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# M.Pharm Practical Notes

## Pharmaceutical Biotechnology Practicals II

Semester 2

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# Protein Identification

Practical 1 - Biological teaching-data practical

## Aim

To identify a model protein using orthogonal analytical evidence.

## Principle

Apparent size, immunoreactivity and peptide-mass or sequence evidence constrain identity differently. Shared size or an assay response is insufficient to identify a protein.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the candidate, source and reference material and retain preparation traceability.
3. Review electrophoretic size and purity together with an appropriate immunochemical or peptide-mapping method.
4. For supplied mass-spectrometric evidence inspect peptide assignments, coverage and identification confidence using the documented database and search settings.
5. Combine supporting and conflicting observations into a justified identity statement.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Sequence coverage (%) = residues covered by accepted peptide assignments / reference sequence length x 100

### Worked Example

Illustrative 120 covered residues in a 200-residue reference give 60% coverage.

Coverage alone does not establish identification confidence; unique peptides, false-match control and sample context matter.

## Result and Interpretation

- Provide a protein-evidence table that distinguishes identity, purity and amount.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Is apparent size sufficient identity?

Different proteins can migrate at similar apparent sizes.

### Why use unique peptides?

They help distinguish the candidate from related proteins.

### Does identification quantify purity?

A separate suitable purity or composition assessment is required.

# Protein Characterisation

Practical 2 - Biological teaching-data practical

## Aim

To assemble structural, physicochemical and functional characterisation of a model protein.

## Principle

Protein quality includes identity, folding, aggregation, variants and activity. Orthogonal methods examine different attributes and may respond differently to the same preparation.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the protein, intended attributes and reference preparation.
3. Review intact or subunit size, charge-related variants and peptide identity through suitable methods.
4. Assess aggregation and secondary-structure or thermal behaviour with method-specific controls.
5. Compare activity with a justified reference and relate differences to structural evidence without assuming causation.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Relative activity (%) = measured activity per stated amount / reference activity per same amount x 100

### Worked Example

Illustrative test specific activity 80 U/mg and reference 100 U/mg give 80% relative activity.

An equal total protein concentration does not imply equal active protein content.

## Result and Interpretation

- Deliver an attribute-method-evidence matrix, limitations and proposed follow-up.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why use orthogonal methods?

No one technique measures every relevant protein attribute.

### What is specific activity?

Activity per defined amount of protein.

### Can aggregation change function?

It may affect behaviour and must be assessed for the particular system.

# Protein Biochemistry and Enzyme Kinetics

Practical 3 - Biological teaching-data practical

## Aim

To estimate enzyme kinetic parameters from controlled initial-rate data.

## Principle

For an appropriate simple enzyme system v = Vmax[S]/(Km + [S]). Initial-rate conditions, substrate depletion and assay linearity must be checked; a fit does not establish that the model is biologically complete.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use supplied benign enzyme data with substrate concentrations and blank-corrected initial rates.
3. Verify the linear measurement interval and rate units and include no-enzyme or other appropriate controls.
4. Fit the nonlinear Michaelis-Menten model and inspect residuals and parameter uncertainty.
5. Discuss whether inhibition, multiple substrates or cooperativity makes the simple model inappropriate.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- v = Vmax[S]/(Km + [S])
- At [S] = Km, v = Vmax/2 for this model.

### Worked Example

Illustrative Vmax = 100 micromol/min and Km = 2 mmol/L. At [S] = 2 mmol/L, v = 50 micromol/min; at 8 mmol/L, v = 80 micromol/min.

## Result and Interpretation

- Report fitted parameters with units, fit limitations and the initial-rate evidence.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### What is Km in the simple model?

The substrate concentration giving half the modelled maximum rate.

### Why use initial rates?

It limits depletion and product-related changes.

### Why prefer direct nonlinear fitting?

Reciprocal transformations can distort the error structure.

# Recombinant DNA Workflow

Practical 4 - Biological teaching-data practical

## Aim

To plan and verify a benign recombinant-DNA workflow.

