.8 indicate protein contamination. The A260/A230 ratio indicates contamination by polysaccharides, EDTA, guanidinium salts, and organic solvents: a ratio of 2.0 to 2.2 is acceptable; lower ratios indicate significant contaminant carryover, which will inhibit PCR and

lanetonline.com

other downstream reactions. DNA concentration is calculated as: concentration (micrograms per ml) = A260 x 50 (for double-stranded DNA) x dilution factor.

Fill in the blank: A DNA preparation with an A260/A280 ratio between _____ and 2.0 is considered free of significant protein contamination; a lower ratio indicates the presence of residual protein or phenol from the extraction procedure.

1.4.2 Assessment of protein yield and integrity

Protein yield is quantified using colorimetric assays that react with protein to produce a colour change measurable by absorbance. The Bradford assay (Coomassie Brilliant Blue G-250 dye; absorbance at 595 nm; bovine serum albumin BSA as standard; range 1 to 20 micrograms per ml; sensitive; fast; compatible with reducing agents but not with detergents at high concentration) is the most widely used method for protein quantification in Nigerian laboratories. The BCA (bicinchoninic acid) assay uses Cu2+ reduction to Cu+ by proteins in alkaline conditions; Cu+ reacts with BCA to form a purple complex measured at 562 nm; more sensitive than Bradford; compatible with more detergents; heat-assisted version (BCA with incubation at 37 to 60 degrees C) gives better sensitivity.

The Lowry assay (Folin-Ciocalteau reagent; absorbance at 750 nm) is an older method still used in some laboratories; it is sensitive but slow and susceptible to interference from reducing agents and detergents. Protein integrity is assessed by SDS-PAGE (described in Series 3): proteins are denatured and separated by size on a polyacrylamide gel; a complete protein extract with many bands across a wide MW range indicates successful extraction; absence of expected bands or unusual smearing indicates degradation or incomplete extraction. For enzyme extracts, a functional assay (enzyme activity measurement; e.g. peroxidase activity, protease activity) is the most meaningful assessment of protein quality because it confirms that the extracted protein is not only present but active.

Fill in the blank: In the Bradford protein assay, _____ Brilliant Blue G-250 dye shifts from red to blue upon binding to protein; the absorbance at 595 nm is proportional to protein concentration and is compared with a BSA standard curve to quantify the protein in the sample.

lanetonline.com

1.4.3 Storage and handling of extracted biomolecules

Proper storage is critical to maintaining the quality of extracted DNA and proteins for future use. Genomic DNA (for PCR and molecular work): store in TE buffer (pH 8.0; EDTA inhibits DNase) at -20 degrees C for short-term (months) or -80 degrees C for long-term (years) storage; avoid repeated freeze-thaw cycles (each cycle causes physical shearing of DNA; maximum 3 to 5 cycles recommended); use low-bind microcentrifuge tubes to minimise DNA adsorption to tube walls. For very long-term archiving (genebanks, forensic samples), DNA can be dried onto FTA cards (cellulose-based cards impregnated with a lysis buffer that preserves DNA at room temperature for years) or stored as ethanol precipitate.

RNA (if extracted for RT-PCR; described in Series 3): extremely labile due to ubiquitous RNases; store at -80 degrees C; use RNase-free water, tubes, and tips; add RNase inhibitor (RNasin) during extraction; process immediately after extraction. Proteins for functional studies: store at -20 degrees C in buffer with 50 percent glycerol (cryoprotectant) to prevent ice crystal formation; add protease inhibitors; for enzymes, store in small aliquots (10 to 50 microlitres) to avoid repeated thawing; stability varies greatly between proteins (some stable for months; others lose activity within hours at room temperature). Proteins for SDS-PAGE: can be stored in SDS sample loading buffer at -20 degrees C or -80 degrees C for months; boil for 5 minutes before loading.

Fill in the blank: Extracted genomic DNA is stored in _____ buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at -20 degrees C for short-term use; the EDTA in the buffer inhibits DNase activity, protecting DNA from degradation during storage.

Pilot questions 1.4

1. Describe what each of the following indicates about a DNA sample: a single high-MW band on agarose gel; a smear below 2 kb; an A260/A280 ratio of 1.6.
2. Explain how DNA concentration is calculated from a nanodrop absorbance reading at 260 nm.
3. Compare the Bradford and BCA assays for protein quantification, stating one advantage of each.
4. State five criteria used to assess the quality of an extracted DNA sample.
5. Explain why repeated freeze-thaw cycles should be avoided when storing genomic DNA.

lanetonline.com

Series Summary

Extraction of DNA and proteins from biological tissues involves cell disruption, removal of contaminants, recovery of the target molecule, and assessment of its quality. Different tissues require different treatments. Plant tissues are difficult because of their cell walls, phenolic compounds, and polysaccharides, so they are commonly ground in liquid nitrogen and treated with CTAB or SDS, beta-mercaptoethanol, and PVPP. Animal tissues are usually homogenised and digested with proteinase K, while blood is commonly processed with spin-column methods. Bacterial cells may require lysozyme pretreatment, whereas fungal tissues are often disrupted by bead milling. The CTAB method of Doyle and Doyle (1987) remains a standard procedure for extracting plant DNA. It involves grinding the tissue in liquid nitrogen, incubating it in CTAB buffer at 65°C, extracting with chloroform:isoamyl alcohol, precipitating the DNA with isopropanol, washing with ethanol, dissolving in TE buffer, and treating with RNase. Protein extraction may be performed under native conditions using PBS, glycerol, DTT, and protease inhibitors for functional studies, or under denaturing conditions using RIPA buffer or SDS for SDS-PAGE. Subcellular components may also be separated by differential centrifugation, with cell debris collected at about 300 × g, nuclei at 1,000–3,000 × g, mitochondria at 10,000–20,000 × g, and ribosomes at about 100,000 × g.

DNA quality is evaluated using agarose gel electrophoresis and spectrophotometry. Good-quality DNA should appear as a single high-molecular-weight band with little or no RNA smear. Pure DNA usually has an A260/A280 ratio of 1.8–2.0 and an A260/A230 ratio of 2.0–2.2. DNA concentration is calculated as **DNA concentration = A260 × 50 × dilution factor (µg/mL)**. Protein concentration may be measured with the Bradford assay at 595 nm using Coomassie G-250 and a BSA standard, or with the BCA assay at 562 nm based on the reduction of Cu^{2+} to Cu^{+}. Protein integrity is assessed by SDS-PAGE and, where necessary, enzyme activity tests. DNA is commonly stored in TE buffer at −20°C to −80°C, while repeated freeze-thaw cycles should be

lanetonline.com

limited to about 3–5. Proteins intended for functional studies may be stored with 50% glycerol at −20°C.

Glossary of Terms

- **Bradford assay:** a colorimetric protein quantification method using Coomassie Brilliant Blue G-250 dye; protein binding causes a colour shift from red to blue measured at 595 nm; compared with a BSA standard curve.
- **BCA assay:** bicinchoninic acid protein quantification assay; proteins reduce Cu2+ to Cu+ in alkaline conditions; Cu+ reacts with BCA to form a purple complex at 562 nm; compatible with more detergents than Bradford.
- **CTAB:** cetyltrimethylammonium bromide; a cationic detergent used in plant DNA extraction; solubilises cell membranes and forms insoluble complexes with polysaccharides at high NaCl concentration, allowing selective DNA recovery.
- **Differential centrifugation:** sequential centrifugation at increasing forces to separate cell organelles by size and density: debris (300 to 600 x g), nuclei (1,000 to 3,000 x g), mitochondria (10,000 to 20,000 x g), microsomes (100,000 x g).
- **EDTA:** ethylene diamine tetraacetic acid; a chelating agent that sequesters divalent cations (Mg2+, Ca2+) required by nucleases and proteases, protecting extracted DNA and protein from enzymatic degradation.
- **FTA card:** cellulose-based paper card impregnated with a lysis/preservation buffer; allows DNA to be dried and stored at room temperature for years without refrigeration; used for field sample collection and long-term archiving.
- **Lysozyme:** an enzyme that cleaves beta-1,4-glycosidic bonds in bacterial peptidoglycan cell walls; used as a pretreatment before chemical lysis to facilitate DNA extraction from Gram-positive bacteria.
- **Nanodrop:** a micro-volume UV spectrophotometer requiring only 1 to 2 microlitres of sample; used to measure DNA/RNA concentration (A260) and purity (A260/A280 and A260/A230 ratios) without dilution.

lanetonline.com

- **Proteinase K:** a broad-spectrum serine protease active in SDS and at 55 to 65 degrees C; used in DNA extraction protocols to digest contaminating proteins from animal tissues, blood, and microbial cells.
- **PVPP:** polyvinylpolypyrrolidone; an insoluble polymer added to plant extraction buffers to adsorb phenolic compounds before they oxidise and bind to proteins or DNA, preventing brown discolouration and inhibition.
- **RIPA buffer:** radioimmunoprecipitation assay buffer; a denaturing lysis buffer (150 mM NaCl, 1 percent NP-40, 0.5 percent SDS, 50 mM Tris-HCl pH 8.0, protease inhibitors) used for total cellular protein extraction for SDS-PAGE.
- **Subcellular fractionation:** the separation of cell components into fractions enriched for specific organelles using differential or density gradient centrifugation; allows isolation of organelle-specific protein populations.
- **TE buffer:** Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0); standard DNA storage buffer; Tris maintains pH 8.0 (prevents acid depurination); EDTA inhibits DNases by chelating Mg2+.
- **A260/A280 ratio:** the ratio of UV absorbance at 260 nm to absorbance at 280 nm; used to assess DNA purity; values of 1.8 to 2.0 indicate pure DNA; lower values indicate protein or phenol contamination.

Concept Check Questions [Series 1]

Objective Questions

1. The cationic detergent used in plant DNA extraction that forms insoluble complexes with polysaccharides at high NaCl concentration is _____. (Linked to SLO 1.3)
2. EDTA protects extracted DNA by chelating _____ cations required by nucleases as cofactors. (Linked to SLO 1.2)
3. A DNA preparation with an A260/A280 ratio of 1.8 to 2.0 is considered _____ of significant protein contamination. (Linked to SLO 1.6)
4. The broad-spectrum serine protease added to animal tissue lysis buffer to digest proteins during DNA extraction is called _____. (Linked to SLO 1.4)

lanetonline.com

5. In differential centrifugation, mitochondria are sedimented at approximately _____ x g after nuclei have been removed. (Linked to SLO 1.5)

Short-Answer Questions

6. State three challenges of DNA extraction from plant tissues and how each is addressed. (Linked to SLO 1.3)
7. Outline the steps of the CTAB method for plant DNA extraction. (Linked to SLO 1.3)
8. Distinguish between native and denaturing protein extraction and state when each is used. (Linked to SLO 1.5)
9. Explain how to assess the quality of extracted DNA using a nanodrop spectrophotometer. (Linked to SLO 1.6)
10. Describe how subcellular fractionation by differential centrifugation separates cell organelles. (Linked to SLO 1.5)

Long-Answer Questions

11. Describe the principles of cell disruption, the key reagents used in extraction buffers, and the steps of the CTAB method for plant DNA extraction. (Linked to SLOs 1.1, 1.2, 1.3)
12. Describe DNA extraction from animal tissues, blood, and bacteria. Explain the principles of protein extraction, subcellular fractionation, and quality assessment of extracted DNA and protein. (Linked to SLOs 1.4, 1.5, 1.6)

Answers to Concept Check Questions [Series 1]

Objective Questions

1. CTAB.
2. Divalent (Mg2+, Ca2+).
3. Free.
4. Proteinase K.
5. 10,000 to 20,000.

Short-Answer Questions

6. Cell wall rigidity: addressed by liquid nitrogen grinding to powder. Phenolic compounds: addressed by beta-mercaptoethanol and PVPP in extraction buffer. Polysaccharides: addressed by

lanetonline.com

CTAB at high NaCl, which forms insoluble complexes with polysaccharides while releasing DNA.

7. Grind leaf in liquid nitrogen; incubate in CTAB buffer (65 degrees C; 30 to 60 min); chloroform:isoamyl alcohol extraction; transfer aqueous phase; add CTAB + isopropanol; precipitate at -20 degrees C; centrifuge; wash with 70 percent ethanol; dissolve in TE; treat with RNase A; assess by nanodrop and agarose gel.
8. Native extraction: mild conditions (PBS, glycerol, DTT, protease inhibitors); used when protein activity must be preserved (enzyme assays, binding studies). Denaturing extraction: SDS, boiling, reducing agents; used for SDS-PAGE analysis when only protein size and quantity are needed.
9. Measure A260 (DNA concentration: A260 x 50 x dilution factor = micrograms per ml). A260/A280 ratio: 1.8 to 2.0 indicates pure DNA; below 1.8 indicates protein or phenol contamination. A260/A230 ratio: 2.0 to 2.2 is acceptable; lower indicates polysaccharide, EDTA, or organic solvent contamination.
10. Step 1 (300 to 600 x g): pellets cell debris and intact cells. Step 2 (1,000 to 3,000 x g): pellets nuclei. Step 3 (10,000 to 20,000 x g): pellets mitochondria and lysosomes. Step 4 (100,000 x g; ultracentrifugation): pellets ribosomes and microsomes; supernatant is cytosol.

Long-Answer Questions

11. Cell disruption methods: mechanical (mortar and pestle + liquid N2; bead mill; sonication; French press); chemical (detergents: SDS, CTAB; chaotropic agents: guanidinium thiocyanate; alkaline lysis: NaOH + SDS). Key buffer reagents: Tris-HCl (pH maintenance); NaCl (ionic stabilisation); EDTA (DNase inhibition by Mg2+/Ca2+ chelation); CTAB or SDS (membrane disruption); beta-mercaptoethanol (prevents phenolic oxidation). Safety: phenol-chloroform in fume hood; nitrile gloves; cryogenic gloves for liquid N2. CTAB steps: grind leaf in liquid N2; add prewarmed CTAB buffer (2% CTAB, 1.4 M NaCl, Tris, EDTA, beta-ME); 65 degrees C 30 to 60 min; chloroform:isoamyl alcohol (24:1); transfer aqueous phase; add CTAB + isopropanol; -20 degrees C precipitation; centrifuge; 70 percent ethanol wash; dissolve in TE; RNase A treatment; assess by nanodrop and gel.
12. Animal tissue DNA: homogenise in SDS lysis buffer; proteinase K (55 degrees C; overnight); phenol-chloroform or spin column; ethanol precipitation; TE. Blood DNA: buffy coat in SDS + proteinase K; commercial spin-column kit (QIAamp; 30 min; no organic solvents). Bacterial DNA: lysozyme pretreatment to digest peptidoglycan; SDS + proteinase K; phenol-chloroform extraction; ethanol precipitation. Protein extraction principles: native (PBS, glycerol, DTT, protease inhibitors) for activity; denaturing (RIPA buffer, SDS) for SDS-PAGE. Plant-specific:

lanetonline.com

PVPP adsorbs phenolics; acetone powder for high-lipid tissues. Subcellular fractionation: differential centrifugation at increasing forces (300 x g debris; 1,000 to 3,000 x g nuclei; 10,000 to 20,000 x g mitochondria; 100,000 x g ribosomes/microsomes); density gradient (Percoll) for chloroplasts. Quality assessment: DNA (agarose gel: single high-MW band; no RNA smear; nanodrop: A260/A280 1.8 to 2.0; A260/A230 2.0 to 2.2; concentration = A260 x 50 x dilution factor); protein (Bradford at 595 nm or BCA at 562 nm; SDS-PAGE for integrity; enzyme activity assay). Storage: DNA in TE at -20 to -80 degrees C; proteins with 50 percent glycerol at -20 degrees C; minimise freeze-thaw.

Answers to Pilot Questions [Series 1]

Part 1.1

1. Cell wall rigidity: address by liquid nitrogen grinding to brittle powder. Phenolic compounds: address by beta-mercaptoethanol and PVPP in extraction buffer to prevent oxidation and adsorb phenolics. Polysaccharides: address by CTAB at high NaCl, which forms insoluble complexes with polysaccharides while DNA remains in solution.
2. Mechanical: mortar and pestle with liquid N2 (tough tissues); bead milling (microorganisms, small volumes). Chemical: SDS or CTAB detergents (solubilise membranes); alkaline lysis (NaOH + SDS; selectively recovers plasmid DNA from bacteria).
3. Tris-HCl: maintains pH 8.0 to prevent acid depurination of DNA. NaCl: provides ionic stabilisation. EDTA: chelates Mg2+/Ca2+; inhibits DNases. CTAB: cationic detergent; disrupts membranes; complexes with polysaccharides at high NaCl. Beta-mercaptoethanol: reducing agent; prevents phenolic oxidation to quinones.
4. NaOH + SDS lyses bacterial membranes and denatures chromosomal DNA and proteins. Neutralisation with potassium acetate precipitates the denatured chromosomal DNA and proteins as an insoluble potassium-SDS complex. Plasmid DNA (covalently closed circular) is small, re-natures rapidly on neutralisation, and remains soluble in the supernatant.
5. Work in fume hood at all times. Wear nitrile gloves and safety goggles. Use sealed centrifuge tubes; avoid splashing. Dispose of phenol-chloroform waste as halogenated organic solvent in labelled waste containers.

Part 1.2

laneronline.com

1. Cell disruption; protein denaturation and removal; RNA removal by RNase A; DNA precipitation with ethanol or isopropanol; washing with 70 percent ethanol; re-suspension in TE or nuclease-free water.
2. CTAB is a cationic detergent that disrupts cell membranes and forms insoluble complexes with polysaccharides at high NaCl (1.4 M); DNA is released into solution while polysaccharides are removed. High NaCl is essential to maintain the CTAB-polysaccharide complex; reducing NaCl below 0.5 M causes CTAB to also precipitate DNA.
3. Tissue is homogenised in SDS lysis buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM EDTA, 1 percent SDS). Proteinase K (100 to 200 micrograms per ml) is added; incubation at 55 degrees C for 3 hours to overnight digests all proteins. Protein is then removed by phenol-chloroform extraction and DNA is precipitated with ethanol.
4. Bacterial peptidoglycan cell walls are not disrupted by detergents alone. Lysozyme cleaves beta-1,4-glycosidic bonds in peptidoglycan, weakening the cell wall; subsequent SDS addition then lyses the weakened membrane. Without lysozyme pretreatment, DNA yield from bacteria is greatly reduced.
5. Phenol-chloroform: effective removal of all proteins; does not require specialised equipment; cheaper reagents. Disadvantage: toxic; requires fume hood. Spin-column: fast (30 min); no organic solvents; consistent quality; suitable for clinical and high-throughput use. Disadvantage: higher reagent cost per sample.

Part 1.3

1. Native: mild buffer (PBS, glycerol, DTT, protease inhibitors); used for enzyme assays and protein-protein interaction studies where activity must be retained. Denaturing: SDS, boiling, reducing agents; used for SDS-PAGE where only protein size and quantity are required.
2. Phenolic compounds: add PVPP to adsorb phenolics and beta-mercaptoethanol to prevent oxidation. Polysaccharides: increase viscosity and interfere with assays; use dilute buffer and centrifugation to remove insoluble polysaccharide. High lipid content (seeds): defatting with cold acetone before aqueous extraction.
3. 300 to 600 x g: cell debris and intact cells. 1,000 to 3,000 x g: nuclei. 10,000 to 20,000 x g: mitochondria and lysosomes. 100,000 x g (ultracentrifugation): ribosomes and microsomes. Supernatant remaining after 100,000 x g = cytosol.
4. Ground plant material is layered on a 40/80 percent Percoll gradient. Centrifugation at approximately 4,000 x g for 5 to 10 minutes. Intact chloroplasts band at the 40/80 percent

lanetonline.com

interface due to their buoyant density. Broken chloroplasts and debris remain at the 40 percent layer. The intact chloroplast band is aspirated, washed, and re-centrifuged.

5. Red blood cells are washed with isotonic saline to remove plasma proteins. Cells are lysed by hypotonic shock (add 4 to 5 volumes ice-cold distilled water; mix) or by freeze-thaw. Centrifuge at 20,000 x g to pellet membrane ghosts. The supernatant (haemolysate) contains predominantly haemoglobin.

Part 1.4

1. Single high-MW band: intact genomic DNA; good quality extraction. Smear below 2 kb: RNA contamination (RNase treatment incomplete) or DNA degradation by nucleases or mechanical shearing. A260/A280 ratio of 1.6: significant protein or phenol contamination; may inhibit downstream reactions; repeat extraction or clean up with ethanol precipitation.
2. DNA concentration (micrograms per ml) = A260 reading x 50 (extinction coefficient for double-stranded DNA) x dilution factor. Example: A260 = 0.25; dilution factor = 10; concentration = 0.25 x 50 x 10 = 125 micrograms per ml.
3. Bradford: fast; simple; BSA standard; 595 nm; compatible with reducing agents. BCA: more sensitive; compatible with more detergent types; less affected by non-protein chromogens; heat-assisted version improves accuracy.
4. A260/A280 ratio (1.8 to 2.0 for pure DNA). A260/A230 ratio (2.0 to 2.2 for pure DNA). DNA concentration (micrograms per ml). Agarose gel: single high-MW band without RNA smear. Absence of visible degradation (no low-MW smear on gel).
5. Each freeze-thaw cycle subjects DNA to physical shearing forces as ice crystals form and melt; repeated cycles progressively fragment high-molecular-weight DNA into smaller pieces, reducing its suitability for applications requiring intact high-MW DNA such as southern blotting and long-read sequencing.

lanetonline.com

References and Attributions [Series 1]

Textual References (Books and External Sources)

- Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11–15.
- Sambrook, J., & Russell, D. W. (2001). Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor Laboratory Press.
- Wilson, K., & Walker, J. (Eds.). (2010). Principles and techniques of biochemistry and molecular biology (7th ed.). Cambridge University Press.
- Green, M. R., & Sambrook, J. (2012). Molecular cloning: A laboratory manual (4th ed.). Cold Spring Harbor Laboratory Press.

Figures, Plates, Tables, and Boxes

- Figure 1.1: Flowchart of the CTAB method for plant DNA extraction. Attribution: AI generated. Suggested OER search string: “CTAB plant DNA extraction protocol flowchart steps procedure OER”.
- Box 1.1: Comparison of DNA and protein extraction methods for different tissue types. Attribution: AI generated. Suggested OER search string: “DNA protein extraction method comparison tissue type reagent table OER”.

lanetonline.com

Course code: BIO 408

Course title: Applied Biotechnology

Course credit load: 2 Units

Series 2: Quantification and Chromatography Techniques

- **Part 2.1: Quantification of Nucleic Acids and Proteins**
  - Sub Part 2.1.1: Spectrophotometric quantification of nucleic acids
  - Sub Part 2.1.2: Fluorometric quantification of nucleic acids
  - Sub Part 2.1.3: Protein quantification methods
- **Part 2.2: Principles of Chromatography**
  - Sub Part 2.2.1: Definition, components, and classification of chromatography
  - Sub Part 2.2.2: Paper chromatography
  - Sub Part 2.2.3: Thin layer chromatography (TLC)
- **Part 2.3: Column Chromatography Techniques**
  - Sub Part 2.3.1: Ion exchange chromatography
  - Sub Part 2.3.2: Gel filtration (size exclusion) chromatography
  - Sub Part 2.3.3: Affinity chromatography
- **Part 2.4: High-Performance Liquid Chromatography and Applications**
  - Sub Part 2.4.1: Principles and components of HPLC
  - Sub Part 2.4.2: Reverse-phase and other HPLC modes

lanetonline.com

- Sub Part 2.4.3: Applications of chromatography in biotechnology

Introduction

Accurate quantification of biomolecules and effective separation by chromatography are two of the most fundamental skills in applied biotechnology. Before any molecular technique can proceed reliably, the researcher must know the concentration and purity of the starting material. Chromatography is the principal technique for separating complex biological mixtures: it underpins protein purification, metabolite analysis, pharmaceutical quality control, food analysis, and forensic science.

This Series covers the quantification of nucleic acids and proteins by spectrophotometric, fluorometric, and colorimetric methods, and then describes the full range of chromatographic techniques from simple paper and thin layer chromatography to column-based methods (ion exchange, gel filtration, affinity) and high-performance liquid chromatography (HPLC). Applications relevant to Nigerian biotechnology, including purification of enzymes for industrial use, analysis of secondary metabolites in medicinal plants, and quality control of food products, are emphasised throughout.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 2.1** quantify nucleic acids and proteins using spectrophotometric, fluorometric, and colorimetric methods and interpret the results (Linked to Part 2.1),
- **SLO 2.2** explain the principles of chromatography and classify chromatographic techniques by stationary phase, mobile phase, and separation mechanism (Linked to Part 2.2),
- **SLO 2.3** describe paper and thin layer chromatography, calculate Rf values, and explain their applications (Linked to Part 2.2),

lanetonline.com

- **SLO 2.4** describe ion exchange, gel filtration, and affinity chromatography and explain the basis of separation in each (Linked to Part 2.3),
- **SLO 2.5** explain the principles and components of HPLC and distinguish between reverse-phase and other HPLC modes (Linked to Part 2.4), and
- **SLO 2.6** describe the applications of chromatography in protein purification, metabolite analysis, food analysis, and forensic science (Linked to Part 2.4).

2.1 Quantification of Nucleic Acids and Proteins

2.1.1 Spectrophotometric quantification of nucleic acids

UV spectrophotometry is the standard method for quantifying nucleic acids in solution. The aromatic rings of the purine and pyrimidine bases absorb UV light with a maximum at 260 nm; Beer-Lambert law applies: A260 = epsilon x c x l, where epsilon is the molar extinction coefficient, c is the molar concentration, and l is the pathlength (1 cm for standard cuvettes). For double-stranded DNA, an absorbance of 1.0 at 260 nm corresponds to approximately 50 micrograms per ml; for single-stranded DNA or RNA, 1.0 at 260 nm corresponds to approximately 40 micrograms per ml; for single-stranded oligonucleotides, approximately 33 micrograms per ml. DNA concentration is therefore calculated as: c (micrograms per ml) = A260 x 50 x dilution factor.

Purity is assessed using absorbance ratios as described in Series 1: A260/A280 of 1.8 to 2.0 indicates pure DNA; 2.0 to 2.2 indicates pure RNA. The A260/A230 ratio (2.0 to 2.2 is acceptable) detects contamination by guanidinium salts, EDTA, polysaccharides, and organic solvents that absorb at 230 nm. The nanodrop spectrophotometer requires only 1 to 2 microlitres of undiluted sample and gives concentration and purity ratios simultaneously; it is the standard quantification tool in modern molecular biology laboratories and is available at IITA, ABU, and University of Ibadan research facilities. Standard UV cuvette spectrophotometry requires 50 to 100 microlitres of sample diluted to an A260 between 0.1 and 1.0 for accuracy.

lanetonline.com

Fill in the blank: For double-stranded DNA, an A260 reading of 1.0 corresponds to a concentration of _____ micrograms per ml; this extinction coefficient is used to calculate DNA concentration from nanodrop or UV spectrophotometer readings.

2.1.2 Fluorometric quantification of nucleic acids

Fluorometric methods offer greater sensitivity and specificity than UV spectrophotometry for nucleic acid quantification, particularly when samples contain contaminants that absorb at 260 nm or when very small amounts of nucleic acid must be measured. Intercalating fluorescent dyes bind specifically to double-stranded or single-stranded nucleic acids and fluoresce intensely only when bound; the fluorescence signal is proportional to nucleic acid concentration. PicoGreen (Invitrogen) is a fluorescent dye with high specificity for double-stranded DNA (dsDNA); it does not fluoresce significantly in the presence of RNA or single-stranded DNA, making it ideal for accurate dsDNA quantification in samples containing RNA contamination. RiboGreen is the equivalent reagent for RNA quantification.

The Qubit fluorometer (Thermo Fisher Scientific) is a compact benchtop instrument that uses single-use fluorescent assay reagents (Qubit dsDNA HS Assay: high sensitivity, 0.2 to 100 ng; Qubit dsDNA BR Assay: broad range, 2 to 1,000 ng) to give accurate DNA concentration values unaffected by RNA, free nucleotides, or protein contamination. Unlike nanodrop, Qubit measures only the target nucleic acid and is therefore more accurate when samples are impure. For total nucleic acid staining, ethidium bromide (EtBr) intercalates into double-stranded DNA and RNA and fluoresces orange-red under UV; SYBR Green I (highly sensitive; 100 times more fluorescent than EtBr with dsDNA; used in real-time PCR; described in Series 3) and SYBR Safe (non-toxic alternative to EtBr for gel staining) are widely used.

Fill in the blank: The Qubit fluorometer uses fluorescent dye reagents that fluoresce only when bound to the target nucleic acid; unlike nanodrop, it is not affected by _____ or protein contamination, making it more accurate for impure DNA samples.

2.1.3 Protein quantification methods

lanetonline.com

The principal colorimetric protein assays are described in Series 1 (Bradford at 595 nm; BCA at 562 nm; Lowry at 750 nm). The Bradford assay (Coomassie Brilliant Blue G-250) is the most widely used in Nigerian research laboratories because it is rapid (5 minutes), sensitive (1 to 20 micrograms per ml linear range; or 200 to 1,500 micrograms per ml for the macro method), and requires only a standard spectrophotometer. All colorimetric assays require a standard curve constructed from known concentrations of a reference protein (bovine serum albumin, BSA; or bovine gamma-globulin); the absorbance of the unknown sample is interpolated from the standard curve to give protein concentration.

For total protein estimation in complex samples (serum, cell lysates), the biuret method (Cu2+ reduced to Cu+ by peptide bonds; absorbance at 540 nm; range 1 to 10 mg per ml; least sensitive but most specific for protein) is used when sensitivity is not a limiting factor. The UV absorbance method at 280 nm (A280 directly proportional to protein concentration due to tyrosine and tryptophan absorption; 1 mg per ml pure protein gives approximately A280 = 1.0 for most proteins, but the extinction coefficient varies widely between proteins) is used for pure protein solutions of known identity. Protein quantification must always be performed in parallel with the intended downstream assay to confirm that assay-compatible concentrations are used.

