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Isolation and Estimation of DNA

Practical 20 - Approved nucleic-acid isolation practical

Aim

To isolate DNA from an approved plant model and assess recovery and integrity.

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.

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.

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