QBOL: Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health

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Development of accurate identification tools for plant pathogens and pests is vital to support European Plant Health Policies. For this project Council Directive 2000/29/EC is important, listing some 300 organisms for which protective measures against introduction into and their spread within the Community needs to be taken. Those threats are now greater than ever because of the increases in the volumes, commodity types and origins of trade, the introduction of new crops, the continued expansion of the EU and the impact of climate change.

Currently identifying pathogens (in particular new emerging diseases) requires a staff with specialised skills in all disciplines (mycology, bacteriology, etc.); which is only possible within big centralised laboratory facilities. Taxonomy, phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are threatened with extinction.

Modern molecular identification/detection techniques may tackle the decline in skills since they often require much less specialist skills to perform, are more amenable for routine purposes and can be used for a whole range of different target organisms. Recently DNA barcoding has arisen as a robust and standardised approach to species identification. QBOL wants now to make DNA barcoding available for plant health diagnostics and to focus on strengthening the link between traditional and molecular taxonomy as a sustainable diagnostic resource.

Within QBOL collections harbouring plantpathogenic Q-organisms will be made available. Informative genes from selected species on the EU Directive and EPPO lists will be DNA barcoded from vouchered specimens. The sequences, together with taxonomic features, will be included in a new internet-based database system. A validation procedure on developed protocols and the database will be undertaken across worldwide partners to ensure robustness of procedures for use in a distributed network of laboratories across Europe.
More information can be found at www.qbol.org.

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Peter Bonants

AESAN/EFSA workshop Seville, 10 February 2010

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Introduction

- Detection (techniques)
- Past / Now / Future
- Qualitative or Quantitative, Single or Multiplex?
- Examples
- EU project QBOL
- Conclusions

Agricultural products endangered

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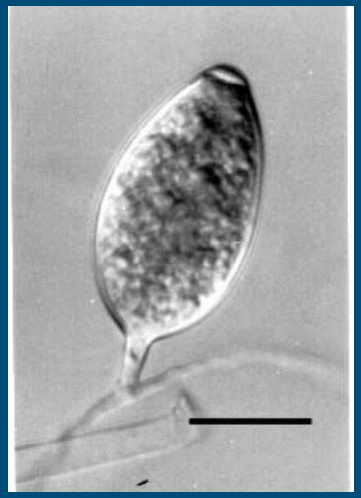
Targets

- Which?
  - Bacteria
  - Viruses
  - Nematodes
  - Fungi
  - Insects
  - Fytoplasms
- Where?
  - in plant
  - in water
  - in soil, compost
  - in air

What is detection?

- Detection is an activity focusing on demonstrating the presence (or absence) of a certain pathogen which is known or suspected to occur in the sample
  - single and multiplex tests
  - specificity, sensitivity
  - diagnostics, monitoring
  - quantitative / qualitative
  - live – dead
  - races – formae speciales

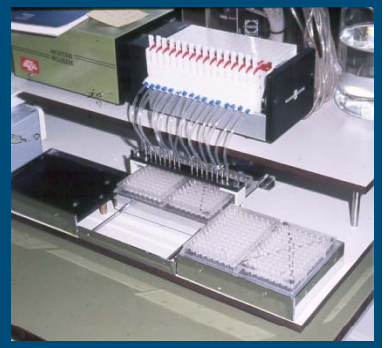
Techniques for identification/detection:



- Based on physiologic characteristics
- Based on biological characteristics
- Based on morphological characteristics (microscopy)
- On protein/carbohydrate level:
  - Antisera
  - Isozyme-patterns