## Principle

A recombinant construct joins defined DNA elements in a documented orientation and reading frame. A map or colony result alone cannot confirm the complete intended sequence.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use a benign reporter or teaching insert and a documented nonpathogenic host/vector system.
3. Draw the construct map and identify promoter, insert boundaries and other required elements without introducing hazardous functions.
4. Review approved assembly and teaching-transformation records and appropriate empty-vector and positive controls.
5. Confirm orientation, junctions and intended sequence by a justified analytical check and maintain construct version traceability.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Expected construct length = retained vector length + inserted sequence length after accounting for the assembly design

### Worked Example

Illustrative retained vector 3,000 bp plus insert 750 bp gives 3,750 bp.

A band near 3.75 kb supports size only; sequence and orientation still require confirmation.

## Result and Interpretation

- Deliver a construct map, control matrix and identity-verification record.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why check orientation?

It can determine whether the intended expression arrangement is present.

### Does selection prove the insert?

It usually establishes only recovery under the selected system.

### Why sequence junctions?

They can reveal assembly errors at the join.

# Protein Expression and Recovery

Practical 5 - Biological teaching-data practical

## Aim

To evaluate expression and recovery of a benign teaching protein.

## Principle

Expression level, solubility, recovery, purity and activity are distinct outcomes. A strong band can represent insoluble or inactive material.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use supplied records or a certified benign reporter system under the approved protocol.
3. Compare nonexpressing and expressing controls and document total, soluble and insoluble fractions.
4. Review the authorised recovery and purification flow with fraction identity and protein-assay controls.
5. Calculate stage recovery and specific activity and assess identity independently.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Protein recovery (%) = recovered target amount / target amount entering the stage x 100
- Purification fold = final specific activity / starting specific activity

### Worked Example

Illustrative starting extract has 100 mg target and purified fraction contains 40 mg: recovery = 40%. Specific activity increasing from 5 to 25 U/mg gives fivefold purification.

## Result and Interpretation

- Present a fraction balance distinguishing target yield, purity and activity.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why examine soluble and insoluble fractions?

Expression does not guarantee soluble recovery.

### Does total protein equal target protein?

The mixture can contain many proteins.

### Why retain activity data?

Structural presence does not prove functional recovery.

# Protein Formulation and Stability

Practical 6 - Biological teaching-data practical

## Aim

To compare protein-formulation stability using orthogonal quality attributes.

## Principle

pH, ionic environment, interfaces and excipients can affect protein aggregation, chemical change and activity. A single total-protein assay can miss these changes.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the benign protein, intended formulation attributes and approved comparison matrix.
3. Review matched pH, excipient and container conditions without changing several factors unknowingly.
4. Assess concentration, aggregates or particles, structural indicators and activity before and after the specified stress.
5. Compare recovery and activity on consistent bases and distinguish reversible physical changes from chemical degradation.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Activity retention (%) = activity after storage / initial activity x 100

### Worked Example

Illustrative initial activity 100 U/mL and final 85 U/mL give 85% retention. Total protein remaining 98% does not negate the activity loss.

## Result and Interpretation

• Recommend a formulation-development direction supported by all measured attributes, not an unverified shelf life.
• Report biological and technical replication separately and explain control performance.

## Precautions

• Use only the approved biological system, containment and decontamination procedure.
• A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
• Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why control interfaces?

Contact and agitation can affect some proteins.

### Why measure aggregates separately?

Total concentration may not distinguish aggregated material.

### Can one stress predict all storage behaviour?

A justified stability programme is needed.

# Biological Database Searching

Practical 7 - Bioinformatics practical

## Aim

To retrieve and cross-check biological database records.

## Principle

Primary sequence records, curated protein records and structural databases have different purposes and levels of review. Stable identifiers, versions, evidence status and retrieval dates support reproducibility.

