LAMP-BART

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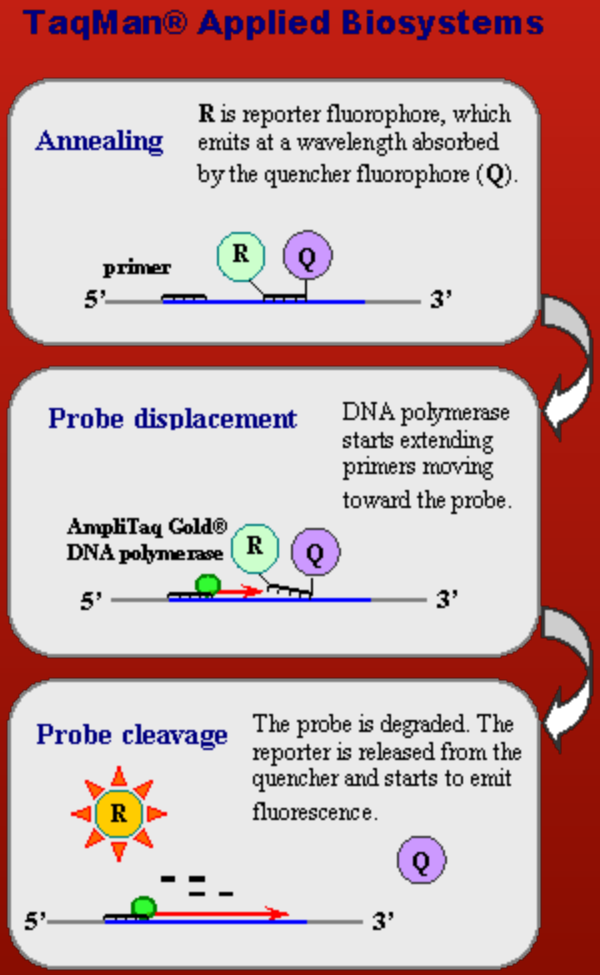
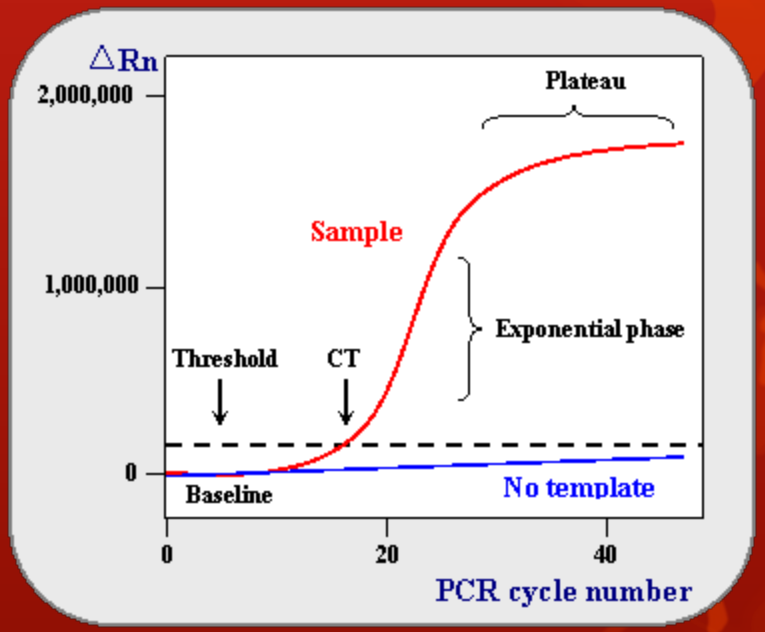
Current molecular diagnostic system

- Detection of target nucleic acid
- quantitative PCR (qPCR)
- Fluorescent dye/fluorescently-labelled oligonucleotides

Quantitative PCR (qPCR)





NCBI 2012. Published online: http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechQPCR.shtml