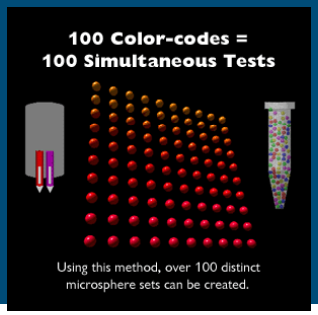
Detection with protein based techniques

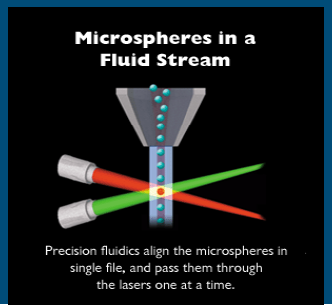
- ELISA

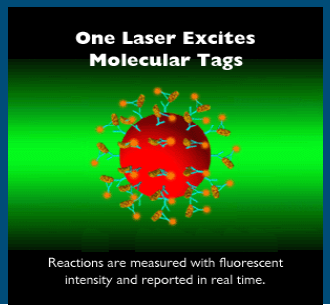


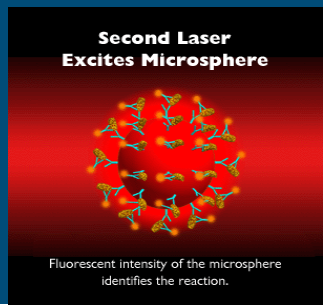
- Immuno fluorescence (IF)
- Immuno fluorescence colony staining (IFC)