## Materials and Requirements

- Public reference records for benign teaching sequences
- Documented analysis software, database release and parameter settings
- Input/output files and validation worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the biological question and retain the input identifier, sequence version, retrieval date and analysis settings.
3. Search by a defined benign gene or protein identifier and organism, then distinguish exact records from name matches.
4. Record accession, sequence version, length, annotation status and cross-references.
5. Compare a sequence record, protein annotation and available structure; investigate mismatched isoforms or numbering.
6. Save reproducible outputs and distinguish a computational prediction from experimental confirmation.
7. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Input / analysis | Setting / evidence | Output | Interpretation |
|---|---|---|---|
| Reference input | Identifier / version | Record | Verify |
| Primary analysis | Parameters | Record | Evaluate |
| Validation / comparison | Independent evidence | Record | Conclude |

## Calculations

- Sequence length = number of valid residues after excluding formatting characters

### Worked Example

Illustrative two records share a gene name but contain 300 and 320 residues. Check isoform and sequence-version evidence before comparing numbered sites.

## Result and Interpretation

• Produce a reproducible analysis record with biological interpretation, uncertainty and an appropriate independent check.

## Precautions

• Use nonpathogenic reference examples without personal sequence data.
• Check sequence alphabet, strand, coordinate and accession versions.
• Do not report a similarity hit or model as experimentally established function.

## Viva Questions and Answers

### Why retain an accession version?

The underlying sequence or annotation can change.

### Is every annotation experimentally confirmed?

Evidence can be curated, predicted or inferred.

### Why check organism?

Shared names can refer to different biological sequences.

# Biological Sequence Analysis

Practical 8 - Bioinformatics practical

## Aim

To align and compare benign nucleotide or protein sequences.

## Principle

Alignment scores depend on substitutions, gaps and the chosen local or global model. Identity is an exact-match proportion; similarity depends on a scoring definition and is not the same as functional equivalence.

## Materials and Requirements

- Public reference records for benign teaching sequences
- Documented analysis software, database release and parameter settings
- Input/output files and validation worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the biological question and retain the input identifier, sequence version, retrieval date and analysis settings.
3. Choose global alignment for overall comparison or local alignment for a region-specific question and justify the choice.
4. Record scoring matrix, gap costs and filtering and inspect the actual aligned positions.
5. Calculate identity and coverage using explicit denominators and evaluate conserved and variable regions.
6. Save reproducible outputs and distinguish a computational prediction from experimental confirmation.
7. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Input / analysis | Setting / evidence | Output | Interpretation |
|---|---|---|---|
| Reference input | Identifier / version | Record | Verify |
| Primary analysis | Parameters | Record | Evaluate |
| Validation / comparison | Independent evidence | Record | Conclude |

## Calculations

- Identity (%) = identical aligned residues / stated alignment-length denominator x 100
- Query coverage (%) = query positions covered / query length x 100

### Worked Example

Illustrative 80 identical positions in an alignment of 100 give 80% identity. If only 100 of a 200-residue query are covered, query coverage is 50%.

## Result and Interpretation

• Produce a reproducible analysis record with biological interpretation, uncertainty and an appropriate independent check.

## Precautions

• Use nonpathogenic reference examples without personal sequence data.
• Check sequence alphabet, strand, coordinate and accession versions.
• Do not report a similarity hit or model as experimentally established function.

## Viva Questions and Answers

### Why report coverage?

A strong short match may cover little of the query.

### What distinguishes local alignment?

It seeks a high-scoring matching region.

### Does high identity always establish function?

Biological context and supporting evidence remain necessary.

# Protein Structure Prediction

Practical 9 - Bioinformatics practical

## Aim

To assess a predicted protein structure with appropriate confidence measures.

## Principle

A predicted coordinate model is a hypothesis. Sequence coverage, local confidence, domain relationships and template or training context affect its use; high local confidence does not prove a biological assembly or ligand-binding pose.

