Diagnostician training

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Workshop outline

Morning Session - qPCR

8:45 Sample preparation overview
9:15 qPCR overview
9:45 Break
10:00 qPCR setup
11:00 qPCR lectures - SDS and *Phytophthora* specific discussion

12:00 Lunch

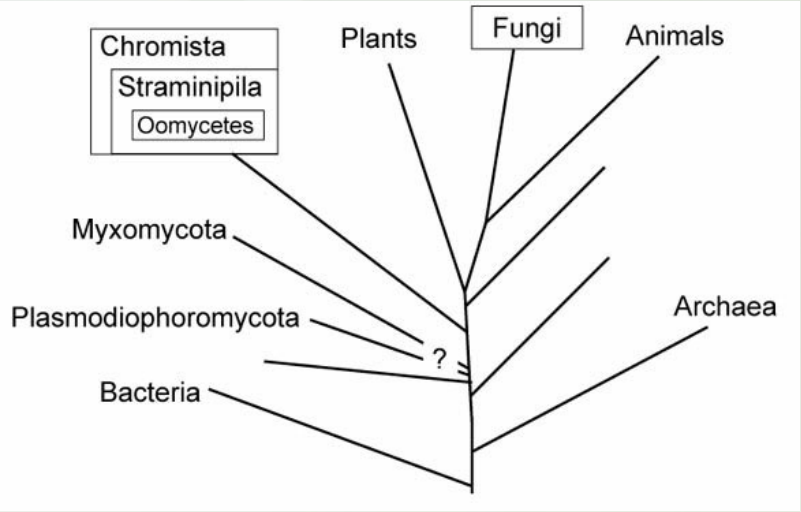
Afternoon Session - Isothermal amplification and RPA

1:00 Review of qPCR results
1:30 RPA overview
2:15 RPA sample prep and reaction setup
3:00 Run RPA reactions
3:30 Break
3:40 Review RPA results
4:00 Q & A session

