Molecular technologies

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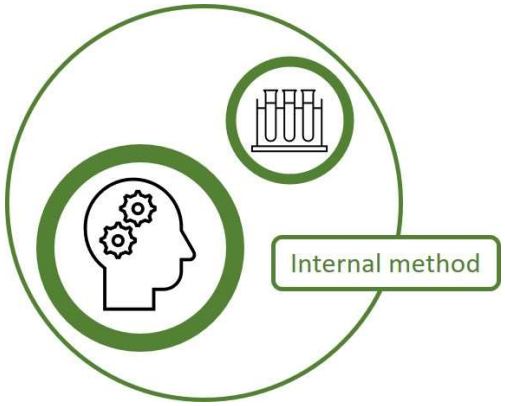
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

How does your lab deal with molecular technology?



- Reference material
- Multiple suppliers
- Suppliers' selection and monitoring
- Quality control on several reagents

Time consuming
High staff effort

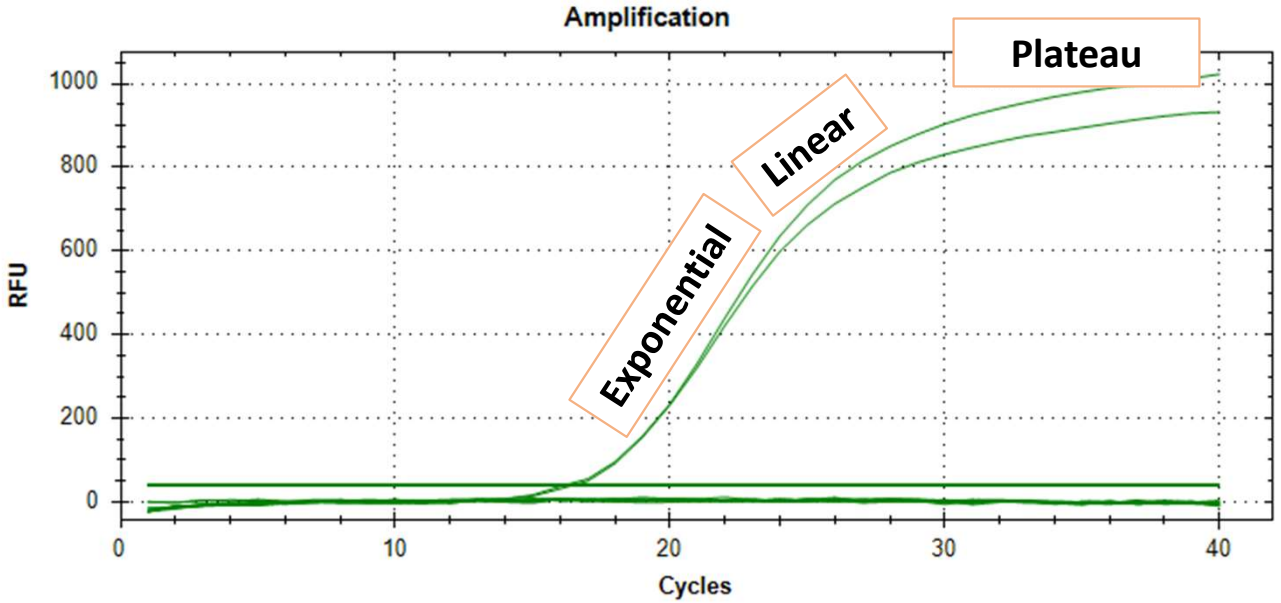
- One supplier
- Quality control on one reagent
- Lot-to-lot consistency

Time saving
Low staff effort

The polymerase reaction
3 phases

A basic PCR reaction has 3 phases:

- Exponential
- Linear (high variability)
- Plateau (End-point: gel detection for traditional methods)