## Materials and Requirements

- Public reference records for benign teaching sequences
- Documented analysis software, database release and parameter settings
- Input/output files and validation worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the biological question and retain the input identifier, sequence version, retrieval date and analysis settings.
3. Confirm the sequence, isoform and intended modelling region.
4. Run or inspect a documented prediction and retain model version and inputs.
5. Map confidence by residue, inspect low-confidence regions and domain relationships, and compare with experimentally determined structures where available.
6. Save reproducible outputs and distinguish a computational prediction from experimental confirmation.
7. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Input / analysis | Setting / evidence | Output | Interpretation |
|---|---|---|---|
| Reference input | Identifier / version | Record | Verify |
| Primary analysis | Parameters | Record | Evaluate |
| Validation / comparison | Independent evidence | Record | Conclude |

## Calculations

- Coverage (%) = modelled target residues / target length x 100

### Worked Example

Illustrative a 250-residue model covers 200 residues: coverage is 80%. A high-confidence core does not justify interpreting the unmodelled 50 residues.

## Result and Interpretation

• Produce a reproducible analysis record with biological interpretation, uncertainty and an appropriate independent check.

## Precautions

• Use nonpathogenic reference examples without personal sequence data.
• Check sequence alphabet, strand, coordinate and accession versions.
• Do not report a similarity hit or model as experimentally established function.

## Viva Questions and Answers

### What is a model-confidence score?

A model-specific estimate of reliability, not direct experimental verification.

### Why inspect domains separately?

Relative domain placement can be uncertain even when local folds are confident.

### Can a predicted fold prove activity?

Functional evidence is separately required.

# Gene Annotation

Practical 10 - Bioinformatics practical

## Aim

To annotate a benign gene region using sequence and evidence records.

## Principle

Gene annotation links sequence coordinates to proposed features such as coding regions, transcripts and functional evidence. An open reading frame alone is not proof of an expressed functional gene.

## Materials and Requirements

- Public reference records for benign teaching sequences
- Documented analysis software, database release and parameter settings
- Input/output files and validation worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the biological question and retain the input identifier, sequence version, retrieval date and analysis settings.
3. Confirm reference assembly, strand and coordinate convention.
4. Identify candidate coding regions and compare transcript, protein and curated homology evidence.
5. Record feature boundaries, translation and evidence strength and flag unresolved start, splice or function assignments.
6. Save reproducible outputs and distinguish a computational prediction from experimental confirmation.
7. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Input / analysis | Setting / evidence | Output | Interpretation |
|---|---|---|---|
| Reference input | Identifier / version | Record | Verify |
| Primary analysis | Parameters | Record | Evaluate |
| Validation / comparison | Independent evidence | Record | Conclude |

## Calculations

- For a simple uninterrupted coding region: amino-acid length is approximately coding nucleotides/3 after accounting for the terminal stop codon.

### Worked Example

Illustrative 903 coding nucleotides including a 3-base stop correspond to 300 amino acids. Introns and alternative transcripts require an appropriate transcript model.

## Result and Interpretation

• Produce a reproducible analysis record with biological interpretation, uncertainty and an appropriate independent check.

## Precautions

• Use nonpathogenic reference examples without personal sequence data.
• Check sequence alphabet, strand, coordinate and accession versions.
• Do not report a similarity hit or model as experimentally established function.

## Viva Questions and Answers

### Why record coordinate convention?

One-based inclusive and zero-based half-open coordinates differ.

### Does an ORF prove expression?

Transcript or protein evidence is needed.

### Why retain evidence status?

Predicted and experimentally supported features have different certainty.

# Phylogenetic Analysis

Practical 11 - Bioinformatics practical

## Aim

To construct and critically interpret a small benign-sequence phylogeny.

## Principle

A phylogeny is an inferred relationship conditional on sequence selection, alignment and model. Support values describe resampling or model-specific support and do not directly represent evolutionary distance or certainty of every biological conclusion.