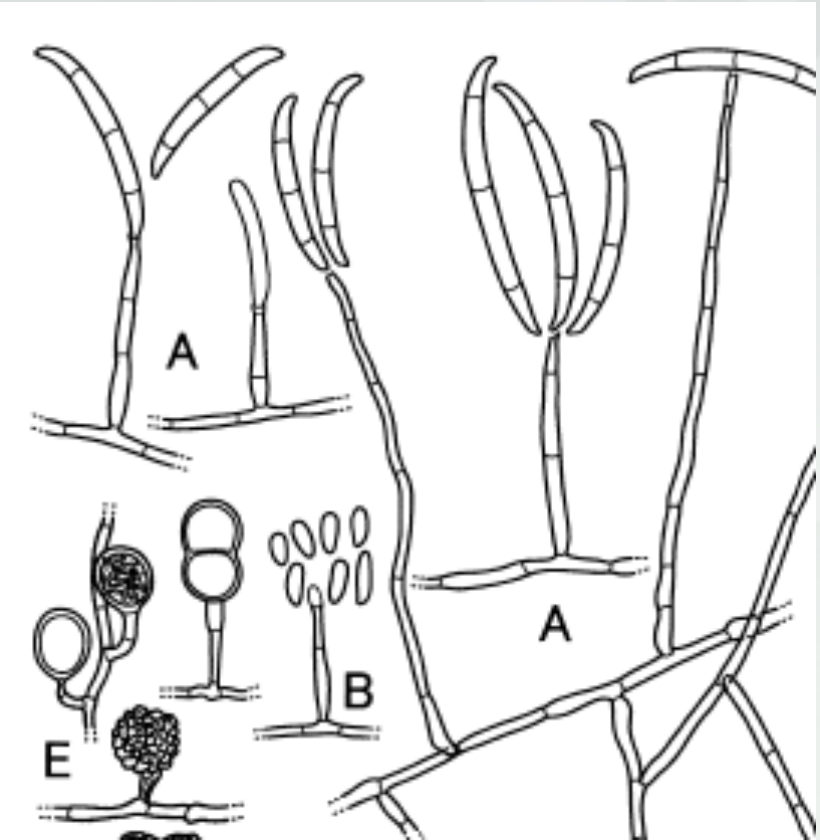
Workshop wrap up - questions and survey

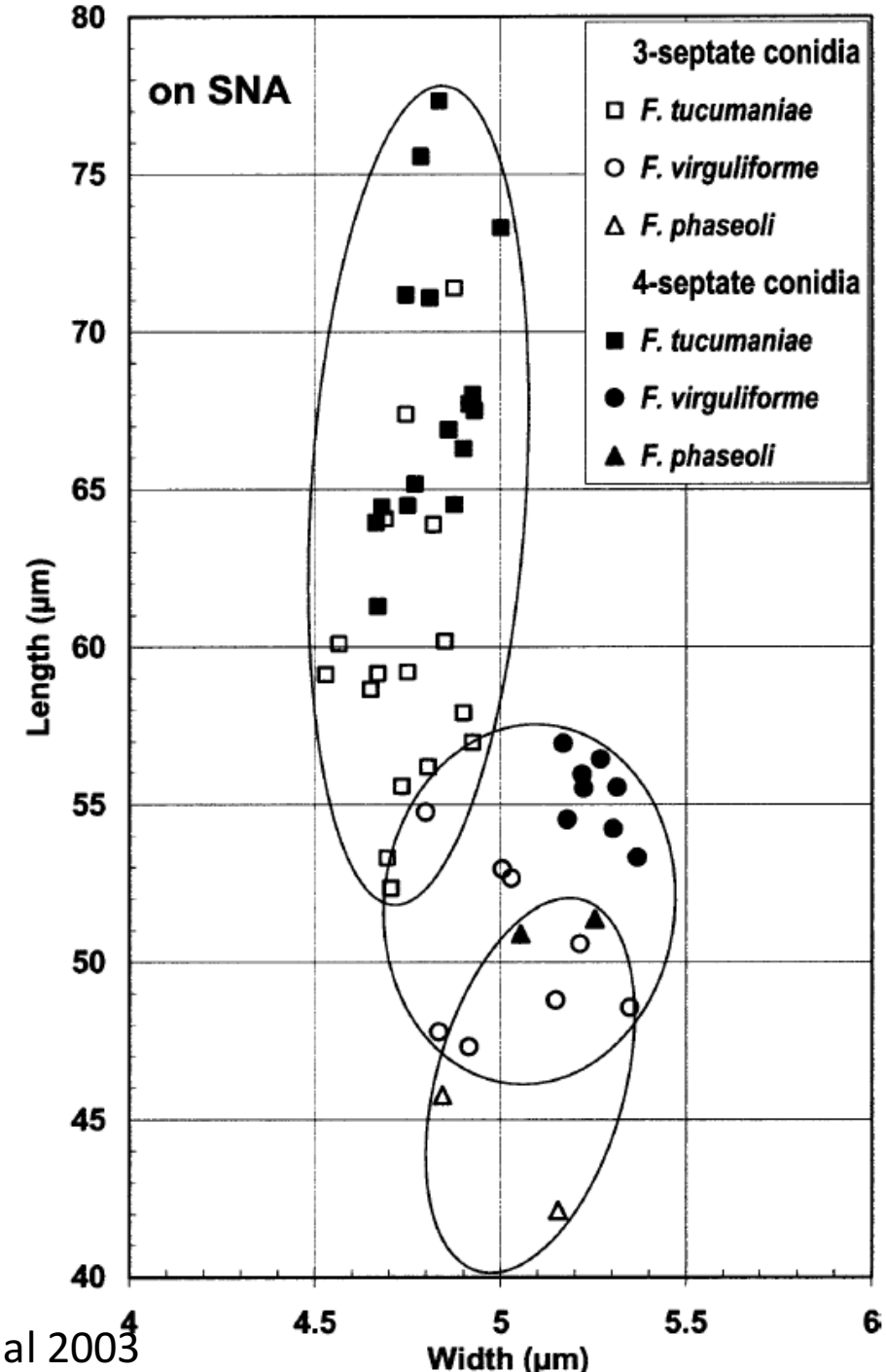
Why use molecular techniques?