The polymerase reaction

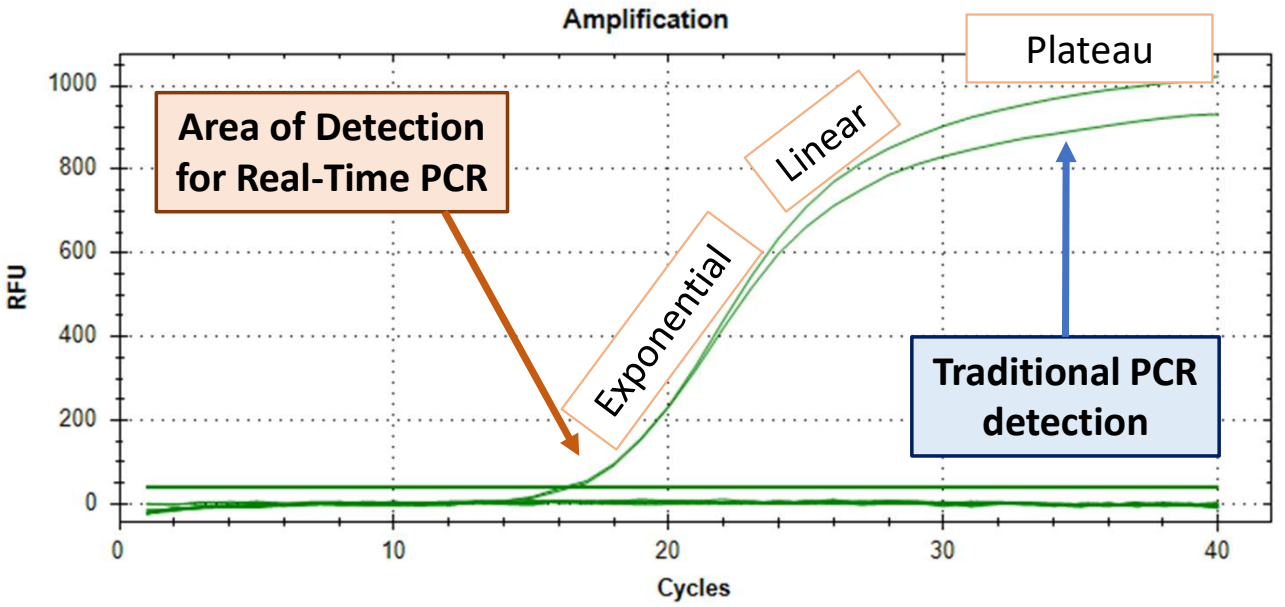
Area of detection

End-point PCR

- Plateau fase: variable results
  - Cloning
  - Sequencing

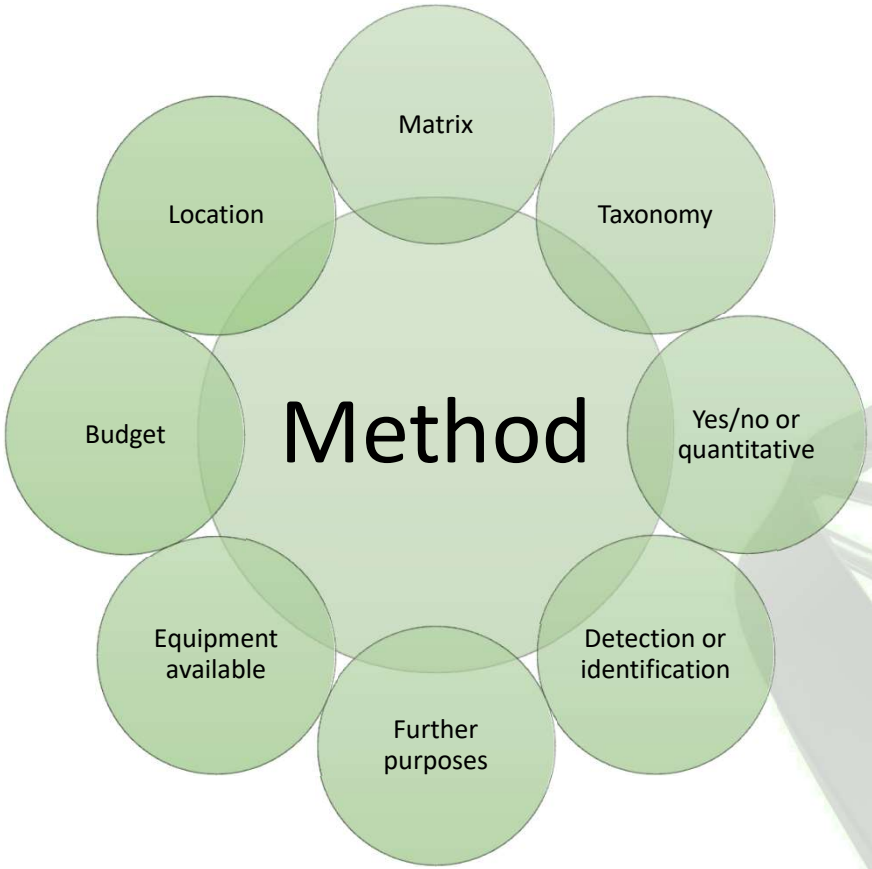
Real-Time PCR

- Exponential fase: precise and accurate
  - Quantification



How to choose the right method?