## Materials and Requirements

- Public reference records for benign teaching sequences
- Documented analysis software, database release and parameter settings
- Input/output files and validation worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the biological question and retain the input identifier, sequence version, retrieval date and analysis settings.
3. Choose comparable homologous regions and justify taxon sampling and any outgroup.
4. Align, inspect ambiguous regions and apply a documented trimming rule.
5. Infer a tree with a justified substitution model, assess support and compare alternative alignments or methods.
6. Save reproducible outputs and distinguish a computational prediction from experimental confirmation.
7. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Input / analysis | Setting / evidence | Output | Interpretation |
|---|---|---|---|
| Reference input | Identifier / version | Record | Verify |
| Primary analysis | Parameters | Record | Evaluate |
| Validation / comparison | Independent evidence | Record | Conclude |

## Calculations

- Bootstrap support (%) = replicate trees containing a split / bootstrap replicates x 100

### Worked Example

Illustrative a split occurs in 850 of 1,000 bootstrap replicates: support = 85%. This is not an 85% probability that an entire historical account is true.

## Result and Interpretation

• Produce a reproducible analysis record with biological interpretation, uncertainty and an appropriate independent check.

## Precautions

• Use nonpathogenic reference examples without personal sequence data.
• Check sequence alphabet, strand, coordinate and accession versions.
• Do not report a similarity hit or model as experimentally established function.

## Viva Questions and Answers

### Why use homologous regions?

Noncomparable sequences can yield misleading alignments and trees.

### What does an outgroup support?

A justified rooting interpretation.

### Does branch length equal elapsed time?

Only with an appropriate calibrated evolutionary model.

# Protein-DNA Binding Studies

Practical 12 - Biological teaching-data practical

## Aim

To interpret protein-DNA binding through controlled mobility-shift data.

## Principle

A bound complex may migrate differently from free labelled DNA. Shift specificity and apparent affinity depend on concentration, stoichiometry, probe and assay conditions.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use a supplied benign protein/probe dataset with free-probe and protein controls.
3. Compare titration lanes with an appropriate unlabelled competitor and a nonbinding or sequence-control comparison.
4. Quantify free and shifted probe in the linear imaging range with consistent background treatment.
5. Fit a justified binding model only when the concentration and equilibrium assumptions are supported.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Fraction bound = shifted-probe signal / (free-probe signal + shifted-probe signal)
- Simple 1:1 model under appropriate conditions: fraction bound = [P_free]/(Kd + [P_free])

### Worked Example

Illustrative free signal 600 and shifted signal 400 give fraction bound 0.40.

At a true free protein concentration of 20 nM, the simple model gives Kd = 20(1 - 0.40)/0.40 = 30 nM; total protein cannot always replace free protein.

## Result and Interpretation

- Report specificity controls and model assumptions with any apparent affinity estimate.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### What supports specificity?

Appropriate competition and sequence controls.

### Why is free concentration relevant?

Binding equilibrium depends on free reactants.

### Does any shifted band identify the protein?

Independent identity and control evidence is needed.

# DNA Isolation Purity and Quantification for PCR

Practical 13 - Approved nucleic-acid isolation practical

## Aim

To qualify an approved DNA preparation for PCR use.

## Principle

Cell disruption releases nucleic acids; controlled lysis and purification separate DNA from proteins, polysaccharides and other contaminants. Precipitated threads alone do not prove DNA identity or purity.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use cauliflower or another authorised plant model; animal-tissue comparisons use supplied records unless separately approved.
3. Record input mass and use the adopted plant-DNA kit or validated lysis and clarification method.
4. Purify through the specified binding/wash/elution or approved precipitation workflow, retaining extraction blanks.
5. Measure DNA concentration and recovered volume and assess integrity by a suitable gel and purity by complementary checks.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- DNA yield per gram = measured DNA mass / input tissue mass
- DNA mass = concentration x elution volume

### Worked Example

Illustrative DNA concentration 80 ng/microlitre in 100 microlitres gives 8,000 ng = 8 micrograms. From 0.20 g plant material, yield is 40 micrograms/g.