- Not reliant on morphology
- Very useful it traits are limited or plastic
- Also useful due to convergent evolution
- Useful for resolving relationships
- May support/resolve morphological taxonomy



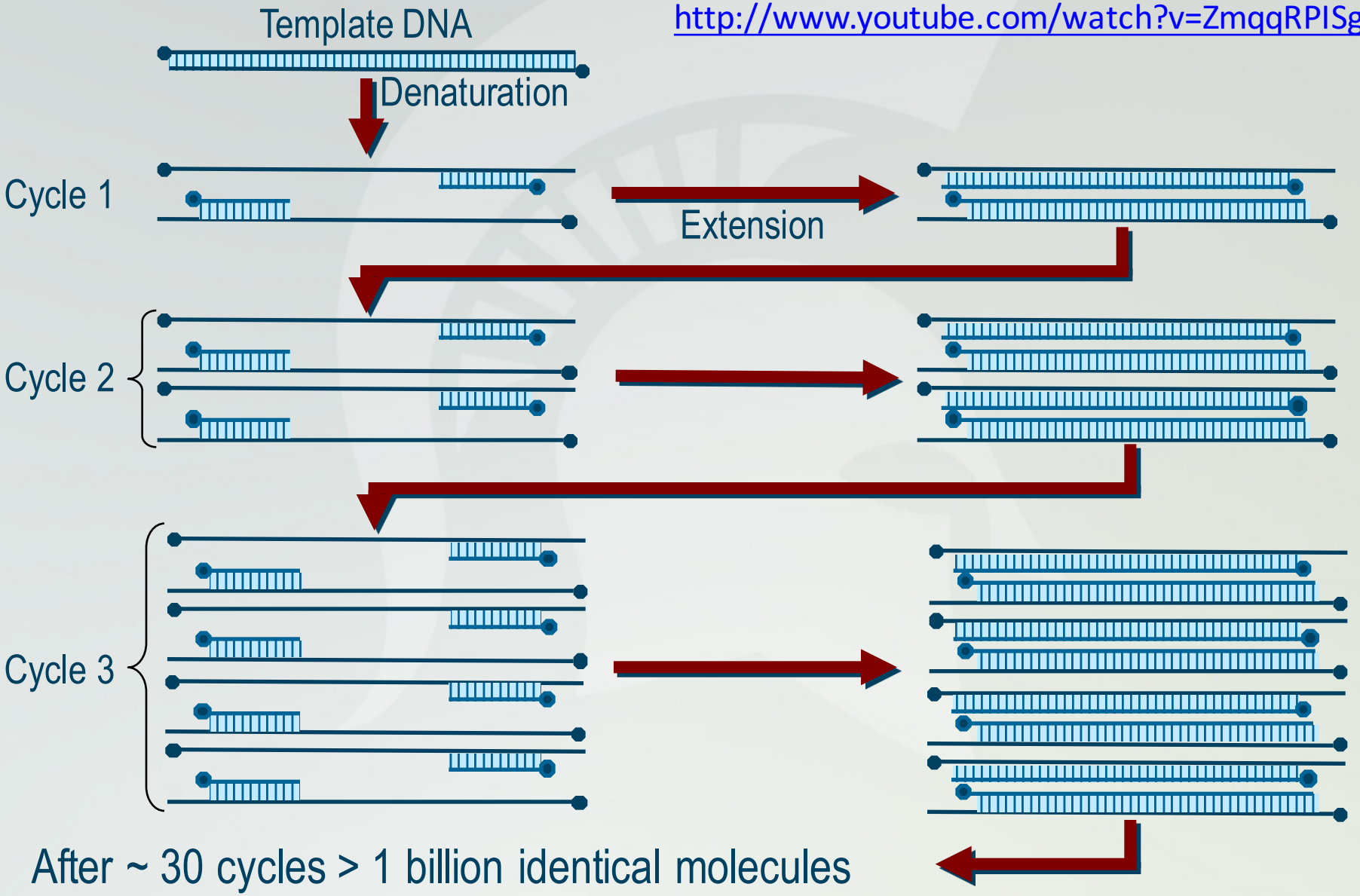
- *Fusarium* spp.
- Overlapping morphology