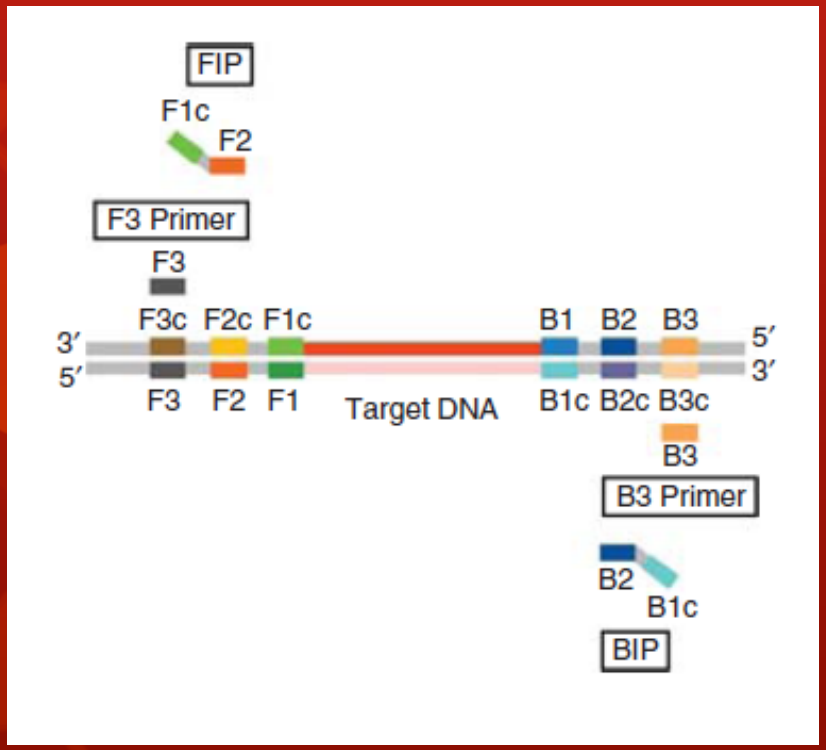
Quantitative PCR (qPCR)

- Strict requirements on assay equipments
  - Temperature cycling
  - Wavelength-specific fluorescent excitation
  - Emission measurement
- Limitations
  - Power consumption
  - Optical arrangements required
- Production of low-cost, simple and robust instruments

LAMP-BART

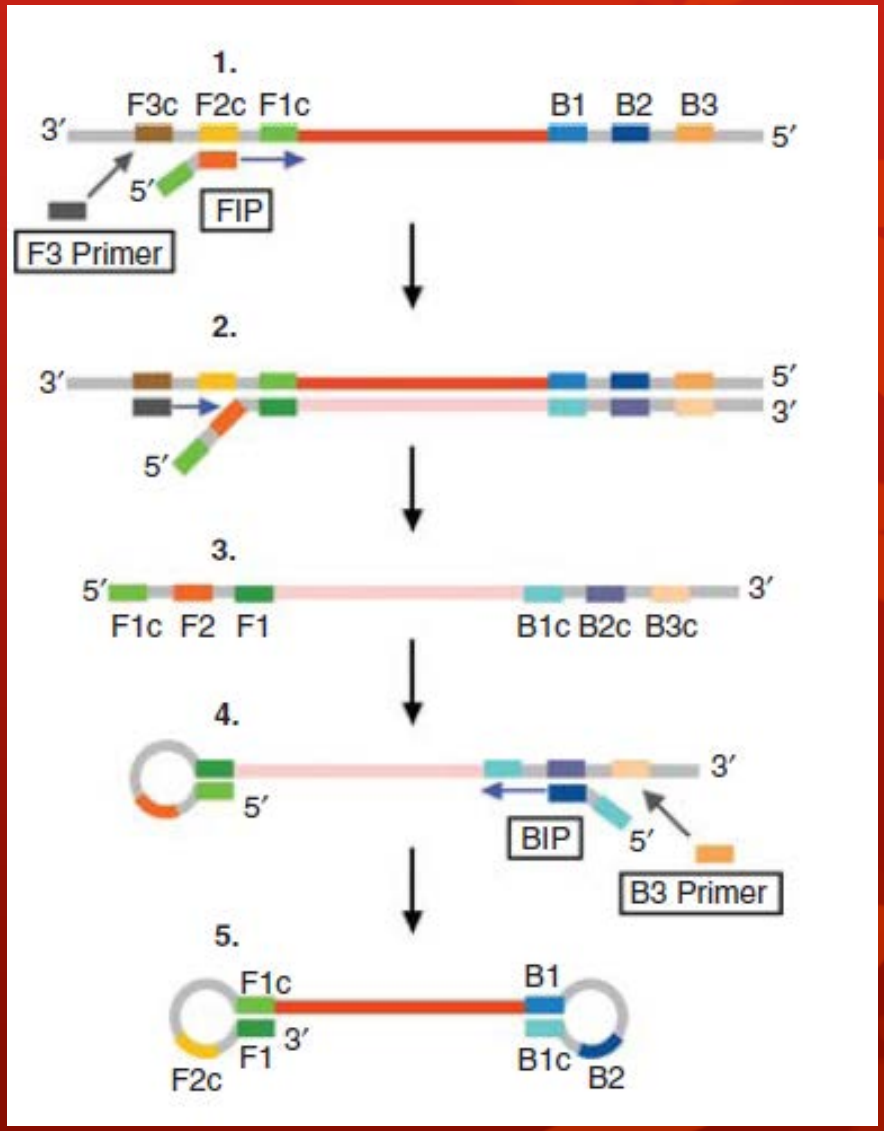
- LAMP
- Loop-mediated amplification
- Alternative amplification method
- Strand-displacing polymerase
- Isothermal amplification (≤65°C)
- 4 primers recognize 6 regions