Scope and context of the analysis



Molecular technologies
End-point & Real-time PCR

End-Point Polymerase Chain Reaction

TARGET

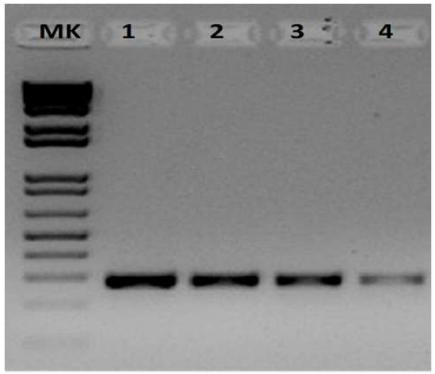
for DNA pathogens:
- End-Point PCR and Nested End-Point PCR

for RNA pathogens:
- End-Point RT-PCR

REVELATION

✓Revelation on agarose gel

✓Possibility of multiplexing



Real-Time Polymerase Chain Reaction

TARGET

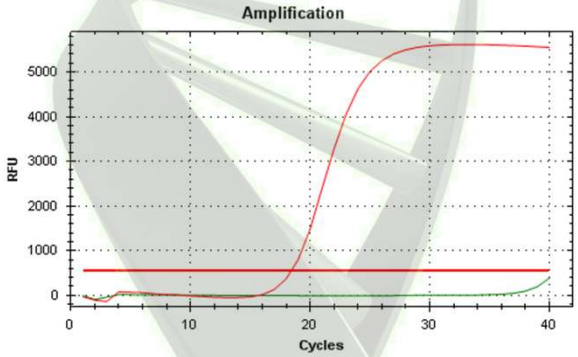
for DNA pathogens:
- Taq-Man® and SYBR-Green®

for RNA pathogens:
- Taq-Man® and SYBR-Green®

REVELATION

✓ Fluorescent dyes reading in real-time

✓Possibility of multiplexing (for Taq-Man®)



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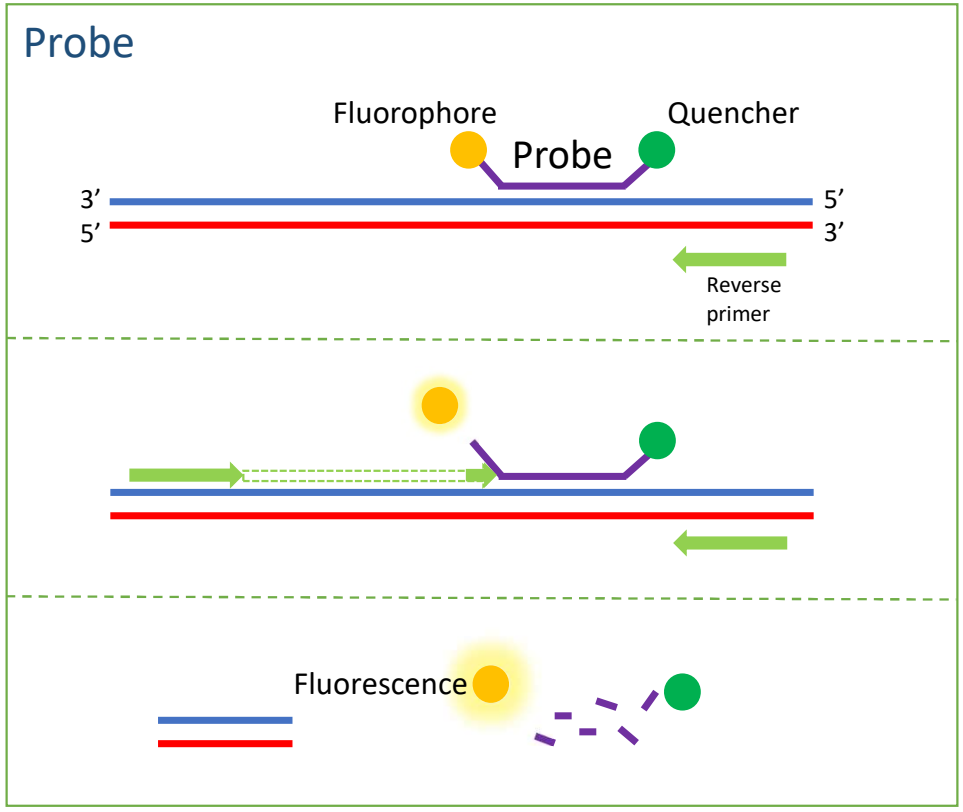
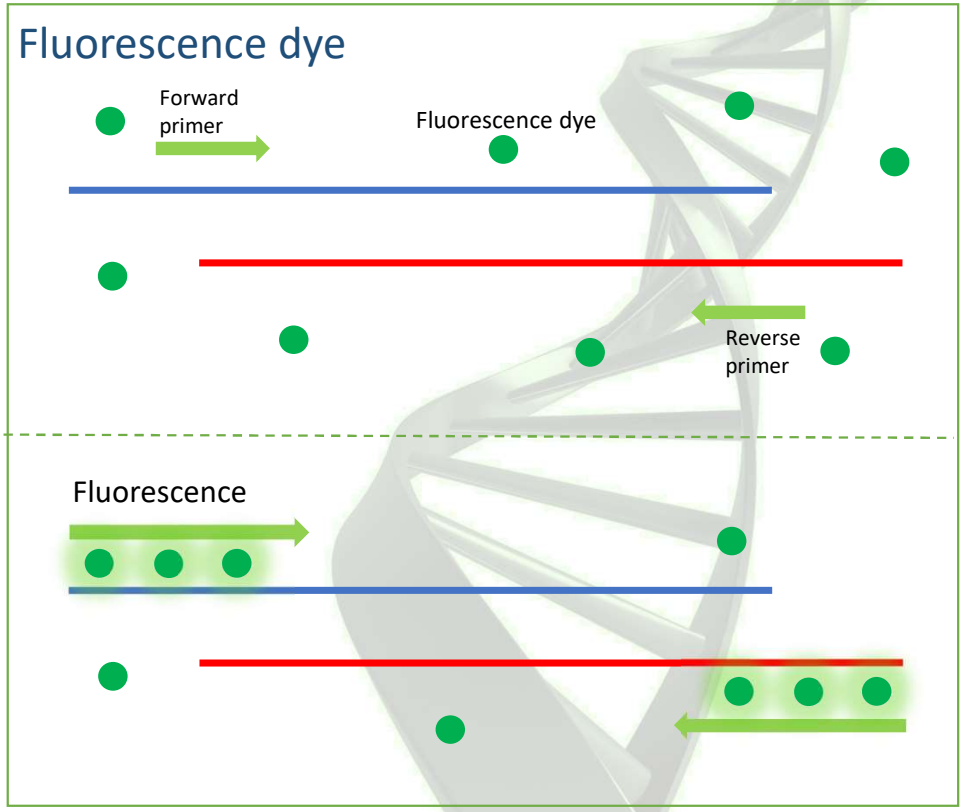
Real-time Polymerase Chain Reaction