Fill in the blank: In the Bradford assay, a standard curve is constructed using known concentrations of _____ serum albumin (BSA); the absorbance of the unknown protein sample at 595 nm is interpolated from this curve to determine protein concentration.

Pilot questions 2.1

1. Calculate the concentration of a DNA sample with A260 = 0.35, measured on a nanodrop (pathlength 1 mm; undiluted sample). Use the dsDNA extinction coefficient.
2. Explain why a Qubit fluorometer gives a more accurate dsDNA measurement than a nanodrop for an impure DNA sample.
3. Describe how a Bradford protein standard curve is constructed and used to quantify an unknown protein.
4. State the A260/A280 and A260/A230 ratios that indicate a pure DNA preparation and explain what low values of each ratio indicate.

lanetonline.com

5. Distinguish between PicoGreen and ethidium bromide as fluorescent DNA stains, stating one advantage of each.

2.2 Principles of Chromatography

2.2.1 Definition, components, and classification of chromatography

Chromatography is a separation technique in which components of a mixture are distributed between two phases: a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or gas that moves through or over the stationary phase). Separation occurs because different components interact with the stationary phase to different extents; components with stronger interaction with the stationary phase move more slowly through the system than those with weaker interaction. The basic components of a chromatographic system are: the stationary phase (silica, cellulose, ion exchange resin, size exclusion resin, affinity ligand-coupled resin, or a reversed-phase bonded silica); the mobile phase (water, organic solvent, buffer); the sample (mixture to be separated); and the detector (UV detector, fluorescence detector, refractive index detector, or mass spectrometer in HPLC; or visual detection for paper and TLC).

Chromatography is classified by: stationary phase (paper, silica TLC plate, column resin); mobile phase (liquid chromatography vs gas chromatography); and separation mechanism (adsorption chromatography; partition chromatography; ion exchange chromatography; size exclusion chromatography; affinity chromatography; reversed-phase chromatography). In adsorption chromatography, solutes adsorb onto the stationary phase surface by van der Waals forces and hydrogen bonding; polar stationary phases (silica) retain polar compounds more strongly. In partition chromatography, solutes partition between the liquid stationary phase and the mobile phase based on their relative solubility in each. Performance is described by the retention factor (k), separation factor (alpha), and plate height (H); high column efficiency (many theoretical plates, N) gives sharp, well-resolved peaks.

lanetonline.com

Fill in the blank: In chromatography, separation occurs because different components interact with the _____ phase to different extents; components with stronger interactions migrate more slowly than those with weaker interactions, allowing their separation over the length of the chromatographic system.

2.2.2 Paper chromatography

Paper chromatography uses cellulose paper as the stationary phase; the mobile phase is an organic solvent or solvent mixture that ascends (ascending paper chromatography) or descends (descending paper chromatography) through the paper by capillary action or gravity. Separation is primarily by partition: solutes partition between the aqueous layer bound to cellulose fibres (stationary) and the moving organic solvent (mobile phase). The technique is simple, inexpensive, and requires no specialised equipment: a glass tank, filter paper (Whatman No. 1 or 3MM), solvent, and a UV lamp or chemical spray reagent are sufficient.

Procedure: a small spot (1 to 2 microlitres) of the sample is applied 1.5 to 2 cm from the bottom of the paper using a micropipette or capillary tube and allowed to dry; the paper is placed in the solvent tank with the spot above the solvent level; the solvent is allowed to ascend until it approaches the top of the paper (30 to 90 minutes depending on solvent and paper length); the paper is removed and the solvent front is marked immediately. Spots are visualised under UV light (for UV-absorbing compounds) or by spraying with ninhydrin (for amino acids; produces purple colour), iodine vapour (for lipids and carbohydrates), or other specific reagents. The Rf (retardation factor) value is calculated as: Rf = distance travelled by spot / distance travelled by solvent front. Rf is characteristic for a compound under defined conditions (solvent, temperature, paper) and is used for identification by comparison with standards run simultaneously. Applications: separation of plant pigments (chlorophylls and carotenoids; butanol:acetic acid:water solvent); amino acid analysis; identification of sugars and vitamins.

Fill in the blank: The _____ (Rf) value in paper and TLC chromatography is calculated as the distance travelled by the compound spot divided by the distance travelled by the solvent front; it is characteristic for a given compound under defined solvent and temperature conditions.

2.2.3 Thin layer chromatography (TLC)

lanetonline.com

Thin layer chromatography (TLC) uses a thin layer of adsorbent (silica gel or aluminium oxide; 0.1 to 0.25 mm thick) coated onto an aluminium, glass, or plastic backing as the stationary phase. TLC is faster than paper chromatography (10 to 30 minutes), gives sharper spots, and offers a wider choice of stationary and mobile phases for different classes of compounds. Normal-phase TLC uses a polar stationary phase (silica gel) and a non-polar mobile phase; polar compounds are retained more strongly. Reversed-phase TLC (RP-TLC) uses a non-polar stationary phase (C18-modified silica) and a polar mobile phase; non-polar compounds are retained more strongly.

TLC procedure: apply the sample as a small spot on the TLC plate 1 to 1.5 cm from the bottom; place the plate in a glass developing chamber pre-equilibrated with the solvent system (chamber lined with filter paper soaked in solvent to saturate the atmosphere); allow solvent to ascend to 1 to 1.5 cm from the top of the plate; remove and mark solvent front immediately. Visualise spots under UV (254 nm or 365 nm); silica plates with fluorescent indicator show dark spots under 254 nm UV where UV-absorbing compounds quench fluorescence. Chemical staining: sulphuric acid charring (universal; carbonises organic compounds on heating); KMnO4 (oxidising compounds show yellow-brown spots on green background); vanillin-sulphuric acid (colour-specific for terpenes and steroids); ninhydrin (amino acids); anisaldehyde-sulphuric acid (terpenoids). TLC is widely used in Nigerian natural product research for rapid screening of medicinal plant extracts and monitoring of organic synthesis reactions.

Fill in the blank: In normal-phase TLC, the stationary phase is polar (_____ gel) and the mobile phase is non-polar; polar compounds interact more strongly with the stationary phase and travel shorter distances (lower Rf) than non-polar compounds.

lanetonline.com

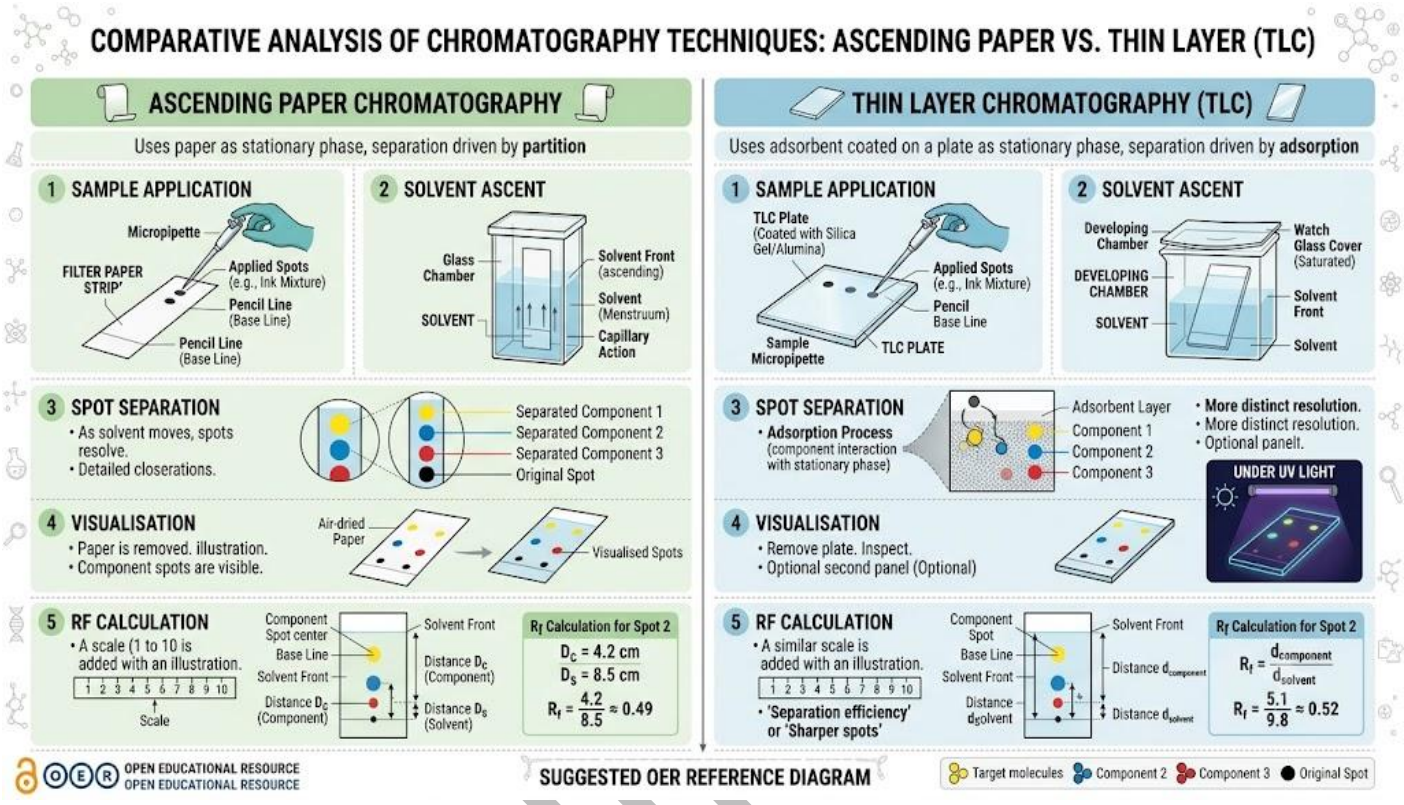


Figure 2.1: Comparison of ascending paper chromatography and TLC procedures with Rf calculation

Pilot questions 2.2

1. Define chromatography and state the two phases present in any chromatographic system.
2. Describe the procedure for ascending paper chromatography to separate amino acids in a plant extract.
3. A compound travels 4.2 cm and the solvent front travels 7.0 cm in a TLC experiment. Calculate the Rf value and explain what it means.
4. Distinguish between normal-phase and reversed-phase TLC in terms of stationary phase, mobile phase, and which compounds are retained more strongly.
5. Name three chemical spray reagents used to visualise spots on TLC plates and state what class of compound each detects.

2.3 Column Chromatography Techniques

lanetonline

2.3.1 Ion exchange chromatography

Ion exchange chromatography (IEX) separates molecules based on differences in their net surface charge at a given pH. The stationary phase is an insoluble resin to which charged groups are covalently attached: cation exchangers carry negatively charged groups (sulphonate: SP; carboxymethyl: CM) that attract positively charged molecules; anion exchangers carry positively charged groups (quaternary ammonium: Q; diethylaminoethyl: DEAE) that attract negatively charged molecules. At a pH above its isoelectric point (pI), a protein carries a net negative charge and will bind to an anion exchanger; at a pH below its pI, it carries a net positive charge and binds to a cation exchanger. Sample proteins that bind to the resin are eluted by increasing the salt concentration (gradient elution) or by changing the pH, both of which reduce electrostatic interactions between protein and resin.

IEX is one of the most widely used initial purification steps for proteins because it is high-capacity, scalable, and inexpensive. The procedure: equilibrate the column with starting buffer (low ionic strength; chosen pH); load the sample; wash with starting buffer to remove unbound proteins; elute bound proteins with a linear NaCl gradient (0 to 1 M) in the same buffer while monitoring absorbance at 280 nm; collect fractions; assay fractions for the target protein (enzyme activity or immunological assay). Proteins elute at different salt concentrations depending on their charge; the target protein is identified in fractions showing the desired activity. Ion exchange resins include Sepharose-based (GE Healthcare) and Toyopearl (Tosoh) products; DEAE-cellulose and CM-cellulose are older resins still used in resource-limited Nigerian laboratories.

Fill in the blank: In anion exchange chromatography, proteins with a net _____ charge at the working pH bind to the positively charged resin groups; they are eluted by increasing the NaCl concentration, which competes with the protein for binding sites on the resin.

2.3.2 Gel filtration (size exclusion) chromatography

Gel filtration chromatography (GFC), also called size exclusion chromatography (SEC), separates molecules based solely on their hydrodynamic size (effective molecular radius). The stationary phase consists of porous beads (cross-linked dextran: Sephadex; cross-linked agarose:

lanetonline.com

Sepharose CL; polyacrylamide: Biogel P; cross-linked dextran-agarose: Superdex) with a defined pore size distribution. Small molecules enter the pores and are retarded; large molecules that cannot enter the pores pass around the beads and elute first (in the void volume, V0). Molecules of intermediate size partially enter the pores and elute between V0 and the total column volume (Vt). Because separation is entirely based on size (not charge or affinity), GFC requires no gradient elution and gives quantitative recovery of native proteins.

Applications of GFC: determination of native molecular weight of proteins (by comparison with standard proteins of known MW; a semi-logarithmic plot of log MW vs elution volume is linear for globular proteins); buffer exchange (desalting: small-pore Sephadex G-25 separates high-MW protein from low-MW buffer salts in minutes; used routinely after IEX elution); removal of aggregates or high-MW contaminants before crystallisation or injection into patients; and fractionation of oligosaccharides, nucleic acids, or synthetic polymers. Commonly used resins: Sephadex G-25 and G-50 for desalting and small protein separation; Sephacryl S-100 to S-400 for protein MW range 1 kDa to 8 MDa; Superose 6 and 12 for high-resolution protein separation on FPLC (fast protein liquid chromatography) systems.

Fill in the blank: In gel filtration chromatography, molecules that are too large to enter the pores of the beads elute first in the _____ volume (V0); small molecules penetrate all pores and elute last; separation is based entirely on hydrodynamic size.

2.3.3 Affinity chromatography

Affinity chromatography exploits the specific, reversible, non-covalent interaction between a target molecule (ligand) and a complementary binding partner (affinity ligand) immobilised on the stationary phase. It provides the highest purification factor of any single chromatographic step, often achieving 100-fold or greater purification in one step. Common affinity systems include: enzyme-substrate or enzyme-inhibitor affinity (e.g. ATP-agarose for kinases; heparin-Sepharose for DNA-binding proteins); lectin affinity (concanavalin A-Sepharose for glycoproteins); antibody affinity (protein A-Sepharose or protein G-Sepharose for immunoglobulin purification; Protein A binds the Fc region of IgG from most mammalian species); metal chelate affinity (immobilised metal ion affinity chromatography, IMAC: Ni2+-

lanetonline.com

NTA resin binds his-tagged recombinant proteins via histidine coordination to Ni2+; eluted with imidazole); and dye-ligand affinity (Cibacron Blue-Sepharose binds albumin, kinases, and dehydrogenases non-specifically).

His-tag affinity (IMAC) is the most widely used affinity purification strategy in recombinant protein production: the gene of interest is cloned into an expression vector with a hexahistidine (6xHis) tag; the expressed fusion protein is loaded onto Ni-NTA resin; impurities are washed away; the his-tagged protein is eluted with 250 to 500 mM imidazole; the his-tag can be removed with an enterokinase or TEV protease cleavage site if needed. IMAC is the first purification step for essentially all recombinant proteins produced in E. coli expression systems. Glutathione S-transferase (GST) tag affinity on glutathione-Sepharose is an alternative affinity system; GST-tagged proteins are eluted with reduced glutathione (10 mM).

Fill in the blank: In IMAC affinity chromatography, hexahistidine (his-tagged) recombinant proteins bind to _____ -NTA resin through coordination between histidine residues and the immobilised nickel ions; they are eluted with imidazole, which competes with histidine for the nickel binding site.

Method	Separation Basis	Stationary Phase	Elution Method	Applications
Ion exchange	Charge differences between molecules	Resin with charged groups (cationic or anionic exchangers)	Increasing salt concentration or changing pH to disrupt ionic interactions	Purification of proteins, nucleic acids, water treatment
Gel filtration (Size exclusion)	Molecular size (larger molecules elute first)	Porous beads (cross-linked polymers like Sephadex)	Buffer flow; molecules separated by exclusion from pores	Protein desalting, molecular weight estimation, separation of biomolecules
Affinity chromatography	Specific binding	Matrix with immobilized	Competitive ligand, pH	Isolation of enzymes,

lanetonline.com

	interactions (ligand–target)	ligands (e.g., antibodies, metal ions, cofactors)	change, or salt to disrupt binding	antibodies, recombinant proteins

Box 2.1: Comparison of ion exchange, gel filtration, and affinity column chromatography

Pilot questions 2.3

1. Explain the basis of separation in ion exchange chromatography and describe how proteins are eluted from an anion exchange column.
2. Distinguish between ion exchange and gel filtration chromatography in terms of separation basis, stationary phase, and elution method.
3. Explain how IMAC (his-tag affinity chromatography) is used to purify a recombinant protein from an E. coli lysate.
4. Describe how gel filtration chromatography can be used to determine the native molecular weight of a protein.
5. State two advantages of affinity chromatography over ion exchange chromatography for protein purification.

2.4 High-Performance Liquid Chromatography and Applications

2.4.1 Principles and components of HPLC

High-performance liquid chromatography (HPLC) is an automated, high-resolution liquid chromatography technique that uses high pressure (50 to 400 bar) to force the mobile phase through a column packed with very small, uniform particles (typically 1.8 to 10 micrometres in diameter). The small particle size greatly increases surface area and interaction between solute and stationary phase, giving much higher resolution and faster separation than conventional gravity-flow column chromatography. A typical HPLC system consists of: (1) solvent reservoir(s): containing the mobile phase(s), degassed to remove dissolved air that would cause baseline noise; (2) high-pressure pump: delivers mobile phase at precise, constant flow rates

lanetonline.com

(typically 0.5 to 2 ml per minute) with RSD below 0.5 percent; (3) sample injector: manual (Rheodyne valve) or autosampler (for high-throughput work); injects 5 to 100 microlitres of sample; (4) column: stainless steel; typically 150 to 250 mm x 4.6 mm internal diameter; packed with silica-based stationary phase particles; (5) detector: UV/Vis (most common; 190 to 800 nm), fluorescence, electrochemical, refractive index, or mass spectrometer (LC-MS); (6) data system: integrates peak areas and produces chromatograms.

A guard column (short column of same stationary phase; 5 to 10 mm) is placed before the analytical column to protect it from particulate matter and strongly retained contaminants. The mobile phase may be delivered isocratically (constant composition) or as a gradient (changing composition over time to elute compounds of increasing hydrophobicity or decreasing polarity). Ultra-high performance liquid chromatography (UHPLC or UPLC; Waters Acquity; sub-2-micrometre particles; up to 1,000 bar) provides even faster, higher-resolution separations in shorter run times (1 to 10 minutes). HPLC systems are available at IITA (Ibadan), University of Ibadan, ABU (Zaria), and major teaching hospitals for research and quality control applications.

Fill in the blank: In HPLC, the use of very small stationary phase particles (1.8 to 10 micrometres) requires a _____ pump to force the mobile phase through the tightly packed column at flow rates of 0.5 to 2 ml per minute; this generates pressures of 50 to 400 bar.

2.4.2 Reverse-phase and other HPLC modes

Reverse-phase HPLC (RP-HPLC) is the most widely used HPLC mode, accounting for approximately 70 percent of all HPLC separations. The stationary phase is non-polar (octadecylsilane, C18; octylsilane, C8; or phenyl groups bonded to silica); the mobile phase is polar (water mixed with organic solvent: methanol or acetonitrile). Non-polar compounds are retained more strongly on the C18 stationary phase and elute later (higher organic solvent concentration needed to displace them). A typical gradient for biomolecule separation starts at 5 percent acetonitrile in water (with 0.1 percent trifluoroacetic acid or formic acid as an ion-pairing agent) and increases linearly to 95 percent acetonitrile over 20 to 30 minutes. RP-HPLC is used for separation of peptides, amino acids, nucleotides, alkaloids, phenolic compounds, lipids, and pharmaceuticals.

lanetonline.com

Other HPLC modes include: normal-phase HPLC (NP-HPLC): polar stationary phase (silica or diol); non-polar mobile phase (hexane, dichloromethane); separates lipids, fat-soluble vitamins, and non-polar natural products; seldom used for biomolecules. Ion exchange HPLC (IEX-HPLC): high-resolution ion exchange on HPLC-grade resins; separates proteins, nucleotides, and organic acids. Size exclusion HPLC (SE-HPLC): high-resolution protein MW analysis on rigid HPLC SEC columns (TSK-GEL, Agilent Bio SEC). Hydrophilic interaction chromatography (HILIC): retains polar compounds on a polar stationary phase using an aqueous-organic mobile phase; used for sugars, nucleosides, and amino acids that are poorly retained by RP-HPLC. Chiral HPLC: uses a chiral stationary phase to separate enantiomers; important in pharmaceutical analysis.

Fill in the blank: In reverse-phase HPLC, the stationary phase is non-polar (typically C18 bonded silica) and the mobile phase is polar (water and _____ or methanol); non-polar compounds are retained more strongly and require higher organic solvent concentration to elute.

2.4.3 Applications of chromatography in biotechnology

Protein purification pipeline: in recombinant protein production, a standard three-step chromatography pipeline is used: (1) IMAC affinity on Ni-NTA resin (capture; high specificity for his-tagged protein; 50 to 100-fold purification); (2) ion exchange chromatography (intermediate purification; removes host cell proteins, DNA, endotoxins; IEX-HPLC on SP-Sepharose or Q-Sepharose); (3) gel filtration (polishing; removes aggregates; ensures homogeneous monomer fraction; Superdex 75 or 200). This three-step pipeline routinely achieves greater than 99 percent purity for injectable-grade recombinant proteins. The fold purification and recovery at each step are tracked in a purification table.

Metabolite and natural product analysis: RP-HPLC is used in Nigerian natural product research to: profile alkaloids, flavonoids, terpenoids, and phenolic acids in medicinal plant extracts; quantify active compounds in standardised herbal extracts; and identify adulterants in herbal medicines by comparison with reference standards. The National Agency for Food and Drug Administration and Control (NAFDAC) uses HPLC for quality control of pharmaceuticals and food products. Forensic applications: HPLC and GC-MS (gas chromatography-mass

lanetonline.com

spectrometry) are used by the NDLEA (National Drug Law Enforcement Agency) and forensic laboratories to identify and quantify controlled substances (cannabis cannabinoids: THC, CBD; opioids; amphetamines) in exhibits and biological samples. Food analysis: HPLC measures food quality parameters including vitamins, mycotoxins (aflatoxins in groundnuts and maize are a major food safety concern in Nigeria), pesticide residues, and food additives.

Fill in the blank: Aflatoxins in Nigerian groundnuts and maize are quantified by _____ HPLC using fluorescence detection after immunoaffinity column clean-up; aflatoxin B1, B2, G1, and G2 are the four principal aflatoxin species monitored for food safety compliance.

Pilot questions 2.4

1. List and describe the six main components of an HPLC system.
2. Distinguish between reverse-phase and normal-phase HPLC in terms of stationary phase, mobile phase, and the types of compounds separated.
3. Describe the three-step chromatography pipeline used for purification of a recombinant his-tagged protein to pharmaceutical grade.
4. Explain how HPLC is used in Nigerian food safety analysis, giving two specific examples.
5. State three advantages of HPLC over conventional gravity-flow column chromatography.

Series Summary

Nucleic acids can be quantified by UV spectrophotometry or fluorescence-based methods. For double-stranded DNA, concentration is calculated as **A260 × 50 × dilution factor = DNA concentration (µg/mL)**. Pure DNA usually has an **A260/A280 ratio of 1.8–2.0** and an **A260/A230 ratio of 2.0–2.2**. Fluorometric methods are more accurate in impure samples and include PicoGreen for double-stranded DNA, RiboGreen for RNA, and the Qubit fluorometer. Ethidium bromide and SYBR Safe are commonly used to visualise nucleic acids in gels. Protein

lanetonline.com

concentration may be measured using Bradford assay at 595 nm, BCA assay at 562 nm, Lowry assay at 750 nm, biuret assay at 540 nm, or direct A280 measurement for pure proteins.

Chromatography separates substances according to how differently they interact with stationary and mobile phases. In paper chromatography and TLC, compounds are identified using **Rf = distance travelled by solute ÷ distance travelled by solvent front**. TLC is faster and gives sharper separation than paper chromatography. Column methods include ion-exchange chromatography for separation by charge, gel filtration for separation by size, and affinity chromatography for highly specific binding, such as Ni-NTA purification of His-tagged proteins. HPLC uses high pressure and small particles for rapid, accurate separation, with reverse-phase C18 columns being most common. Its applications include protein purification, alkaloid analysis, aflatoxin measurement, and forensic drug testing.

Glossary of Terms

- **Affinity chromatography:** a chromatographic technique that exploits specific, reversible, non-covalent interaction between a target molecule and an immobilised ligand; provides the highest purification factor per step; examples include IMAC (his-tag), protein A (antibodies), and GST-glutathione.
- **Bradford assay:** colorimetric protein quantification using Coomassie Brilliant Blue G-250; protein binding shifts absorbance from 465 nm to 595 nm; BSA standard curve; range 1 to 20 micrograms per ml; the most widely used protein assay in molecular biology laboratories.
- **Gel filtration chromatography:** size exclusion chromatography in which molecules are separated by hydrodynamic size; large molecules elute first in the void volume; small molecules elute last after penetrating all pores; used for MW determination and desalting.
- **HPLC:** high-performance liquid chromatography; uses high-pressure pumps to force mobile phase through columns packed with small particles (1.8 to 10 micrometres); achieves high resolution and fast separation; modes include RP-HPLC, IEX-HPLC, SE-HPLC, and HILIC.

lanetonline.com

- **IMAC:** immobilised metal ion affinity chromatography; uses metal-chelate resin (Ni-NTA) to bind his-tagged recombinant proteins; elution with imidazole; the standard first-step purification for proteins expressed in E. coli.
- **Ion exchange chromatography:** separation based on electrostatic interaction between charged stationary phase groups and oppositely charged molecules; anion exchangers (DEAE, Q) bind negative proteins; cation exchangers (CM, SP) bind positive proteins; eluted by salt gradient.
- **Mobile phase:** the liquid or gas phase that moves through or over the stationary phase, carrying solute molecules with it at a rate inversely related to their interaction with the stationary phase.
- **PicoGreen:** a fluorescent dye with high specificity for double-stranded DNA; fluoresces intensely only when intercalated into dsDNA; used for accurate dsDNA quantification in samples containing RNA or single-stranded DNA contamination.
- **Qubit fluorometer:** a compact fluorometer using single-use fluorescent assay reagents for accurate quantification of dsDNA, ssDNA, or RNA; unaffected by contaminating RNA, protein, or free nucleotides; more accurate than nanodrop for impure samples.
- **Rf value:** retardation factor; the ratio of the distance travelled by a compound spot to the distance travelled by the solvent front in paper or thin layer chromatography; characteristic for a compound under defined conditions; used for identification.
- **Reverse-phase HPLC (RP-HPLC):** the most common HPLC mode; non-polar stationary phase (C18 bonded silica); polar mobile phase (water/acetonitrile or water/methanol); non-polar compounds retained more strongly; used for separation of peptides, alkaloids, phenolics, lipids, and pharmaceuticals.
- **Stationary phase:** the fixed phase in a chromatographic system; interacts with solute molecules to different extents, causing differential migration and separation; examples include cellulose (paper), silica gel (TLC), ion exchange resin, and C18-bonded silica (RP-HPLC).
- **Thin layer chromatography (TLC):** a rapid analytical chromatographic technique using a thin layer of silica gel or alumina on an aluminium or glass backing; faster and sharper than paper chromatography; spots visualised by UV, chemical staining, or charring.

lanetonline.com

- **Void volume (V0):** in gel filtration chromatography, the volume of mobile phase outside the beads in the column; molecules too large to enter any pores elute in V0 and appear as the first peak in the chromatogram.

Concept Check Questions [Series 2]

Objective Questions

1. For double-stranded DNA, an A260 reading of 1.0 corresponds to a concentration of _____ micrograms per ml. (Linked to SLO 2.1)
2. The ratio of the distance travelled by a compound to the distance travelled by the solvent front in paper or TLC chromatography is called the _____ value. (Linked to SLO 2.3)
3. In gel filtration chromatography, molecules too large to enter the pores elute first in the _____ volume. (Linked to SLO 2.4)
4. His-tagged recombinant proteins bind to _____ -NTA resin in IMAC affinity chromatography and are eluted with imidazole. (Linked to SLO 2.4)
5. In reverse-phase HPLC, the stationary phase is non-polar (typically _____ bonded silica) and the mobile phase is polar. (Linked to SLO 2.5)

Short-Answer Questions

6. Explain how DNA concentration is calculated from a nanodrop reading and state what A260/A280 and A260/A230 ratios indicate. (Linked to SLO 2.1)
7. Describe the procedure for ascending TLC and explain how spots are visualised. (Linked to SLO 2.3)
8. Compare ion exchange and gel filtration chromatography in terms of separation basis and elution method. (Linked to SLO 2.4)
9. Describe the three-step chromatography pipeline for purification of a recombinant his-tagged protein. (Linked to SLO 2.6)
10. State three advantages of HPLC over gravity-flow column chromatography. (Linked to SLO 2.5)

Long-Answer Questions

lanetonline.com

11. Describe the methods available for quantification of nucleic acids and proteins. Compare paper chromatography and TLC in terms of stationary phase, procedure, Rf calculation, and applications. (Linked to SLOs 2.1, 2.2, 2.3)
12. Describe ion exchange, gel filtration, and affinity chromatography. Explain the principles, components, and modes of HPLC, and discuss its applications in Nigerian biotechnology, food safety, and forensic science. (Linked to SLOs 2.4, 2.5, 2.6)

Answers to Concept Check Questions [Series 2]

Objective Questions

1. 50.
2. Rf.
3. Void (V0).
4. Ni.
5. C18.