LAMP



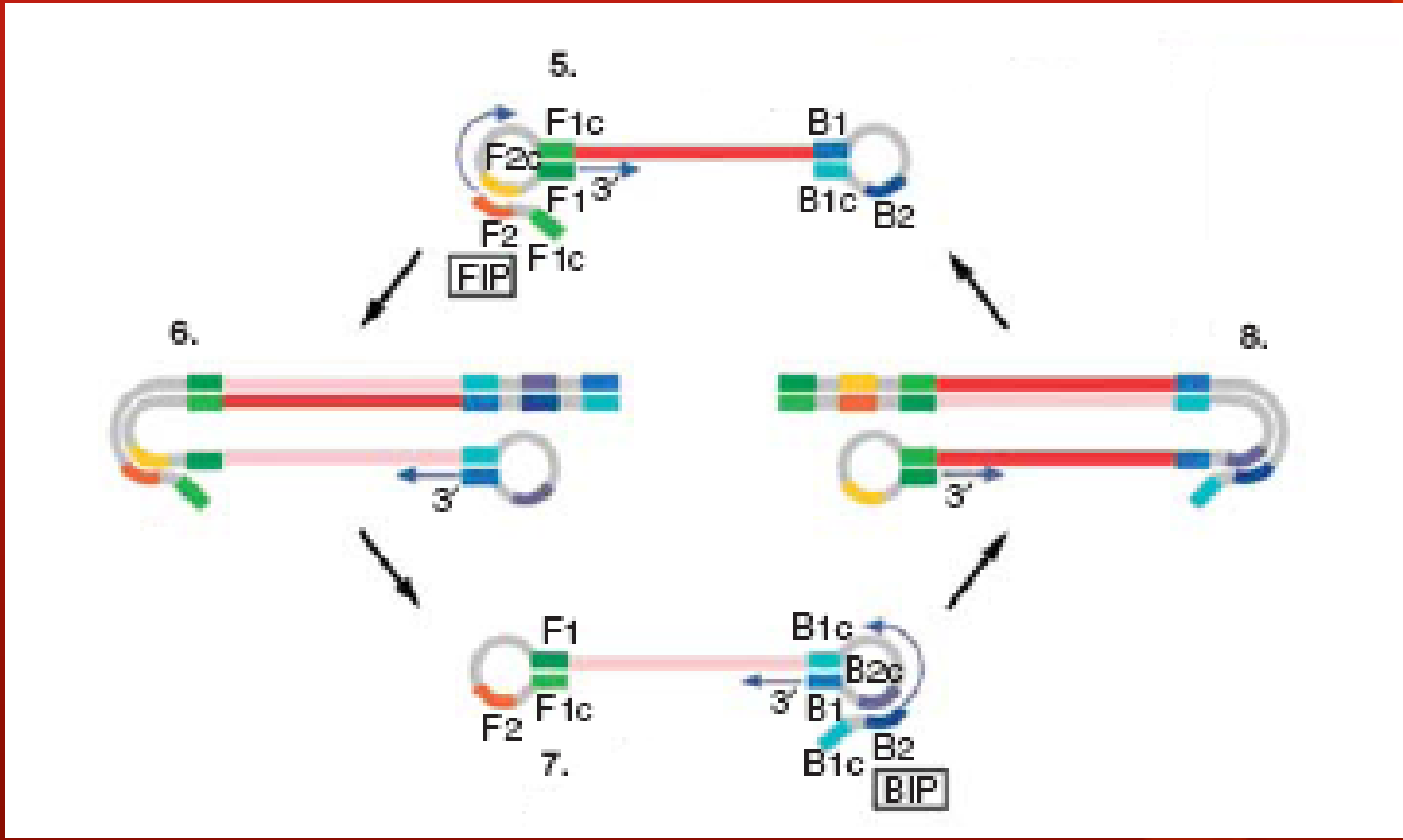
Tomita *et al*. (2008)