- Luminex system





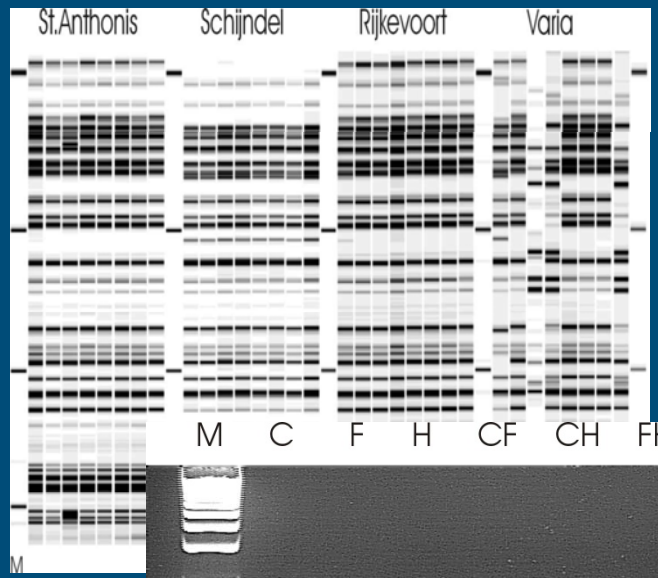


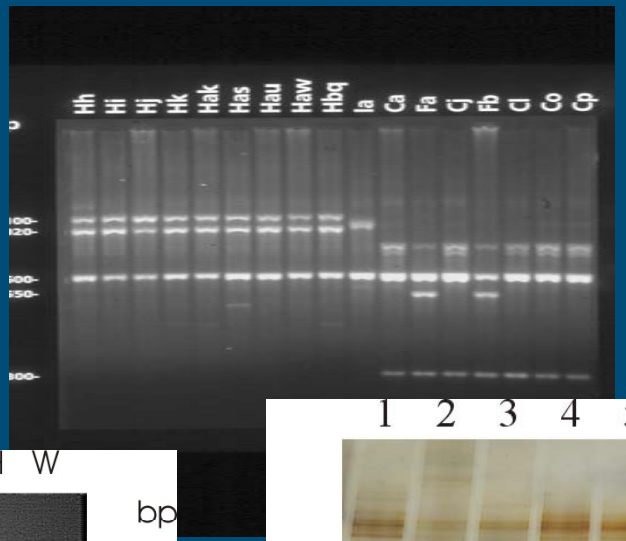


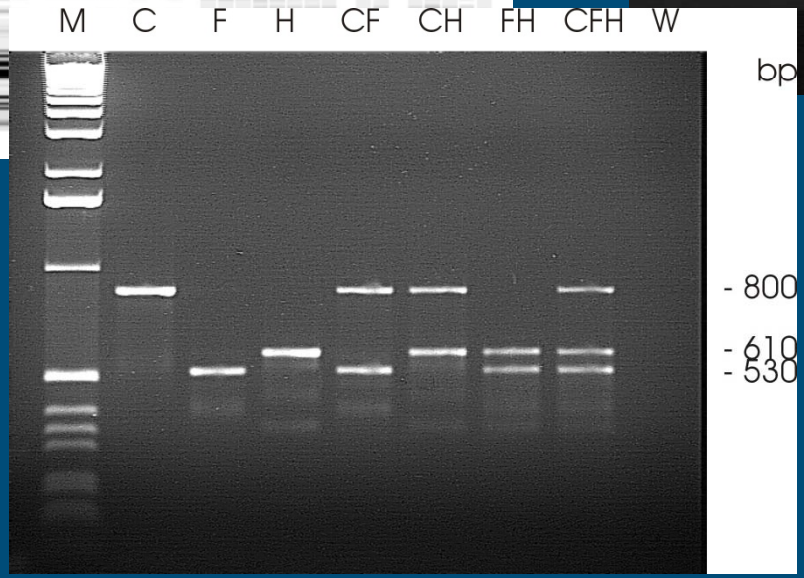
DNA/RNA based techniques

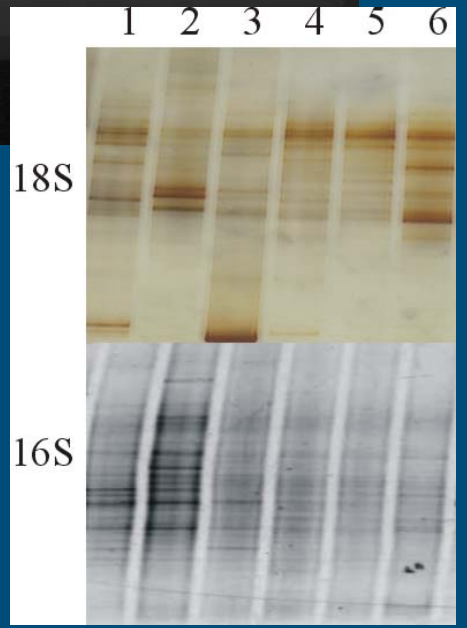
- AFLP
- RAPD-PCR
- SCAR-PCR
- REP-PCR
- ISSR
- SSR
- RFLP
- (RT)-PCR
- DGGE

