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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.

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