Short-Answer Questions

6. Concentration (micrograms per ml) = A260 x 50 x dilution factor. A260/A280 ratio: 1.8 to 2.0 = pure DNA; below 1.8 = protein or phenol contamination. A260/A230 ratio: 2.0 to 2.2 = acceptable; below 2.0 = contamination by salts, EDTA, or organic solvent.
7. Apply sample spot 1 to 1.5 cm from bottom of TLC plate; place in chamber pre-equilibrated with solvent; allow solvent to ascend to near top; mark solvent front; dry plate. Visualise: UV lamp (254 or 365 nm) for UV-absorbing compounds; chemical spray (ninhydrin for amino acids; KMnO4 for oxidisable compounds; sulphuric acid charring for all organics).
8. Ion exchange: separates by charge; proteins bind to resin at low ionic strength; eluted by NaCl gradient or pH change. Gel filtration: separates by size; large molecules elute first in void volume; no gradient needed; isocratic elution with buffer.

lanetonline.com

9. Step 1: IMAC on Ni-NTA (captures his-tagged protein; 50 to 100-fold purification; elute with imidazole). Step 2: ion exchange HPLC (removes host cell proteins, DNA, endotoxins). Step 3: gel filtration SEC (polishing; removes aggregates; ensures homogeneous monomer).
10. Higher resolution due to small particle size. Faster separation (minutes vs hours). Automated gradient delivery and fraction collection. Quantitative peak area integration by data system. Reproducible and precise retention times for compound identification.

Long-Answer Questions

11. Nucleic acid quantification: UV spectrophotometry (A260 x 50 = dsDNA micrograms per ml; A260/A280 1.8 to 2.0; A260/A230 2.0 to 2.2; nanodrop requires 1 to 2 microlitres). Fluorometric (PicoGreen: dsDNA-specific; Qubit: accurate for impure samples; EtBr: gel staining). Protein quantification: Bradford (Coomassie G-250; 595 nm; 1 to 20 micrograms per ml; BSA standard); BCA (562 nm; more detergent-tolerant); Lowry (750 nm; slow); A280 (pure known protein). Paper chromatography: cellulose stationary phase; organic solvent mobile phase; partition separation; ascending procedure (spot at baseline; tank with solvent; ascent 30 to 90 min; mark solvent front; visualise with ninhydrin/UV/iodine); Rf = spot distance / solvent distance; applications: amino acids, plant pigments, sugars. TLC: silica gel or alumina on Al/glass backing; faster (10 to 30 min); normal-phase (polar silica, non-polar mobile: retains polar compounds) or reversed-phase (C18, aqueous mobile: retains non-polar compounds); visualised by UV (254/365 nm), sulphuric acid charring, $KMnO_4$, ninhydrin, anisaldehyde; Rf same formula; applications: medicinal plant screening, reaction monitoring.
12. Ion exchange: charge-based separation; anion exchanger (DEAE, Q) binds negative proteins; cation exchanger (CM, SP) binds positive proteins; eluted by NaCl gradient (0 to 1 M) or pH change; high capacity; good for initial capture. Gel filtration: size-based separation; large molecules elute in void volume (V0); small molecules elute at total volume (Vt); intermediate molecules elute between V0 and Vt; isocratic elution; applications: MW determination, desalting (Sephadex G-25), aggregate removal (Superdex). Affinity: specific binding to immobilised ligand; IMAC Ni-NTA (his-tagged proteins; elute with imidazole); protein A (antibodies; elute with low pH); GST-glutathione (GST-tagged proteins; elute with reduced glutathione); highest purification

lanetonline.com

factor per step. HPLC: components (solvent reservoir; high-pressure pump; injector; column 1.8 to 10 micrometre particles; UV/fluorescence detector; data system); modes: RP-HPLC (C18; water/acetonitrile; most common; peptides, alkaloids, lipids); NP-HPLC (silica; hexane; lipids); IEX-HPLC (protein separation); SE-HPLC (MW analysis); HILIC (sugars, nucleosides). Applications: protein purification pipeline (IMAC + IEX + SEC); natural product profiling (alkaloids, flavonoids, terpenoids in medicinal plants); NAFDAC food safety (aflatoxin B1/B2/G1/G2 in groundnut/maize by fluorescence HPLC after immunoaffinity clean-up); NDLEA forensic (THC, CBD, opioids by RP-HPLC or GC-MS).

Answers to Pilot Questions [Series 2]

Part 2.1

1. Nanodrop pathlength is 1 mm (0.1 cm); correction factor = 10 for 1 cm pathlength. A260 (corrected to 1 cm) = 0.35 x 10 = 3.5. Concentration = 3.5 x 50 = 175 micrograms per ml. (Note: most nanodrop instruments automatically correct for pathlength and report the 1 cm equivalent directly.)
2. Nanodrop measures total A260 absorbance, including contributions from RNA, free nucleotides, and aromatic contaminants. Qubit reagents (PicoGreen-based) fluoresce only when bound to dsDNA and do not respond to RNA, free nucleotides, or protein, giving a fluorescence signal proportional only to dsDNA concentration.
3. Prepare 5 to 7 BSA standards (0, 2, 4, 8, 12, 16, 20 micrograms per ml in the same buffer as the unknown); add Bradford reagent to each standard and to the unknown; incubate 5 minutes at room temperature; measure A595 for all; plot A595 vs BSA concentration; draw best-fit line; read unknown concentration from the line by interpolation.
4. A260/A280 of 1.8 to 2.0: pure DNA. Below 1.8: protein or phenol contamination (absorbs at 280 nm). A260/A230 of 2.0 to 2.2: acceptable purity. Below 2.0:

lanetonline.com

contamination by guanidinium salts, EDTA, polysaccharides, or organic solvents (absorb at 230 nm), which inhibit downstream enzymatic reactions.

5. PicoGreen: specific for dsDNA; does not fluoresce with RNA or ssDNA; accurate in mixed samples; advantage: specificity. EtBr: intercalates into both dsDNA and RNA; widely available; inexpensive; used for gel staining; advantage: universal nucleic acid stain for gel visualisation.

Part 2.2

1. Chromatography: a separation technique in which components of a mixture distribute between a stationary phase and a mobile phase based on differential interactions. Two phases: stationary phase (solid or supported liquid; does not move) and mobile phase (liquid or gas; moves through the stationary phase carrying solutes).
2. Spot amino acid mixture and reference standards 2 cm from bottom of Whatman paper; allow to dry. Place paper in tank with butanol:acetic acid:water (4:1:5 upper phase) solvent; allow to ascend 15 to 20 cm. Remove paper; mark solvent front; dry in fume hood. Spray with ninhydrin solution; heat at 100 degrees C for 5 minutes; amino acids appear as purple-pink spots. Calculate Rf for each spot and compare with reference standards.
3. Rf = 4.2 / 7.0 = 0.60. This means the compound travelled 60 percent of the distance covered by the solvent front; Rf of 0.60 is characteristic of this compound under the defined solvent system and temperature, and can be compared with reference standards for identification.
4. Normal-phase TLC: polar stationary phase (silica gel); non-polar mobile phase (hexane, ethyl acetate); polar compounds retained more strongly (lower Rf); used for lipids, fat-soluble vitamins, non-polar natural products. Reversed-phase TLC: non-polar stationary phase (C18 silica); polar mobile phase (water/methanol); non-polar compounds retained more strongly (lower Rf); used for phenolics, alkaloids, and polar-organic compounds.
5. Ninhydrin: amino acids and primary amines (purple-pink colour). KMnO4: oxidisable compounds including alkenes, alcohols, and aldehydes (yellow-brown spots on green background). Vanillin-sulphuric acid: terpenoids, steroids, and terpene alcohols (various colours on heating).

lanetonline.com

Part 2.3

1. Proteins with opposite charge to the resin bind electrostatically at low ionic strength. Unbound proteins are washed away with starting buffer. Bound proteins are eluted by increasing NaCl concentration (0 to 1 M gradient); Na+ ions compete with protein cationic groups for binding sites on anion exchanger resin, progressively displacing proteins with increasing salt. Proteins elute at characteristic NaCl concentrations based on their charge density.
2. Ion exchange: separates by charge; proteins bind and are eluted by salt gradient. Gel filtration: separates by size; no binding to resin; large molecules elute first in void volume; isocratic elution; does not concentrate protein. Ion exchange: good initial capture step; high capacity. Gel filtration: good polishing step; also used for desalting and MW determination.
3. Express his-tagged protein in E. coli; lyse cells by sonication or French press in lysis buffer (50 mM NaH2PO4 pH 8.0, 300 mM NaCl, 10 mM imidazole). Load lysate onto Ni-NTA resin pre-equilibrated with lysis buffer. Wash with wash buffer (50 mM imidazole) to remove non-specifically bound proteins. Elute his-tagged protein with elution buffer (250 to 500 mM imidazole). Assess purity by SDS-PAGE.
4. Load sample onto gel filtration column (e.g. Superdex 200) pre-equilibrated with running buffer. Run molecular weight standards of known MW simultaneously or previously under identical conditions. Record elution volume (Ve) for each protein. Plot log(MW) vs Ve for standards to generate a linear calibration curve. Read Ve for unknown protein; interpolate log(MW) from calibration curve; calculate MW.
5. Highest purification factor per step (50 to greater than 1,000-fold in one step). Specificity: captures only the target molecule even from a crude lysate. Less buffer and column volumes needed. Fewer steps to achieve high purity compared with ion exchange alone.

Part 2.4

1. Solvent reservoir (contains degassed mobile phase). High-pressure pump (delivers mobile phase at constant flow rate; 0.5 to 2 ml per min; 50 to 400 bar). Sample injector (manual valve or autosampler; 5 to 100 microlitres injection volume). Column (stainless steel; 150

lanetonline.com

to 250 mm; 1.8 to 10 micrometre particles; specific stationary phase). Detector (UV/Vis; fluorescence; refractive index; or mass spectrometer). Data system (integrates chromatogram; calculates peak areas and retention times).

2. RP-HPLC: non-polar stationary phase (C18 bonded silica); polar mobile phase (water/acetonitrile or water/methanol); non-polar compounds retained more strongly; separates peptides, alkaloids, lipids, phenolics, pharmaceuticals. NP-HPLC: polar stationary phase (silica); non-polar mobile phase (hexane, dichloromethane); polar compounds retained more strongly; separates lipid-soluble vitamins, waxes, and non-polar natural products.
3. Step 1: IMAC on Ni-NTA resin (capture his-tagged protein; 50 to 100-fold purification; elute with 250 to 500 mM imidazole). Step 2: ion exchange HPLC on SP or Q-Sepharose (intermediate purification; removes host cell proteins, nucleic acids, endotoxins; NaCl gradient elution). Step 3: size exclusion HPLC on Superdex 75 or 200 (polishing; removes aggregates and ensures monomer homogeneity; isocratic elution).
4. Aflatoxin analysis: RP-HPLC with fluorescence detection after immunoaffinity column clean-up quantifies aflatoxins B1, B2, G1, G2 in groundnut and maize extracts; NAFDAC uses this for food safety compliance monitoring. Pharmaceutical quality control: HPLC verifies identity, purity, and concentration of active pharmaceutical ingredients in tablets, syrups, and injectables distributed in Nigeria.
5. Higher resolution: small particle size increases theoretical plate number (N), giving sharper, better-resolved peaks. Faster run time: high-pressure flow through small particles reduces analysis time from hours to minutes. Quantitative automation: autosampler, gradient programmer, and data system allow unattended, reproducible analysis of large sample batches.

References and Attributions [Series 2]

Textual References (Books and External Sources)

- Wilson, K., & Walker, J. (Eds.). (2010). Principles and techniques of biochemistry and molecular biology (7th ed.). Cambridge University Press.

lanetonline.com

- Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). Introduction to modern liquid chromatography (3rd ed.). Wiley.
- Stahl, E. (Ed.). (1969). Thin layer chromatography: A laboratory handbook (2nd ed.). Springer.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein. Analytical Biochemistry, 72, 248–254.

Figures, Plates, Tables, and Boxes

- Figure 2.1: Comparison of ascending paper chromatography and TLC procedures with Rf calculation. Attribution: AI generated. Suggested OER search string: “paper chromatography TLC procedure Rf value comparison diagram OER”.
- Box 2.1: Comparison of ion exchange, gel filtration, and affinity column chromatography. Attribution: AI generated. Suggested OER search string: “ion exchange gel filtration affinity chromatography comparison table stationary phase elution OER”.

lanetonline.com

Course code: BIO 408

Course title: Applied Biotechnology

Course credit load: 2 Units

Series 3: Electrophoresis and DNA Amplification

- **Part 3.1: Principles and Types of Electrophoresis**
  - Sub Part 3.1.1: Principles of electrophoresis
  - Sub Part 3.1.2: Agarose gel electrophoresis
  - Sub Part 3.1.3: SDS-PAGE and native PAGE
- **Part 3.2: Advanced Electrophoretic Techniques**
  - Sub Part 3.2.1: Two-dimensional gel electrophoresis (2-DE)
  - Sub Part 3.2.2: Capillary electrophoresis
  - Sub Part 3.2.3: Southern, Northern, and Western blotting
- **Part 3.3: Polymerase Chain Reaction (PCR)**
  - Sub Part 3.3.1: Principles and components of PCR
  - Sub Part 3.3.2: PCR cycle and optimisation
  - Sub Part 3.3.3: Variants of PCR
- **Part 3.4: Real-Time PCR and Its Applications**
  - Sub Part 3.4.1: Principles of real-time (quantitative) PCR
  - Sub Part 3.4.2: Detection chemistries: SYBR Green and TaqMan

lanetonline.com

- Sub Part 3.4.3: Applications of PCR and qPCR in biotechnology

Introduction

Electrophoresis and PCR are the two most frequently performed techniques in molecular biology laboratories. Electrophoresis separates charged molecules in an electric field and is used to analyse DNA fragment sizes, assess protein molecular weights, monitor purification, and detect specific nucleic acid or protein targets by blotting. The polymerase chain reaction (PCR) amplifies specific DNA sequences from minute quantities of template, enabling gene detection, cloning, sequencing, genotyping, and pathogen diagnosis from samples that contain far too little material for direct analysis.

This Series covers the principles of electrophoresis from agarose gel and SDS-PAGE through two-dimensional proteomics and capillary electrophoresis to Southern, Northern, and Western blotting. It then describes PCR in full, from the components and thermocycling protocol through optimisation and variants (RT-PCR, multiplex PCR, nested PCR, digital PCR) to quantitative real-time PCR (qPCR) with SYBR Green and TaqMan chemistries and the diverse applications of PCR in Nigerian disease diagnostics, crop breeding, food safety, and forensic science.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 3.1** explain the principles of electrophoresis and describe the procedure for agarose gel and SDS-PAGE electrophoresis (Linked to Part 3.1),
- **SLO 3.2** describe two-dimensional gel electrophoresis, capillary electrophoresis, and Southern, Northern, and Western blotting (Linked to Part 3.2),
- **SLO 3.3** describe the components and thermocycling protocol of PCR and explain how each component contributes to the reaction (Linked to Part 3.3),

lanetonline.com

- **SLO 3.4** describe the variants of PCR (RT-PCR, multiplex, nested, digital) and state when each is used (Linked to Part 3.3),
- **SLO 3.5** explain the principles of real-time qPCR, distinguish between SYBR Green and TaqMan detection, and interpret Ct values (Linked to Part 3.4), and
- **SLO 3.6** describe the applications of electrophoresis, PCR, and qPCR in Nigerian biotechnology, diagnostics, plant breeding, food safety, and forensic science (Linked to Parts 3.2, 3.4).

3.1 Principles and Types of Electrophoresis

3.1.1 Principles of electrophoresis

Electrophoresis is the migration of charged molecules through a medium (liquid or gel) under the influence of an applied electric field. The rate of migration depends on: the net charge of the molecule (higher charge gives faster migration toward the opposite electrode); the size and shape of the molecule (smaller, more compact molecules migrate faster through a porous gel matrix); and the properties of the medium (pore size, viscosity, electric field strength). Negatively charged molecules (DNA, RNA; nucleic acids are uniformly negatively charged due to phosphate backbone) migrate toward the anode (positive electrode). In SDS-treated proteins, the negative SDS coating overrides the protein's own charge, making all proteins migrate toward the anode at rates determined only by size.

The support medium provides a molecular sieve that separates molecules by size. Common media are: agarose gel (polysaccharide; forms a porous network on gelation; used for DNA and RNA separation; pore size adjusted by agarose concentration: 0.5 to 3 percent); polyacrylamide gel (formed by polymerisation of acrylamide and bis-acrylamide; finer pore size than agarose; used for protein and small DNA/RNA separation; pore size adjusted by acrylamide concentration and crosslinker ratio); and capillary tubes filled with polymer solution (capillary electrophoresis). The electrical parameters are: voltage (V); current (I); and power (P = V x I); running at constant

lanetonline.com

voltage gives faster initial migration that slows as resistance increases with ion depletion; running at constant current is often preferred for uniform results.

Fill in the blank: In agarose gel electrophoresis of DNA, all DNA molecules carry a uniform negative charge due to their phosphate backbone and migrate toward the _____ (positive electrode); smaller fragments migrate faster through the gel pores than larger fragments.

3.1.2 Agarose gel electrophoresis

Agarose gel electrophoresis is the standard method for separating and analysing DNA and RNA fragments. Procedure: (1) prepare agarose gel by dissolving 0.8 to 2 percent agarose in TAE (Tris-acetate-EDTA) or TBE (Tris-borate-EDTA) running buffer by heating; cool to approximately 60 degrees C; add ethidium bromide (0.5 micrograms per ml) or SYBR Safe; pour into gel tray with comb; allow to set (30 minutes at room temperature); (2) remove comb; place gel in electrophoresis tank filled with running buffer; (3) mix DNA samples with 6x loading dye (bromophenol blue; xylene cyanol; glycerol to increase density); load into wells; load appropriate DNA ladder (1 kb, 100 bp, or Lambda HindIII depending on expected fragment sizes); (4) run at 80 to 120 V for 30 to 60 minutes; (5) visualise under UV transilluminator (EtBr) or blue-light illuminator (SYBR Safe); photograph gel.

Agarose concentration determines resolution: 0.5 to 0.8 percent resolves large fragments (2 to 20 kb); 1 percent is general-purpose (200 bp to 10 kb); 2 to 3 percent resolves small fragments (50 to 500 bp). DNA size is estimated by comparing band migration to molecular weight ladder bands on a semi-log plot of log(bp) vs migration distance, which is approximately linear for most gels. Ethidium bromide intercalates into double-stranded DNA and RNA and fluoresces orange-red under UV. Safety: EtBr is mutagenic (intercalates into DNA); handle with nitrile gloves; dispose of EtBr-containing gels and solutions as hazardous chemical waste according to institutional protocols; SYBR Safe and GelRed are safer alternatives. A DNA ladder of known fragment sizes must always be included to allow size estimation.

Fill in the blank: A _____ percent agarose gel is appropriate for general-purpose DNA analysis in the 200 bp to 10 kb range; lower concentrations (0.5 to 0.8 percent) are used for large fragments and higher concentrations (2 to 3 percent) for small fragments below 500 bp.

lanetonline.com

3.1.3 SDS-PAGE and native PAGE

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) separates proteins by molecular weight. SDS (sodium dodecyl sulphate) is an anionic detergent that binds to proteins at approximately 1.4 g SDS per gram protein, coating the polypeptide chain uniformly with negative charges and denaturing its secondary and tertiary structure, producing rod-like SDS-protein complexes whose charge-to-mass ratio is approximately constant. When beta-mercaptoethanol or DTT is included (reducing conditions), disulphide bonds are also broken, separating multi-subunit proteins into individual polypeptide chains. Because charge-to-mass ratio is approximately constant, SDS-PAGE separates proteins purely by size (smaller proteins migrate faster). Proteins are detected by staining with Coomassie Brilliant Blue R-250 (destained with methanol:acetic acid:water; sensitivity approximately 0.2 to 0.5 micrograms per band) or silver stain (100-fold more sensitive than Coomassie; detects nanogram quantities).

SDS-PAGE procedure: denature protein samples by mixing with SDS sample buffer (SDS; glycerol; bromophenol blue; beta-mercaptoethanol) and boiling for 5 minutes; load onto vertical polyacrylamide gel (stacking gel 4 to 5 percent; resolving gel 8 to 15 percent depending on protein MW range); run at 120 to 200 V until dye front reaches the bottom; stain and destain. Molecular weight is estimated by plotting log(MW) vs migration distance and interpolating from a standard protein ladder. Native PAGE omits SDS; proteins migrate in their native charge and shape; used to study protein-protein interactions, enzyme activity in gels, and native quaternary structure. Gradient gels (4 to 20 percent acrylamide gradient) resolve proteins across a wide MW range in a single gel.

Fill in the blank: In SDS-PAGE, _____ (sodium dodecyl sulphate) binds to denatured proteins at a constant ratio of approximately 1.4 g per gram protein, giving all proteins a uniform negative charge proportional to their mass, so that migration rate reflects only molecular weight.

Pilot questions 3.1

1. Explain why DNA migrates toward the anode during agarose gel electrophoresis and why smaller fragments migrate faster.

lanetonline.com

2. Describe the procedure for agarose gel electrophoresis of a PCR product, from gel preparation to visualisation.
3. Explain how SDS denatures proteins and why this allows SDS-PAGE to separate proteins by molecular weight.
4. State the safety precautions required when working with ethidium bromide and suggest a safer alternative.
5. Distinguish between SDS-PAGE (reducing) and native PAGE and state when each is used.

3.2 Advanced Electrophoretic Techniques

3.2.1 Two-dimensional gel electrophoresis (2-DE)

Two-dimensional gel electrophoresis (2-DE) separates proteins in two independent dimensions: isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second. IEF separates proteins by their isoelectric point (pI): in a pH gradient immobilised on a gel strip (immobilised pH gradient, IPG strip; Biorad ReadyStrip or GE Amersham; pH range 3 to 10 or narrower range strips for improved resolution), each protein migrates until it reaches the pH at which its net charge is zero (its pI) and stops. After IEF, the IPG strip is equilibrated in SDS buffer and placed on top of an SDS-PAGE gel; in the second dimension, proteins are separated by size. The resulting two-dimensional gel maps contain spots at positions defined by both pI (horizontal axis) and MW (vertical axis), potentially resolving thousands of proteins from a complex mixture.

2-DE is the primary technique for comparative proteomics: comparing the protein expression profiles (proteomes) of two or more conditions (diseased vs healthy tissue; treated vs untreated cells; two crop varieties). Spots are detected by Coomassie, silver stain, or fluorescent dyes (DIGE: difference gel electrophoresis, in which two proteomes labelled with different fluorescent dyes are co-separated on the same gel; differences are detected by two-colour imaging). Spots of interest are excised, digested with trypsin, and identified by mass spectrometry (MALDI-TOF MS or LC-MS/MS). Limitations of 2-DE include: poor resolution of

lanetonline.com

very hydrophobic proteins (membrane proteins), very basic proteins (pI above 10), very large (above 200 kDa) or very small (below 10 kDa) proteins; and high labour requirement. Modern shotgun proteomics (LC-MS/MS without gel separation) is increasingly replacing 2-DE for comprehensive proteome analysis.

Fill in the blank: In two-dimensional gel electrophoresis, proteins are first separated by their _____ point (pI) using isoelectric focusing on an immobilised pH gradient strip, then by molecular weight in the second dimension by SDS-PAGE.

3.2.2 Capillary electrophoresis

Capillary electrophoresis (CE) performs electrophoretic separations in narrow fused-silica capillaries (50 to 100 micrometres internal diameter; 25 to 75 cm length) filled with buffer or polymer solution. Very high voltages (10 to 30 kV) are applied across the capillary, generating high electric field strengths (200 to 600 V per cm); the narrow capillary dissipates the Joule heat efficiently, allowing high-resolution separations in short times (2 to 30 minutes). Detection is typically by UV absorbance or fluorescence through a window in the capillary wall. Electroosmotic flow (EOF), the bulk flow of liquid toward the cathode driven by the electric field acting on the diffuse double layer at the negatively charged capillary wall, carries all solutes (regardless of charge) toward the detector, while electrophoretic mobility superimposes differential migration rates.

CE modes include: capillary zone electrophoresis (CZE: separation in free solution by charge-to-size ratio); capillary gel electrophoresis (CGE: separation in polymer-filled capillary by size; used for DNA fragment analysis and protein sizing); capillary isoelectric focusing (CIEF: IEF in capillary; high-resolution pI determination); and micellar electrokinetic chromatography (MEKC: surfactant micelles in the capillary create a pseudo-stationary phase for separation of neutral compounds). Capillary electrophoresis is used in DNA fragment analysis (microsatellite sizing; STR profiling in forensics; automated sequencing; ABI 3730 sequencer uses CE with fluorescent dye-labelled DNA and polymer-filled capillary array), protein analysis, pharmaceutical quality control, and chiral drug separation. The National Forensic Science

lanetonline.com

Laboratory uses CE-based STR profiling for DNA fingerprinting in Nigerian criminal investigations.

Fill in the blank: In capillary gel electrophoresis (CGE), DNA fragments are separated by size through a _____ -filled capillary; this is the basis of automated DNA sequencing and STR (short tandem repeat) profiling used in forensic DNA identification.

3.2.3 Southern, Northern, and Western blotting

Blotting techniques transfer separated molecules from an electrophoresis gel onto a membrane (nitrocellulose or PVDF) where they are immobilised and detected by hybridisation (nucleic acids) or antibody binding (proteins). Southern blotting (E.M. Southern, 1975) detects specific DNA sequences: genomic DNA is digested with restriction enzymes; fragments are separated by agarose gel electrophoresis; DNA is denatured in situ (NaOH); transferred to nylon or nitrocellulose membrane by capillary transfer, vacuum transfer, or electroblotting; UV-crosslinked (baking) to fix DNA to membrane; hybridised with a labelled DNA probe (radioactive 32P-labelled, digoxigenin-labelled, or biotin-labelled) complementary to the target sequence; detected by autoradiography or chemiluminescence. Southern blotting is used for restriction fragment length polymorphism (RFLP) analysis, GMO detection, and confirmation of gene integration in transgenic organisms.

Northern blotting detects specific RNA sequences; RNA is size-separated on a denaturing agarose gel (formaldehyde-containing gel to disrupt RNA secondary structure); transferred to membrane; hybridised with a probe specific to the mRNA of interest; detection indicates transcript presence, size, and relative abundance. Western blotting (immunoblotting) detects specific proteins: proteins are separated by SDS-PAGE; transferred to PVDF or nitrocellulose membrane; blocked with non-fat milk or BSA to prevent non-specific antibody binding; incubated with primary antibody specific to the target protein; incubated with secondary antibody conjugated to horseradish peroxidase (HRP) or alkaline phosphatase; detected by chemiluminescent (ECL) or colorimetric substrate. Western blotting is used for confirming recombinant protein expression, diagnosing viral infections (HIV Western blot confirmatory test), and studying protein expression levels.

lanetonline.com

Fill in the blank: In Western blotting, the _____ antibody binds specifically to the target protein immobilised on the membrane; the secondary antibody conjugated to HRP or alkaline phosphatase then binds to the primary antibody and generates a detectable chemiluminescent or colorimetric signal.

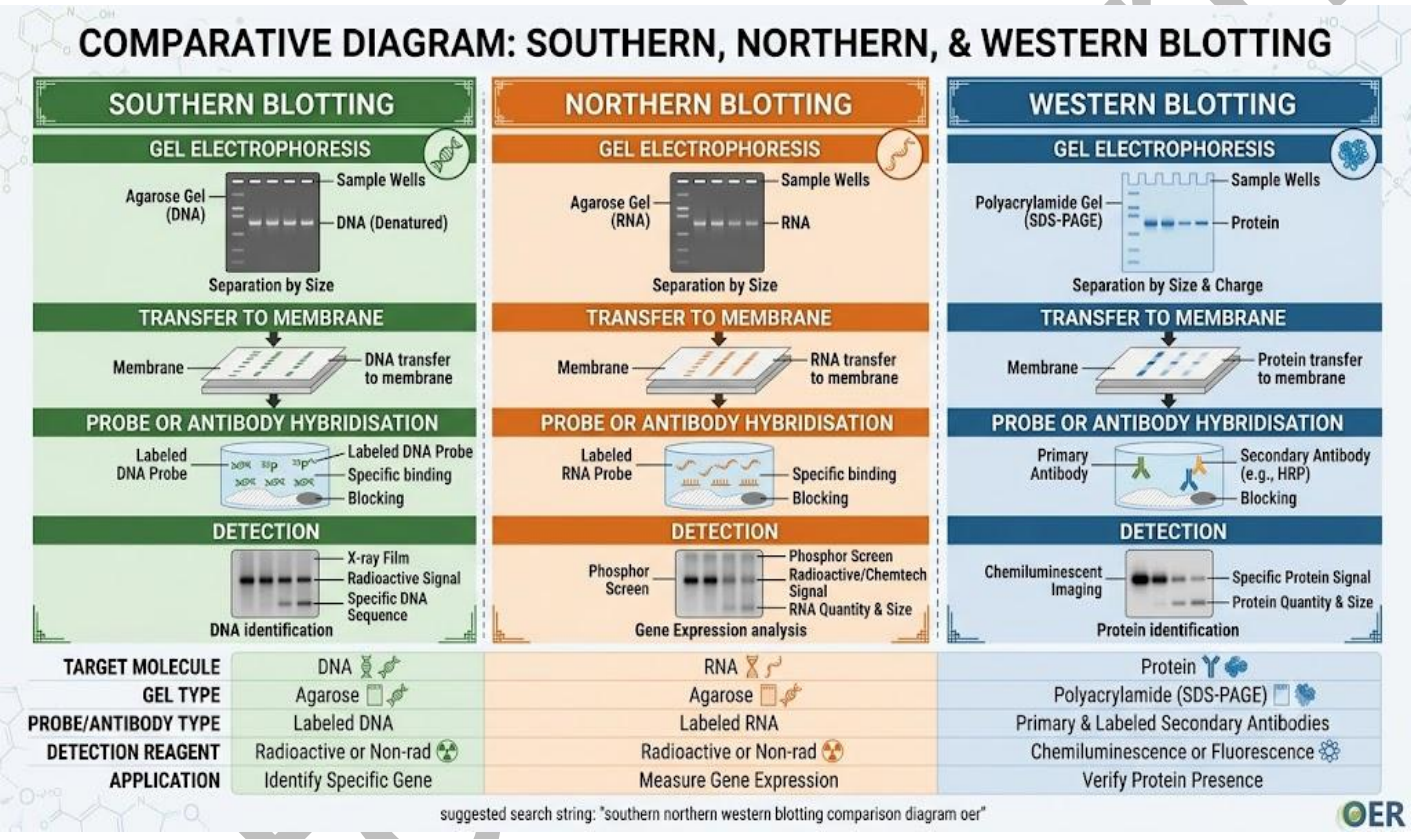


Figure 3.1: Comparison of Southern, Northern, and Western blotting procedures

Pilot questions 3.2

1. Describe the principle of isoelectric focusing (IEF) and explain how it separates proteins in the first dimension of 2-DE.
2. State two advantages of capillary electrophoresis over conventional slab gel electrophoresis.
3. Describe the steps of Southern blotting and state two applications in biotechnology.
4. Distinguish between Northern and Western blotting in terms of target molecule, gel type, detection reagent, and application.
5. Explain the role of blocking in Western blotting and state the reagents commonly used.