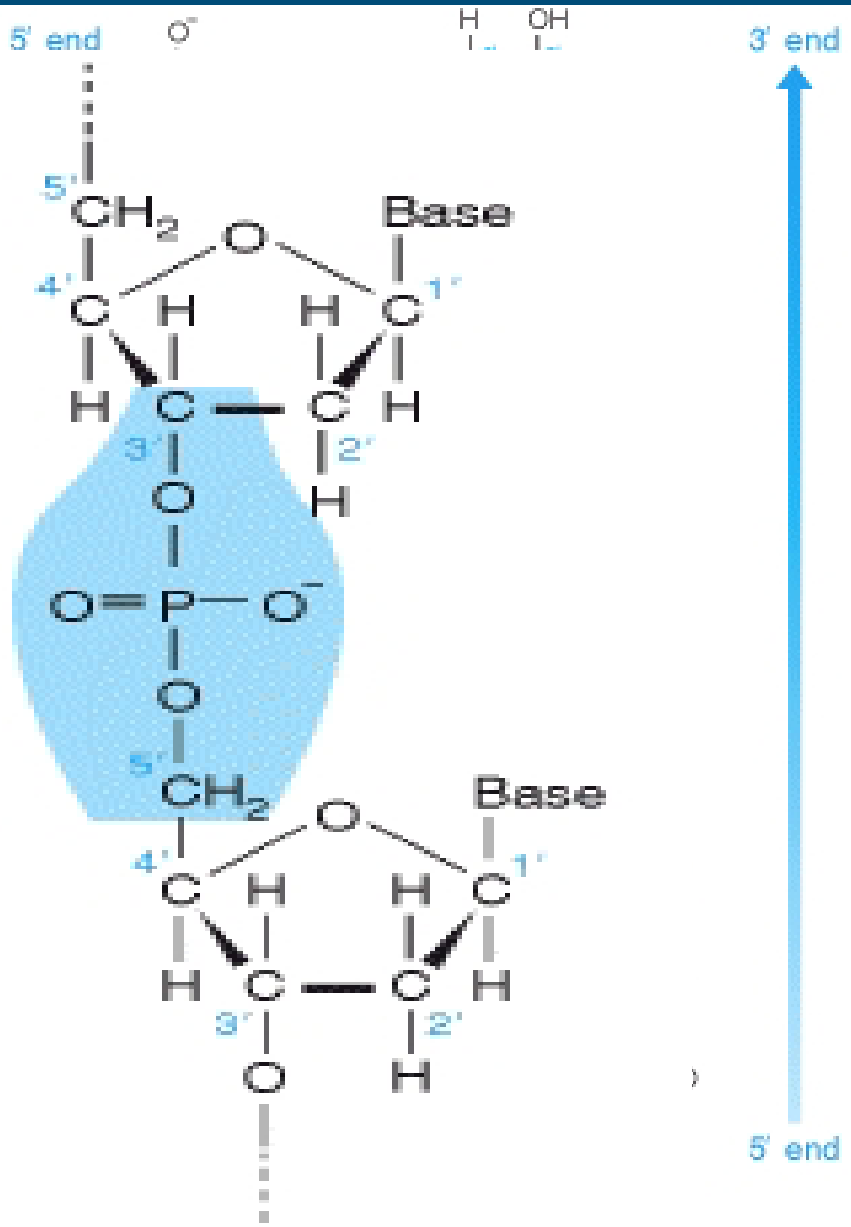
- NASBA
- TaqMan
- Padlock probes

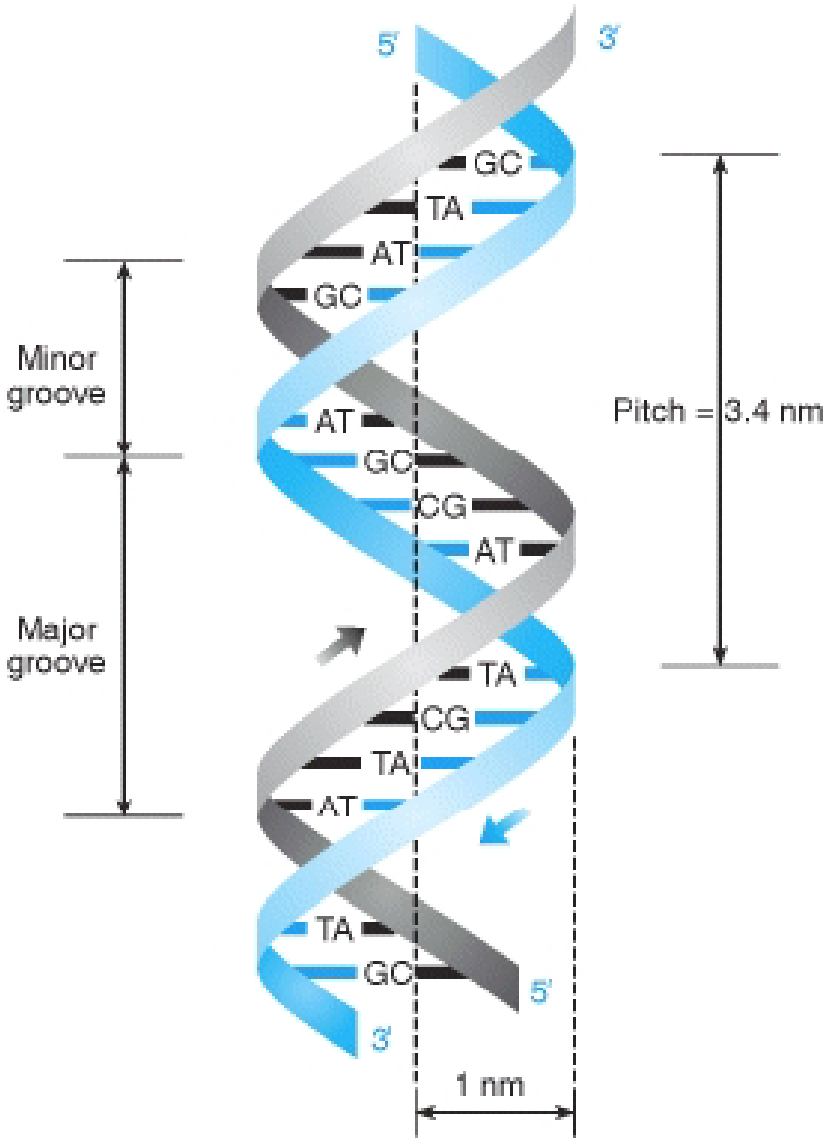




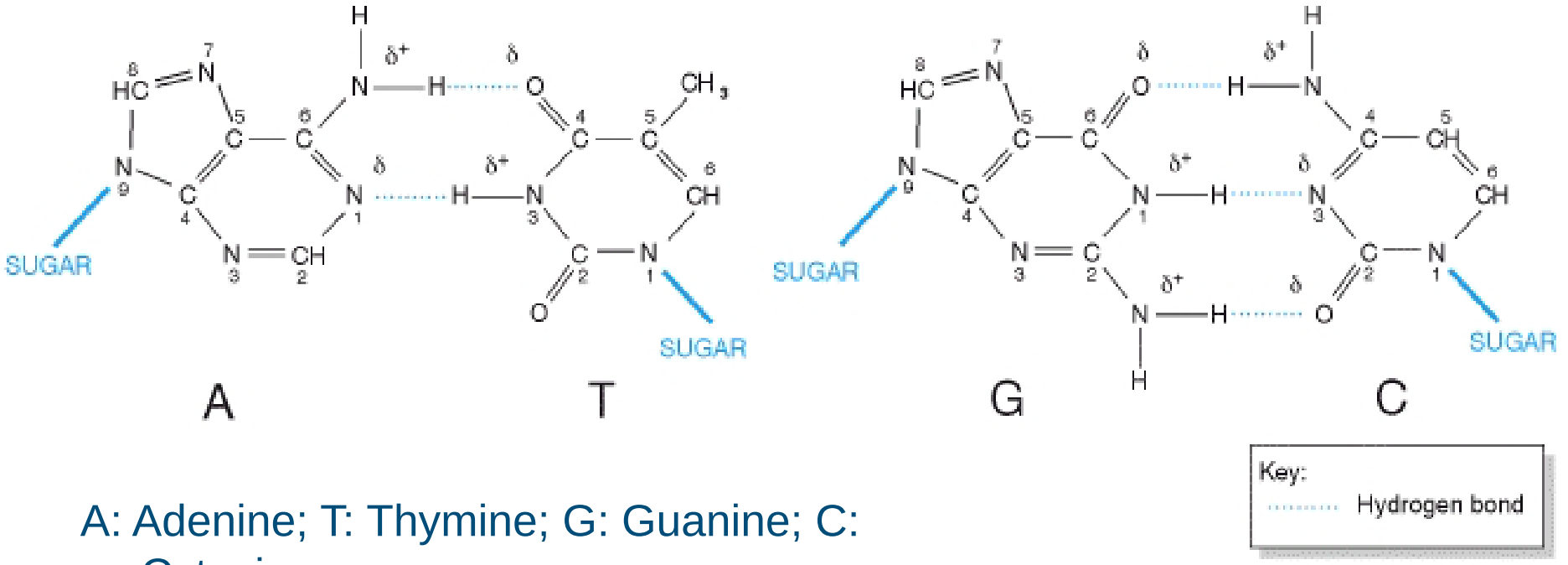





5' end
3' end
Base
Base
5' end

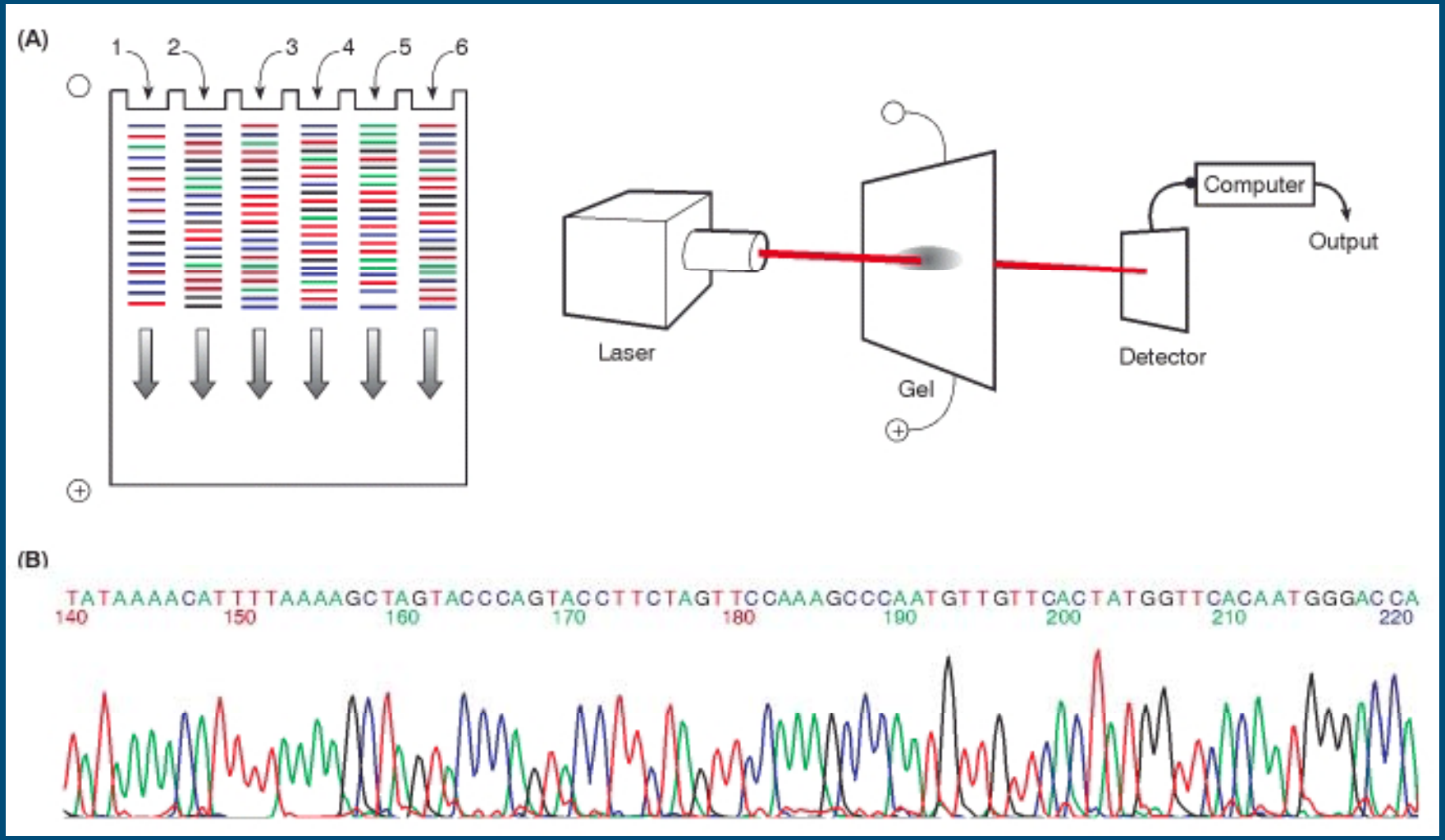

5'
3'
GC
TA
AT
GC
AT
GC
CG
AT
TA
CG
TA
AT
TA
GC
5'
3'
Minor groove
Major groove
Pitch = 3.4 nm
1 nm

What is DNA?



A: Adenine; T: Thymine; G: Guanine; C: Cytosine

Gene sequence analysis



Gene sequence analysis

Sanger sequencing:

500-900 bp per run of 2 hrs

454 sequencing:

Generates more than 100 million bases per run of 7.5 hrs with an average yield of more than 400,000 reads per run

Solexa sequencing:

40 Mb per run in 25-30 bp fragments

SOLiD sequencing:

Generates up till 6 Gigabases and 240M tags per run

Detection techniques: molecular

- Determine DNA sequence of the perpetrator
- DNA sequence difference between target and non-target
  - often ITS, 16S, 18S, 28S, b-tub, EF1a, Cox1