Probes vs Fluorescence dye





Molecular methods
Highlights

	End point PCR	Real-Time PCR Probe based	Real-Time PCR Dye based
Sensitivity	☆☆☆	★★☆	★☆☆
Time	☆☆☆	★★☆	★★☆
Quantitative	semi	★☆☆	★☆☆
High throughput	☆☆☆	★☆☆	★☆☆
Easy in use	☆☆☆	★☆☆	★☆☆
Money	★☆☆	★★☆	★★☆
Expertise neccessary	HIGH	LOW	MEDIUM

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Molecular technologies

Advantages and limitations

- Fast development of new tests
- Only available technology for some pests (bacteria, fungi, nematodes and phytoplasma)
- Higher sensitivity than other technologies
  Quantification possible
- High througput
- Fast assays
- Assay can be automated
- Equipments and reagents costs decrease

- Equipped laboratory
- Long sample preparation
- High costs
- Expertise needed
- Lack of referenced commercial kits
- Lack of TPS and ring-test organisation

Molecular technologies

Do's and dont's

- Follow the manufacturer's instructions
- Use sterilised pipettes and tubes to avoid contaminations
- Mix all reagents thoroughly before adding to the plate
- Control the DNA/RNA extraction
- **Fast assays**
- **Assay can be automated**
- **Equipments and reagents costs decrease**

- **Equipped laboratory**
- **Long sample preparation**
- **High costs**
- **Expertise needed**
- **Lack of referenced commercial kits**
- **Lack of TPS and ring-test organisation**

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Sampling and samples preparation

- Homogeneous samples
- Storage
- Biological relevant
  - When to take action/monitor
- Importance of DNA extraction method
  - Quantification
  - Sequencing
  - DNA loss
  - PCR inhibitors
- Sometimes it’s not the PCR but the Extraction

Molecular data analysis

- Positive/negative amplification control
- DNA extraction control
- Melting curve (fluorescent dye only)
- Quantification? Framework (calibration line, EC)

Validation restriction

- Reference material
- Cross-reaction
- Variation between equipment and labs
- Spiking → Real samples

Thank you for your attention!

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