Report the actual assay specificity and extraction-blank result.

### PCR suitability

• Assess concentration, integrity, inhibitor carryover and extraction-blank performance.
• A dilution or spiked amplification-control comparison can help identify inhibition. Keep the downstream no-template and positive controls separate.

## Result and Interpretation

• Present extraction flow, recovery, purity indicators and gel evidence.
• Report biological and technical replication separately and explain control performance.

## Precautions

• Use only the approved biological system, containment and decontamination procedure.
• A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
• Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why avoid harsh handling after lysis?

It can shear high-molecular-weight DNA.

### Why include an extraction blank?

It checks contamination introduced during purification.

### Why do plant matrices need care?

Polysaccharides and secondary metabolites can interfere.

# PCR Instrument Operation and Program Review

Practical 14 - Approved PCR practical

## Aim

To plan and interpret PCR amplification of a benign teaching target.

## Principle

PCR repeats denaturation, primer annealing and extension to amplify a defined region. Primer specificity, template quality and reaction controls determine whether an observed product supports the intended target.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use a known benign plant, yeast or certified teaching-DNA target and the selected polymerase protocol.
3. Record primer identities, expected product size, template input, reagent master mix and the protocol-specific thermal programme.
4. Include no-template, positive-template and extraction controls where applicable; separate preparation and post-amplification handling.
5. Run the authorised programme and assess product size and nonspecific bands by a suitable method, retaining all controls.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Idealised copies after n perfect doublings = initial copies x 2^n; real PCR efficiency and plateau limit this model.

### Worked Example

Illustrative 100 starting copies after 10 perfect doublings would give 102,400 copies.

A band endpoint does not establish that this ideal amplification occurred or provide reliable original-copy quantitation.

## Result and Interpretation

- Deliver a reaction map, thermal programme rationale, control results and target-specific interpretation.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why include a no-template control?

It checks reagent or handling contamination.

### Why must annealing be target specific?

Primer binding depends on sequence and reaction chemistry.

### Does endpoint band brightness give original copy number?

Not without a validated quantitative method.

# Reverse-Transcription PCR and Quantitative Data

Practical 15 - Approved PCR practical

## Aim

To interpret reverse-transcription PCR and distinguish it from real-time quantitative PCR.

## Principle

Reverse transcription converts RNA into cDNA before amplification. Real-time PCR measures signal during cycling; RT-PCR and qPCR are distinct terms, although reverse transcription can be combined with qPCR.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Use a documented benign RNA target with concentration, integrity and DNA-contamination assessment.
2. Apply the selected reverse-transcriptase protocol and record RNA input and priming strategy.
3. Include a no-reverse-transcriptase control, no-template control and suitable positive control.
4. Amplify the resulting cDNA using the authorised target-specific programme and assess product specificity.
5. If quantitative real-time data are used, evaluate efficiency, reference normalisation and Cq quality before relative-expression analysis.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- For a justified equal-efficiency relative-expression model: fold change = 2^(-delta-delta-Cq)

### Worked Example

Illustrative target-minus-reference delta Cq is 5 in control and 3 in treated material. Delta-delta-Cq = -2, giving a fourfold relative expression under the model assumptions.

The estimate is invalid if reference stability, specificity or efficiency assumptions fail.

## Result and Interpretation

- Deliver a reaction map, thermal programme rationale, control results and target-specific interpretation.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### What does reverse transcription produce?

cDNA from RNA.

### Why use a no-RT control?

It checks whether DNA contributes to the amplification signal.

### Is RT-PCR always real-time PCR?

No; reverse transcription and real-time detection describe different operations.

# Primer Design with Software

Practical 16 - Bioinformatics practical

## Aim

To design and assess primers for a benign teaching target.

## Principle

Primer sequence, orientation, melting behaviour and complementarity influence amplification. A predicted primer pair needs specificity, product and reaction-condition validation.