Aoki et al 2003

Fundamentals of PCR



http://www.youtube.com/watch?v=ZmqqRPISg0g

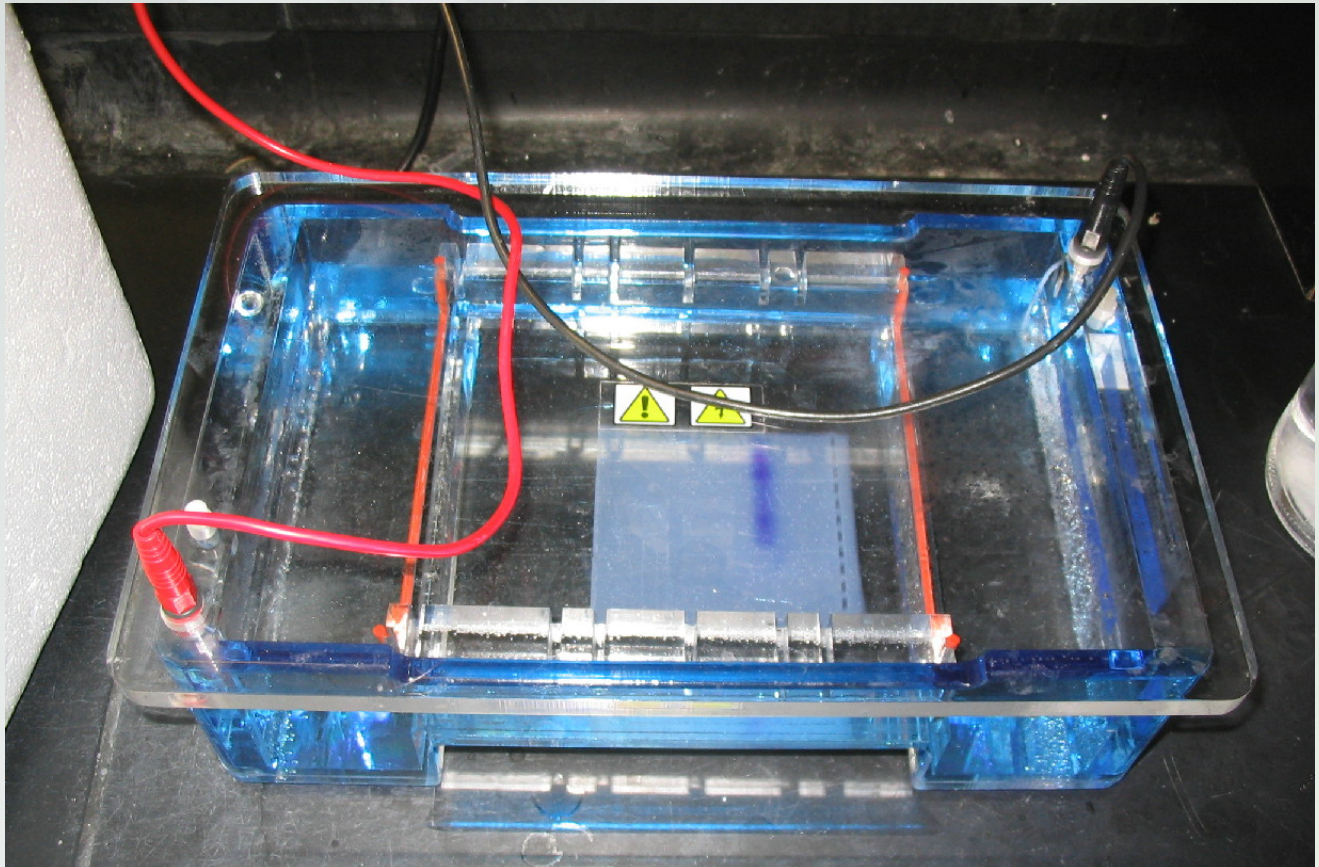
After ~ 30 cycles > 1 billion identical molecules