1. Starting-structure producing step



LAMP

2. Cycling amplification step



Tomita *et al*. (2008)

LAMP-BART

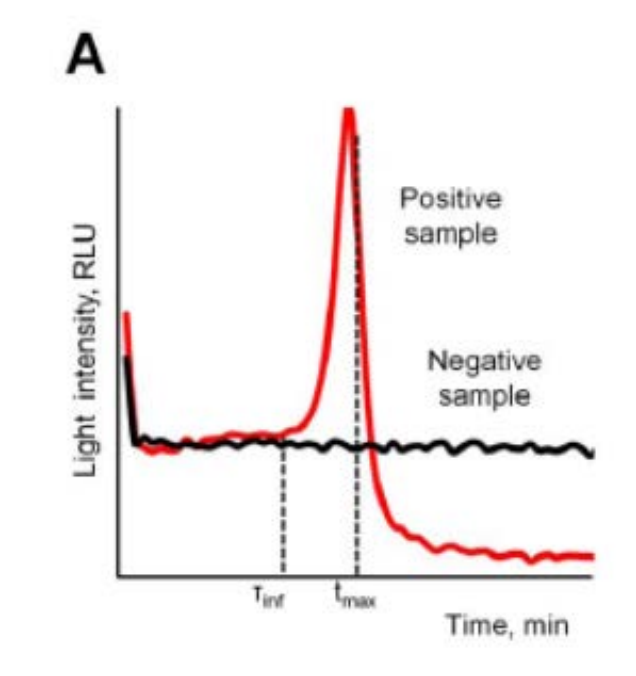
- BART
- Bioluminescent Assay in Real-Time
- Alternative amplification detection method
- Inorganic pyrophosphate (PP_i)
- By-product of all amplification

BART

Gandelman *et al (*2010)

- 1 molecule released each nucleotide addition
- Proportional to amount of polynucleotide synthesized
- Starting template concentration

LAMP-BART

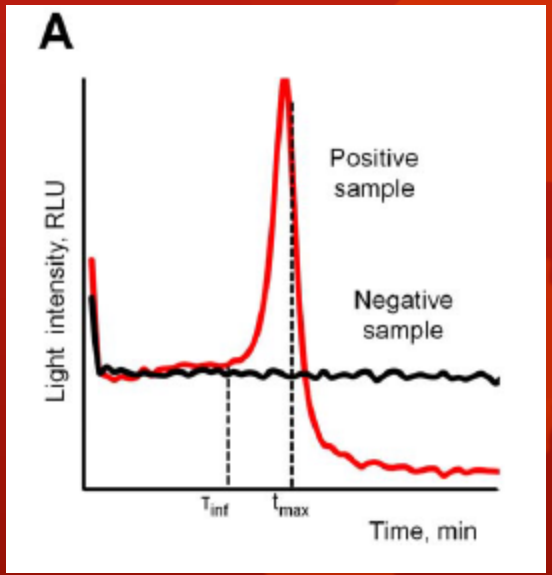


Gandelman *et al (*2010)

