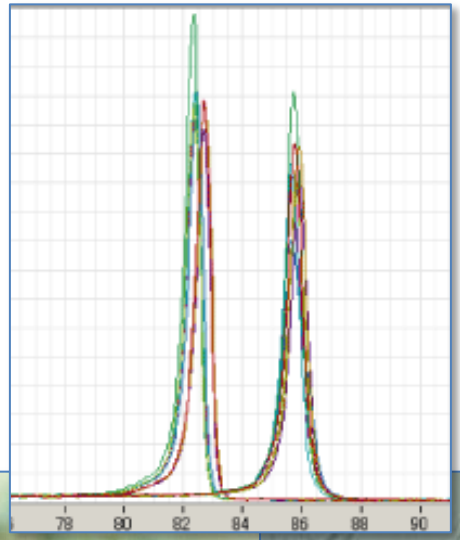
Molecular diagnostics for the fast identification of organisms

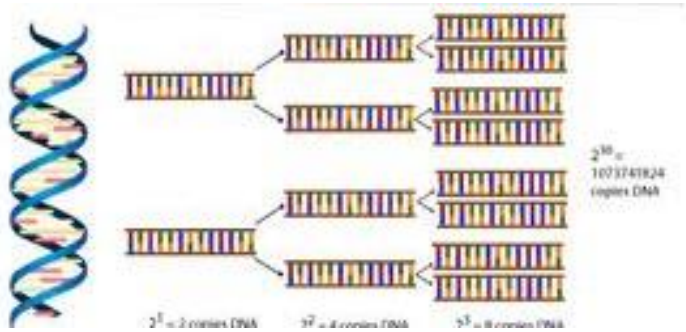


typical analysis through PCR: time

- DNA extraction

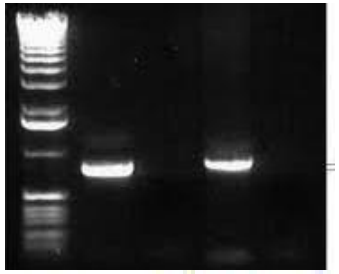
2-12 hours

- DNA target amplification(PCR)



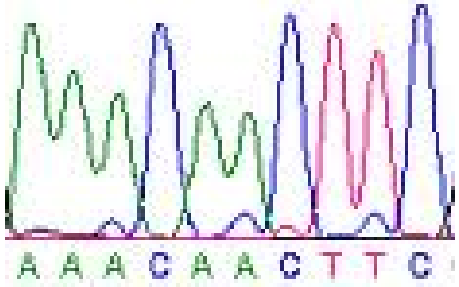
2 hours

- Visualization of PCR results



1 hour

- Further analyses(restriction/sequencing)