PCR

PCR Thermocycler

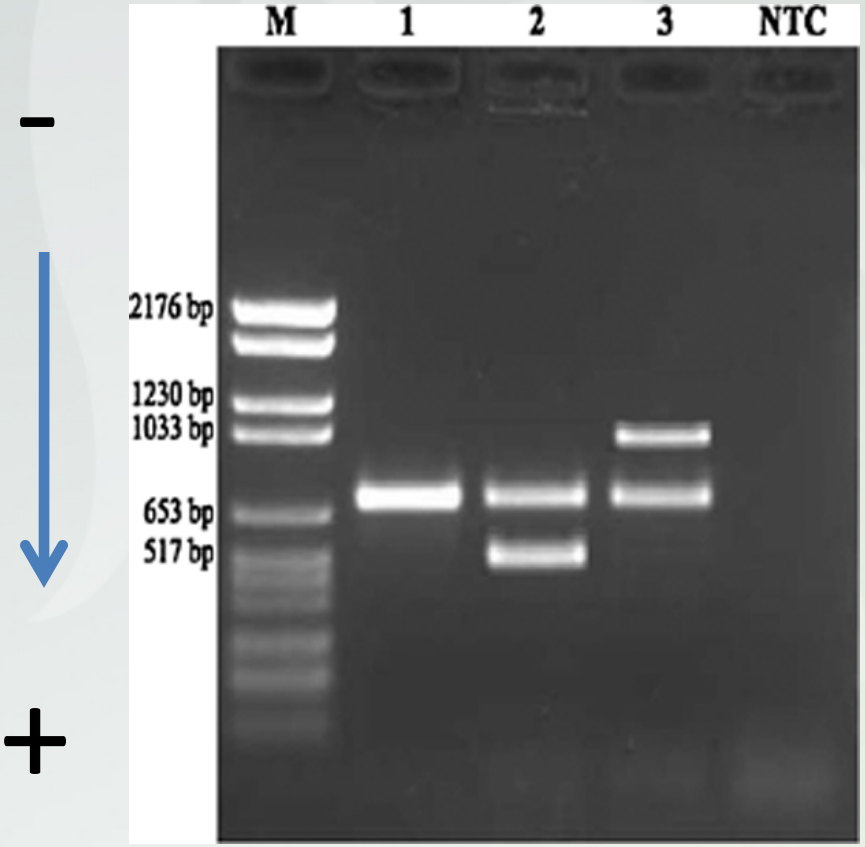
Agarose gel electrophoresis

- DNA is separated by migration through a gel due to voltage gradient, small molecules travel faster than larger ones



Agarose gel electrophoresis

- Using a DNA ladder of known size we can determine the size of our PCR product



Real-time PCR instrument (examples)