55°C 1hr

LAMP-BART

- qPCR curve vs BART curve
- Sigmoidal curve vs sharp peak
- Rapid reduction in bioluminescence after the peak
  - APS exhausted
  - PPi inhibits luciferase



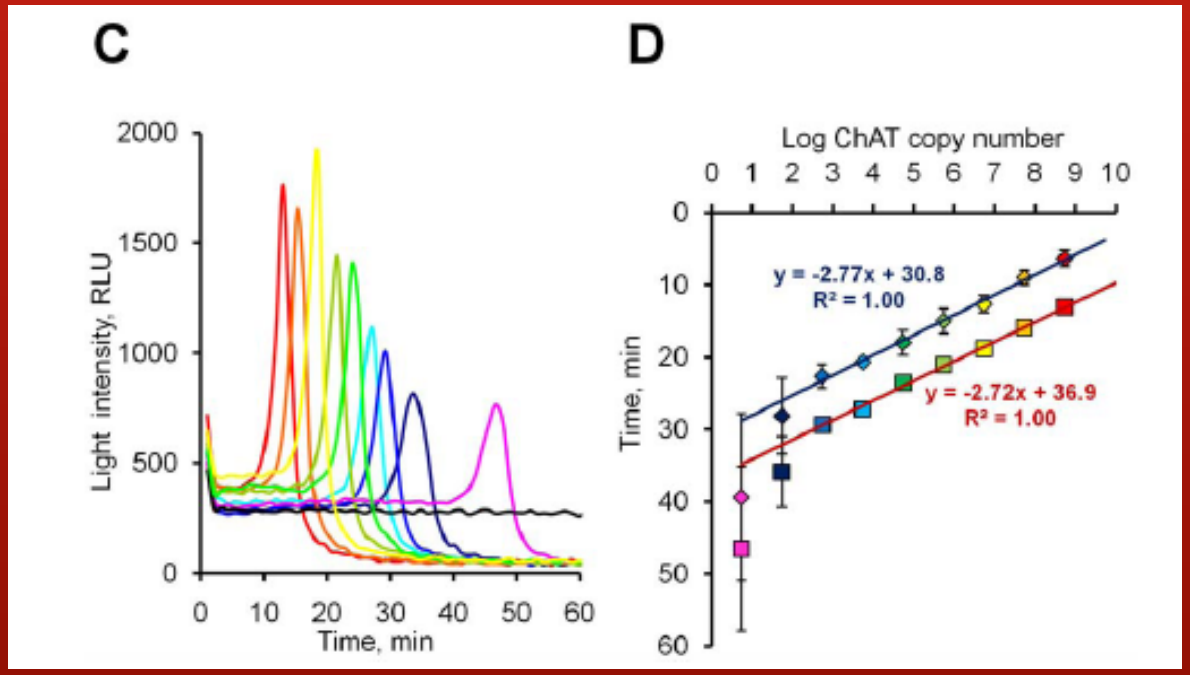
$(DNA)_n + dNTP \rightarrow (DNA)_{n+1} + PP_i$ — *DNA polymerase* (1)

$PP_i + APS \rightarrow ATP + SO_4^{2-}$ — *ATP sulfurylase* (2)

$ATP + O_2 + luciferin \rightarrow Oxyluciferin + AMP + PP_i + CO_2 + light$ — *Firefly luciferase* (3)

Gandelman *et al* (2010)

Quantitative LAMP-BART



Gandelman *et al (*2010)