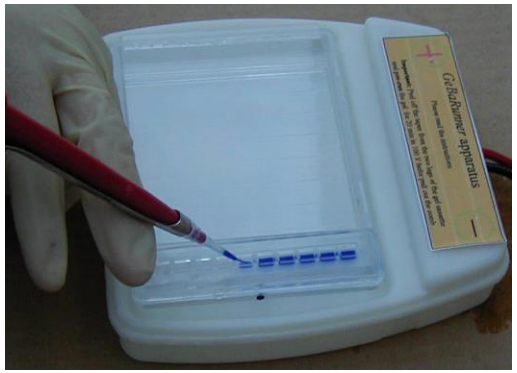


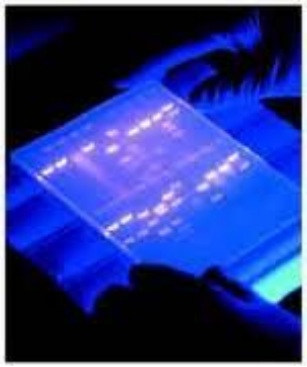
4-48 hours

typical analysis through PCR: tools

Extraction

PCR





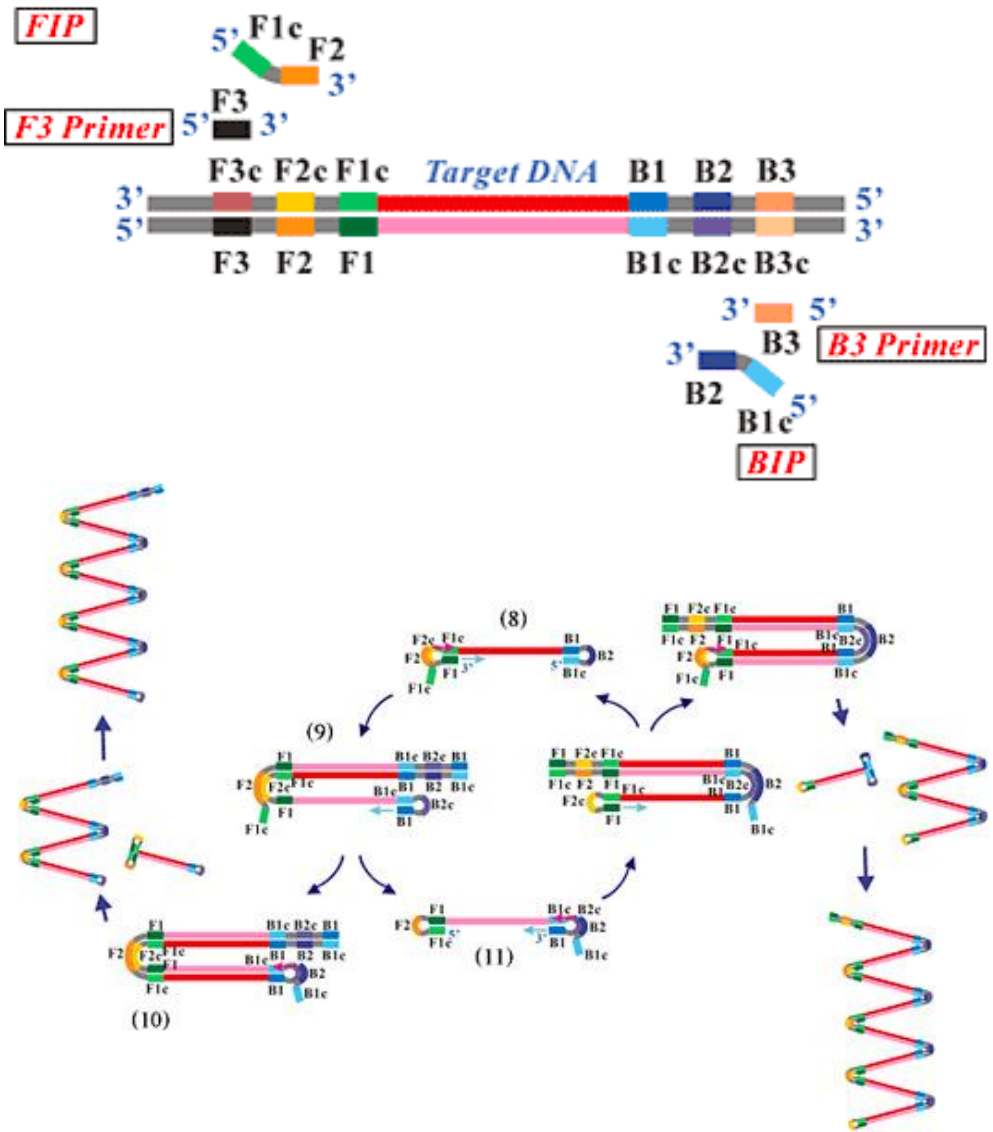
Monitoring with fast molecular tools

Development of DNA extraction/amplification protocol :

- easy
- fast
- sensible
- applicable on both DNA and RNA
- available in the field

LAMP – Loop mediated Isothermal Amplification

- amplification at costant temperature (65°C)
- High efficiency and stability of the reaction: 10^9- 10^{10} copies of the target in 10- 40 minutes
- High specificity

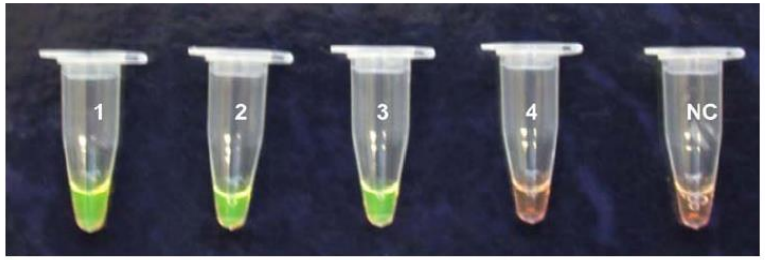


LAMP: further features

- Efficiency like Real Time/nested PCR
- Less prone to inibition due to contaminations
- More stable reagents (no need of fridge/freezer during transport)
- No need of lab instruments to visualize results