## Materials and Requirements

- Public reference records for benign teaching sequences
- Documented analysis software, database release and parameter settings
- Input/output files and validation worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Define the biological question and retain the input identifier, sequence version, retrieval date and analysis settings.
3. Select a known benign target accession and exact intended product coordinates.
4. Use documented design software to assess length, GC content, melting estimates, self-dimers and cross-dimers.
5. Check both primers against the intended reference context, retain expected product size and validate with appropriate amplification controls.
6. Save reproducible outputs and distinguish a computational prediction from experimental confirmation.
7. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Input / analysis | Setting / evidence | Output | Interpretation |
|---|---|---|---|
| Reference input | Identifier / version | Record | Verify |
| Primary analysis | Parameters | Record | Evaluate |
| Validation / comparison | Independent evidence | Record | Conclude |

## Calculations

- GC (%) = (G + C residues)/primer length x 100
- Simple Wallace estimate for a short primer: Tm approximately 2(A + T) + 4(G + C) degrees C; use the appropriate software thermodynamic model for final design.

### Worked Example

Illustrative a 20-base primer with 10 G/C residues has 50% GC and a simple Wallace estimate of 60 degrees C. This is not a prescribed annealing temperature.

## Result and Interpretation

• Produce a reproducible analysis record with biological interpretation, uncertainty and an appropriate independent check.

## Precautions

• Use nonpathogenic reference examples without personal sequence data.
• Check sequence alphabet, strand, coordinate and accession versions.
• Do not report a similarity hit or model as experimentally established function.

## Viva Questions and Answers

### Why inspect primer dimers?

They can consume reagents and create nonspecific signal.

### Is calculated Tm the annealing temperature?

Reaction chemistry and validation determine the programme.

### Why retain accession coordinates?

They define the actual target and expected product.

# DNA Amplification with Random and Specific Primers

Practical 17 - Approved PCR practical

## Aim

To compare target-specific and random-primer amplification records for benign teaching DNA.

## Principle

PCR repeats denaturation, primer annealing and extension to amplify a defined region. Primer specificity, template quality and reaction controls determine whether an observed product supports the intended target.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use a known benign plant, yeast or certified teaching-DNA target and the selected polymerase protocol.
3. Record primer identities, expected product size, template input, reagent master mix and the protocol-specific thermal programme.
4. Include no-template, positive-template and extraction controls where applicable; separate preparation and post-amplification handling.
5. Run the authorised programme and assess product size and nonspecific bands by a suitable method, retaining all controls.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Idealised copies after n perfect doublings = initial copies x 2^n; real PCR efficiency and plateau limit this model.

### Worked Example

Illustrative 100 starting copies after 10 perfect doublings would give 102,400 copies.

A band endpoint does not establish that this ideal amplification occurred or provide reliable original-copy quantitation.

### Primer-system comparison

• Target-specific primers define an expected locus and product. Random-primer patterns require stringent reproducibility controls and should not be treated as a locus-specific sequence result.
• Record primer identity, polymerase, thermal programme and repeatability independently for each system; do not optimise unknown pathogenic sequences.

## Result and Interpretation

• Deliver a reaction map, thermal programme rationale, control results and target-specific interpretation.
• Report biological and technical replication separately and explain control performance.

## Precautions

• Use only the approved biological system, containment and decontamination procedure.
• A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
• Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why include a no-template control?

It checks reagent or handling contamination.

### Why must annealing be target specific?

Primer binding depends on sequence and reaction chemistry.

### Does endpoint band brightness give original copy number?

Not without a validated quantitative method.

# Southern Hybridisation

Practical 18 - Biological teaching-data practical

## Aim

To interpret Southern-hybridisation records for a benign DNA target.

## Principle

Southern analysis separates DNA, transfers it to a membrane and detects fragments that hybridise with a defined probe. Fragment patterns depend on the digest, target and probe specificity.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use supplied images or an approved benign target and nonhazardous detection system.
3. Map digest, gel, transfer, probe and control lanes with their documented conditions.
4. Assess digestion completeness, transfer and detection controls and align size references.
5. Interpret detected fragment sizes against the expected map and consider incomplete digestion or nonspecific signal.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Detected fragment size is estimated from the calibrated ladder within its valid range.