3.3 Polymerase Chain Reaction (PCR)

3.3.1 Principles and components of PCR

PCR (polymerase chain reaction; Mullis et al., 1987; Nobel Prize in Chemistry 1993) is an in vitro method for amplifying a specific DNA sequence exponentially. Starting from as few as a single copy of the target sequence, PCR can generate millions to billions of copies in 2 to 3 hours. The reaction exploits the ability of a thermostable DNA polymerase to extend a short oligonucleotide primer hybridised to a denatured single-stranded DNA template. A standard PCR reaction (25 to 50 microlitres) contains: (1) template DNA (1 to 100 ng genomic DNA; or 0.1 to 10 ng plasmid DNA; or cDNA from RT-PCR); (2) forward primer and reverse primer (18 to 25 nucleotides each; complementary to opposite strands flanking the target sequence; typically 0.1 to 0.5 micromolar each); (3) thermostable DNA polymerase (Taq polymerase from Thermus aquaticus; 0.5 to 2.5 units; lacks 3’ to 5’ proofreading exonuclease; error rate approximately 1 error per 10^5 bp per cycle; or high-fidelity polymerases with proofreading: Pfu, Phusion, Q5 with error rates 10 to 100 times lower); (4) deoxyribonucleoside triphosphates (dNTPs: dATP, dCTP, dGTP, dTTP; typically 200 micromolar each); (5) PCR buffer (10x buffer supplied with enzyme; contains Tris-HCl pH 8.3 to 8.8; KCl; and MgCl2 at 1.5 to 2.5 mM; Mg2+ is an essential cofactor for Taq polymerase); (6) nuclease-free water to the final volume.

The function of each component: template provides the sequence to be copied; primers define the boundaries of the amplicon and provide a 3’-OH for polymerase extension; Taq polymerase synthesises new DNA from the primer 3’ end using dNTPs as substrates in the 5’ to 3’ direction; dNTPs are the building blocks; Mg2+ activates the polymerase and stabilises the template-primer duplex; buffer maintains optimal pH and ionic conditions. Primer design is critical: primers should have a Tm (melting temperature) of 50 to 65 degrees C; GC content of 40 to 60 percent; no significant self-complementarity (which causes primer dimers) or complementarity between forward and reverse primers; and 3’ end stability (G or C at 3’ end). Primer Tm is estimated as: Tm = 2(A+T) + 4(G+C) degrees C (basic Wallace rule) or by nearest-neighbour thermodynamics (Oligo Calc, Primer3 tools).

lanetonline.com

Fill in the blank: Taq DNA polymerase, isolated from the thermophilic bacterium Thermus _____, is used in PCR because it remains active at the high temperatures (94 to 95 degrees C) used for DNA denaturation, eliminating the need to add fresh enzyme after each cycle.

3.3.2 PCR cycle and optimisation

A standard PCR programme consists of: (1) initial denaturation: 94 to 95 degrees C for 2 to 5 minutes (fully denatures template and activates hot-start polymerase); (2) cycling (25 to 35 cycles): denaturation at 94 to 95 degrees C for 15 to 30 seconds (separates double-stranded DNA into single strands); annealing at 50 to 65 degrees C (typically 5 degrees C below the primer Tm) for 20 to 30 seconds (primers hybridise to complementary sequences on template strands); extension at 72 degrees C for 1 minute per kb of expected product (Taq polymerase synthesises new DNA at approximately 1 kb per minute); (3) final extension: 72 degrees C for 5 to 10 minutes (completes partially extended products); (4) hold at 4 to 15 degrees C.

During the first cycle, long extension products of variable length are produced; from the third cycle onward, the predominant product is the defined amplicon bounded by both primers (the specific band). Amplicon copies double approximately each cycle; after n cycles, approximately 2^n copies of the target sequence are present (exponential amplification; in practice, efficiency is 85 to 100 percent per cycle). Common PCR optimisation problems and solutions: no band (increase template or primer concentration; increase MgCl2; reduce annealing temperature; increase extension time; check primer design); multiple bands (increase annealing temperature by 2 to 5 degrees C; reduce template; reduce primer concentration; use hot-start polymerase); smear (excessive template; primer dimers; too many cycles; reduce template or cycles; redesign primers). Hot-start PCR (antibody-blocked or chemical modification of Taq) prevents non-specific amplification at low temperatures during reaction setup.

Fill in the blank: In a PCR thermal cycle, the annealing temperature is typically set 5 degrees C below the primer _____ (Tm); if the annealing temperature is too low, non-specific bands appear; if too high, primer binding is inhibited and no product is obtained.

3.3.3 Variants of PCR

lanetonline.com

Reverse transcription PCR (RT-PCR): RNA is first converted to complementary DNA (cDNA) by reverse transcriptase (Moloney Murine Leukemia Virus MMLV-RT or AMV-RT) using either an oligo-dT primer (for polyadenylated mRNA), a specific reverse primer, or random hexamers; the cDNA is then used as template for conventional PCR. RT-PCR is used for: detection of RNA viruses (e.g. SARS-CoV-2; yellow fever virus; Lassa fever virus); gene expression analysis (detecting specific mRNA transcripts); and generating cDNA libraries for cloning. Multiplex PCR: two or more primer pairs targeting different sequences are included in the same reaction; amplicons of different sizes are distinguished on an agarose gel; used for simultaneous detection of multiple pathogens, gender determination, or deletion analysis.

Nested PCR: two rounds of PCR using two sets of primers; the first (outer) primer pair amplifies a large region containing the target; the second (inner, nested) primer pair amplifies a smaller region within the first amplicon; dramatically increases sensitivity and specificity; used when target DNA is present in very low copy number (HIV provirus; Mycobacterium tuberculosis in clinical samples). Touchdown PCR: annealing temperature starts high (above Tm) and decreases by 0.5 to 1 degree C per cycle over the first 10 to 20 cycles, then remains constant for remaining cycles; increases specificity by favouring exact primer-template matches in early cycles. Digital PCR (dPCR): the reaction is partitioned into thousands to millions of individual droplets or chambers before PCR; each partition either contains the target (positive) or does not (negative); absolute quantification without a standard curve; highly sensitive (detects single copies); used for rare mutation detection and copy number variation analysis. LAMP (loop-mediated isothermal amplification): amplification at constant temperature (60 to 65 degrees C) using Bst polymerase and four to six primers; does not require a thermocycler; visible result by turbidity or colorimetric indicator; increasingly used for field diagnostics in Nigeria.

Fill in the blank: In _____ PCR (RT-PCR), RNA is first converted to complementary DNA (cDNA) by reverse transcriptase before being amplified by PCR; this allows detection of RNA viruses such as Lassa fever virus and SARS-CoV-2 in clinical samples.

Pilot questions 3.3

1. List the six components of a standard PCR reaction and state the function of each.

lanetonline.com

2. Describe the three steps of a PCR thermal cycle and explain what happens molecularly at each step.
3. Explain what happens if the PCR annealing temperature is set too low or too high.
4. Distinguish between RT-PCR and nested PCR and state one application of each.
5. Explain the principle of digital PCR and state one advantage over conventional qPCR.

3.4 Real-Time PCR and Its Applications

3.4.1 Principles of real-time (quantitative) PCR

Real-time PCR (quantitative PCR, qPCR) monitors the accumulation of PCR product during each cycle using a fluorescent signal, allowing quantification of the starting amount of target DNA or RNA. In conventional PCR, product is analysed only at the endpoint (after all cycles); in qPCR, fluorescence is measured after each extension step, generating an amplification curve. The amplification plot shows three phases: the baseline phase (fluorescence below the detection threshold; no signal above background in early cycles); the exponential phase (fluorescence increases exponentially as product accumulates; the most informative phase for quantification); and the plateau phase (reagents become limiting; amplification slows and stops).

The threshold cycle (Ct; also called Cq, cycle of quantification) is defined as the cycle at which the fluorescence signal crosses a defined threshold above baseline. Samples with more starting template reach the threshold earlier (lower Ct); samples with less template reach it later (higher Ct). For every doubling of initial template amount, Ct decreases by approximately 1 cycle (100 percent efficiency). Absolute quantification uses a standard curve generated from serial dilutions of a known copy number standard; the Ct of the unknown is interpolated from the standard curve to give copy number. Relative quantification compares the expression of a target gene to a reference (housekeeping) gene (e.g. beta-actin, GAPDH, 18S rRNA) using the 2^(-delta delta Ct) method; fold-change between experimental and control conditions is calculated without a standard curve.

lanetonline.com

Fill in the blank: The _____ cycle (Ct) in qPCR is the cycle at which the fluorescence signal crosses the detection threshold; a lower Ct indicates more starting template because the threshold is reached earlier in the exponential amplification phase.

3.4.2 Detection chemistries: SYBR Green and TaqMan

SYBR Green I is a fluorescent intercalating dye that binds non-specifically to all double-stranded DNA; fluorescence increases proportionally to the amount of dsDNA produced. It is inexpensive and requires no probe design: any primer pair can be used. However, SYBR Green detects any dsDNA including primer dimers and non-specific amplification products, which can produce false-positive or inaccurate signals. A melting curve analysis (dissociation curve) is performed after qPCR by slowly heating the product from 65 degrees C to 95 degrees C while monitoring fluorescence: the specific amplicon melts at a characteristic temperature (Tm) determined by its GC content and length, appearing as a single peak; primer dimers melt at a lower temperature; multiple peaks indicate non-specific products. Melting curve analysis is essential for validating SYBR Green qPCR results.

TaqMan probes are sequence-specific oligonucleotide probes (18 to 30 nucleotides) labelled with a fluorescent reporter dye at the 5’ end (e.g. FAM, VIC, or HEX) and a quencher at the 3’ end (e.g. TAMRA, BHQ). When intact, the probe’s fluorescence is quenched by FRET (fluorescence resonance energy transfer) between reporter and quencher. During PCR extension, Taq polymerase’s 5’ to 3’ exonuclease activity cleaves the probe as the polymerase extends through it, separating reporter from quencher; freed reporter dye fluoresces, generating signal proportional to amplicon accumulation. TaqMan is more specific than SYBR Green because signal is generated only by cleavage of the exact probe sequence; it is used for allelic discrimination (SNP genotyping), pathogen detection, and multiplex qPCR (multiple probes with different fluorescent labels detect different targets simultaneously). TaqMan assays are more expensive because each target requires a custom probe.

Fill in the blank: In TaqMan qPCR, the fluorescent signal is generated when Taq polymerase’s 5’ to 3’ _____ activity cleaves the probe during extension, separating the reporter dye from

laneonline.com

the quencher; this provides higher specificity than SYBR Green because signal is generated only from the exact probe sequence.

3.4.3 Applications of PCR and qPCR in biotechnology

Disease diagnostics: PCR-based diagnostics are widely used in Nigerian clinical and public health laboratories. RT-PCR is the gold standard for detection of RNA viruses: Lassa fever virus (NCDC National Reference Laboratory, Abuja); SARS-CoV-2 (COVID-19 diagnosis; RT-qPCR on nasopharyngeal swabs; exponentially expanded during 2020 to 2021 in NCDC-supported laboratories); yellow fever virus (Lagos University Teaching Hospital; UCH Ibadan); and monkeypox virus (MPXV; qPCR using OPG197 target gene). TaqMan qPCR with specific probes is used for HIV viral load monitoring (target: gag gene; used to guide antiretroviral therapy decisions) and hepatitis B and C viral load in liver clinics across Nigeria.

Plant breeding and crop biotechnology: PCR is used for marker-assisted selection (MAS; Series 4 BIO402); SSR and KASP (kompetitive allele-specific PCR) markers are used to select for disease resistance genes (CMD2 in cassava; blast resistance in rice at NCRI) and drought tolerance QTLs in breeding lines. RT-qPCR measures gene expression during stress responses (drought; pathogen infection) in research at IITA and Nigerian universities. GMO detection: PCR tests targeting transgenic constructs (35S CaMV promoter; NOS terminator; event-specific sequences) are used by NAFDAC and the National Biosafety Management Agency (NBMA) to screen food products and seed consignments for undeclared GMO content. Forensic science: STR (short tandem repeat) profiling using multiplex PCR with fluorescently labelled primers and capillary electrophoresis separation generates DNA fingerprints for individual identification; used for paternity testing and criminal investigations by the Nigeria Police Force Forensic Department and NFSL (National Forensic Science Laboratory).

Fill in the blank: _____ (kompetitive allele-specific PCR) is a qPCR-based SNP genotyping assay widely used in crop breeding for marker-assisted selection; it simultaneously detects two alleles at a SNP locus using allele-specific primers labelled with different fluorescent dyes.

laneronline.com

PCR Variant	Key Feature	Application
Conventional PCR	Amplifies DNA using thermal cycling	General DNA amplification, cloning, genotyping
RT-PCR	Reverse transcription of RNA into cDNA before amplification	Gene expression analysis, detection of RNA viruses
Multiplex PCR	Multiple primer sets amplify several targets simultaneously	Pathogen detection, genetic testing, forensic analysis
Nested PCR	Two successive PCR reactions with inner and outer primers	Increased specificity, detection of low-abundance DNA
Touchdown PCR	Gradual lowering of annealing temperature to improve primer binding	Enhances specificity and yield in difficult templates
Digital PCR	Partitioning sample into many reactions for absolute quantification	Rare mutation detection, precise quantification of nucleic acids
LAMP **(Loop-mediated isothermal amplification)**	Isothermal amplification using multiple primers	Rapid diagnostics, point-of-care testing, field applications

Box 3.1: Comparison of PCR variants and their applications

Pilot questions 3.4

1. Explain what a Ct value represents in qPCR and describe how it is used for relative quantification using the 2^(-delta delta Ct) method.
2. Compare SYBR Green and TaqMan qPCR detection, stating one advantage and one disadvantage of each.
3. Explain why a melting curve analysis is performed after SYBR Green qPCR.
4. Describe two applications of PCR in Nigerian disease diagnostics.
5. Explain how STR profiling by multiplex PCR and capillary electrophoresis is used in forensic DNA identification.

lanetonline.com

Series Summary

Electrophoresis separates charged molecules through a gel using an electric field. Agarose gel electrophoresis is mainly used for DNA and RNA, with smaller fragments moving faster than larger ones and fragment sizes estimated using a DNA ladder. SDS-PAGE separates proteins mainly by molecular weight after SDS denaturation, while native PAGE separates proteins according to their natural charge, size, and shape. Two-dimensional electrophoresis combines isoelectric focusing, which separates proteins by isoelectric point, with SDS-PAGE, which separates them by molecular weight. Capillary electrophoresis provides rapid, high-resolution separation and is widely used in DNA sequencing and forensic STR analysis. Blotting methods include Southern blotting for DNA, Northern blotting for RNA, and Western blotting for proteins.

PCR, developed by Kary Mullis, amplifies a selected DNA region using a template, primers, Taq polymerase, dNTPs, magnesium chloride, and buffer. Each cycle involves denaturation at about 94–95°C, primer annealing at about 50–65°C, and extension at 72°C, producing approximately **2^n copies after n cycles**. Major variants include RT-PCR for RNA analysis, multiplex PCR for several targets, nested PCR for rare targets, digital PCR for absolute quantification, and LAMP for rapid field testing. Quantitative PCR measures fluorescence during amplification, and the cycle threshold value, or Ct, indicates the starting amount of template: a lower Ct means more starting material. SYBR Green detects all double-stranded DNA, while TaqMan uses target-specific probes. PCR is widely applied in disease diagnosis, viral-load testing, GMO detection, marker-assisted selection, and forensic profiling.

Glossary of Terms

- **Agarose gel electrophoresis:** separation of DNA or RNA by size through a porous agarose matrix under an electric field; smaller fragments migrate faster; fragments visualised by EtBr or SYBR Safe fluorescence under UV.

lanetonline.com

- **Capillary electrophoresis (CE):** electrophoretic separation in narrow fused-silica capillaries at high voltage; fast; high-resolution; used for DNA sequencing, STR profiling, protein analysis, and pharmaceutical quality control.
- **Ct (threshold cycle):** in qPCR, the cycle number at which the fluorescence signal crosses the detection threshold; inversely proportional to the amount of starting template; used for quantification.
- **Digital PCR (dPCR):** PCR performed in thousands of individual partitioned droplets or chambers; each partition gives a positive or negative result; enables absolute quantification without a standard curve; detects rare mutations and copy number variation.
- **LAMP:** loop-mediated isothermal amplification; DNA amplification at constant temperature (60 to 65 degrees C) using Bst polymerase and 4 to 6 primers; does not require a thermocycler; suited to field diagnostics.
- **Melting curve analysis:** a post-qPCR step in which temperature is gradually increased while monitoring fluorescence; the specific PCR product melts at a characteristic Tm; used to validate SYBR Green qPCR by distinguishing specific products from primer dimers.
- **PCR (polymerase chain reaction):** an in vitro method for exponentially amplifying a specific DNA sequence using thermostable DNA polymerase, primers, dNTPs, and repetitive thermal cycling; invented by Mullis et al. (1987); Nobel Prize 1993.
- **qPCR (quantitative real-time PCR):** PCR in which fluorescent signal is monitored during each cycle; enables quantification of starting DNA or RNA amount from the Ct value; uses SYBR Green or TaqMan probe detection.
- **RT-PCR:** reverse transcription PCR; RNA is first converted to cDNA by reverse transcriptase, then amplified by PCR; used to detect RNA viruses (Lassa fever, SARS-CoV-2) and to measure mRNA expression.
- **SDS-PAGE:** sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SDS denatures proteins and confers uniform negative charge proportional to mass; proteins separated by molecular weight; detected by Coomassie or silver stain.

lanetonline.com

- **Southern blotting:** transfer of electrophoretically separated DNA fragments from an agarose gel to a membrane, followed by hybridisation with a labelled probe to detect specific sequences; used for RFLP analysis and GMO detection.
- **TaqMan probe:** a dual-labelled oligonucleotide probe (reporter dye at 5' end; quencher at 3' end) used in qPCR; Taq polymerase's 5' to 3' exonuclease cleaves the probe during extension, releasing the reporter dye and generating signal.
- **Western blotting:** transfer of SDS-PAGE-separated proteins to a membrane, followed by detection with specific primary and secondary antibodies; used to confirm protein expression and diagnose infections (HIV Western blot).
- **2-DE (two-dimensional gel electrophoresis):** combines isoelectric focusing (first dimension; pI separation) with SDS-PAGE (second dimension; MW separation); resolves thousands of proteins as discrete spots; used for comparative proteomics.

Concept Check Questions [Series 3]

Objective Questions

1. In SDS-PAGE, _____ binds to denatured proteins at approximately 1.4 g per gram, giving all proteins a uniform negative charge proportional to their mass. (Linked to SLO 3.1)
2. In two-dimensional gel electrophoresis, the first dimension separates proteins by their _____ point using isoelectric focusing. (Linked to SLO 3.2)
3. Taq DNA polymerase is derived from the thermophilic bacterium Thermus _____. (Linked to SLO 3.3)
4. A lower Ct value in qPCR indicates _____ starting template, because the fluorescence threshold is reached earlier. (Linked to SLO 3.5)
5. In TaqMan qPCR, the fluorescent signal is generated when Taq polymerase's 5' to 3' _____ activity cleaves the probe, separating reporter dye from quencher. (Linked to SLO 3.5)

lanetonline.com

Short-Answer Questions

- Describe the procedure for agarose gel electrophoresis of a PCR product. (Linked to SLO 3.1)
- Distinguish between Southern and Western blotting in terms of target molecule, gel type, and detection reagent. (Linked to SLO 3.2)
- List the six components of a PCR reaction and state the function of each. (Linked to SLO 3.3)
- Compare SYBR Green and TaqMan qPCR, stating one advantage and one disadvantage of each. (Linked to SLO 3.5)
- State three applications of PCR or qPCR in Nigerian biotechnology. (Linked to SLO 3.6)

Long-Answer Questions

1. Describe the principles of electrophoresis. Explain the procedures for agarose gel electrophoresis and SDS-PAGE, and describe Southern and Western blotting. (Linked to SLOs 3.1, 3.2)
2. Describe the components and thermal cycle of PCR. Explain the variants of PCR and the principles of qPCR including SYBR Green and TaqMan detection. Discuss applications in Nigerian disease diagnostics, crop breeding, and forensic science. (Linked to SLOs 3.3, 3.4, 3.5, 3.6)

Answers to Concept Check Questions [Series 3]

Objective Questions

6. SDS.
7. Isoelectric (pI).
8. Aquaticus.
9. More.
10. Exonuclease.

lanetonline.com

Short-Answer Questions

11. Dissolve 1 percent agarose in TAE buffer; cool to 60 degrees C; add EtBr or SYBR Safe; pour into tray with comb; allow to set. Remove comb; place in tank with buffer. Mix samples with loading dye; load with DNA ladder. Run at 80 to 120 V for 30 to 60 min. Visualise under UV transilluminator; photograph.
12. Southern: target is DNA; separated on agarose gel; detected by labelled DNA probe hybridisation; used for RFLP, GMO detection. Western: target is protein; separated by SDS-PAGE; detected by primary antibody and enzyme-conjugated secondary antibody; used for protein expression confirmation and HIV diagnosis.
13. Template (provides sequence to copy); forward and reverse primers (define amplicon boundaries; provide 3'-OH for extension); Taq polymerase (synthesises new DNA at 72 degrees C); dNTPs (building blocks); MgCl2 (essential Taq cofactor); PCR buffer (maintains optimal pH and ionic conditions).
14. SYBR Green: cheaper; no probe design needed; detects all dsDNA; requires melting curve to confirm specificity; disadvantage: detects primer dimers. TaqMan: sequence-specific; suitable for multiplex qPCR; no melting curve needed; disadvantage: custom probe required; more expensive per assay.
15. RT-qPCR for SARS-CoV-2 and Lassa fever virus detection at NCDC reference laboratories. TaqMan HIV viral load monitoring for antiretroviral therapy management. STR multiplex PCR with capillary electrophoresis for forensic DNA fingerprinting at NFSL.

Long-Answer Questions

1. Electrophoresis principles: charged molecules migrate in electric field; rate depends on charge (DNA negative; migrates to anode), size (smaller migrates faster through gel pores), and medium pore size. Agarose gel electrophoresis: 0.8 to 2 percent agarose in TAE/TBE; EtBr or SYBR Safe; load samples with loading dye + DNA ladder; run 80 to 120 V for 30 to 60 min; visualise under UV; size estimated from ladder. SDS-PAGE: SDS denatures protein; confers uniform negative charge proportional to mass; stacking gel (4 to 5 percent) concentrates samples; resolving gel (8 to 15 percent) separates by MW; run 120 to 200 V; stain with Coomassie or silver. Southern blotting: digest genomic DNA

lanetonline.com

with restriction enzymes; agarose gel electrophoresis; denature in NaOH; transfer to membrane; UV-crosslink; hybridise with labelled probe; detect by autoradiography or chemiluminescence; applications: RFLP, GMO detection, transgene confirmation. Western blotting: SDS-PAGE; transfer to PVDF/nitrocellulose; block with milk or BSA; primary antibody; secondary antibody-HRP; ECL detection; applications: protein expression, HIV confirmatory diagnosis.

2. PCR components: template; forward and reverse primers (18 to 25 nt; define amplicon); Taq polymerase (from Thermus aquaticus; thermostable; 1 error per 10^5 bp); dNTPs (200 micromolar each); MgCl2 (1.5 to 2.5 mM; Taq cofactor); buffer. Thermal cycle: denaturation (94 to 95 degrees C; separates strands); annealing (50 to 65 degrees C; primers hybridise; set 5 degrees C below Tm); extension (72 degrees C; 1 min/kb; Taq synthesises new strand). After n cycles: approximately 2^n copies; product doubles each cycle from cycle 3 onward. Variants: RT-PCR (reverse transcriptase converts RNA to cDNA first; detects RNA viruses); multiplex (multiple primer pairs; multiple targets simultaneously); nested (two PCR rounds; inner primers in second round; sensitivity for rare targets); touchdown (decreasing annealing temperature for specificity); digital PCR (partitioned droplets; absolute quantification; rare mutation detection); LAMP (isothermal 60 to 65 degrees C; Bst polymerase; field diagnostics). qPCR: fluorescence monitored each cycle; Ct = cycle at threshold crossing; lower Ct = more template; 2^(-delta delta Ct) for relative quantification. SYBR Green: cheap; intercalates all dsDNA; melting curve needed. TaqMan: 5’ reporter + 3’ quencher probe; exonuclease cleavage releases signal; specific; multiplex; costly. Nigerian applications: NCDC RT-qPCR for Lassa fever (L gene target) and SARS-CoV-2 (N and RdRp genes); HIV viral load (TaqMan gag gene; Roche COBAS system at LUTH, LAUTECH, etc.); NAFDAC GMO screening (35S promoter PCR); IITA KASP MAS for CMD2 and CBSD resistance; NFSL STR profiling (NGM Select kit; 15 STR loci; multiplex PCR + CE) for criminal case evidence.

lanetonline.com

Answers to Pilot Questions [Series 3]

Part 3.1

6. DNA has a uniformly negative charge due to the phosphate backbone; it therefore migrates toward the positively charged anode. Smaller fragments experience less resistance passing through the gel pores and migrate faster; larger fragments are more impeded by the pore matrix and migrate slower.
7. Prepare 1 percent agarose gel in TAE; cool; add EtBr or SYBR Safe; pour and set. Remove comb; place in tank with TAE buffer. Mix PCR product with 6x loading dye; load alongside 1 kb DNA ladder. Run at 80 to 100 V for 30 to 45 min. Visualise under UV (EtBr) or blue light (SYBR Safe); photograph.
8. SDS binds to denatured protein polypeptide chains at approximately 1.4 g SDS per gram protein, uniformly coating the chain with negative charges proportional to chain length (mass). This removes the protein's own net charge contribution, making charge-to-mass ratio approximately constant for all proteins; therefore migration rate through the polyacrylamide gel reflects only molecular weight.
9. Wear nitrile gloves at all times when handling EtBr solutions or gels. Work in a designated EtBr area. Do not allow EtBr solutions to contact skin (mutagenic). Dispose of EtBr gels and solutions in labelled EtBr waste containers for incineration or chemical treatment. Safer alternative: SYBR Safe or GelRed, which are less toxic and can be disposed of as general laboratory waste.
10. SDS-PAGE (reducing): SDS denatures protein; DTT or beta-ME breaks disulphide bonds; proteins separated only by MW; used for determining protein MW and assessing purity of denatured samples. Native PAGE: no SDS; proteins retain native structure; separated by both charge and shape; used to study protein-protein interactions, enzyme activity in gel, and native quaternary structure.

Part 3.2

11. IEF separates proteins by pI using a continuous pH gradient immobilised on a gel strip (IPG strip). When voltage is applied, proteins migrate through the pH gradient; each

lanetonline.com

protein moves until it reaches the position where the pH equals its pI, at which point its net charge is zero and migration ceases. Proteins with different pI values stop at different positions along the strip, achieving separation.

12. Faster separation (minutes vs hours for slab gels). Higher resolution due to high field strength (200 to 600 V per cm). Much smaller sample volumes (nanolitres to microlitres). Automated detection and data analysis.
13. Digest genomic DNA with restriction enzyme(s). Run fragments on agarose gel. Denature DNA in gel with NaOH. Transfer DNA to nylon membrane (capillary or vacuum transfer). UV crosslink to fix DNA. Hybridise with labelled probe overnight. Wash away unbound probe. Detect by autoradiography or chemiluminescence. Applications: RFLP genetic mapping; confirmation of transgene integration in GMO plants; gene copy number analysis.
14. Northern: target = RNA (mRNA); denaturing agarose gel (formaldehyde); RNA transferred to membrane; hybridised with DNA or RNA probe specific to mRNA; indicates transcript size and expression level. Western: target = protein; SDS-PAGE; transferred to PVDF or nitrocellulose; detected with primary antibody then secondary antibody-HRP; indicates protein size and abundance.
15. Blocking prevents non-specific binding of primary and secondary antibodies to the membrane surface, which would generate high background signal. Reagents: 5 percent non-fat dried milk in TBST (Tris-buffered saline with Tween-20) is most common and cheapest; 1 to 5 percent BSA in TBST is used for phospho-protein detection because milk contains casein (a phosphoprotein) that can interfere with anti-phospho antibodies.