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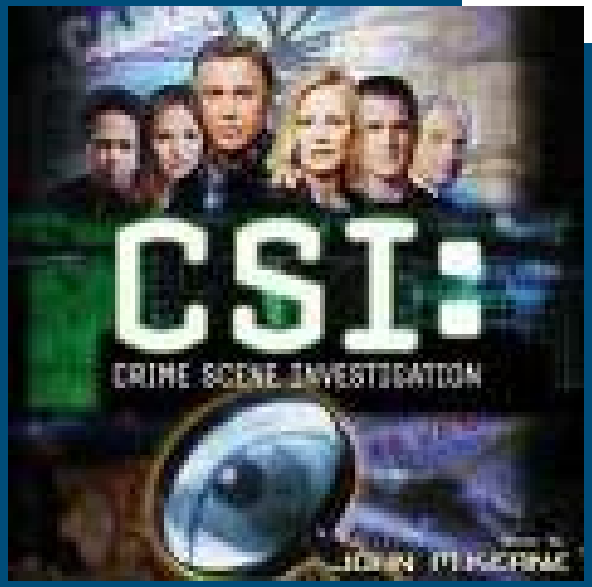
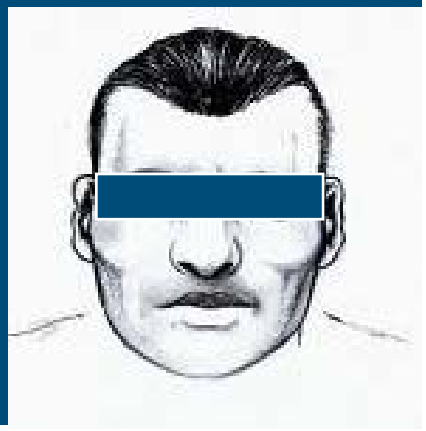
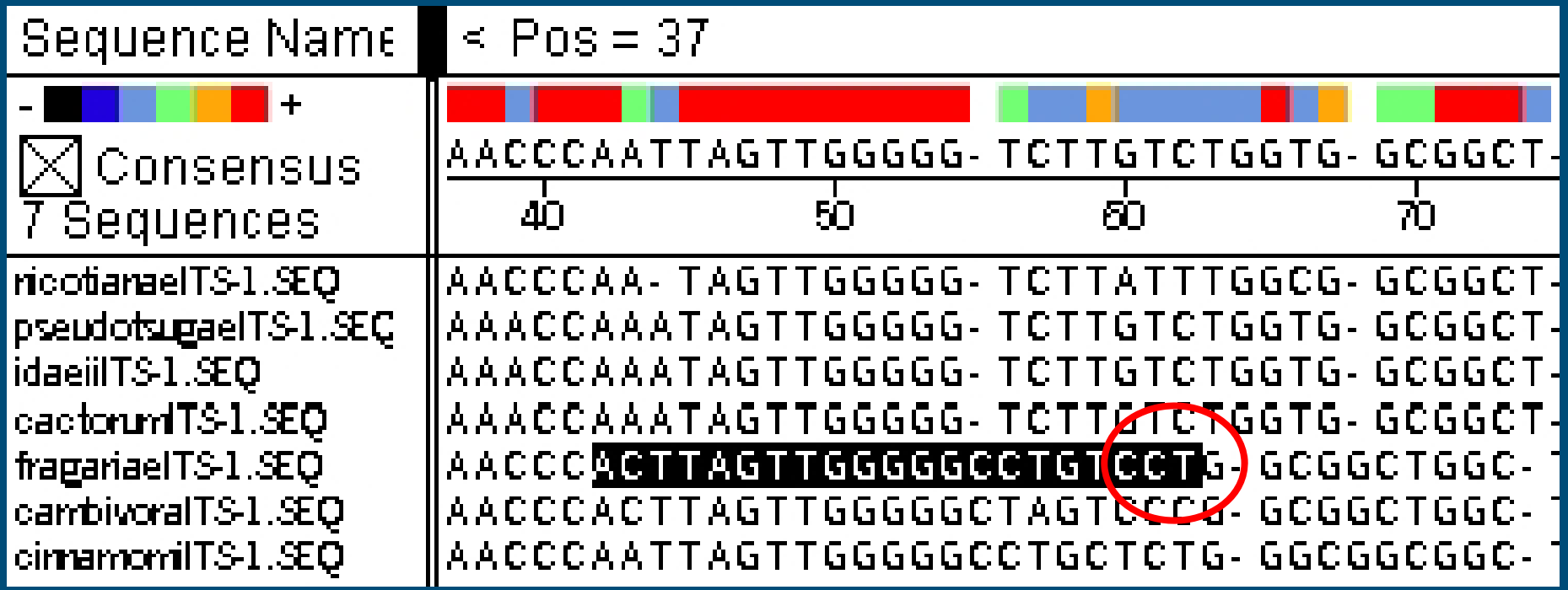
Detection techniques: molecular



