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Reverse-Transcription PCR and Quantitative Data

Practical 15 - Approved PCR practical

Aim

To interpret reverse-transcription PCR and distinguish it from real-time quantitative PCR.

Principle

Reverse transcription converts RNA into cDNA before amplification. Real-time PCR measures signal during cycling; RT-PCR and qPCR are distinct terms, although reverse transcription can be combined with qPCR.

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 benign RNA target with concentration, integrity and DNA-contamination assessment.
2. Apply the selected reverse-transcriptase protocol and record RNA input and priming strategy.
3. Include a no-reverse-transcriptase control, no-template control and suitable positive control.
4. Amplify the resulting cDNA using the authorised target-specific programme and assess product specificity.
5. If quantitative real-time data are used, evaluate efficiency, reference normalisation and Cq quality before relative-expression analysis.

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

- For a justified equal-efficiency relative-expression model: fold change = 2^(-delta-delta-Cq)

Worked Example

Illustrative target-minus-reference delta Cq is 5 in control and 3 in treated material. Delta-delta-Cq = -2, giving a fourfold relative expression under the model assumptions.

The estimate is invalid if reference stability, specificity or efficiency assumptions fail.

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

What does reverse transcription produce?

cDNA from RNA.

Why use a no-RT control?

It checks whether DNA contributes to the amplification signal.

Is RT-PCR always real-time PCR?

No; reverse transcription and real-time detection describe different operations.

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