LAMP: results visualization

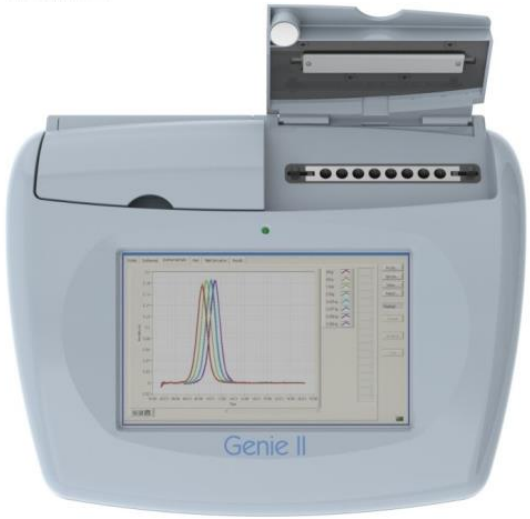
Fluorescence



Turbidity

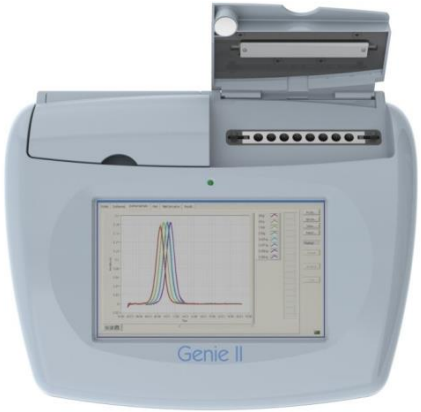
Color change

Genie II



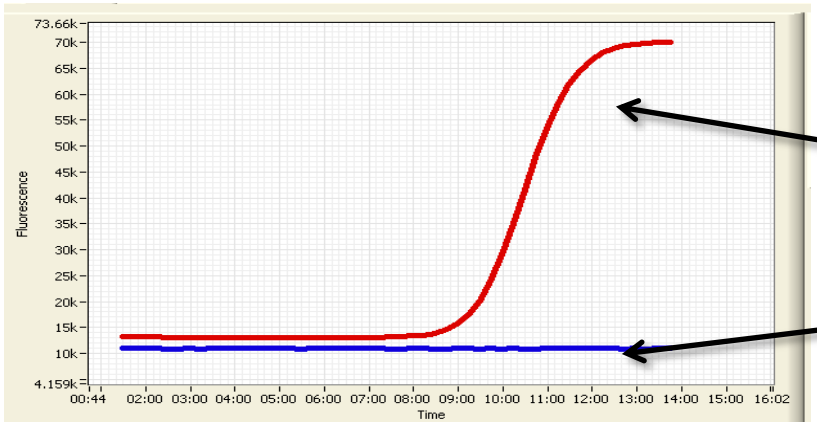
Amplification and visualization in the same tool:

Genie II



- **16 wells**
- **Results in real time**
- **No need of a PC**
- **Rechargeable batteries lasting several hours**

- Results as positive/negative after ≈ 30 min

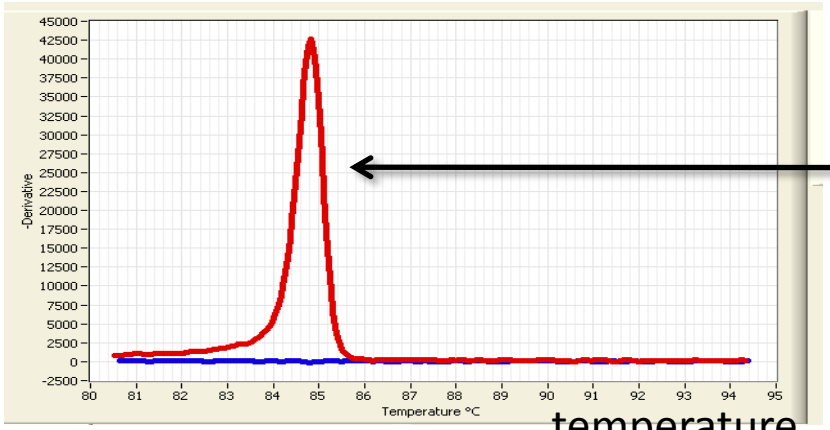


time

Positive outcome = presence of the target organism

Negative outcome=no target organism

- check of specificity through annealing temperature



temperature

Annealing curve

LAMP validation

- **Sensibility test** : detection limits

- **Specificity test**: ability to doscriminate between target organism and related species

- LAMP primers specific for *Torymus sinensis*
- Amplification of *T. sinensis* but not of other *Torymus* species
- Test used to verify the identity of released parasitoids

Torymus sinensis?

Yes

No

~30 minuti

First screening:

Fast molecular tools

Confirmation screening:

Classical molecular tools (lab)