### Worked Example

Illustrative expected restriction fragments are 2 and 5 kb, but the probe lies only in the 5 kb fragment. One 5 kb signal can therefore be consistent with the map.

Unexpected bands require further evidence rather than automatic assignment to extra gene copies.

## Result and Interpretation

- Provide a probe/restriction map, labelled image and evidence-limited target interpretation.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### What is detected?

Fragments complementary to the probe under the selected conditions.

### Why verify transfer?

Poor transfer can create a false absence of signal.

### Why can extra bands appear?

Incomplete digestion, nonspecific hybridisation or genuine structural differences may contribute.

# Western Blotting

Practical 19 - Biological teaching-data practical

## Aim

To quantify a target protein from a controlled Western-blot dataset.

## Principle

Western blotting combines separation, transfer and antibody-based detection. Specificity and a nonsaturated linear response are needed; normalisation corrects selected loading effects but cannot repair poor specificity.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use approved protein records with target and loading or total-protein controls.
3. Assess full-lane separation, transfer controls, antibody specificity and the absence of saturated exposure.
4. Apply consistent regions and background subtraction and normalise with a justified stable loading measure.
5. Compare independent biological replicates and retain uncropped images and processing settings.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Normalised target = background-corrected target signal / justified loading signal
- Relative fold = normalised test / normalised reference

### Worked Example

Illustrative target/loading signals are 200/100 in control and 300/100 in test: normalised values 2 and 3 give a 1.5-fold difference.

Do not interpret a saturated band or unstable loading control quantitatively.

## Result and Interpretation

- Report target specificity, linear range, normalisation and biological variability.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why keep uncropped images?

They preserve lane context and additional signals.

### What invalidates densitometry?

Saturation or an unvalidated response range can distort ratios.

### Why distinguish biological replicates?

They represent independent biological observations rather than repeated readings.

# Gene Transformation of a Teaching Strain

Practical 20 - Approved teaching transformation practical

## Aim

To assess a benign teaching-plasmid transformation and calculate efficiency.

## Principle

Transformation introduces defined DNA into a recipient. Colonies recovered under a specified selection or screening system require identity confirmation; efficiency depends on DNA, cell and recovery conditions.

## Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

## Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Use only a certified nonpathogenic teaching strain and approved benign plasmid with no hazardous insert.
3. Follow the manufacturer or institutional transformation protocol without substituting unknown vectors or organisms.
4. Review no-DNA, competent-cell and positive-plasmid controls and record the total recovery and plated fraction.
5. Count valid teaching plates, calculate efficiency and verify candidate identity with a justified benign assay.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

## Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

| Preparation / control | Condition / method | Raw observation | Calculated / interpreted result |
|---|---|---|---|
| Negative / vehicle control | Specify | Record | Evaluate |
| Positive / reference control | Specify | Record | Evaluate |
| Test replicates | Specify | Record | Calculate |

## Calculations

- Transformation efficiency (CFU/microgram) = colonies x dilution correction x total recovery volume / plated volume / input DNA mass in micrograms

### Worked Example

Illustrative 100 colonies arise from one tenth of the undiluted recovery after input of 0.010 microgram DNA. Estimated efficiency = 100 x 10 / 0.010 = 100,000 CFU/microgram.

Selection survival alone does not prove the intended insert or sequence.

## Result and Interpretation

- Report strain/plasmid identity, controls, plated fraction and efficiency with the identity-check result.
- Report biological and technical replication separately and explain control performance.

## Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

## Viva Questions and Answers

### Why use a no-DNA control?

It checks background recovery under the selected system.

### Why correct plated fraction?

Only part of the recovered material may have been counted.

### Does a colony confirm sequence?

A suitable identity check is still needed.

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