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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.

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