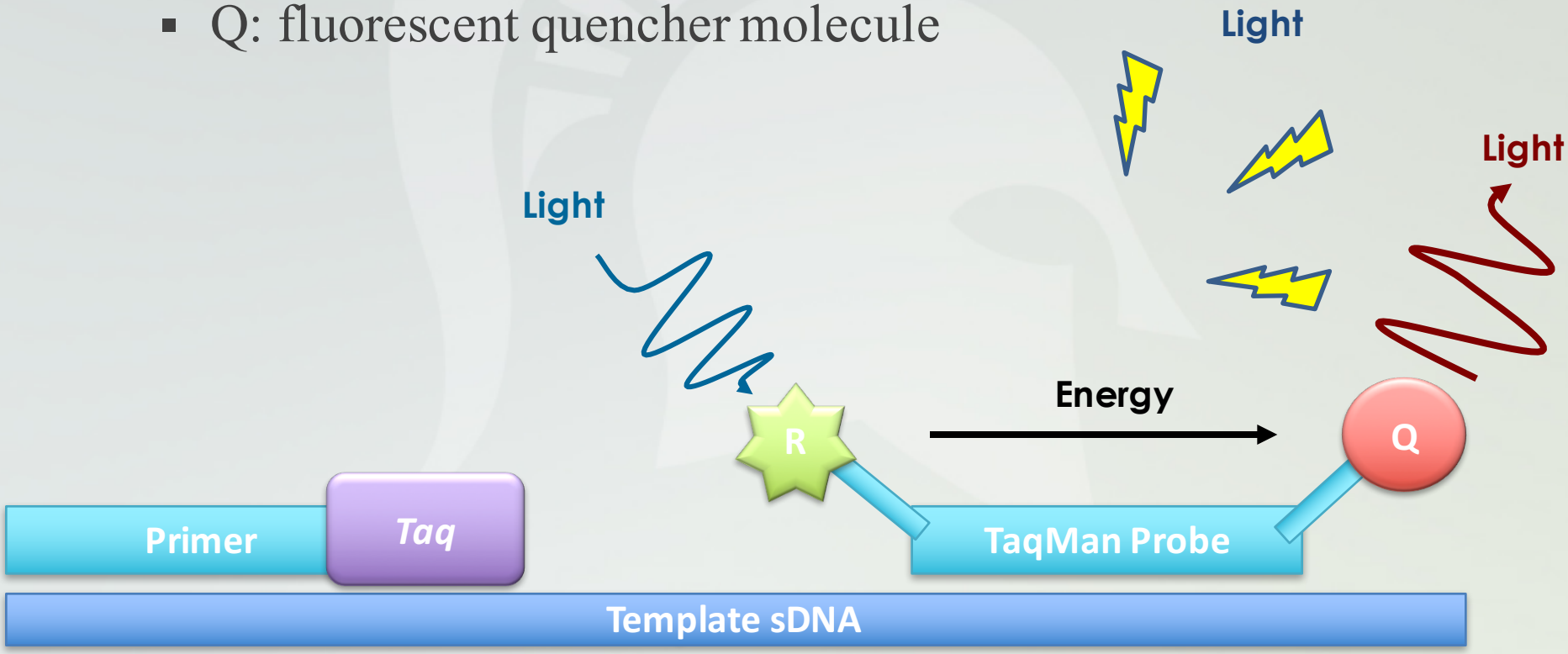
Bio-Rad

Cepheid

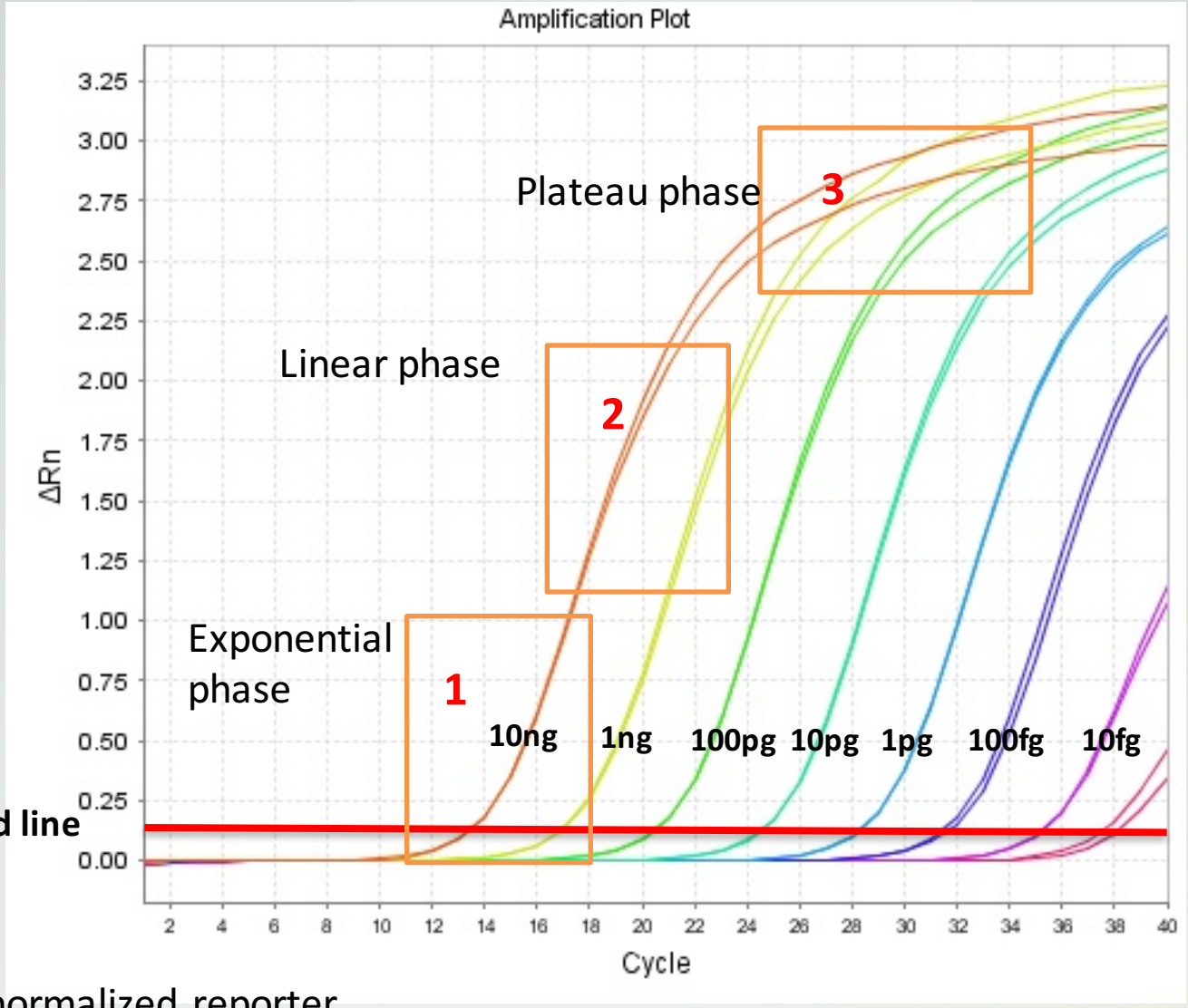
Applied Biosystems

Real-time qPCR assay

- **Hydrolysis probe chemistry**
  - R: reporter fluorescent molecule
  - Q: fluorescent quencher molecule