Copy Number: 10^8-10

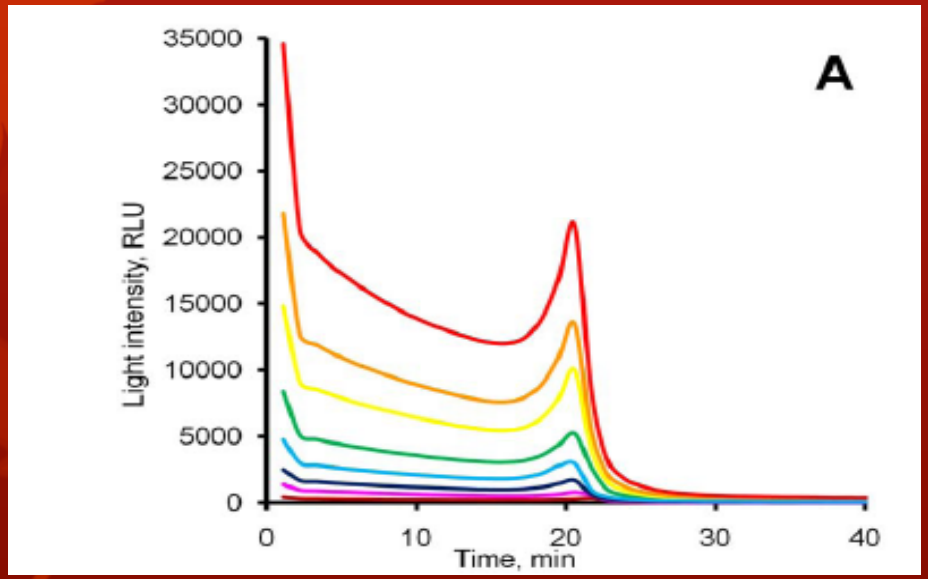
Linear correlation, similar to correlation of Ct value to DNA template load in qPCR

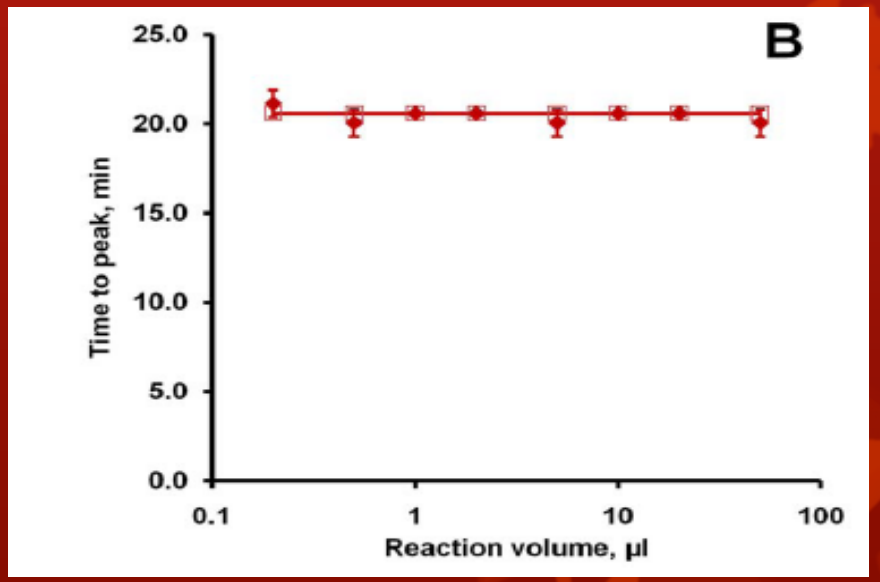
Quantitative LAMP-BART

- Time required for same amount PP_i to be released = amount of target nucleic acid
- Time-to-peak ɑ starting target gene conc.

Quantitative LAMP-BART

- Amplification with same DNA conc. but different reaction volume
- Light intensity, reduction in volume
- Time-to-peak unchanged
- Depend on time-to-peak, not absolute light intensity output





Gandelman *et al* (2010)

Application of LAMP-BART

- Detection of *Chlamydia trachomatis* (*CT*) in clinical specimens
- 105 clinicial urine specimens of unknown *CT* status
- Bacterial DNA isolated
- Compared with qPCR result