Part 3.3

1. Template (provides the target sequence). Forward and reverse primers (hybridise to opposite strands flanking the target; define amplicon; provide 3’-OH). Taq DNA polymerase (synthesises new DNA from primers at 72 degrees C). dNTPs (dATP, dCTP, dGTP, dTTP; building blocks for new strand). MgCl2 (essential cofactor for Taq; stabilises template-primer duplex). PCR buffer (maintains pH 8.3 to 8.8 and ionic strength for optimal polymerase activity).

lanetonline.com

2. Denaturation (94 to 95 degrees C): hydrogen bonds broken; double-stranded DNA separates into two single strands; template exposed. Annealing (50 to 65 degrees C; 5 degrees C below Tm): primers hybridise to complementary sequences on each single-stranded template; stable primer-template duplex formed. Extension (72 degrees C): Taq polymerase synthesises new DNA strand from primer 3'-OH, incorporating dNTPs in the 5' to 3' direction at approximately 1 kb per minute.
3. Too low: primers bind non-specifically to partially complementary sequences; multiple non-specific bands appear on gel. Too high: primers do not bind stably even to the correct target sequence; no product or very faint band obtained.
4. RT-PCR: RNA converted to cDNA by reverse transcriptase; cDNA amplified by PCR; application: detection of RNA viruses such as Lassa fever virus or SARS-CoV-2 in clinical samples. Nested PCR: two rounds using outer then inner primer pairs; second round increases specificity; application: detection of Mycobacterium tuberculosis or HIV provirus at very low copy numbers in clinical specimens.
5. Digital PCR partitions the PCR reaction into thousands of individual droplets or chambers before thermal cycling. Each partition either contains the target sequence (amplifies; positive) or does not (no amplification; negative). After PCR, the fraction of positive partitions is counted; the absolute copy number is calculated using Poisson statistics without reference to a standard curve. Advantage over conventional qPCR: provides absolute quantification; is unaffected by PCR efficiency variation; detects rare mutations at frequencies below 0.1 percent.

Part 3.4

1. Ct is the cycle number at which the amplification fluorescence signal crosses the user-defined threshold. For relative quantification (2^(-delta delta Ct)): delta Ct = Ct(target gene) - Ct(reference gene) for each sample. Delta delta Ct = delta Ct(experimental) - delta Ct(control). Fold-change = 2^(-delta delta Ct). This normalises target gene expression to a stable housekeeping gene and calculates fold-change relative to the control condition.
2. SYBR Green: advantage: cheap; no probe needed; any primer pair works. Disadvantage: detects all dsDNA including primer dimers; requires melting curve to confirm specificity.

laietonline.com

TaqMan: advantage: high specificity (only cleaved probe generates signal); suitable for multiplex qPCR. Disadvantage: custom-designed labelled probe required for each target; more expensive.

3. SYBR Green intercalates into all dsDNA including non-specific products and primer dimers. After qPCR, a melting curve (slow heating from 65 to 95 degrees C with continuous fluorescence monitoring) is performed; the specific amplicon melts at a characteristic Tm (single sharp peak); primer dimers melt at a lower temperature; multiple peaks or a low-Tm peak indicate non-specific amplification that may have contributed to the Ct value.
4. RT-qPCR for SARS-CoV-2 (N and RdRp gene targets; nasopharyngeal swab RNA; Ct below 37 considered positive; NCDC national COVID-19 testing programme). TaqMan qPCR for Lassa fever virus (L segment target; plasma RNA; NCDC National Reference Laboratory, Abuja; used to confirm Lassa fever diagnosis and monitor treatment response).
5. Genomic DNA is extracted from blood or buccal swab. Multiple STR (short tandem repeat) loci are co-amplified using multiplex PCR with fluorescently labelled primers (different colours per locus). Amplified STR alleles (different sizes) are separated by capillary electrophoresis on an ABI sequencer. Each locus generates two allele peaks (heterozygote) or one (homozygote); allele sizes are read from an internal size standard. The resulting multi-locus STR profile is compared with a reference sample or a criminal database (AFIS) to establish identity or exclude a suspect.

lanetonline.com

References and Attributions [Series 3]

Textual References (Books and External Sources)

1. Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology, 51, 263–273.
2. Sambrook, J., & Russell, D. W. (2001). Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor Laboratory Press.
3. Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology, 98(3), 503–517.
4. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-delta delta Ct) method. Methods, 25(4), 402–408.

Figures, Plates, Tables, and Boxes

5. Figure 3.1: Comparison of Southern, Northern, and Western blotting procedures. Attribution: AI generated. Suggested OER search string: “Southern Northern Western blotting comparison procedure diagram steps OER”.
6. Box 3.1: Comparison of PCR variants and their applications. Attribution: AI generated. Suggested OER search string: “PCR variants types RT-PCR multiplex nested digital LAMP comparison table applications OER”.

lanetonline.com

Course code: BIO 408

Course title: Applied Biotechnology

Course credit load: 2 Units

Series 4: Genomic Sequencing and Cloning

- **Part 4.1: DNA Sequencing Technologies**
  - Sub Part 4.1.1: Sanger (chain termination) sequencing
  - Sub Part 4.1.2: Next-generation sequencing (NGS) platforms
  - Sub Part 4.1.3: Third-generation sequencing and nanopore technology
- **Part 4.2: Genome Analysis and Bioinformatics**
  - Sub Part 4.2.1: Genome assembly, annotation, and databases
  - Sub Part 4.2.2: Sequence alignment and BLAST searching
  - Sub Part 4.2.3: Transcriptomics and metagenomics
- **Part 4.3: Principles of Molecular Cloning**
  - Sub Part 4.3.1: Restriction enzymes and ligation
  - Sub Part 4.3.2: Cloning vectors: plasmids, bacteriophage, and cosmids
  - Sub Part 4.3.3: Transformation, selection, and screening of recombinants
- **Part 4.4: Expression Systems and Advanced Cloning**
  - Sub Part 4.4.1: Prokaryotic expression systems
  - Sub Part 4.4.2: Eukaryotic expression systems
  - Sub Part 4.4.3: Advanced cloning strategies and CRISPR applications

lanetonline.com

Introduction

DNA sequencing and molecular cloning are fundamental techniques in recombinant DNA technology. DNA sequencing determines the exact order of nucleotides in DNA, RNA-derived cDNA, or complete genomes. It is used to study gene function, identify mutations, monitor pathogen evolution, and develop molecular diagnostic or therapeutic approaches. Molecular cloning allows a selected DNA sequence to be isolated, copied, modified, and expressed in a host cell. This supports the production of recombinant proteins, development of genetically modified organisms, and investigation of gene function.

This Series examines the development of sequencing technologies from Sanger sequencing to next-generation and nanopore long-read methods, together with the bioinformatics tools used to analyse sequence data. It also explains the major stages of molecular cloning, including restriction digestion, ligation, vector selection, transformation, and screening of recombinant cells. In addition, it introduces prokaryotic and eukaryotic expression systems and advanced methods such as Gibson assembly and CRISPR-Cas9 genome editing, with emphasis on their applications in Nigerian biotechnology research.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 4.1** describe Sanger sequencing and explain how dideoxynucleotides cause chain termination (Linked to Part 4.1),
- **SLO 4.2** compare next-generation and third-generation sequencing platforms and describe their applications (Linked to Part 4.1),
- **SLO 4.3** explain genome assembly, annotation, sequence alignment, and BLAST searching (Linked to Part 4.2),
- **SLO 4.4** describe restriction enzyme cutting, ligation, and the types of cloning vectors (Linked to Part 4.3),

lanetonline.com

- **SLO 4.5** explain transformation, blue-white selection, and colony PCR screening of recombinants (Linked to Part 4.3), and
- **SLO 4.6** describe prokaryotic and eukaryotic expression systems and advanced cloning strategies including Gibson assembly and CRISPR-Cas9 (Linked to Part 4.4).

4.1 DNA Sequencing Technologies

4.1.1 Sanger (chain termination) sequencing

Sanger sequencing (Frederick Sanger, 1977; Nobel Prize in Chemistry 1980) was the dominant sequencing method for over three decades. It exploits the ability of 2',3'-dideoxynucleoside triphosphates (ddNTPs) to terminate DNA strand elongation: when a ddNTP is incorporated by DNA polymerase instead of the corresponding dNTP, no 3'-OH group is available for the next phosphodiester bond, and extension stops. In a sequencing reaction, a single-stranded DNA template is extended from a primer by DNA polymerase in the presence of all four dNTPs and a small proportion of one fluorescently labelled ddNTP (in modern dye-terminator Sanger sequencing, all four ddNTPs are labelled with different fluorescent dyes in a single reaction). Extension products of all lengths are produced, each terminating at a specific nucleotide; the products are separated by size (one base resolution) using capillary electrophoresis, and the fluorescent dye on each terminal ddNTP is read in order of size to give the sequence.

Modern automated Sanger sequencing (ABI 3130 or 3730 capillary sequencer) uses BigDye Terminator chemistry: a single PCR-like sequencing reaction contains template, primer, Taq polymerase, all four dNTPs, and low concentrations of four differently labelled ddNTPs (ddATP: green; ddCTP: blue; ddGTP: black/dark blue; ddTTP: red). The products are purified (ethanol precipitation or ExoSAP-IT treatment to remove excess primer and dNTPs), injected into polymer-filled capillaries, separated electrophoretically, and detected by laser-induced fluorescence. Read length is typically 600 to 1,000 bp; accuracy is above 99.9 percent. Sanger sequencing is still the gold standard for confirming single genes, sequencing PCR products and cloning inserts, and verifying CRISPR editing outcomes. Cost per sample in Nigeria is

lanetonline.com

approximately USD 5 to 15 per reaction through commercial sequencing services in Lagos, Ibadan, and Abuja.

Fill in the blank: In Sanger sequencing, chain termination occurs when a _____ (ddNTP) is incorporated instead of the corresponding dNTP; the absence of a 3’-OH group prevents addition of the next nucleotide, stopping extension at that position.

4.1.2 Next-generation sequencing (NGS) platforms

Next-generation sequencing (NGS; also called massively parallel sequencing or second-generation sequencing) performs millions to billions of sequencing reactions simultaneously, reducing the cost of sequencing a human genome from USD 3 billion (Human Genome Project; 2003) to under USD 1,000 (2014). The dominant NGS platform is Illumina (HiSeq, NovaSeq, NextSeq, MiSeq): library preparation involves fragmenting DNA, ligating sequencing adapters to both ends, and amplifying fragments on a flow cell surface by bridge amplification to form clonal clusters; each cluster is sequenced by synthesis using reversible dye-terminator chemistry (one fluorescently labelled, 3’-blocked nucleotide incorporated at a time; imaged; cleaved; next cycle); read lengths are typically 150 bp paired-end; error rate approximately 0.1 to 0.3 percent; throughput 10 Gb to 6 Tb per run depending on instrument.

Other NGS platforms: Ion Torrent (Thermo Fisher): sequencing by synthesis using pH change detection (H+ released during nucleotide incorporation detected by semiconductor chip); no fluorescence; fast; moderate throughput; used for amplicon sequencing and targeted panels. MGI/BGI (DNBSEQ): DNA nanoballs on patterned array; used in large genomic centres including Africa CDC genomic surveillance programmes. NGS applications in Nigeria: SARS-CoV-2 whole-genome sequencing for variant tracking (NCDC and partner institutions; Illumina MiSeq; sequencing of approximately 5 percent of confirmed cases during 2021 to 2023); pathogen genomics (Lassa fever virus; monkeypox; meningococcal disease; typhoid fever); crop genome sequencing (cassava, cowpea, yam genomes available in public databases); human population genomics (AWI-Gen study; H3Africa Consortium; whole-genome sequencing of Nigerian population samples for disease association studies).

lanetonline.com

Fill in the blank: In Illumina NGS, DNA fragments are first ligated to _____ adapters and amplified on a flow cell surface by bridge amplification to form clonal clusters; each cluster is sequenced by synthesis using reversible dye-terminator chemistry.

4.1.3 Third-generation sequencing and nanopore technology

Third-generation sequencing platforms read single DNA molecules directly without amplification, producing much longer reads than NGS (tens to hundreds of kilobases). Pacific Biosciences (PacBio) SMRT (single molecule real-time) sequencing: DNA polymerase is immobilised at the bottom of a zero-mode waveguide (ZMW) well; as each nucleotide is incorporated, its fluorescent label generates a pulse of light before being cleaved; real-time fluorescence is monitored; read lengths of 10 to 25 kb average (up to 100 kb); accuracy 99.9 percent (HiFi/CCS reads using circular consensus sequencing); used for de novo genome assembly, structural variant detection, and full-length isoform sequencing.

Oxford Nanopore Technologies (ONT) MinION: DNA passes through a protein nanopore embedded in a synthetic membrane; as each nucleotide translocates through the pore, it causes a characteristic change in ionic current; the current signal is decoded by a neural network (basecalling) to give the DNA sequence. The MinION is a USB device weighing 90 grams, sequencing up to 50 Gb per flow cell; it is the only sequencer deployable in the field without laboratory infrastructure. Read lengths range from a few hundred bases to megabases (ultra-long reads). Raw accuracy is 98 to 99.5 percent; accuracy improves to above 99.9 percent with high-coverage assembly. The MinION has been used in Nigeria for: SARS-CoV-2 sequencing in field settings (ACEGID, Redeemer's University; IHVN laboratories); Lassa fever virus genomic surveillance; and meningitis outbreak investigation. Direct RNA sequencing (without cDNA conversion) is possible with ONT, preserving base modifications (m6A, m5C) information.

Fill in the blank: The Oxford Nanopore MinION sequences DNA by detecting changes in _____ current as each nucleotide passes through a protein nanopore; it is the only sequencing platform deployable in remote field settings without laboratory infrastructure.

Pilot questions 4.1

laneteonline.com

- Explain the principle of Sanger chain termination sequencing and the role of ddNTPs.
- Describe Illumina NGS library preparation and sequencing by synthesis.
- State two advantages of Oxford Nanopore MinION over Illumina for pathogen sequencing in Nigeria.
- Compare Sanger sequencing and NGS in terms of read length, throughput, cost, and accuracy.
- Name two NGS applications in Nigeria and state the institution involved in each.

4.2 Genome Analysis and Bioinformatics

4.2.1 Genome assembly, annotation, and databases

Genome assembly reconstructs the complete genome sequence from short sequencing reads. De novo assembly (without a reference genome) uses algorithms that find overlaps between reads and join them into contigs (contiguous sequences), then scaffolds (contigs linked by paired-end read information). Short Illumina reads (150 bp) are assembled using overlap-layout-consensus (OLC) assemblers (SPAdes; Velvet; ABySS); long nanopore or PacBio reads allow much better resolution of repetitive regions and produce assemblies close to chromosome-level with fewer gaps (Flye; Canu assemblers). Reference-guided assembly maps reads to an existing reference genome; used when a reference is available (human, rice, cassava).

Genome annotation assigns biological function to predicted features in an assembled genome: gene prediction identifies open reading frames (ORFs) using ab initio gene finders (AUGUSTUS; SNAP) and evidence-based methods (RNA-seq transcript evidence; protein homology from related species); functional annotation assigns gene names and GO (Gene Ontology) terms by sequence similarity search (BLAST; InterProScan against PFAM protein domain database). Key genomic databases: NCBI GenBank (nucleotide sequences; ncbi.nlm.nih.gov/genbank); EMBL-EBI (European Molecular Biology Laboratory-European Bioinformatics Institute); UniProt (protein sequences and functions; uniprot.org); Ensembl (annotated vertebrate genomes); Phytozome (plant genomes; phytozome.jgi.doe.gov; cassava

lanetonline.com

genome: Manihot esculenta v8.1; cowpea genome: Vigna unguiculata v1.1); CoGe (comparative genomics). Nigerian researchers deposit sequences in GenBank under NCBI BioProject accessions.

Fill in the blank: Genome _____ assigns biological function to predicted genes in an assembled genome sequence, using gene prediction algorithms, BLAST homology searches, and protein domain databases such as PFAM and InterPro.

4.2.2 Sequence alignment and BLAST searching

Sequence alignment identifies regions of similarity between two or more sequences. Pairwise alignment compares two sequences; multiple sequence alignment (MSA) aligns three or more sequences simultaneously. Global alignment (Needleman-Wunsch algorithm) aligns entire sequences end-to-end; used when sequences are similar in length and overall composition. Local alignment (Smith-Waterman algorithm) finds the highest-scoring locally similar region; more appropriate for distantly related or partial sequences. Scoring matrices (BLOSUM62 for protein alignment; PAM matrices) assign scores to each pair of aligned residues based on evolutionary substitution frequencies; gap penalties discourage insertion of gaps.

BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990; NCBI) is the most widely used sequence similarity search tool. BLASTn searches a nucleotide query against a nucleotide database; BLASTp searches a protein query against a protein database; BLASTx translates a nucleotide query and searches protein databases; tBLASTn searches a protein query against translated nucleotide databases. Key output statistics: the E-value (expect value) is the number of alignments with that score or better expected by chance in a database of that size; E-value below 0.001 (10^-3) is generally considered significant; E-value below 10^-10 indicates strong similarity. Percent identity (percentage of aligned positions with identical residues) and query coverage (percentage of the query length covered by the alignment) also characterise alignment quality. BLAST results are used to: assign function to newly sequenced genes; identify organisms from partial sequences (16S rRNA BLAST for bacteria); and find homologues for primer design or cloning.

lanetonline.com

Fill in the blank: In BLAST output, the _____ value indicates the number of alignments with that score or better expected by chance in the database; a value below 10^-3 is considered significant, and values below 10^-10 indicate strong sequence similarity.

4.2.3 Transcriptomics and metagenomics

Transcriptomics is the large-scale analysis of all RNA transcripts (the transcriptome) in a cell or tissue under defined conditions. RNA-seq (RNA sequencing) uses NGS to sequence cDNA libraries made from total RNA or poly-A-selected mRNA: RNA is extracted; poly-A selection or rRNA depletion enriches for mRNA; RNA is fragmented and converted to cDNA; sequencing adapters are ligated; NGS is performed; reads are mapped to a reference genome or assembled de novo; read counts per gene are used to quantify expression; differential expression analysis (DESeq2; edgeR software) identifies genes significantly up- or down-regulated between conditions. RNA-seq is used for: gene expression profiling in plant stress responses (drought, pathogen infection) at IITA and Nigerian universities; cancer transcriptomics; and identification of novel transcripts and splice variants.

Metagenomics is the direct sequencing of DNA extracted from a complex environmental sample (soil, water, gut contents, clinical sample) without prior culturing of individual organisms. Shotgun metagenomics sequences all DNA in the sample; taxonomic assignment of reads identifies all organisms present; functional annotation identifies metabolic pathways encoded by the community. 16S rRNA amplicon sequencing (amplifying and sequencing the hypervariable V3-V4 region of the bacterial 16S rRNA gene) is a targeted metagenomic approach that identifies bacterial community composition cheaply; widely used for gut microbiome analysis. Nigerian applications: soil microbiome characterisation in cassava and cocoa farms (IITA Ibadan; University of Ibadan); gut microbiome studies in Nigerian populations (University of Lagos, ABU); water quality metagenomics for surveillance of antimicrobial resistance genes in Lagos waterways (NCDC collaborations).

Fill in the blank: _____ sequencing uses NGS to quantify all mRNA transcripts in a sample; read counts per gene are compared between conditions to identify differentially expressed genes using tools such as DESeq2 or edgeR.

lanetonline.com

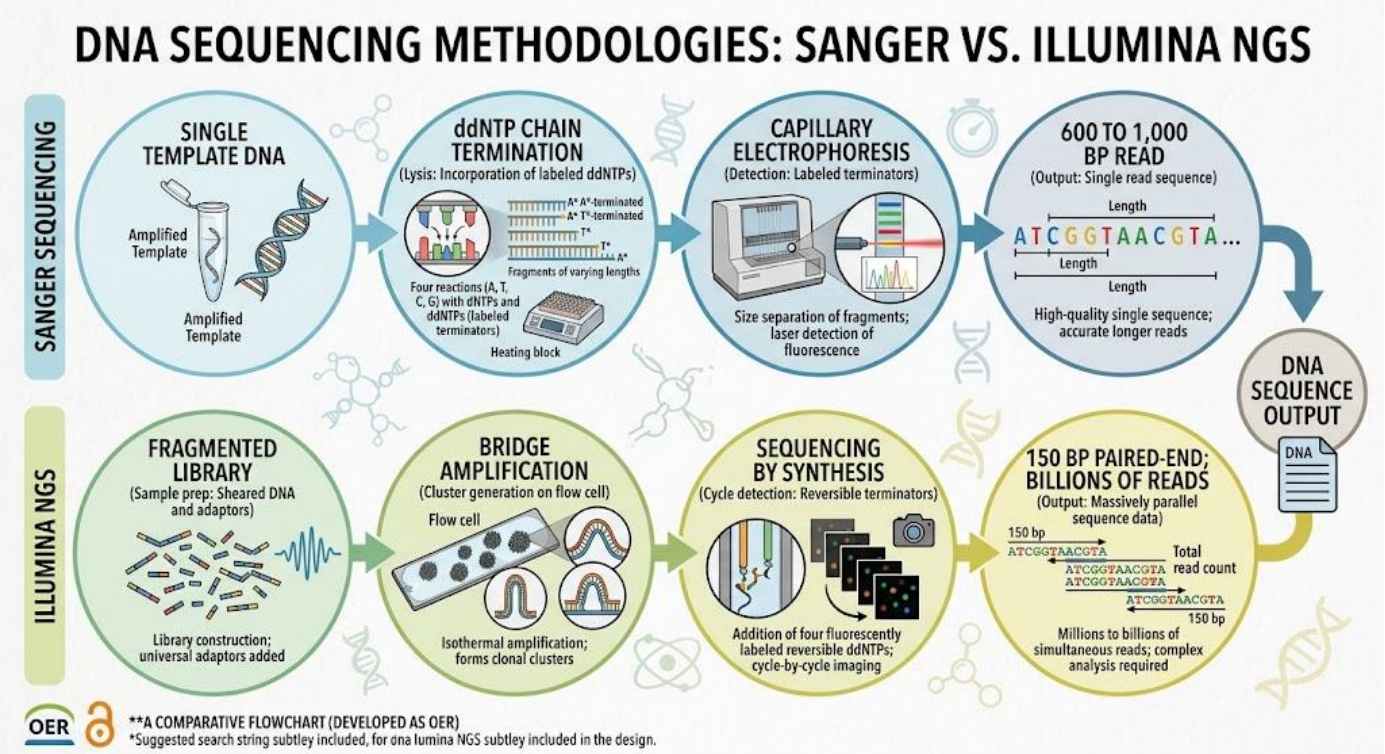


Figure 4.1: Comparison of Sanger sequencing and Illumina NGS workflows

Pilot questions 4.2

- Distinguish between de novo genome assembly and reference-guided assembly, stating when each is used.
- Explain the meaning of an E-value in BLAST output and state what E-value threshold is considered significant.
- Describe the steps of RNA-seq from RNA extraction to differential expression analysis.
- Define metagenomics and distinguish between shotgun metagenomics and 16S rRNA amplicon sequencing.
- Name two genomic databases and state what type of sequence information each holds.

4.3 Principles of Molecular Cloning

4.3.1 Restriction enzymes and ligation

lanetonline

Restriction endonucleases (restriction enzymes) are bacterial enzymes that recognise specific short DNA sequences (recognition sites; typically 4 to 8 bp) and cleave both strands of double-stranded DNA within or adjacent to the recognition sequence. Type II restriction enzymes (the most useful for cloning) cleave within their recognition sequence at defined positions; they are named for the organism from which they were isolated (EcoRI from Escherichia coli strain RY13; BamHI from Bacillus amyloliquefaciens; HindIII from Haemophilus influenzae strain Rd). Cleavage may produce: 5' overhang (sticky ends; e.g. EcoRI: 5'-G^AATTC-3' produces 5'-AATT overhangs); 3' overhang (e.g. PstI); or blunt ends (no overhang; e.g. SmaI: 5'-CCC^GGG-3'). Compatible sticky ends (produced by the same or isocaudomeric enzymes) are highly efficient in ligation; blunt end ligation is less efficient.

DNA ligase (T4 DNA ligase from bacteriophage T4) catalyses the formation of phosphodiester bonds between the 3'-OH and 5'-phosphate ends of adjacent DNA strands in the presence of ATP. In cloning, the insert DNA (target gene) and the vector are both cut with the same restriction enzyme(s) to produce compatible ends; ligase joins insert and vector to form a recombinant DNA molecule. Directional cloning uses two different restriction enzymes to cut the insert and vector, ensuring the insert can only be ligated in one orientation. The molar ratio of insert to vector for optimal ligation is typically 3:1 to 5:1. Ligation reactions are incubated at 16 degrees C overnight (cohesive end ligation) or at room temperature for 10 minutes with rapid ligation kits. The proportion of recombinant vs non-recombinant ligation products depends on insert to vector ratio, enzyme efficiency, and dephosphorylation of the vector (with calf intestinal alkaline phosphatase, CIP, or shrimp alkaline phosphatase, SAP) to reduce re-ligation of vector without insert.

Fill in the blank: Vector dephosphorylation with _____ intestinal alkaline phosphatase (CIP) removes the 5'-phosphate from linearised vector ends, preventing self-ligation and increasing the proportion of recombinant clones obtained after transformation.

4.3.2 Cloning vectors: plasmids, bacteriophage, and cosmids

A cloning vector is a DNA molecule capable of autonomous replication in a host cell that is used to carry an insert DNA of interest. A plasmid vector must have: an origin of replication (ori;

lanetonline.com

allows autonomous replication in the host); one or more selectable markers (antibiotic resistance genes: ampicillin resistance AmpR; kanamycin resistance KanR; chloramphenicol resistance CamR; allow selection of transformed cells on antibiotic-containing agar); and a multiple cloning site (MCS; polylinker; a short sequence containing recognition sites for many restriction enzymes in a non-essential region, allowing insertion of foreign DNA without disrupting replication or selection). Common plasmid vectors: pUC19 (2.7 kb; AmpR; MCS within lacZ-alpha gene; blue-white selection; high-copy number; standard cloning vector); pBluescript (3.0 kb; AmpR; lacZ; T3 and T7 promoters flanking MCS; used for in vitro transcription); pET series (Novagen; for E. coli protein expression under T7 promoter).

For large inserts, specialised vectors are used: bacteriophage lambda vectors (can accommodate inserts of 9 to 23 kb; used in genomic library construction); cosmids (hybrid of plasmid and phage; contain phage cos sites for packaging into phage heads; carry inserts of 35 to 45 kb; used for genomic libraries); BAC (bacterial artificial chromosome; based on E. coli F-plasmid; inserts 100 to 300 kb; used for physical mapping and sequencing large genomes; key vector in human genome project); YAC (yeast artificial chromosome; inserts 100 kb to 1 Mb; used for very large genomic regions). For expression in mammalian cells, viral vectors (lentiviral, adenoviral, AAV) are used; for plant transformation, Ti plasmid of Agrobacterium tumefaciens (described in Part 4.4). Expression vectors additionally carry a promoter, ribosome binding site (Shine-Dalgarno sequence for prokaryotes), and transcription terminator flanking the MCS.

Fill in the blank: The multiple cloning site (MCS) of a plasmid vector is a short sequence containing recognition sites for many _____ enzymes; it is located within or adjacent to a selectable marker gene so that insertion of foreign DNA can be detected by disruption of that marker.

4.3.3 Transformation, selection, and screening of recombinants

Transformation introduces foreign DNA into bacterial host cells. Chemical transformation uses chemically competent cells prepared by treating E. coli (DH5-alpha; TOP10; XL1-Blue) with CaCl2 and MgCl2 to make cells transiently permeable to DNA; ligation mix is added to competent cells; heat shock at 42 degrees C for 30 to 45 seconds temporarily opens membrane

lanetonline.com

pores allowing DNA entry; cells are recovered in SOC medium at 37 degrees C for 45 to 60 minutes before plating. Electroporation uses a brief high-voltage pulse (1.8 to 2.5 kV; 25 microfarads; 200 ohms) to create transient pores in the bacterial membrane; more efficient than chemical transformation (10^9 to 10^10 transformants per microgram DNA vs 10^6 to 10^8 for chemical); requires electro-competent cells and electroporation cuvettes.

Selection: transformed cells are plated on LB agar containing the appropriate antibiotic (e.g. ampicillin 100 micrograms per ml for AmpR vectors); only cells that took up a vector grow as colonies; untransformed cells are killed by the antibiotic. Screening for recombinants: blue-white selection (alpha-complementation): pUC19 and pBluescript carry a truncated lacZ-alpha gene encoding the alpha fragment of beta-galactosidase; intact lacZ-alpha (no insert) produces blue colonies in the presence of IPTG (inducer) and X-Gal (chromogenic beta-gal substrate; cleaved to produce blue product); insertion into MCS disrupts lacZ-alpha; recombinant clones produce white colonies. Colony PCR: a small amount of colony material is added directly to a PCR reaction using primers flanking the MCS or spanning the insert; a positive band of the expected insert size confirms recombinant; negative (no insert) gives only vector band or no band. Recombinant clones are confirmed by restriction enzyme digestion of miniprep plasmid DNA and by Sanger sequencing of the insert.

