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Gene Amplification by PCR

Practical 20 - Approved PCR practical

Aim

To plan and interpret PCR amplification of a benign teaching target.

Principle

PCR repeats denaturation, primer annealing and extension to amplify a defined region. Primer specificity, template quality and reaction controls determine whether an observed product supports the intended target.

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 a known benign plant, yeast or certified teaching-DNA target and the selected polymerase protocol.
3. Record primer identities, expected product size, template input, reagent master mix and the protocol-specific thermal programme.
4. Include no-template, positive-template and extraction controls where applicable; separate preparation and post-amplification handling.
5. Run the authorised programme and assess product size and nonspecific bands by a suitable method, retaining all controls.
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

- Idealised copies after n perfect doublings = initial copies x 2^n; real PCR efficiency and plateau limit this model.

Worked Example

Illustrative 100 starting copies after 10 perfect doublings would give 102,400 copies.

A band endpoint does not establish that this ideal amplification occurred or provide reliable original-copy quantitation.

Result and Interpretation

- Deliver a reaction map, thermal programme rationale, control results and target-specific 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

Why include a no-template control?

It checks reagent or handling contamination.

Why must annealing be target specific?

Primer binding depends on sequence and reaction chemistry.

Does endpoint band brightness give original copy number?

Not without a validated quantitative method.

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