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Gene Transformation of a Teaching Strain

Practical 20 - Approved teaching transformation practical

Aim

To assess a benign teaching-plasmid transformation and calculate efficiency.

Principle

Transformation introduces defined DNA into a recipient. Colonies recovered under a specified selection or screening system require identity confirmation; efficiency depends on DNA, cell and recovery 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 only a certified nonpathogenic teaching strain and approved benign plasmid with no hazardous insert.
3. Follow the manufacturer or institutional transformation protocol without substituting unknown vectors or organisms.
4. Review no-DNA, competent-cell and positive-plasmid controls and record the total recovery and plated fraction.
5. Count valid teaching plates, calculate efficiency and verify candidate identity with a justified benign assay.
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

- Transformation efficiency (CFU/microgram) = colonies x dilution correction x total recovery volume / plated volume / input DNA mass in micrograms

Worked Example

Illustrative 100 colonies arise from one tenth of the undiluted recovery after input of 0.010 microgram DNA. Estimated efficiency = 100 x 10 / 0.010 = 100,000 CFU/microgram.

Selection survival alone does not prove the intended insert or sequence.

Result and Interpretation

- Report strain/plasmid identity, controls, plated fraction and efficiency with the identity-check result.
- 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 no-DNA control?

It checks background recovery under the selected system.

Why correct plated fraction?

Only part of the recovered material may have been counted.

Does a colony confirm sequence?

A suitable identity check is still needed.

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