Fill in the blank: In blue-white selection, white colonies contain a _____ plasmid (insert disrupts lacZ-alpha; no active beta-galactosidase; X-Gal not cleaved); blue colonies contain empty vector (intact lacZ-alpha; X-Gal cleaved to blue product).

Step	Process	Details / Purpose
1. Restriction digestion	Digest insert PCR product and vector with same restriction enzyme(s)	Generate compatible ends for ligation
2. Vector dephosphorylation	Treat vector with CIP (calf intestinal phosphatase)	Prevent self-ligation of vector
3. Ligation	Ligate insert into vector using T4 DNA ligase (16 °C overnight; insert:vector = 3:1)	Create recombinant plasmid

lanetonline.com

4. Transformation	Introduce plasmid into competent *E. coli* by heat shock	Propagate recombinant DNA
5. Plating	Plate cells on LB + ampicillin + IPTG + X-Gal	Enable antibiotic selection and blue-white screening
6. Colony selection	Pick white colonies (recombinants)	Identify clones with insert
7. Colony PCR	Amplify DNA from colonies	Verify presence of insert
8. Miniprep & digest	Isolate plasmid DNA and perform restriction digest	Confirm insert orientation and size
9. Sanger sequencing	Sequence plasmid DNA	Verify insert sequence accuracy

Box 4.1: Flowchart of a standard molecular cloning procedure using restriction enzyme cloning

Pilot questions 4.3

1. Explain why restriction enzymes that produce sticky ends are preferred over blunt end cutters for cloning.
2. State three essential components that every cloning vector must contain and explain the function of each.
3. Describe the procedure for transforming competent E. coli cells by heat shock and explain what happens during heat shock.
4. Explain the principle of blue-white selection and describe the result expected on an X-Gal plate after cloning into pUC19.
5. Describe how colony PCR is used to screen for recombinant clones after transformation.

4.4 Expression Systems and Advanced Cloning

4.4.1 Prokaryotic expression systems

Prokaryotic expression systems commonly use *Escherichia coli* to produce recombinant proteins quickly and at relatively low cost. Expression vectors contain strong inducible promoters,

lanetonline.com

allowing cells to grow before protein production begins and reducing the harmful effects of toxic proteins. The most widely used system is the T7 promoter system found in pET vectors. The target gene is placed downstream of the T7 promoter, while *E. coli* BL21(DE3) carries the T7 RNA polymerase gene under the control of the lac promoter. Addition of IPTG induces the production of T7 RNA polymerase, which then transcribes the cloned gene at a high rate. Recombinant protein may account for about 10–30% of the total cellular protein.

Common challenges include inclusion bodies, codon bias, lack of post-translational modifications, and endotoxin contamination. Inclusion bodies can be reduced by lowering the induction temperature to **16–25°C**, using less IPTG, or co-expressing chaperones such as GroEL/GroES. Codon bias may be corrected with a codon-optimised gene or a Rosetta strain containing rare tRNAs. Because *E. coli* cannot perform complex modifications such as glycosylation or GPI-anchor formation, eukaryotic hosts are preferred for proteins requiring these processes. Endotoxin contamination is controlled using removal columns and confirmed with the LAL test. In Nigerian research laboratories, *E. coli* is used to produce diagnostic antigens, laboratory enzymes, and research proteins for biochemical studies.

Fill in the blank: In the T7/pET E. coli expression system, protein expression is induced by adding _____ (IPTG), which induces expression of T7 RNA polymerase in the BL21(DE3) host; T7 RNA polymerase then drives high-level transcription of the cloned gene from the T7 promoter.

4.4.2 Eukaryotic expression systems

Eukaryotic expression systems are required for proteins that need post-translational modifications (glycosylation; phosphorylation; disulphide bond formation) or correct eukaryotic folding for biological activity. Yeast (Saccharomyces cerevisiae; Pichia pastoris): grows on inexpensive media; performs N-glycosylation (though with different glycan patterns than mammalian); GRAS (generally recognised as safe) organisms for food-grade protein production. Pichia pastoris uses the AOX1 (alcohol oxidase) promoter induced by methanol; high-level secretion of recombinant protein into medium; used commercially for insulin, hepatitis B vaccine antigen, and industrial enzymes. Insect cell/baculovirus system (Sf9 or Hi5 cells + recombinant

lanetonline.com

baculovirus): gene of interest replaces viral polyhedrin gene; baculovirus infects insect cells and produces very high levels of recombinant protein; performs most mammalian post-translational modifications; used for viral-like particles (VLPs) and vaccine antigens.

Mammalian cell expression (CHO, HEK293, COS cells): gold standard for therapeutic proteins requiring authentic glycosylation; stable transfection using plasmid vectors with selection markers (G418/neomycin; hygromycin; puromycin) generates stable cell lines; transient transfection with lipofection or PEI (polyethylenimine) for rapid protein production. All FDA-approved biologic drugs (monoclonal antibodies, EPO, tissue plasminogen activator) are produced in CHO or other mammalian cells. Plant expression systems: Agrobacterium-mediated transformation introduces a gene of interest (flanked by T-DNA border sequences) into plant nuclear genome under a strong plant promoter (35S CaMV promoter; ubiquitin promoter); transgenic plants expressing recombinant proteins ('pharming') are used for edible vaccine antigens and industrial enzymes; Bt cowpea and Bt cotton in Nigeria express insecticidal Cry proteins under 35S CaMV promoter in plant cells.

Fill in the blank: Mammalian cell expression in _____ (Chinese Hamster Ovary) cells is the gold standard for producing therapeutic recombinant proteins such as monoclonal antibodies that require authentic human-like glycosylation for biological activity and safety.

4.4.3 Advanced cloning strategies and CRISPR applications

Gibson assembly (Daniel Gibson, 2009) allows seamless assembly of multiple DNA fragments without restriction enzymes. Fragments with 20 to 40 bp overlapping sequences are incubated with a mixture of three enzymes: T5 exonuclease (degrades from 5' ends, creating single-stranded 3' overhangs that can hybridise with complementary overhangs of adjacent fragments); Phusion DNA polymerase (fills in gaps); Taq DNA ligase (seals nicks). Gibson assembly can join 2 to 10 fragments simultaneously; is seamless (no restriction site scars); and is the standard method for constructing synthetic genes, assembling multiple gene cassettes, and building complex genetic circuits. The HiFi Assembly (NEB) and SLIC (sequence and ligation-independent cloning) are commercial variants.

lanetonline.com

CRISPR-Cas9 (described in Series 2 BIO402) is used in conjunction with cloning for: knocking out genes by introducing frameshift mutations (indels); knocking in specific sequences by homology-directed repair (HDR) using a donor DNA template cloned into a delivery vector; large chromosomal deletions by using two guide RNAs flanking a region; and base editing (converting C to T or A to G at a target position without double-strand breaks; CBE: cytosine base editor; ABE: adenine base editor). In Nigeria, CRISPR applications in research include: editing of the SWEET gene promoter in cassava for bacterial blight resistance (IITA in collaboration with Innovative Genomics Institute, UC Berkeley); editing of eIF4E gene in cowpea for potyvirus resistance (IITA); and developing CRISPR-based diagnostics (SHERLOCK; DETECTR) that use CRISPR effectors (Cas13 for RNA detection; Cas12a for DNA detection) to detect pathogen nucleic acids with high sensitivity at point-of-care.

Fill in the blank: Gibson assembly joins multiple DNA fragments seamlessly using three enzymes: T5 exonuclease (creates 3' overhangs), Phusion polymerase (fills gaps), and Taq DNA _____ (seals nicks); it requires 20 to 40 bp overlapping sequences at the junctions between adjacent fragments.

Pilot questions 4.4

1. Describe the T7/pET E. coli expression system and explain how IPTG induces protein expression.
2. State three problems commonly encountered in E. coli expression and describe one solution for each.
3. Explain why mammalian cell expression systems are used instead of E. coli for therapeutic protein production.
4. Describe the principle of Gibson assembly and state two advantages over restriction enzyme cloning.
5. Explain how CRISPR-Cas9 is used to knock in a specific gene sequence using homology-directed repair (HDR).

lanetonline.com

Series Summary

This Series covered DNA sequencing technologies and their bioinformatics applications. Sanger sequencing uses ddNTP chain termination and capillary electrophoresis to produce highly accurate reads of about 600–1,000 base pairs, making it suitable for confirming individual genes. Next-generation sequencing, especially Illumina technology, uses bridge amplification and reversible dye terminators to generate billions of short paired-end reads and has been applied to SARS-CoV-2 variant surveillance. Third-generation platforms produce much longer reads: PacBio SMRT sequencing can generate reads of about 10–25 kb with high consensus accuracy, while the portable Oxford Nanopore MinION detects changes in ionic current as DNA passes through nanopores and is suitable for field-based sequencing. Bioinformatics analysis includes reference-guided or de novo genome assembly, gene annotation, BLAST searches, RNA-seq analysis with tools such as DESeq2 and edgeR, and microbiome studies using shotgun metagenomics or 16S rRNA amplicon sequencing. In BLAST analysis, an E-value below **10^-3** generally indicates a meaningful sequence match.

Molecular cloning involves cutting DNA with Type II restriction enzymes such as EcoRI, BamHI, and HindIII, joining fragments with T4 DNA ligase, and introducing the recombinant vector into host cells by heat shock or electroporation. Plasmid vectors such as pUC19 and pET contain an origin of replication, selectable marker, and multiple cloning site, while lambda phages, cosmids, and BACs carry larger DNA inserts. Recombinant cells are identified through antibiotic selection, blue-white screening, colony PCR, and Sanger sequencing. Expression hosts include *E. coli* for rapid and inexpensive protein production, *Pichia pastoris* for secretion and glycosylation, insect cells for vaccine antigens, CHO cells for therapeutic antibodies, and plants for agricultural biotechnology. Advanced approaches include Gibson assembly for seamless joining of multiple DNA fragments and CRISPR-based techniques for gene knockout, knock-in, base editing, molecular diagnostics, and crop improvement.

Glossary of Terms

lanetonline.com

1. **BAC (bacterial artificial chromosome):** a cloning vector based on the E. coli F-plasmid; accommodates DNA inserts of 100 to 300 kb; used for physical mapping and sequencing of large genomes.
2. **BLAST:** Basic Local Alignment Search Tool; a sequence similarity search algorithm that compares a query sequence against a database; E-value below 10^-3 indicates significant similarity; types include BLASTn (nucleotide), BLASTp (protein), and BLASTx (translated nucleotide).
3. **Blue-white selection:** a screening method for recombinant plasmid clones; insertion of foreign DNA into the MCS of pUC19 disrupts lacZ-alpha; colonies with insert are white (no beta-galactosidase activity; X-Gal not cleaved); empty vector colonies are blue.
4. **CIP (calf intestinal alkaline phosphatase):** an enzyme that removes 5'-phosphate groups from linearised vector ends, preventing self-ligation and increasing the proportion of recombinant clones after transformation.
5. **CRISPR-Cas9:** a programmable genome editing system using a guide RNA and Cas9 nuclease to make site-specific double-strand breaks; used for gene knockout, knock-in (HDR), base editing, and CRISPR-based diagnostics.
6. **Dideoxynucleotide (ddNTP):** a nucleotide analogue lacking the 3'-OH group; when incorporated by DNA polymerase, it terminates chain elongation; used in Sanger sequencing to generate fragments terminating at each nucleotide position.
7. **Gibson assembly:** a one-step, seamless multi-fragment DNA assembly method using T5 exonuclease, Phusion polymerase, and Taq ligase acting on fragments with overlapping 20 to 40 bp sequences; no restriction enzymes or scars required.
8. **IPTG:** isopropyl beta-D-thiogalactopyranoside; a non-metabolisable inducer of the lac operon; used to induce T7 RNA polymerase expression in BL21(DE3) cells, driving recombinant protein production from pET vectors.
9. **MinION:** Oxford Nanopore Technologies' USB-format sequencer that sequences single DNA molecules by detecting ionic current changes as nucleotides pass through a protein nanopore; produces long reads (up to megabases); field-deployable.

lanetonline.com

10. **Multiple cloning site (MCS):** a short polylinker sequence in a cloning vector containing recognition sites for many restriction enzymes; located within or adjacent to a selectable marker gene (lacZ-alpha in pUC19) to allow detection of insert by marker disruption.
11. **Next-generation sequencing (NGS):** massively parallel DNA sequencing that performs millions to billions of reactions simultaneously; Illumina (reversible dye-terminators; 150 bp reads) is the dominant platform; dramatically lower cost than Sanger.
12. **Restriction endonuclease:** a bacterial enzyme that recognises a specific DNA sequence and cleaves both strands; Type II enzymes cleave within the recognition sequence producing sticky (5’ or 3’ overhang) or blunt ends; used in molecular cloning.
13. **T4 DNA ligase:** a bacteriophage T4 enzyme that catalyses formation of phosphodiester bonds between adjacent 3’-OH and 5’-phosphate ends in ATP-dependent reactions; used to join insert and vector DNA in cloning.
14. **T7/pET expression system:** an E. coli expression system in which the gene of interest is transcribed by T7 RNA polymerase (induced by IPTG in BL21(DE3) cells) from a T7 promoter in a pET vector; produces high-level recombinant protein.

Concept Check Questions [Series 4]

Objective Questions

1. In Sanger sequencing, chain termination occurs when a _____ (ddNTP) is incorporated instead of the corresponding dNTP. (Linked to SLO 4.1)
2. In Illumina NGS, DNA fragments are amplified on a flow cell surface by _____ amplification to form clonal clusters. (Linked to SLO 4.2)
3. In BLAST, an E-value below _____ is generally considered a significant sequence match. (Linked to SLO 4.3)
4. Vector self-ligation is prevented by treating linearised vector with _____ (CIP) to remove 5’-phosphate groups. (Linked to SLO 4.4)
5. In the T7/pET expression system in E. coli, recombinant protein expression is induced by adding _____. (Linked to SLO 4.6)

lanetonline.com

Short-Answer Questions

- Explain the principle of Sanger chain termination sequencing and the role of ddNTPs. (Linked to SLO 4.1)
- Describe the three essential components of a plasmid cloning vector and state the function of each. (Linked to SLO 4.4)
- Explain blue-white selection and describe the expected result on an X-Gal plate after cloning into pUC19. (Linked to SLO 4.5)
- Describe the principle of Gibson assembly and state two advantages over restriction enzyme cloning. (Linked to SLO 4.6)
- Explain why mammalian CHO cells are used instead of E. coli for production of therapeutic monoclonal antibodies. (Linked to SLO 4.6)

Long-Answer Questions

1. Compare Sanger sequencing, NGS, and nanopore sequencing. Explain genome annotation, BLAST searching, and the applications of RNA-seq and metagenomics in Nigerian biotechnology. (Linked to SLOs 4.1, 4.2, 4.3)
2. Describe the principles of molecular cloning including restriction enzyme digestion, ligation, transformation, and screening. Describe the E. coli and CHO cell expression systems and explain Gibson assembly and CRISPR-Cas9 knock-in. (Linked to SLOs 4.4, 4.5, 4.6)

Answers to Concept Check Questions [Series 4]

Objective Questions

6. Dideoxynucleotide.
7. Bridge.
8. 10^-3 (0.001).
9. CIP.

lanetonline.com

10. IPTG.

Short-Answer Questions

11. Sanger sequencing uses ddNTPs that lack a 3’-OH group. When Taq polymerase incorporates a ddNTP instead of a dNTP, no phosphodiester bond can form with the next nucleotide, terminating chain elongation. With four differently labelled ddNTPs in one reaction, fragments terminating at every nucleotide position are produced; CE separation by size and laser detection of terminal dye gives the sequence.
12. Origin of replication (ori): allows autonomous plasmid replication in the host cell without chromosomal integration. Antibiotic resistance marker (e.g. AmpR): allows selection of transformed cells on antibiotic-containing agar; untransformed cells are killed. Multiple cloning site (MCS): cluster of restriction enzyme recognition sites; allows insertion of foreign DNA by restriction enzyme cloning.
13. pUC19 carries a truncated lacZ-alpha gene in the MCS. Intact lacZ-alpha (empty vector) produces active beta-galactosidase that cleaves X-Gal to a blue product; colonies are blue. Insertion of foreign DNA into MCS disrupts lacZ-alpha; no functional enzyme; X-Gal not cleaved; colonies are white. On IPTG + X-Gal plates: recombinant clones (with insert) are white; empty vector clones are blue.
14. Gibson assembly joins multiple DNA fragments with 20 to 40 bp overlapping ends using three enzymes in one reaction: T5 exonuclease chews back 5’ ends exposing single-stranded 3’ overhangs; complementary overhangs anneal; Phusion polymerase fills gaps; Taq ligase seals nicks. Advantages: (1) seamless (no restriction site scars); (2) can join multiple fragments (2 to 10) simultaneously.
15. CHO cells perform authentic human-like glycosylation (addition of complex N-glycans to asparagine residues) required for therapeutic mAb efficacy, half-life, and safety. E. coli cannot glycosylate proteins; a CHO-produced mAb would have correct folding, disulphide bonds, and glycans; an E. coli-produced version would be aglycosylated with reduced effector function and potentially immunogenic.

Long-Answer Questions

1. Sanger: ddNTP chain termination; single template; BigDye terminator; CE separation; 600 to 1,000 bp; 99.9 percent accuracy; gold standard for single gene/insert verification.

laretonline.com

NGS (Illumina): fragment library; bridge amplification into clusters; sequencing by synthesis (reversible dye-terminators); 150 bp paired-end; billions of reads per run; 0.1 to 0.3 percent error; used for SARS-CoV-2 whole-genome sequencing (NCDC), crop genomics, human population genomics (H3Africa). Nanopore (ONT MinION): single-molecule; ionic current through nanopore; long reads (up to megabases); USB field device; used for SARS-CoV-2 sequencing at ACEGID (Redeemers University) and Lassa fever surveillance. Genome annotation: gene prediction (AUGUSTUS; evidence from RNA-seq and protein homology); functional annotation (BLAST against UniProt; InterProScan for PFAM domains; GO term assignment); Phytozome holds cassava (v8.1) and cowpea (v1.1) genomes. BLAST: E-value below 10^-3 = significant; below 10^-10 = strong similarity; BLASTn/BLASTp/BLASTx for nucleotide/protein queries; used to assign gene function, identify organisms from 16S sequences, find primer regions. RNA-seq: RNA extraction; poly-A selection; cDNA library; NGS; map to reference; DESeq2/edgeR for differential expression; used at IITA for cassava drought stress gene expression. Metagenomics: shotgun (all DNA in sample; taxonomic + functional analysis); 16S rRNA amplicon (V3-V4; bacterial community); used for soil microbiome in cassava farms (IITA) and gut microbiome studies (University of Lagos, ABU).

2. Cloning: restriction enzyme (Type II; EcoRI produces 5'-AATT sticky ends; SmaI produces blunt ends); T4 ligase (ATP; 16 degrees C; joins compatible ends; 3:1 insert:vector); CIP dephosphorylation of vector prevents self-ligation. Vectors: plasmid (ori + antibiotic resistance + MCS; pUC19 for cloning; pET for expression); lambda phage (9 to 23 kb); cosmid (35 to 45 kb); BAC (100 to 300 kb). Transformation: heat shock (CaCl2-competent E. coli; 42 degrees C; 30 to 45 s; SOC recovery; plate on LB+Amp) or electroporation (1.8 to 2.5 kV; more efficient). Selection: AmpR on LB+Amp. Screening: blue-white (IPTG+X-Gal; white = recombinant); colony PCR (primers flanking MCS; band = insert present); miniprep + restriction digest + Sanger to confirm. E. coli/pET expression: BL21(DE3); T7 promoter; IPTG induction; 10 to 30 percent total cell protein; problems: inclusion bodies (lower temp; chaperones), codon bias (Rosetta), no glycosylation. CHO: authentic glycosylation for therapeutic mAbs; stable transfection; used for all FDA-approved biologics. Gibson assembly: overlapping fragments; T5 exonuclease + Phusion + Taq ligase; seamless; joins 2 to 10 fragments;

lanetonline.com

used for synthetic gene assembly. CRISPR knock-in (HDR): design gRNA to target locus near desired insertion; clone into Cas9 expression vector; provide donor template with homology arms flanking the insertion sequence; co-transfect gRNA/Cas9 + donor into cells; HDR incorporates donor at cut site; screen by PCR and sequencing.

Answers to Pilot Questions [Series 4]

Part 4.1

6. A ddNTP lacks the 3'-OH group. When incorporated by DNA polymerase, no new phosphodiester bond can form with the next dNTP, stopping extension. With four differently fluorescently labelled ddNTPs at low concentration, the reaction produces a ladder of fragments each terminating at a specific nucleotide; CE separation and laser detection reads the dye colour at each position to give the sequence.
7. Fragment genomic DNA (sonication or enzymatic); repair fragment ends and add 3'-A overheads; ligate sequencing adapters to both ends; PCR amplify library; load onto flow cell; bridge amplification generates clonal clusters; sequence by synthesis (reversible dye-terminator nucleotides incorporated one at a time; imaged after each cycle; dye and 3'-block cleaved; next cycle); read fluorescence colour at each position for all clusters simultaneously.
8. Field-deployable (USB device; no laboratory needed; used in remote outbreak settings in Nigeria). Long reads (tens of kilobases; resolves complex genomic regions and structural variants that 150 bp Illumina reads cannot span).
9. Sanger: 600 to 1,000 bp read; 1 read per run; 99.9 percent accuracy; low cost per sample for small numbers; gold standard for single gene. NGS (Illumina): 150 bp paired-end; billions of reads per run; 0.1 to 0.3 percent raw error; very low cost per base for large-scale projects; cannot resolve long repeats.
10. SARS-CoV-2 whole-genome sequencing (NCDC and partner institutions; Illumina MiSeq; for variant surveillance). Lassa fever virus genome sequencing (ACEGID, Redeemer's University; ONT MinION; for outbreak investigation and phylogenetics).

Part 4.2

lanetonline.com

11. De novo assembly: assembles genome without reference; uses overlap algorithms (SPAdes for short reads; Flye for long reads); used for new species or when reference is absent or too divergent. Reference-guided assembly: maps reads to existing reference genome; faster and more accurate when a close reference is available; used for variant calling in clinical or population genomics studies.
12. E-value = expected number of alignments with that score or better by chance in the database. Below 10^-3 (0.001) is generally significant. Below 10^-10 indicates strong similarity with high confidence that the match is biologically meaningful rather than chance.
13. Extract total RNA; select poly-A mRNA (oligo-dT beads) or deplete rRNA; fragment RNA; convert to cDNA by reverse transcription; ligate sequencing adapters; PCR amplify; NGS (Illumina 150 bp paired-end); map reads to reference genome (HISAT2 or STAR); count reads per gene (HTSeq or featureCounts); normalise; differential expression analysis (DESeq2 or edgeR) identifies significantly up- or down-regulated genes between conditions.
14. Metagenomics: sequencing DNA extracted directly from an environmental sample without culturing. Shotgun metagenomics: sequences all DNA in sample; provides both taxonomic composition and functional gene information. 16S rRNA amplicon sequencing: amplifies and sequences only the bacterial 16S rRNA gene V3-V4 region; cheaper; identifies bacterial community composition but not function.
15. NCBI GenBank (nucleotide sequences; genome records; gene sequences from all organisms). UniProt (protein sequences with functional annotation; reviewed Swiss-Prot and unreviewed TrEMBL sections; uniprot.org).

Part 4.3

1. Sticky ends (4 to 6 nucleotide overhangs) form hydrogen bonds with complementary overhangs on the insert, increasing the probability that insert and vector stay paired long enough for T4 ligase to seal both nicks. Blunt end ligation requires no hydrogen bonding to hold ends together; the two ends must be perfectly flush; ligation efficiency is 10 to 100 times lower and requires higher enzyme concentration or PEG additives.

lanetonline.com

2. Origin of replication (ori): autonomous plasmid replication in host; maintains plasmid through cell divisions. Antibiotic resistance gene (e.g. AmpR): selects only transformed cells on antibiotic plates; untransformed cells die. Multiple cloning site (MCS): restriction enzyme recognition sites; allows insertion of foreign DNA by restriction enzyme cloning into a convenient non-essential region.
3. Mix ligation product with ice-cold chemically competent E. coli cells; incubate on ice 20 to 30 minutes; heat shock at 42 degrees C for 30 to 45 seconds (brief heat pulse opens transient membrane channels/pores allowing DNA entry); return to ice for 2 minutes; add SOC medium; incubate 37 degrees C 45 to 60 minutes (recovery; allows AmpR gene expression); spread on LB + ampicillin plates; incubate overnight at 37 degrees C.
4. pUC19 MCS is inserted within the lacZ-alpha gene encoding the alpha fragment of beta-galactosidase. On IPTG + X-Gal plates: intact lacZ-alpha (empty vector) produces alpha fragment; complementation with host omega fragment gives functional beta-galactosidase; X-Gal is cleaved to insoluble blue product; colony is blue. Disrupted lacZ-alpha (insert in MCS): no functional alpha; no beta-galactosidase activity; X-Gal not cleaved; colony is white. After cloning: most white colonies contain insert; blue colonies are empty vector re-ligations.
5. Pick a small amount from each colony with a sterile toothpick or pipette tip; add directly to a 25 microlitres PCR reaction containing primers flanking the MCS (vector-based M13 Forward/M13 Reverse primers) or spanning insert-vector junction; run standard PCR; load on agarose gel alongside expected size markers; recombinant clones give a band at the expected insert + MCS size; empty vector gives a smaller band (MCS only) or no band.

Part 4.4

1. Gene of interest cloned into pET vector downstream of T7 promoter. Host: E. coli BL21(DE3) carries T7 RNA polymerase gene integrated into chromosome under lac promoter control. IPTG is added to growing culture; IPTG binds lac repressor, de-repressing lac promoter; T7 RNA polymerase is expressed; T7 RNA polymerase binds T7 promoter in pET vector (E. coli RNA polymerase cannot); high-level transcription of cloned gene; protein accumulates to 10 to 30 percent total cell protein.

lanetonline.com

2. Inclusion body formation: reduce induction temperature to 16 to 25 degrees C and/or reduce IPTG concentration; co-express chaperones GroEL/GroES. Codon bias: synthesise codon-optimised gene (using IDT or Twist Bioscience codon optimisation tools) or use Rosetta E. coli strain containing plasmid with rare codon tRNAs. Lack of glycosylation: switch to eukaryotic expression system (Pichia pastoris for N-glycosylation; CHO for complex human-like glycans).
3. E. coli cannot glycosylate proteins; therapeutic mAbs require complex N-glycans for Fc receptor binding (effector function; ADCC), serum half-life (FcRn recycling), and to avoid immunogenicity in patients. CHO cells perform complex N-glycosylation closely resembling human patterns, produce correctly folded disulphide-linked IgG molecules, and are the FDA-validated cell line for biologic drug manufacture.
4. Fragments to be joined are designed with 20 to 40 bp overlapping sequences at their junctions (added by PCR with overhanging primers or by synthetic gene design). All fragments are mixed with Gibson assembly enzyme mix (T5 exonuclease; Phusion polymerase; Taq ligase). T5 exonuclease degrades 5' ends creating 3' single-stranded overhangs; complementary overhangs anneal; Phusion fills gaps; Taq ligase seals nicks; incubate 50 degrees C for 15 to 60 minutes. Advantages: seamless (no restriction site scars in final construct); joins 2 to 10 fragments in one reaction.
5. Design a gRNA targeting a sequence near the desired insertion site. Clone gRNA sequence into Cas9 expression vector (pX459 or similar). Design donor DNA template with: left homology arm (500 to 1,000 bp matching sequence immediately left of cut site); desired insert sequence; right homology arm (500 to 1,000 bp matching sequence immediately right of cut site). Co-transfect gRNA/Cas9 vector + donor template into target cells. Cas9 makes a double-strand break at the target site; HDR pathway uses the donor template to repair the break, incorporating the insert. Screen colonies by PCR with primers flanking the insertion site; confirm by Sanger sequencing.

References and Attributions [Series 4]

Textual References (Books and External Sources)

1. Sambrook, J., & Russell, D. W. (2001). Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor Laboratory Press.

lanetonline.com

2. Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences USA, 74(12), 5463–5467.
3. Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., & Smith, H. O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. Nature Methods, 6(5), 343–345.
4. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410.

Figures, Plates, Tables, and Boxes

5. Figure 4.1: Comparison of Sanger sequencing and Illumina NGS workflows. Attribution: AI generated. Suggested OER search string: “Sanger sequencing NGS comparison workflow flowchart Illumina OER”.
6. Box 4.1: Flowchart of a standard molecular cloning procedure. Attribution: AI generated. Suggested OER search string: “molecular cloning procedure flowchart restriction enzyme ligation transformation blue white selection OER”.

lanetonline.com

Course code: BIO 408

Course title: Applied Biotechnology

Course credit load: 2 Units

Series 5: Applications of Biotechnology

- **Part 5.1: Agricultural Biotechnology**
  - Sub Part 5.1.1: Transgenic crop development and genetically modified organisms (GMOs)
  - Sub Part 5.1.2: Molecular breeding and crop improvement in Nigeria
  - Sub Part 5.1.3: Microbial biotechnology in agriculture
- **Part 5.2: Medical and Pharmaceutical Biotechnology**
  - Sub Part 5.2.1: Recombinant vaccines and diagnostics
  - Sub Part 5.2.2: Monoclonal antibodies and biopharmaceuticals
  - Sub Part 5.2.3: Pharmacogenomics and personalised medicine
- **Part 5.3: Industrial and Environmental Biotechnology**
  - Sub Part 5.3.1: Industrial enzymes and fermentation biotechnology
  - Sub Part 5.3.2: Biofuels and bioenergy
  - Sub Part 5.3.3: Bioremediation and environmental monitoring
- **Part 5.4: Biosafety, Ethics, and Regulatory Frameworks**
  - Sub Part 5.4.1: Biosafety principles and laboratory safety levels
  - Sub Part 5.4.2: Ethical issues in biotechnology

lanetonline.com

- Sub Part 5.4.3: Nigerian and international regulatory frameworks for biotechnology

Introduction

The preceding four series of this course have built the technical toolkit of applied biotechnology: extraction, quantification, chromatography, electrophoresis, PCR, sequencing, and cloning. This final Series surveys how these tools are deployed in four major application domains: agriculture (transgenic crops, molecular breeding, microbial inoculants); medicine and pharmaceuticals (recombinant vaccines, diagnostics, monoclonal antibodies, pharmacogenomics); industry and the environment (fermentation, industrial enzymes, biofuels, bioremediation); and the biosafety, ethical, and regulatory context within which all biotechnology is practised.