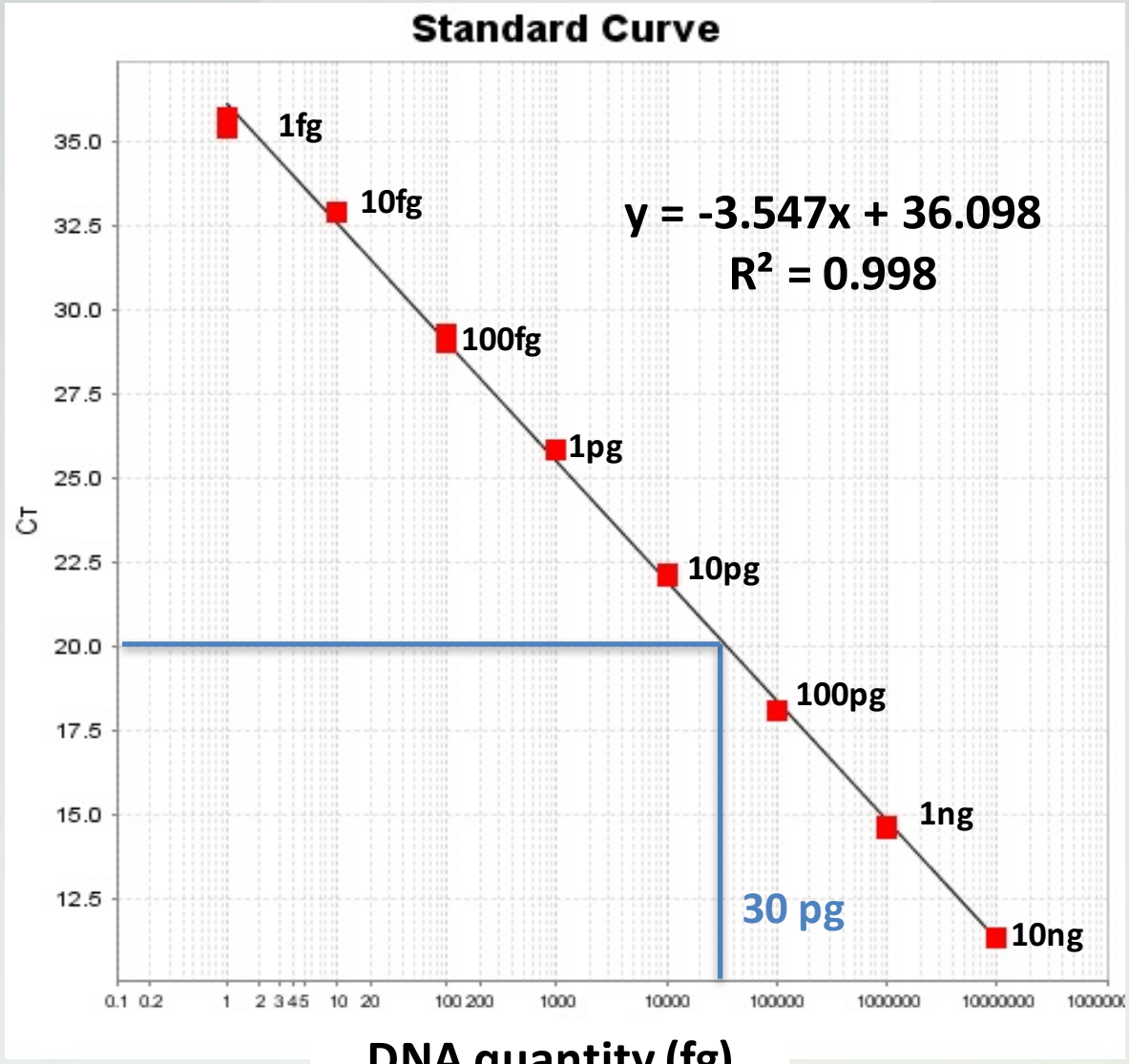


qPCR Serial Dilution Standard Curve



ΔRn: Delta normalized reporter

Unknown Sample Quantification



Real-time PCR Advantages

- Quantifiable result
- Larger dynamic range ~ 7 orders of magnitude
- Quicker time to result than end-point PCR
- Low-medium sample throughput
  - i.e. single tube reactions, 96/384 well plates, OpenArray - 3072 wells