Application of LAMP-BART

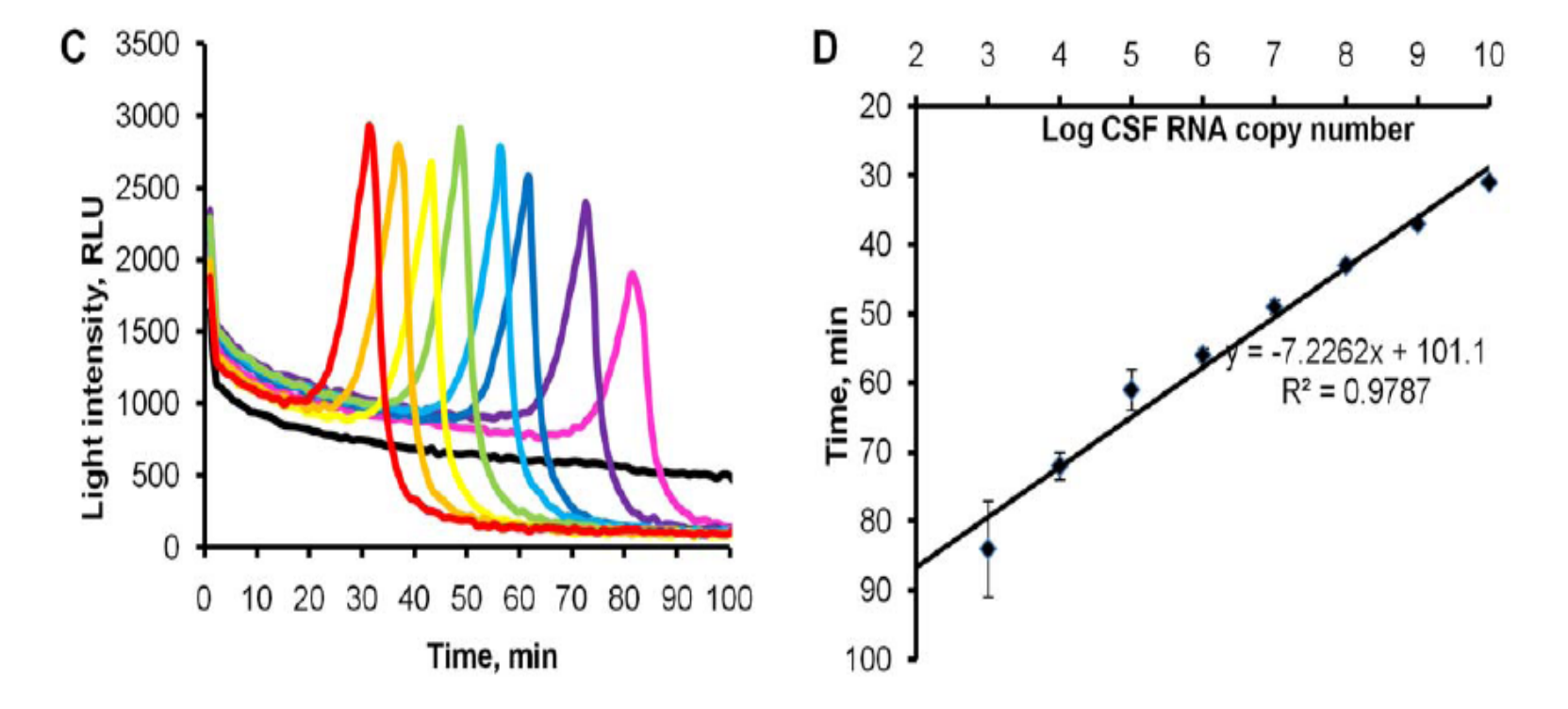
	LAMP-BART	qPCR
Total number of samples	105	105
CT-positive samples	43 (t_{max}<60 min)	45 (Ct≤40 cycles)
CT-negative samples	62	60 (Ct>40)
Sensitivity, %	95.6	100
Specificity, %	100	100
Assay time	60 min	2.5 hours
Mean t_{max} or Ct/equivalent time	33.6 min	35.2 cycles ~1 h 46 min

Gandelman *et al (*2010)

Application of RT-LAMP-BART

- Classical swine fever virus
- Detection of RNA template
- Purified RNA amplified in closed-tube one-step format
- Reverse transcription, LAMP amplification, BART detection reagents