Nigeria is engaged with all these application areas, from the approval and cultivation of Bt cowpea to the expansion of molecular diagnostics for infectious diseases, the growth of the fermentation industry, and the development of a national biosafety framework. This Series provides both the scientific basis of each application and the specific Nigerian context, including the institutions, regulations, and challenges relevant to practising biotechnologists in Nigeria.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 5.1** describe the development of transgenic crops, give Nigerian examples, and explain the role of molecular breeding tools in crop improvement (Linked to Part 5.1),
- **SLO 5.2** describe the applications of microbial biotechnology in Nigerian agriculture (Linked to Part 5.1),
- **SLO 5.3** describe recombinant vaccines, diagnostics, monoclonal antibodies, and pharmacogenomics and their relevance to Nigerian healthcare (Linked to Part 5.2),

lanetonline.com

- **SLO 5.4** describe industrial biotechnology applications including fermentation, industrial enzymes, biofuels, and bioremediation (Linked to Part 5.3),
- **SLO 5.5** explain the biosafety levels used in containment of biological hazards (Linked to Part 5.4), and
- **SLO 5.6** describe the ethical issues in biotechnology and the regulatory frameworks governing biotechnology in Nigeria and internationally (Linked to Part 5.4).

5.1 Agricultural Biotechnology

5.1.1 Transgenic crop development and genetically modified organisms (GMOs)

Transgenic crops (genetically modified organisms, GMOs) are plants into which one or more genes from another organism have been stably introduced using recombinant DNA technology. The principal methods of plant transformation are: Agrobacterium tumefaciens-mediated transformation (most common for dicots including cassava, cowpea, soybean, and tobacco; Agrobacterium naturally transfers a segment of its Ti plasmid T-DNA into plant cells; the gene of interest is cloned between T-DNA border sequences; Agrobacterium co-cultivated with plant explants; transgenic calli or shoots selected on hygromycin or kanamycin medium; regenerated plants confirmed by PCR and Southern blot); and biolistic transformation (gene gun; gold or tungsten microprojectiles coated with DNA are accelerated into plant tissue at high velocity; used for monocots like maize, rice, wheat, and banana that are recalcitrant to Agrobacterium).

The first commercialised transgenic crop was the Flavr Savr tomato (Caltech; 1994; delayed ripening by antisense polygalacturonase). Major global transgenic traits: herbicide tolerance (Roundup Ready soybean, maize, cotton; Monsanto; 35S promoter driving CP4 EPSPS gene from Agrobacterium cp4; confers tolerance to glyphosate herbicide); Bt insect resistance (Bt maize, cotton, cowpea; expressing Bacillus thuringiensis Cry proteins toxic to Lepidoptera or Coleoptera larvae); virus resistance (papaya ringspot virus-resistant papaya; Hawaii; 1998; coat protein-mediated resistance); Golden Rice (beta-carotene in endosperm; two transgenes from daffodil and maize; addresses vitamin A deficiency). In Nigeria: Bt cowpea (pod borer resistance; Cry1Ab; approved by NBMA and NAFDAC in 2021; developed by IITA and IAR in

lanetonline.com

collaboration with AATF; first biotech crop cultivated by Nigerian smallholders); Bt cotton is under regulatory evaluation by the NBMA.

Fill in the blank: Bt cowpea, the first biotech crop approved for cultivation by Nigerian smallholders, expresses the _____ (Cry1Ab) protein from Bacillus thuringiensis; it was approved by the National Biosafety Management Agency (NBMA) and NAFDAC in 2021 after multi-season confined field trials.

5.1.2 Molecular breeding and crop improvement in Nigeria

Molecular breeding uses DNA markers and genomic information to accelerate conventional plant breeding without introducing transgenes. The key tools are: marker-assisted selection (MAS; using SSR or SNP markers linked to resistance genes or QTLs to select genotypes without phenotypic screening; described in BIO402 Series 4); genomic selection (GS; using genome-wide SNP arrays to predict breeding values; IITA uses GS for cassava yield, dry matter, and disease resistance); marker-assisted backcrossing (MABC; introgressing specific genes into elite backgrounds using flanking markers for foreground and background selection; IITA uses MABC to introduce CMD2 and CBSD resistance into high-yielding West African cassava varieties). KASP (kompetitive allele-specific PCR) genotyping platforms (LGC Biosearch) are widely used at IITA and partner institutions for cost-effective high-throughput MAS on thousands of breeding lines per season.

QTL (quantitative trait locus) mapping identifies genomic regions associated with quantitative traits by analysing correlations between marker genotypes and phenotypes in segregating populations (F2, BC, RIL populations). GWAS (genome-wide association study) uses linkage disequilibrium in diverse germplasm panels to map trait-associated SNPs at higher resolution than QTL mapping. IITA has used GWAS to identify SNPs associated with cassava mosaic disease resistance, provitamin A content, and starch quality; IAR has used QTL mapping for groundnut late leaf spot resistance and yield components. Doubled haploid (DH) technology uses anther culture or microspore culture to produce completely homozygous (doubled haploid) plants from F1 hybrids in one generation, eliminating the 6 to 8 generations of selfing normally needed for inbred line development; used for maize line development at IITA and NCRI.

lanetonline.com

Fill in the blank: _____ (genome-wide association study) uses linkage disequilibrium in a diverse panel of germplasm accessions to associate SNP marker genotypes with phenotypic traits at high resolution; it has been used at IITA to identify SNPs associated with cassava mosaic disease resistance and provitamin A content.

5.1.3 Microbial biotechnology in agriculture

Microbial biotechnology contributes to Nigerian agriculture through: biofertilisers (microbial inoculants applied to seeds or soil to improve nutrient availability); biological nitrogen fixation (BNF; Rhizobium, Bradyrhizobium, Mesorhizobium inoculation of legume seeds; Bradyrhizobium japonicum inoculants for soybean increase grain yield by 300 to 700 kg per ha in nitrogen-deficient soils of the Guinea savanna; used in IITA-promoted soybean packages for Nigerian farmers; Rhizobium inoculants for cowpea are produced at IITA Ibadan); phosphate-solubilising bacteria (Bacillus megaterium, Pseudomonas fluorescens; solubilise inorganic phosphate for plant uptake; relevant for acid soils of southern Nigeria); mycorrhizal fungi (arbuscular mycorrhiza form symbiotic associations with over 80 percent of crop plant roots; enhance P and Zn uptake; improve drought tolerance; commercial AM inoculants are used in tree crop nurseries).

Biopesticides are microbial or microbially derived agents used for pest and disease control: Bacillus thuringiensis (Bt) spray formulations (DiPel; Thuricide) are used against caterpillars in vegetable and maize production; B. subtilis and Trichoderma harzianum-based fungicides are applied as seed treatments or soil drenches against Fusarium, Pythium, and Sclerotium root rots in cowpea and groundnut; the entomopathogenic fungus Metarhizium anisopliae controls cassava green mite and grasshoppers. Biocontrol agents for post-harvest mycotoxin management: the competitive exclusion biocontrol product Aflasafe (IITA; contains atoxigenic Aspergillus flavus strains that colonise maize and groundnut ahead of toxigenic strains, reducing aflatoxin contamination by 80 to 99 percent) is registered and in use in Nigeria, Kenya, Tanzania, Senegal, and Burkina Faso. IITA manufactures Aflasafe in Nigeria and distributes through the Aflasafe Technology Transfer and Commercialisation (ATTC) project.

lanetonline.com

Fill in the blank: _____ is an IITA-developed biocontrol product containing atoxigenic strains of Aspergillus flavus that competitively exclude toxigenic strains from colonising maize and groundnut; it reduces aflatoxin contamination by 80 to 99 percent and is registered for use in Nigeria.

Pilot questions 5.1

- Describe how Agrobacterium tumefaciens is used to produce a transgenic plant and state which crops it is most suitable for.
- Define marker-assisted selection (MAS) and state two crops in Nigeria where it is used and the traits targeted.
- Explain the principle and agricultural benefits of Bradyrhizobium inoculation in Nigerian soybean production.
- Describe how Aflasafe works to control aflatoxin contamination in Nigerian crops.
- Distinguish between a transgenic crop (GMO) and a crop developed by molecular breeding and give one Nigerian example of each.

5.2 Medical and Pharmaceutical Biotechnology

5.2.1 Recombinant vaccines and diagnostics

Recombinant vaccines use one or more antigens produced by recombinant DNA technology rather than whole killed or attenuated pathogens, improving safety and enabling large-scale production. Types of recombinant vaccines: subunit vaccines (purified recombinant protein antigen; e.g. hepatitis B vaccine: recombinant HBsAg produced in Saccharomyces cerevisiae or CHO cells; meningococcal vaccines; HPV vaccines: VLPs of L1 capsid protein produced in yeast or insect cells; widely used in Nigerian EPI schedule); viral vector vaccines (gene encoding the antigen cloned into a replication-incompetent viral vector such as adenovirus; e.g. Johnson & Johnson Janssen COVID-19 vaccine: adenovirus 26 vector expressing SARS-CoV-2 spike protein; Oxford-AstraZeneca ChAdOx1: chimpanzee adenovirus vector; widely deployed in Nigeria 2021 to 2022); mRNA vaccines (synthetic mRNA encoding antigen in lipid nanoparticle; Pfizer-BioNTech; Moderna; self-amplifying mRNA vaccines under development).

lanetonline.com

Recombinant diagnostics: recombinant antigens produced in E. coli or yeast are used as capture antigens in ELISA (enzyme-linked immunosorbent assay) and rapid diagnostic tests (RDTs) for: HIV (HIV-1 gp41 and gp120/gp160; HIV-2 gp36; used in anti-HIV ELISA and rapid antibody tests); hepatitis B (HBsAg detection ELISA; anti-HBs antibody ELISA); malaria (Plasmodium falciparum HRP-2 antigen; used in PfHRP2 rapid malaria RDTs; widely used at primary healthcare level across Nigeria); tuberculosis (ESAT-6 and CFP-10 antigens; interferon-gamma release assays, IGRAs). Lateral flow immunoassay (LFA; rapid test strip): colloidal gold-labelled antibody detects antigen in sample; migrates to test line where immobilised capture antibody retains gold-antibody-antigen complexes; visible coloured line. NAFDAC regulates diagnostic kits imported or manufactured in Nigeria; the NCDC coordinates national diagnostic laboratory network for infectious disease surveillance.

Fill in the blank: The hepatitis B vaccine is a _____ vaccine produced by expressing recombinant HBsAg (hepatitis B surface antigen) in yeast; it is included in Nigeria's Expanded Programme on Immunisation (EPI) and has dramatically reduced new infections in vaccinated cohorts.

5.2.2 Monoclonal antibodies and biopharmaceuticals

Monoclonal antibodies (mAbs) are monospecific immunoglobulins produced by a single B-cell clone, all binding the same epitope with identical affinity. They are produced by the hybridoma technology (Kohler and Milstein, 1975; Nobel Prize 1984): an immunised mouse spleen cell (producing antibody of desired specificity) is fused with a myeloma cell (immortal tumour cell) using polyethylene glycol; hybridoma cells are selected in HAT medium (hypoxanthine, aminopterin, thymidine; kills unfused myeloma cells; spleen cells die naturally); individual hybridoma clones are screened by ELISA for the desired antibody; positive clones are expanded; mAb is purified by protein A or protein G affinity chromatography. Recombinant mAbs for therapeutic use are now produced in CHO cells from cloned antibody genes rather than hybridomas.

mAb applications in medicine: cancer therapy (rituximab: anti-CD20; for B-cell non-Hodgkin lymphoma; trastuzumab: anti-HER2; for breast cancer; bevacizumab: anti-VEGF; for colorectal

lanetonline.com

and lung cancer); autoimmune disease (adalimumab: anti-TNF-alpha; for rheumatoid arthritis; psoriasis); infectious disease (palivizumab: anti-RSV F protein; for RSV prophylaxis in premature infants; COVID-19: sotrovimab; casirivimab + imdevimab). Biopharmaceuticals include recombinant hormones (insulin produced in E. coli; growth hormone; EPO for anaemia); coagulation factors (Factor VIII and IX for haemophilia; produced in CHO cells or BHK cells); tissue plasminogen activator (tPA; for stroke treatment; CHO cells). In Nigeria, most biopharmaceuticals are imported; domestic production capacity is limited; the National Institute for Pharmaceutical Research and Development (NIPRD) in Abuja is developing local production of selected recombinant vaccines and diagnostics.

Fill in the blank: Monoclonal antibodies for therapeutic use are produced in _____ (Chinese Hamster Ovary) cells, which perform the complex N-glycosylation required for Fc effector function and long serum half-life via FcRn recycling.

5.2.3 Pharmacogenomics and personalised medicine

Pharmacogenomics is the study of how genetic variation affects individual responses to drugs, enabling prediction of drug efficacy and adverse reactions based on a patient's genotype. Key examples: CYP2D6 and CYP2C19 (cytochrome P450 enzymes; metabolise codeine, antidepressants, antipsychotics, antimalarials; polymorphic: poor metabolisers (PM), extensive metabolisers (EM), ultrarapid metabolisers (UM) have very different plasma drug levels from the same dose; CYP2D6 PM patients given codeine may experience opioid toxicity; CYP2D6 UM patients may not respond to codeine); TPMT (thiopurine methyltransferase; metabolises azathioprine and 6-mercaptopurine; deficient patients accumulate toxic metabolites; dose reduction required); G6PD deficiency (X-linked; common in Nigeria: approximately 20 percent of Nigerian males carry a deficient allele; primaquine, dapsone, nitrofurantoin trigger haemolytic anaemia in G6PD-deficient individuals; testing before prescription is recommended).

Warfarin dosing is influenced by polymorphisms in VKORC1 (target enzyme) and CYP2C9 (metabolising enzyme); African populations carry different VKORC1 and CYP2C9 variant frequencies than European populations, requiring African-specific dosing algorithms. The H3Africa (Human Heredity and Health in Africa) Consortium, which includes Nigerian partner

lanetonline.com

institutions (University of Ibadan, ABU Zaria, LUTH, UCH), is building genome-wide association study (GWAS) data for African populations to identify genetic risk factors for hypertension, type 2 diabetes, kidney disease, and stroke prevalent in Nigeria; this data will eventually inform pharmacogenomic decision-making for Nigerian patients. Point-of-care pharmacogenomic testing (e.g. Spartan RX CYP2C19 system for clopidogrel dosing in cardiac patients) is beginning to be deployed in tertiary hospitals in Lagos and Abuja.

Fill in the blank: _____ deficiency, which affects approximately 20 percent of Nigerian males, can cause haemolytic anaemia when affected individuals are given primaquine or other oxidative drugs; testing for G6PD status before prescribing these drugs is therefore clinically important in Nigeria.

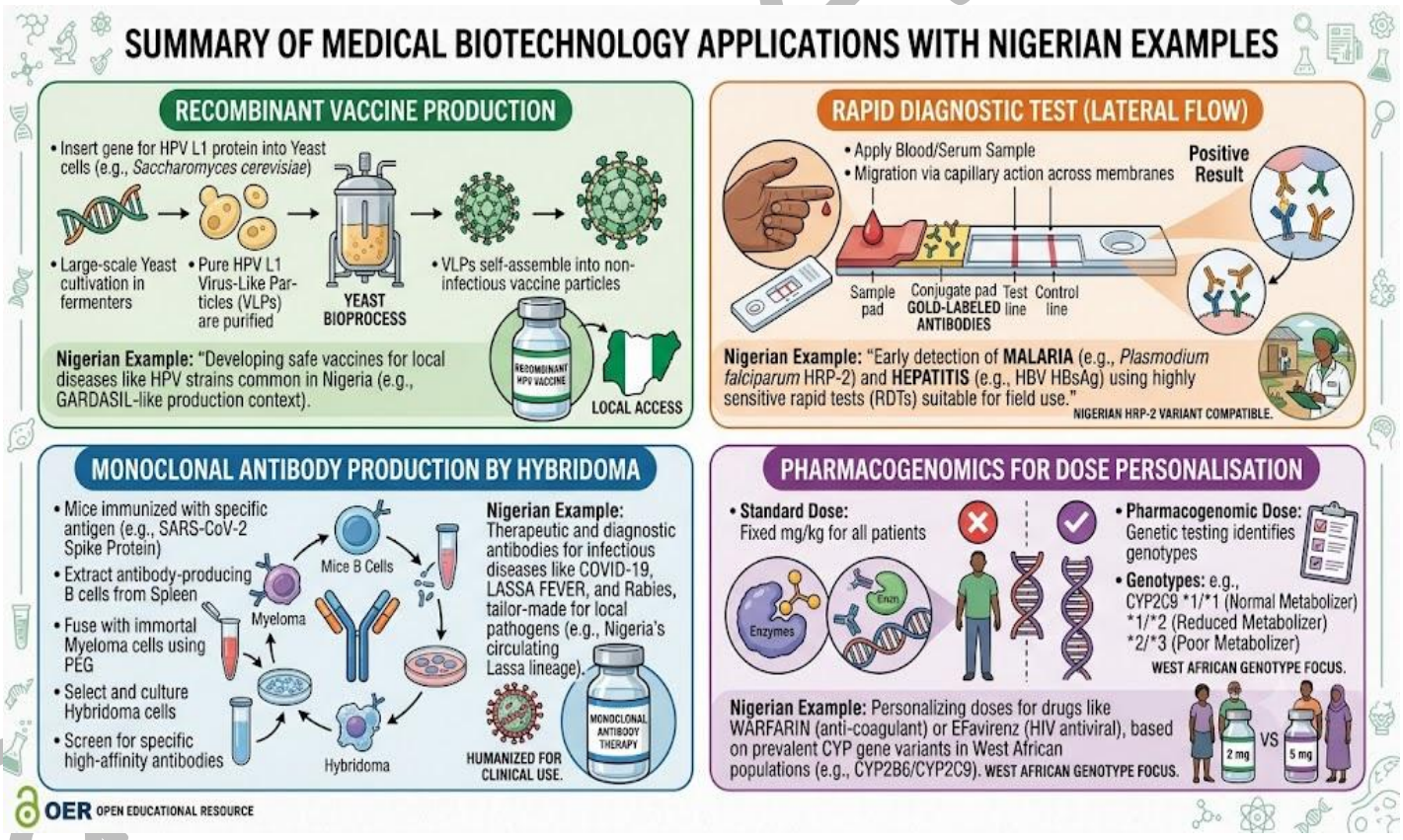


Figure 5.1: Four applications of medical biotechnology with Nigerian examples

Pilot questions 5.2

- Distinguish between a subunit vaccine and a viral vector vaccine and give one Nigerian example of each.
- Describe hybridoma technology and explain how monoclonal antibodies are produced.
- Explain how G6PD deficiency and CYP2D6 polymorphism illustrate the importance of pharmacogenomics in Nigerian clinical practice.
- Describe how a lateral flow rapid diagnostic test (RDT) detects a pathogen antigen in a patient sample.
- State two biopharmaceuticals produced by recombinant DNA technology, name the expression host for each, and state the disease each treats.

5.3 Industrial and Environmental Biotechnology

5.3.1 Industrial enzymes and fermentation biotechnology

Industrial biotechnology uses microorganisms and enzymes to produce chemicals, materials, and fuels from biological raw materials. Fermentation is the controlled growth of microorganisms in a bioreactor to produce a desired product. Submerged liquid fermentation (SmF) in stirred-tank bioreactors (CSTR) is the standard mode for antibiotic, amino acid, and enzyme production; solid-state fermentation (SSF) grows fungi on solid substrate (rice bran, cassava peel, corn cobs) and is increasingly used for enzyme production, biofuel pre-treatment, and production of fermented Nigerian foods. In Nigeria, the fermented food industry (dawadawa: Parkia biglobosa fermented with Bacillus subtilis; ogi/akamu: maize fermented by Lactobacillus; fufu/garri: cassava fermented by Lactobacillus and Leuconostoc; iru: oil bean fermented with Bacillus; burukutu/pito: sorghum or maize beer fermented with Saccharomyces cerevisiae and lactic acid bacteria) represents a significant biotechnological activity traditionally practised at household and artisanal scale.

Industrial enzymes are produced by microbial fermentation: amylases (alpha and glucoamylase; Aspergillus niger, Bacillus licheniformis; used in starch hydrolysis for glucose syrup, beer

lanetonline.com

brewing, and cassava starch processing in Nigeria); cellulases (Trichoderma reesei; used for cellulose degradation in biofuel production and textile processing); proteases (subtilisin from Bacillus subtilis; used in detergent formulations, leather processing, and meat tenderising); lipases (Candida rugosa; used in food flavour development, biodiesel production, and pharmaceutical synthesis); pectinases (Aspergillus niger; used in fruit juice clarification in the Nigerian beverage industry); and xylanases (used in animal feed and pulp bleaching). The International Institute of Tropical Agriculture (IITA) and Obafemi Awolowo University (OAU) Ile-Ife have conducted research on production of industrial enzymes (pectinases, cellulases) from cassava peel and other Nigerian agricultural wastes using SSF.

Fill in the blank: _____ fermentation (SSF) grows fungi on solid agricultural substrates such as cassava peel or corn cobs and is used in Nigeria for production of industrial enzymes (cellulases, pectinases) from low-cost agroindustrial waste materials.

5.3.2 Biofuels and bioenergy

Biofuels are transportation fuels produced from biological materials (biomass). First-generation biofuels use food crop sugars or starch: bioethanol from sugarcane (Brazil; largest producer), maize (USA), or cassava starch (Nigeria has explored cassava-to-ethanol using Saccharomyces cerevisiae fermentation after enzymatic saccharification with glucoamylase). Second-generation biofuels use lignocellulosic biomass (agricultural residues: maize stover, rice straw, cassava stems, sugar cane bagasse): pretreatment (dilute acid; steam explosion; alkali) disrupts the lignin-hemicellulose-cellulose matrix; enzymatic saccharification with cellulase and xylanase converts cellulose and hemicellulose to fermentable sugars; S. cerevisiae or xylose-fermenting yeasts (Pichia stipitis) ferment sugars to ethanol. Biodiesel is produced by transesterification of vegetable oils (palm oil; jatropha; soybean) or waste cooking oil with methanol using an alkali or lipase catalyst; Nigerian palm oil (Elaeis guineensis; Nigeria is the world's fourth-largest producer) is a potential biodiesel feedstock.

Biogas (methane) is produced by anaerobic digestion of organic waste: organic material (animal manure, market waste, municipal solid waste, cassava effluent) is digested by a mixed microbial community in an anaerobic digester; acetoclastic methanogens (Methanosaeta, Methanosarcina)

lanetonline.com

convert acetate to CH4 and CO2; biogas (55 to 65 percent CH4) is used for cooking, lighting, and electricity generation. Small-scale biogas digesters are deployed in rural communities across Nigeria through FMARD (Federal Ministry of Agriculture and Rural Development) programmes and NGOs; large-scale biogas plants treating cassava processing waste (starch factory effluent containing high COD of 50,000 to 100,000 mg per litre) have been installed at cassava starch factories in Ogun and Oyo states. Algal biofuels (biodiesel from microalgae lipids; hydrogen from photosynthetic algae) are at research stage globally; University of Lagos and FUTA have pilot-scale microalgae cultivation systems for biofuel research.

Fill in the blank: Biogas is produced by _____ digestion of organic waste; methanogenic archaea convert acetate and H2 to methane (CH4); small-scale biogas digesters in rural Nigeria use livestock manure and organic waste to produce fuel for cooking and lighting.

5.3.3 Bioremediation and environmental monitoring

Bioremediation uses living organisms (primarily microorganisms, but also plants: phytoremediation) to degrade or immobilise environmental contaminants. In situ bioremediation treats contamination at the site without excavation; ex situ involves removing contaminated material for treatment elsewhere. Key mechanisms: aerobic biodegradation (bacteria and fungi use organic pollutants as carbon and energy sources; effective for petroleum hydrocarbons, BTEX, PAHs); anaerobic biodegradation (reductive dechlorination of chlorinated solvents by Dehalococcoides; methanogenic degradation of aromatic compounds); bioaugmentation (addition of specific degrading microorganisms to contaminated site); biostimulation (addition of nutrients or electron donors/acceptors to stimulate indigenous microbial activity).

Nigeria has severe contamination of the Niger Delta from decades of oil spills (estimated 9 to 13 million barrels spilled between 1958 and 2008; UNEP Ogoniland assessment 2011 documented hydrocarbon pollution of soil and groundwater); bioremediation is a key component of Niger Delta cleanup alongside mechanical soil removal and phytoremediation. Rhamnolipid-producing Pseudomonas aeruginosa and alkane-degrading Alcanivorax, Marinobacter, and Rhodococcus erythropolis strains isolated from Niger Delta soil have been characterised for bioremediation potential at University of Port Harcourt, UNILAG, and Covenant University. Phytoremediation:

lanetonline.com

Vetiver grass (Vetiveria zizanioides) and sunflower (Helianthus annuus) accumulate heavy metals in shoots; Chromolaena odorata and Mucuna pruriens are used for petroleum hydrocarbon-contaminated soil remediation in Nigerian studies. Environmental monitoring uses PCR-based methods (16S rRNA metagenomics) to assess microbial community shifts as indicators of contamination level and remediation progress.

Fill in the blank: _____ is the use of living microorganisms to degrade environmental contaminants; in the Niger Delta, hydrocarbon-degrading bacteria such as Alcanivorax and Rhodococcus erythropolis are used to break down crude oil components in contaminated soil and water.

Application	**Organism / Product**	**Substrate / Process**	**Nigerian Context**
Fermented foods	Lactic acid bacteria, yeasts	Cassava (for garri, fufu), sorghum, millet	Staple foods like garri, ogi, and palm wine rely on traditional fermentation
Industrial enzymes	Amylases, proteases, cellulases	Produced by fungi (*Aspergillus*, *Trichoderma*) or bacteria	Used in brewing, textiles, leather, and detergent industries in Nigeria
Bioethanol	Yeast (*Saccharomyces cerevisiae*)	Fermentation of sugarcane, cassava, maize	Nigeria explores cassava-based ethanol as renewable fuel and export commodity
Biogas	Methanogenic archaea	Anaerobic digestion of organic waste, manure	Rural communities and farms use biogas for cooking and electricity generation

lanetonline.com

Bioremediation	Hydrocarbon-degrading bacteria (*Pseudomonas*, *Bacillus*)	Breakdown of oil spills and industrial pollutants	Critical for Niger Delta oil spill cleanup and environmental restoration

Box 5.1: Industrial and environmental biotechnology applications in the Nigerian context

Pilot questions 5.3

1. Name four traditionally fermented Nigerian foods and state the principal microorganism responsible for each fermentation.
2. Describe the steps required to produce bioethanol from cassava using second-generation technology.
3. Explain the difference between biostimulation and bioaugmentation in bioremediation.
4. Describe the environmental biotechnology challenge in the Niger Delta and name two microorganisms used in bioremediation studies.
5. Name three industrial enzymes produced by microbial fermentation, state the producing microorganism for each, and give one industrial application of each in Nigeria.

5.4 Biosafety, Ethics, and Regulatory Frameworks

5.4.1 Biosafety principles and laboratory safety levels

Biosafety refers to the practices, procedures, and physical containment measures that prevent unintentional exposure to biological agents or their accidental release from the laboratory. The WHO/CDC biosafety level (BSL) classification defines four containment levels based on the hazard of the agents worked with. BSL-1: agents not known to cause disease in healthy adults (E. coli K-12; non-pathogenic Saccharomyces cerevisiae; standard teaching laboratory); open bench work; gloves and lab coat; hand washing. BSL-2: agents causing moderate disease; transmitted by ingestion or mucous membrane contact (Staphylococcus aureus; Salmonella; hepatitis B and C; HIV; SARS-CoV-2 for routine diagnostic work); limited access; biological safety cabinet

lanetonline.com

(BSC Class II) for aerosol-generating procedures; face protection; autoclave for decontamination.

BSL-3: agents that may cause serious or lethal disease transmitted by inhalation; treatment available (Mycobacterium tuberculosis; Coxiella burnetii; yellow fever virus; West Nile virus; monkeypox virus; Lassa fever virus in some protocols); negative pressure laboratory; HEPA-filtered exhaust air; self-closing double-door entry; respirator (N95 or PAPR); all procedures in BSC Class II or III. BSL-4: agents causing lethal disease for which no treatment or vaccine exists (Ebola; Marburg; Lassa fever at higher concentrations; smallpox; Crimean-Congo haemorrhagic fever); maximum containment; positive pressure suits or Class III BSC; dedicated air handling; shower out; autoclave pass-through. In Nigeria, the NCDC National Reference Laboratory and IHVN have BSL-2 and BSL-3 certified facilities; Lassa fever virus work is conducted at BSL-3 or BSL-3+ facilities under strict protocols. No BSL-4 facility currently exists in Nigeria; samples of the most dangerous agents are referred to international collaborating laboratories.

Fill in the blank: BSL-_____ laboratories are required for working with agents such as Mycobacterium tuberculosis and Lassa fever virus that may cause serious disease transmitted by inhalation; they require negative pressure, HEPA filtration, double-door entry, and respirator use.

