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Plasmid Isolation and Quality Assessment

Practical 22 - Approved nucleic-acid isolation practical

Aim

To assess isolation and quality of a defined benign teaching plasmid.

Principle

An approved plasmid-purification workflow enriches plasmid DNA while reducing chromosomal DNA, proteins and other contaminants. Supercoiled, nicked and linear forms migrate differently; an uncut plasmid cannot be sized directly against linear DNA standards.

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 nonpathogenic teaching strain and benign plasmid under the approved risk assessment.
2. Follow the selected kit procedure for cell harvest, controlled lysis, neutralisation and clarification; avoid genomic-DNA shearing into the preparation.
3. Purify and elute using the specified binding and wash conditions with appropriate blanks.
4. Assess concentration and plasmid forms by suitable methods; confirm identity with a justified restriction or sequence check.
5. Record recovered mass and fitness for the intended benign downstream use.

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.

Plasmid interpretation

- Use a linearised or otherwise validated reference when estimating plasmid size. Retain map, sequence version and identity-check records.

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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