Application of RT-LAMP-BART

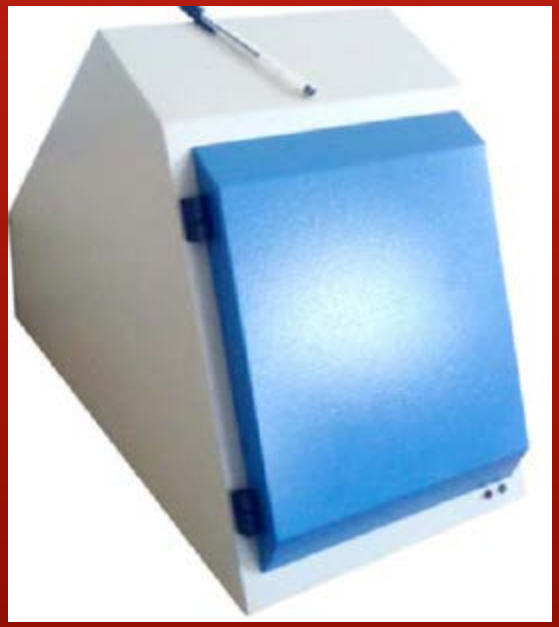


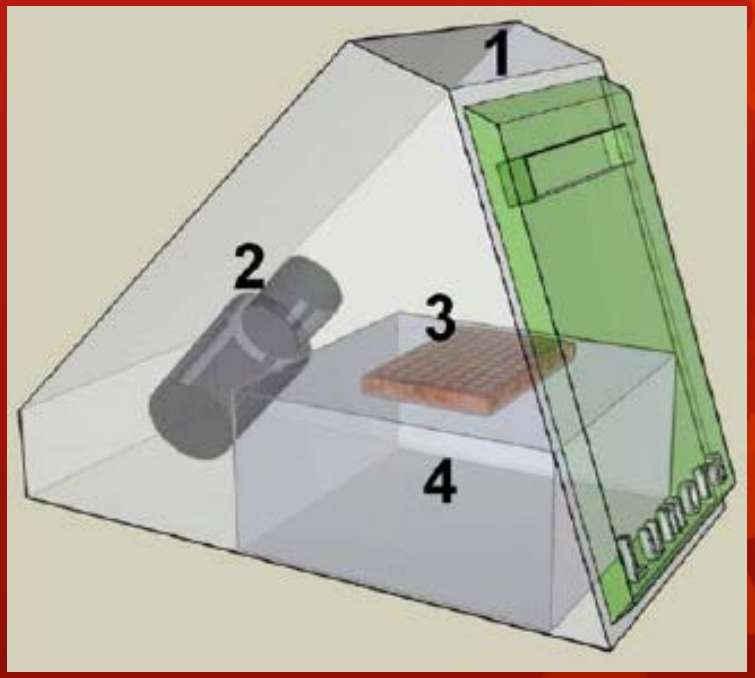
Gandelman *et al* (2010)

Copy Number: 10^{10}-10^{3}

Coupled RT-LAMP-BART quantifiaction and detection of viral RNA genome for diagnostics

Instruments for LAMP-BART





Gandelman *et al (*2010)

Advantages of LAMP-BART

- Coupled amplification and detection system in single closed tube
- Reduce risk of contamination
- Effectively used on patient-derived samples, tolerance to inhibitors in patient sample
- Straightforward, simple, rapid
- LAMP: 4 primers, 6 recognition sites, high specificity
- Cost-effective
  - Simple light detector
  - No need temperature cycle, wavelength-specific fluorescent excitation, emission measurement
  - Require only a constant temp. maintained by heat block
- Quantitation rely on time-to-peak but not absolute intensity
  - Tolerance to contaminating ATP or PP_i
  - Rate of change not absolute level determined

Possible drawback of LAMP-BART

- Single signal, not possible for mutiplex PCR

Conclusion

- Novel coupled amplification and detection
- Single close-tubed format
- Simple, fast, cost-effect quantitative assay

References

- NCBI. 2012. Published online: http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechQPCR.shtml
- Tomita *et al*. 2008. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. Nature Protocols 3, - 877 - 882
- Gandelman *et al*. 2010. Novel Bioluminescent Quantitative Detection of Nucleic Acid Amplification in Real-Time. PloS ONE, 5(11): e14155
- Kwok J and Kwong KM. 2012. Loop-mediated isothermal amplification for detection of HLA-B*58:01 allele. Tissue Antigens., ISSN 0001-2815
- Kiddle *et al*. 2012. GMO detection using a bioluminescent real time reporter (BART) of loop mediated isothermal amplification (LAMP) suitable for field use. BMC Biotechnol. 30;12:15