5.4.2 Ethical issues in biotechnology

Biotechnology raises ethical issues across several domains. GMO ethics: concerns include potential unintended effects on non-target organisms (e.g. Bt protein toxicity to Monarch butterfly larvae; extensively studied; risk assessed as low at field densities); gene flow from transgenic to wild relatives (e.g. Bt cotton pollen potentially crossing to wild Gossypium species; managed by refugia); monopolisation of seed supply by large corporations limiting farmer choice and autonomy (e.g. terminator technology: genetic use restriction technology; never commercialised but raised global concern); labelling and consumer right to choose (the EU requires labelling of foods containing above 0.9 percent GMO; Nigeria requires NAFDAC approval but GMO food labelling regulations are still being developed under NBMA guidance). Human genetics ethics: concerns in genetic testing (access to results; insurance and employment

lanetonline.com

discrimination based on genetic data; paternity testing implications; genetic determinism); gene therapy (somatic vs germline editing; heritable genome modifications raise concerns about designer babies and eugenics; CRISPR germline editing of human embryos by He Jiankui in 2018 was widely condemned as premature and unethical); biopiracy (the patenting of genetic resources from biodiversity-rich countries without benefit-sharing; the Nagoya Protocol under the Convention on Biological Diversity addresses access and benefit-sharing, ABS, for genetic resources; Nigeria ratified the Nagoya Protocol in 2016).

Animal welfare in biotechnology: use of laboratory animals in transgenic research and testing must comply with the 3Rs principle (Replace with in vitro or computational methods where possible; Reduce the number of animals used; Refine procedures to minimise suffering). NAFDAC and university ethics committees in Nigeria require ethical approval for animal experiments. Intellectual property (IP): patents on genes, processes, and transgenic organisms raise issues of access for developing-country researchers and breeders; the TRIPS Agreement (Trade-Related Intellectual Property Rights; WTO) requires member states including Nigeria to provide IP protection for biological inventions; however, public sector institutions (IITA; NCRI; IAR) and CGIAR centres publish most of their discoveries as open-access public goods to maximise access for smallholder farmers.

Fill in the blank: The _____ Protocol under the Convention on Biological Diversity governs access to genetic resources and requires that benefits arising from their use be shared equitably with the country of origin; Nigeria ratified this protocol in 2016 to protect its biological resources from biopiracy.

5.4.3 Nigerian and international regulatory frameworks for biotechnology

Nigeria's primary biotechnology regulatory instrument is the National Biosafety Management Agency (NBMA) Act (2015) and its associated regulations. NBMA is responsible for: risk assessment and approval of confined field trials, open release, and commercial cultivation of GM crops; import and transit of GMOs; inspection and monitoring of approved biotechnology activities; public participation in the biosafety decision-making process. The NBMA Act established the National Biosafety Committee (NBC) as a technical advisory body. NAFDAC

lanetonline.com

(National Agency for Food and Drug Administration and Control) regulates the safety of GM food and feed products under the NAFDAC Act; joint NBMA-NAFDAC evaluation is required for GM crops intended for human consumption.

International frameworks governing biotechnology include: the Cartagena Protocol on Biosafety (2000; supplementary to the Convention on Biological Diversity; governs transboundary movement of living modified organisms, LMOs; requires advance informed agreement, AIA, from the importing country before first transboundary movement of LMOs for intentional environmental release; Nigeria ratified in 2003); the Nagoya Protocol on Access and Benefit-Sharing (2010; governs access to genetic resources and traditional knowledge and equitable sharing of benefits; Nigeria ratified 2016); WHO biosafety guidelines (Laboratory Biosafety Manual, 4th edition, 2020; guidance on containment levels, protective equipment, and waste management). The Codex Alimentarius Commission (FAO/WHO) establishes international standards for safety assessment of GM foods; Nigerian food safety standards align with Codex. The African Union has developed the African Model Law on Biosafety as a framework for member states. Nigeria's NBMA has approved Bt cowpea (2021), confined trials of drought-tolerant maize (WEMA project: Water Efficient Maize for Africa; conventional and transgenic varieties), and is evaluating Bt cotton applications.

Fill in the blank: The _____ Protocol on Biosafety (2000) governs the transboundary movement of living modified organisms (LMOs) and requires the exporting country to obtain advance informed agreement (AIA) from the importing country before the first intentional release of an LMO into the environment.

Pilot questions 5.4

1. Describe the four biosafety levels (BSL-1 to BSL-4) and state one example organism and one containment requirement for each.
2. Explain two ethical concerns associated with GMO crop development and deployment in Nigeria.
3. Describe the role of NBMA in regulating biotechnology in Nigeria and state two crops it has evaluated or approved.

lanetonline.com

4. Explain what the Cartagena Protocol on Biosafety requires and state when Nigeria ratified it.
5. Explain the 3Rs principle in animal experimentation and state why it is important in Nigerian biotechnology research.

Series Summary

This Series covered the major applications of biotechnology in agriculture and medicine. Agricultural biotechnology includes the development of transgenic crops through *Agrobacterium*-mediated or biolistic transformation, with examples such as Bt cowpea approved in Nigeria in 2021, Bt cotton, herbicide-tolerant crops, and virus-resistant varieties. Molecular breeding uses tools such as SSR and KASP markers, marker-assisted selection, marker-assisted backcrossing, genomic selection, GWAS, and doubled-haploid technology in crop improvement programmes involving cassava, maize, and cowpea. Microbial biotechnology supports agriculture through *Bradyrhizobium* inoculants for soybean, phosphate-solubilising bacteria, Bt-based biopesticides, *Trichoderma* biocontrol, and Aflasafe, which uses non-toxigenic *Aspergillus flavus* strains to reduce aflatoxin contamination. Medical biotechnology includes recombinant vaccines, virus-like particles, viral-vector vaccines, recombinant antigens for ELISA and rapid diagnostic tests, monoclonal antibody production through hybridoma and CHO-cell systems, and pharmacogenomic studies involving genes such as CYP2D6 and G6PD, including population research under H3Africa.

Industrial biotechnology includes submerged and solid-state fermentation, the production of Nigerian foods such as dawadawa, fufu, ogi, and burukutu, and the manufacture of enzymes such as amylases, cellulases, and pectinases from *Aspergillus* and *Bacillus*. It also covers biofuel production from cassava starch, lignocellulosic materials, palm oil, cassava effluent, and animal manure. Bioremediation applies microorganisms such as *Pseudomonas*, *Alcanivorax*, and *Rhodococcus erythropolis*, as well as Vetiver grass, to clean oil-contaminated environments in the Niger Delta. Biosafety measures range from BSL-1 to BSL-4 containment, with specialised BSL-3 facilities used for dangerous pathogens such as Lassa fever virus. In Nigeria, GMO

lanetonline.com

activities are regulated by the NBMA Act of 2015, while the Cartagena and Nagoya Protocols guide the movement of living modified organisms and access to genetic resources. Ethical concerns include gene flow, corporate control, labelling, germline editing, biopiracy, animal welfare under the 3Rs, intellectual-property rights, and the need to balance commercial interests with public-good research.

Glossary of Terms

1. **Aflasafe:** an IITA biocontrol product containing atoxigenic strains of Aspergillus flavus that competitively exclude toxigenic aflatoxin-producing strains from colonising maize and groundnut; reduces aflatoxin contamination by 80 to 99 percent in Nigeria and other African countries.
2. **Anaerobic digestion:** the breakdown of organic matter by a consortium of microorganisms in the absence of oxygen, producing biogas (methane and CO2) and digestate; used in Nigeria for treatment of cassava processing waste and livestock manure.
3. **Bioaugmentation:** the addition of specific microbial strains or consortia to a contaminated site to enhance biodegradation of pollutants; used in oil-contaminated Niger Delta soils.
4. **Bioremediation:** the use of living organisms (bacteria, fungi, or plants) to degrade, transform, or immobilise environmental contaminants; key strategy for Niger Delta crude oil contamination.
5. **Biostimulation:** the addition of nutrients (nitrogen, phosphorus) or electron donors/acceptors to stimulate the activity of indigenous microbial communities at a contaminated site, enhancing natural biodegradation.
6. **BSL (biosafety level):** a classification of laboratory containment requirements based on the hazard of the biological agents handled; BSL-1 (lowest) to BSL-4 (highest; for agents causing lethal disease with no treatment).
7. **Cartagena Protocol on Biosafety:** a supplementary protocol to the Convention on Biological Diversity governing the transboundary movement of living modified

lanetonline.com

organisms (LMOs); requires advance informed agreement (AIA) from importing countries; Nigeria ratified in 2003.

8. **G6PD deficiency:** a common X-linked enzyme deficiency affecting approximately 20 percent of Nigerian males; affected individuals develop haemolytic anaemia when given oxidative drugs including primaquine, dapsone, and nitrofurantoin.
9. **Hybridoma:** a cell hybrid produced by fusing an antibody-producing B lymphocyte with a myeloma cell; immortal and secretes monoclonal antibody of a single defined specificity; the basis of mAb production (Kohler and Milstein, 1975).
10. **NBMA (National Biosafety Management Agency):** the Nigerian federal agency established by the NBMA Act (2015) to regulate the development, production, import, export, transit, containment, release, and use of GMOs and their products in Nigeria.
11. **Nagoya Protocol:** a supplementary protocol to the Convention on Biological Diversity (2010) governing access to genetic resources and traditional knowledge and the equitable sharing of benefits with the country of origin; Nigeria ratified in 2016.
12. **Pharmacogenomics:** the study of how an individual's genetic variation affects drug metabolism, efficacy, and adverse reactions; enables personalised dosing; relevant examples in Nigeria include CYP2D6 polymorphism and G6PD deficiency.
13. **Subunit vaccine:** a vaccine containing one or more purified recombinant protein antigens without whole pathogen; produced in yeast, insect, or CHO cells; examples include hepatitis B (HBsAg) and HPV (L1 VLP) vaccines used in the Nigerian EPI schedule.
14. **Ti plasmid:** the tumour-inducing plasmid of Agrobacterium tumefaciens; its T-DNA is naturally transferred into plant cells; disarmed Ti plasmid vectors are used to deliver transgenes into dicotyledonous crop plants.
15. **WEMA (Water Efficient Maize for Africa):** a public-private partnership developing drought-tolerant maize varieties (conventional and transgenic) for smallholder farmers in sub-Saharan Africa; confined field trials have been conducted in Nigeria under NBMA oversight.

lanetonline.com

Concept Check Questions [Series 5]

Objective Questions

1. Bt cowpea, the first biotech crop approved for Nigerian smallholders, expresses the _____ insecticidal protein from Bacillus thuringiensis. (Linked to SLO 5.1)
2. _____ is an IITA biocontrol product containing atoxigenic Aspergillus flavus strains that reduce aflatoxin contamination in maize and groundnut by 80 to 99 percent. (Linked to SLO 5.2)
3. BSL-_____ laboratories handle agents such as Mycobacterium tuberculosis and Lassa fever virus that may cause serious disease transmitted by inhalation. (Linked to SLO 5.5)
4. The Nigerian federal agency responsible for approving GMO crop trials and commercial cultivation under the 2015 Act is the National Biosafety _____ Agency. (Linked to SLO 5.6)
5. G6PD deficiency affects approximately _____ percent of Nigerian males and can cause haemolytic anaemia when affected individuals are given primaquine or other oxidative drugs. (Linked to SLO 5.3)

Short-Answer Questions

- Describe how Agrobacterium tumefaciens is used to produce a transgenic dicot crop. (Linked to SLO 5.1)
- Distinguish between a subunit vaccine and a viral vector vaccine and give one example of each used in Nigeria. (Linked to SLO 5.3)
- Describe the steps of bioethanol production from cassava using second-generation technology. (Linked to SLO 5.4)
- Explain the Cartagena Protocol on Biosafety and state two of its key requirements. (Linked to SLO 5.6)
- State two ethical concerns specific to GMO crops and explain how each is addressed by regulatory frameworks. (Linked to SLO 5.6)

Long-Answer Questions

lanetonline.com

1. Describe the applications of agricultural biotechnology in Nigeria, covering transgenic crops, molecular breeding, and microbial biotechnology. (Linked to SLOs 5.1, 5.2)
2. Describe the applications of medical, industrial, and environmental biotechnology relevant to Nigeria. Explain the biosafety levels, ethical issues, and the regulatory frameworks governing biotechnology in Nigeria. (Linked to SLOs 5.3, 5.4, 5.5, 5.6)

Answers to Concept Check Questions [Series 5]

Objective Questions

6. Cry1Ab.
7. Aflasafe.
8. 3.
9. Management.
10. 20.

Short-Answer Questions

11. Gene of interest cloned between T-DNA left and right border sequences in a binary vector. Disarmed Agrobacterium (Ti plasmid lacking oncogenes) carrying the binary vector is co-cultivated with plant explants (leaf discs, hypocotyl segments, immature embryos). T-DNA integrates into plant nuclear genome. Transgenic calli or shoots selected on medium containing antibiotic (hygromycin or kanamycin) and Agrobacterium-eliminating antibiotic (cefotaxime). Regenerated plants confirmed by PCR for transgene and Southern blot for integration pattern. Most suitable for dicots; cassava, cowpea, soybean, tobacco.
12. Subunit vaccine: purified recombinant protein antigen; Nigerian example: hepatitis B vaccine (recombinant HBsAg produced in yeast; used in EPI schedule). Viral vector vaccine: antigen gene cloned into replication-incompetent viral vector; Nigerian example: Oxford-AstraZeneca COVID-19 vaccine (ChAdOx1 chimpanzee adenovirus vector expressing SARS-CoV-2 spike protein; widely deployed in Nigeria 2021 to 2022).

lanetonline.com

13. Pretreatment of cassava lignocellulosic biomass (stems, peel) with dilute acid or steam explosion to disrupt lignin-hemicellulose-cellulose structure. Enzymatic saccharification with cellulase and xylanase to convert cellulose and hemicellulose to fermentable sugars (glucose, xylose). Fermentation with Saccharomyces cerevisiae (glucose) or xylose-fermenting yeast (Pichia stipitis) at 30 to 35 degrees C for 48 to 72 hours. Distillation to concentrate ethanol to fuel grade (99.5 percent). Blending with petrol (E10 to E85) for use as transportation fuel.
14. The Cartagena Protocol on Biosafety governs transboundary movements of living modified organisms (LMOs). Two key requirements: (1) Advance Informed Agreement (AIA): before the first intentional transboundary transfer of an LMO for environmental release, the exporter must notify the importing country and obtain its agreement before the transfer. (2) Risk assessment: the importing country must conduct an evidence-based risk assessment of potential adverse effects on biodiversity and human health before granting approval.
15. Gene flow: pollen from transgenic crops may transfer transgenes to wild relatives; addressed by requiring isolation distances, refugia, and monitoring programmes as conditions of NBMA approval. Consumer choice and labelling: consumers may wish to avoid GM food; addressed by requiring NAFDAC approval and developing mandatory labelling regulations for products containing above a defined threshold of GM content.

Long-Answer Questions

1. Transgenic crops: Agrobacterium (T-DNA integration; Ti plasmid; for dicots: cassava, cowpea) or biolistics (gold microprojectiles; for monocots: maize, rice). Traits: Bt insect resistance (Cry1Ab; pod borer; Bt cowpea approved NBMA/NAFDAC 2021); herbicide tolerance; virus resistance. Bt cotton under NBMA evaluation. Molecular breeding: MAS (SSR, KASP markers; CMD2 cassava mosaic resistance; CBSD resistance; IITA); genomic selection (genome-wide SNP; cassava yield and dry matter; IITA); MABC (introgresses target gene using foreground + background markers); GWAS (SNPs associated with traits in diverse panels; IITA cassava); doubled haploid (anther culture; maize at IITA/NCRI). Microbial biotechnology: Bradyrhizobium japonicum inoculants for soybean (300 to 700 kg per ha yield gain; BNF; IITA-promoted packages); phosphate-solubilising bacteria (Bacillus, Pseudomonas; acid soils); mycorrhizal inoculants;

lanetonline.com

biopesticides (Bt spray; Trichoderma seed treatment against root rots; Metarhizium against cassava green mite); Aflasafe (atoxigenic A. flavus competitive exclusion; 80 to 99 percent aflatoxin reduction; registered Nigeria; manufactured IITA).

2. Medical: recombinant vaccines (HBsAg subunit in yeast; HPV VLPs; viral vector COVID-19: ChAdOx1 deployed Nigeria 2021 to 2022; mRNA vaccines Pfizer/Moderna); diagnostics (recombinant antigen ELISA and RDTs; PfHRP2 malaria RDT; lateral flow principle; NAFDAC-approved kits); mAbs (hybridoma technology; CHO production; rituximab, trastuzumab, adalimumab; COVID-19 mAbs; biopharmaceuticals: insulin in E. coli; coagulation factors in CHO; NIPRD developing local production); pharmacogenomics (CYP2D6 poor metabolisers; G6PD deficiency 20 percent Nigerian males; H3Africa GWAS Nigerian populations). Industrial: fermentation (SmF vs SSF; dawadawa/ogi/fufu/burukutu traditional fermented foods; industrial enzymes: amylases Aspergillus, cellulases Trichoderma, pectinases Aspergillus; cassava peel SSF research IITA/OAU); biofuels (first-gen: cassava starch ethanol; second-gen: pretreatment + cellulase saccharification + fermentation; biodiesel from palm oil transesterification; biogas from anaerobic digestion of cassava effluent and manure; FMARD rural digesters); bioremediation (Niger Delta oil spill: aerobic degradation by Pseudomonas, Alcanivorax, Rhodococcus erythropolis; phytoremediation: Vetiver grass heavy metals; 16S rRNA metagenomics for monitoring). Biosafety levels: BSL-1 (E. coli K-12; open bench; gloves); BSL-2 (Salmonella, HIV; BSC Class II; face protection; autoclave); BSL-3 (M. tuberculosis, Lassa fever; negative pressure; HEPA; respirator; NCDC/IHVN certified); BSL-4 (Ebola, Marburg; positive pressure suit; none in Nigeria). Ethics: GMO (gene flow; corporate control; labelling; consumer choice); human genetics (germline editing condemned; biopiracy; Nagoya Protocol ABS); 3Rs animal experiments; TRIPS vs open access (IITA CGIAR public goods). Regulation: NBMA Act 2015 (risk assessment; approval confined trials and open release; approved Bt cowpea 2021; evaluating Bt cotton; WEMA drought-tolerant maize); NAFDAC (GM food safety); Cartagena Protocol (ratified Nigeria 2003; transboundary LMO AIA); Nagoya Protocol (ratified 2016; ABS for genetic resources); Codex Alimentarius (GM food safety standards).

lanetonline.com

Answers to Pilot Questions [Series 5]

Part 5.1

6. Gene of interest is cloned between T-DNA left and right border sequences in a binary vector. Disarmed Agrobacterium carrying the binary vector is co-cultivated with plant explants (leaf discs, cotyledons). T-DNA transfers to and integrates randomly into the plant nuclear genome. Transgenic callus/shoots are selected on antibiotic medium; regenerated plants confirmed by PCR and Southern blot. Most suitable for dicots: cassava, cowpea, soybean, tobacco, cotton.
7. MAS uses molecular markers (SSR or SNP/KASP) tightly linked to target genes to select for those genes by genotyping rather than phenotyping. Nigeria examples: (1) Cassava at IITA: CMD2 gene (cassava mosaic disease resistance) selected using flanking SSR and KASP markers instead of whitefly inoculation disease scoring. (2) Rice at NCRI Badeggi: blast resistance genes (Pi9, Pita) pyramided using flanking SNP markers to avoid disease screening for each gene separately.
8. Bradyrhizobium japonicum forms root nodules on soybean, fixing atmospheric N2 to NH4+ via nitrogenase enzyme. Fixed nitrogen directly fertilises the soybean crop, reducing or eliminating need for inorganic nitrogen fertiliser. In nitrogen-deficient Guinea savanna soils of Nigeria, inoculation increases soybean grain yield by 300 to 700 kg per ha compared with uninoculated plants; yield response is greatest on first-time soybean fields with no resident Bradyrhizobium.
9. Aflasafe contains four atoxigenic (non-aflatoxin-producing) strains of Aspergillus flavus; when applied to the soil surface around maize or groundnut plants at flowering, these strains colonise maize ears and groundnut pods and peg zones ahead of naturally occurring toxigenic A. flavus strains; competitive exclusion displaces toxigenic strains; aflatoxin contamination of harvested grain is reduced by 80 to 99 percent.
10. Transgenic crop (GMO): a foreign gene is inserted into the plant genome using recombinant DNA technology (Agrobacterium or biolistics); Nigerian example: Bt cowpea (Cry1Ab gene from B. thuringiensis; approved NBMA 2021). Molecular

lanetonline.com

breeding: no foreign gene introduced; DNA markers guide selection of superior genotypes from within the species' natural gene pool; Nigerian example: CMD2-resistant high-yielding cassava lines developed at IITA by MABC using SSR/KASP markers.

Part 5.2

11. Subunit vaccine: one or more purified recombinant protein antigens; no replication; safe; requires adjuvant. Nigerian example: hepatitis B vaccine (recombinant HBsAg in yeast; EPI schedule). Viral vector vaccine: antigen gene delivered in a replication-incompetent viral vector; strong cellular and humoral immune response; no adjuvant needed. Nigerian example: Oxford-AstraZeneca COVID-19 vaccine (ChAdOx1 chimpanzee adenovirus vector; spike protein antigen; mass deployment Nigeria 2021 to 2022).
12. Immunise mouse with desired antigen; collect spleen cells (producing antibody of desired specificity). Fuse spleen cells with myeloma cells using PEG; plate in HAT medium (kills unfused myeloma; spleen cells die naturally; only hybridomas survive). Screen hybridoma clones by ELISA for antibody binding to antigen. Expand positive clones. Produce mAb in mass culture. Purify by protein A/G affinity chromatography.
13. G6PD deficiency (approximately 20 percent Nigerian males): G6PD-deficient red blood cells are vulnerable to oxidative stress; primaquine (for malaria radical cure) and dapsone generate oxidative metabolites that destroy G6PD-deficient cells, causing haemolytic anaemia; testing for G6PD status before prescribing primaquine prevents this avoidable adverse reaction. CYP2D6 polymorphism: poor metabolisers accumulate codeine to toxic levels; ultrarapid metabolisers convert codeine rapidly to morphine, risking overdose in neonates of ultrarapid mothers; pharmacogenomic testing guides safe analgesic prescribing.
14. Patient sample (blood, urine, nasal swab) applied to sample pad. Liquid migrates along nitrocellulose membrane by capillary flow. If target antigen present, it binds to colloidal gold-labelled antibody in the conjugate pad. Gold-labelled antibody-antigen complex migrates to test line where immobilised capture antibody retains it; visible coloured line = positive. Excess gold-labelled antibody continues to control line (always appears); no test line = negative.

lanetonline.com

15. Insulin (recombinant human insulin; expressed in E. coli or Saccharomyces cerevisiae; treats type 1 and type 2 diabetes; widely prescribed in Nigeria). Hepatitis B surface antigen vaccine (HBsAg; expressed in Saccharomyces cerevisiae; prevents hepatitis B infection; included in Nigerian EPI schedule).

Part 5.3

1. Dawadawa: Parkia biglobosa (locust bean) fermented by Bacillus subtilis (alkaline fermentation; increases protein digestibility). Ogi/akamu: maize fermented by Lactobacillus plantarum and L. fermentum (lactic acid fermentation; reduces pH; increases digestibility). Fufu: cassava fermented by Lactobacillus and Leuconostoc species (wet fermentation; softens root; reduces HCN). Burukutu/pito: sorghum or maize beer fermented by Saccharomyces cerevisiae and lactic acid bacteria.
2. Pretreatment: cassava lignocellulosic biomass (stems, peels) treated with dilute sulphuric acid (1 to 2 percent; 120 to 180 degrees C; 15 to 30 min) or steam explosion to disrupt lignin and expose cellulose. Saccharification: add cellulase (Trichoderma reesei Celluclast) + xylanase + beta-glucosidase; incubate 50 degrees C; 48 to 72 h; cellulose and hemicellulose hydrolysed to glucose and xylose. Fermentation: add Saccharomyces cerevisiae (glucose) and/or Pichia stipitis (xylose); 30 to 35 degrees C; 48 to 72 h; ethanol produced. Distillation: remove ethanol to above 95 percent; molecular sieve dehydration to 99.5 percent fuel-grade ethanol.
3. Biostimulation: adds nutrients (N, P) or electron acceptors (oxygen, nitrate, sulphate) to contaminated site to enhance activity of indigenous hydrocarbon-degrading microorganisms already present; no external organisms added. Bioaugmentation: introduces known efficient degrading microorganism strains (e.g. Alcanivorax, Rhodococcus erythropolis) from outside the site to supplement or replace the indigenous community when natural degraders are absent or insufficient.
4. Niger Delta has extensive crude oil contamination from pipeline spills, illegal bunkering, and oil well blowouts; UNEP 2011 Ogoniland assessment documented contamination of soil (metres deep) and groundwater with BTEX and PAHs. Two microorganisms: Alcanivorax borkumensis (obligate hydrocarbonoclastic bacterium; degrades alkanes; blooms during oil spills; isolated from Niger Delta). Rhodococcus erythropolis (versatile

lanetonline.com

aerobic bacterium; degrades both aliphatic and aromatic hydrocarbons; produces biosurfactant; used in bioremediation studies at University of Port Harcourt).

5. Alpha-amylase (Bacillus licheniformis or Aspergillus oryzae; produced by submerged fermentation; hydrolyses starch to dextrins; used in cassava starch processing to produce glucose syrup for confectionery and beverage industries in Nigeria). Pectinase (Aspergillus niger; SSF on cassava peel or citrus waste; degrades pectin in fruit cell walls; used for juice clarification in fruit drink production and cassava pulp processing). Cellulase (Trichoderma reesei; submerged fermentation; degrades cellulose; used for second-generation bioethanol production from cassava biomass and cotton textile processing).

Part 5.4

1. BSL-1: agents not causing disease in healthy adults (E. coli K-12); containment: open bench work, gloves, lab coat. BSL-2: agents causing moderate disease (Salmonella, hepatitis B, HIV); containment: BSC Class II for aerosols, face protection, autoclave. BSL-3: agents causing serious inhalation-transmitted disease with available treatment (M. tuberculosis, Lassa fever virus); containment: negative pressure, HEPA filtration, respirator (N95), double-door entry. BSL-4: agents causing lethal disease with no treatment (Ebola, Marburg); containment: positive pressure full suit or Class III BSC, shower-out, pass-through autoclave.
2. Gene flow from transgenic to wild relatives: Bt cotton pollen may cross-pollinate wild Gossypium species; addressed by NBMA requiring isolation distances and gene flow monitoring as conditions of approval and post-release monitoring programmes. Corporate control of seed supply: large companies may patent and control key transgenic traits, restricting farmer access; addressed in Nigeria by IITA and IAR (CGIAR public sector) developing open-access Bt cowpea with AATF; seed multiplied and distributed through national seed companies at competitive prices.
3. NBMA (National Biosafety Management Agency) was established by the NBMA Act 2015; it regulates all activities involving GMOs in Nigeria: reviews biosafety dossiers; conducts risk assessments; approves confined field trials and open environmental releases; monitors compliance; coordinates public participation. Two crops: Bt cowpea

lanetonline.com

(Cry1Ab pod borer resistance; approved for commercial cultivation 2021; first biotech crop for Nigerian smallholders). WEMA drought-tolerant maize (confined field trials approved under NBMA oversight; conventional and transgenic drought-tolerant varieties for Guinea savanna smallholders).

4. The Cartagena Protocol on Biosafety (2000) governs transboundary movement of living modified organisms (LMOs). Key requirements: (1) Advance Informed Agreement (AIA): before the first intentional transboundary movement of an LMO for intentional environmental release, the exporting country must notify and the importing country must evaluate and either grant or refuse import; (2) Risk assessment: importing country must conduct a science-based risk assessment of potential adverse effects on biodiversity and human health. Nigeria ratified in 2003.
5. Replace: substitute animal experiments with in vitro (cell culture, organoids), computational (in silico), or ex vivo (tissue explants) methods where scientifically valid; reduces total animal use. Reduce: use the minimum number of animals necessary to obtain statistically valid results; use appropriate experimental designs and power calculations. Refine: modify procedures to minimise pain, suffering, and distress; use appropriate anaesthesia, analgesia, and humane endpoints. Importance in Nigeria: required by university ethics committees and NAFDAC before approving animal experiments; aligns Nigerian research with international standards and reduces costs; promotes alternative technologies (cell culture, computational) that strengthen Nigerian research capacity.

References and Attributions [Series 5]

Textual References (Books and External Sources)

1. IITA. (2021). Bt cowpea approval in Nigeria. International Institute of Tropical Agriculture, Ibadan.
2. UNEP. (2011). Environmental assessment of Ogoniland. United Nations Environment Programme, Nairobi.

lanetonline.com

3. WHO. (2020). Laboratory biosafety manual (4th ed.). World Health Organization, Geneva.
4. Cartagena Protocol on Biosafety. (2000). Convention on Biological Diversity. Montreal.
5. Kohler, G., & Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. Nature, 256(5517), 495–497.

Figures, Plates, Tables, and Boxes

6. Figure 5.1: Four applications of medical biotechnology with Nigerian examples. Attribution: AI generated. Suggested OER search string: “medical biotechnology recombinant vaccine diagnostic monoclonal antibody pharmacogenomics diagram OER”.
7. Box 5.1: Industrial and environmental biotechnology applications in the Nigerian context. Attribution: AI generated. Suggested OER search string: “industrial biotechnology fermentation enzymes biofuel bioremediation Nigeria applications table OER”.

lanetonline.com