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DNA Fragmentation by Agarose Electrophoresis

Practical 24 - Electrophoresis practical

Aim

To assess supplied DNA-fragmentation gel patterns with suitable controls.

Principle

DNA cleavage can alter size distribution and produce ladders or smears. A pattern is not specific for one cell-death mechanism and is affected by extraction, nuclease activity and gel 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. Select an appropriate fragment range, gel, buffer and approved stain; record electrophoresis settings.
3. Use a size ladder, extraction or loading controls and the supplied DNA preparations.
4. Run only through the authorised electrical and imaging procedure; retain the original unsaturated image.
5. Fit log10 fragment size against migration within the supported ladder range and estimate unknown linear-fragment sizes.
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

- Example calibration: log10(size in bp) = intercept + slope x migration distance

Worked Example

Illustrative log10(bp) = 4.0 - 0.030d with d in mm. At d = 40 mm, size is 10^2.8 = 631 bp approximately.
Do not apply this linear-fragment calibration directly to uncut supercoiled plasmids.

Fragmentation interpretation

- Compare untreated, reference and test lanes at a justified DNA-input basis. Retain extraction blanks and the full image.

• Report the pattern descriptively and seek orthogonal apoptosis or damage evidence rather than diagnosing mechanism from a smear.

Result and Interpretation

• Provide a labelled lane map, control assessment and approximate fragment sizes with calibration limits.
• 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 ladder?

It provides size references in the same run.

Why avoid saturated images?

They obscure quantitative intensity and band detail.

Why can plasmid forms migrate differently?

Conformation changes mobility independently of length.

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