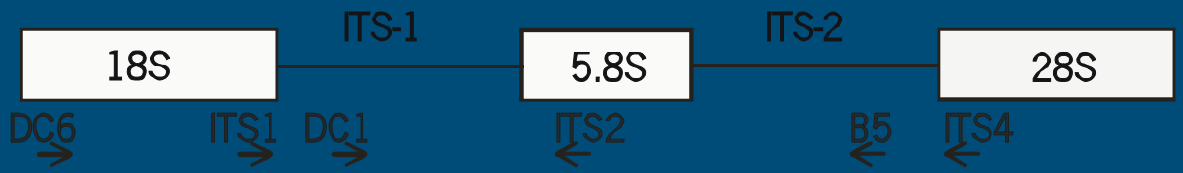


PCR: Sequence difference perpetrator

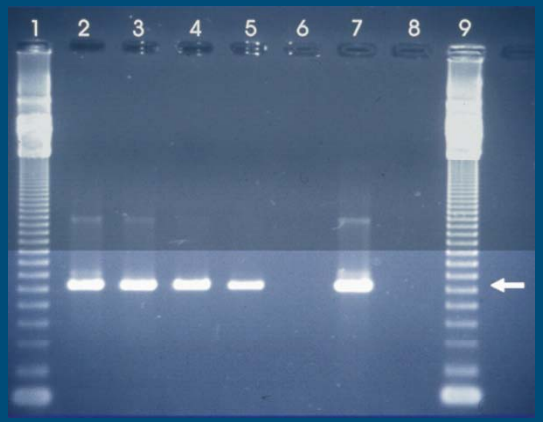


Specific sequence difference of the perpetrator *Phytophthora fragariae*

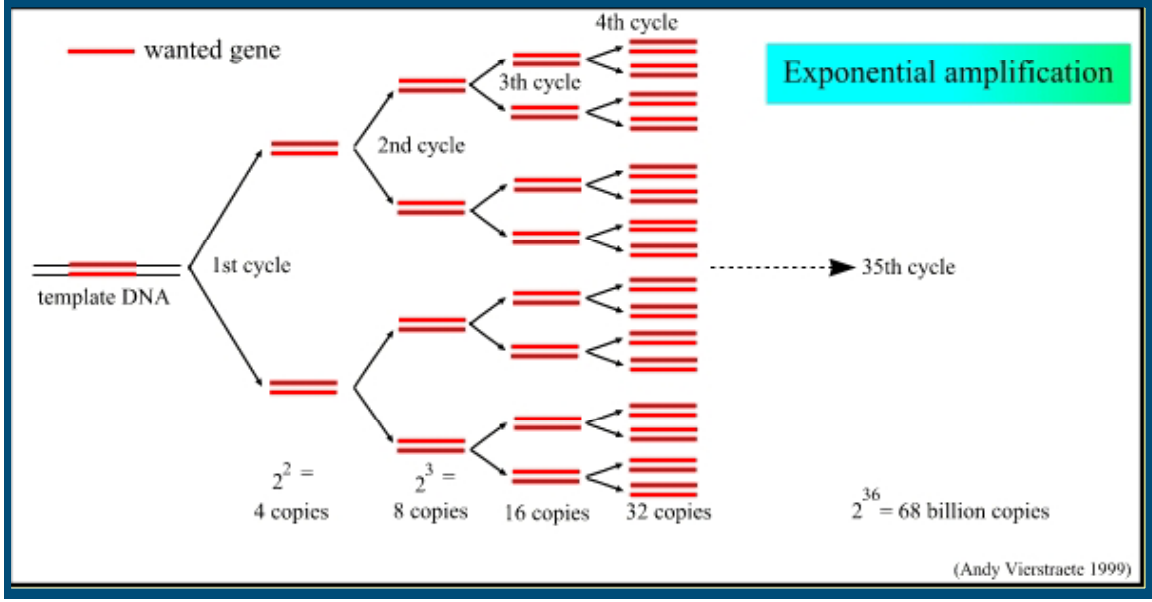
PCR *Phytophthora fragariae*



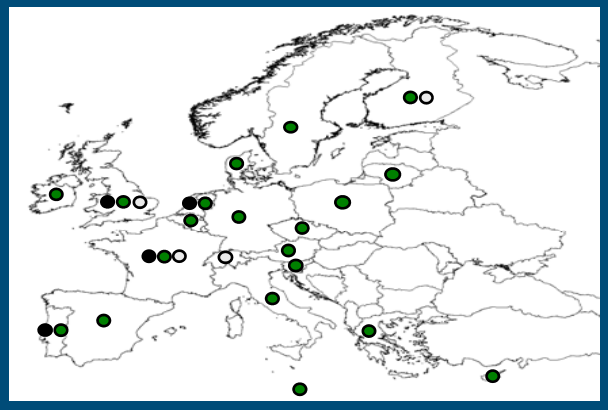
- ITS scheme for *P. fragariae*



- PCR zoospores *P. fragariae*



EU-Portcheck: On-site detection



- TaqMan PCR protocols
- 10 Q-organisms
- DNA extraction protocols
- On-site system

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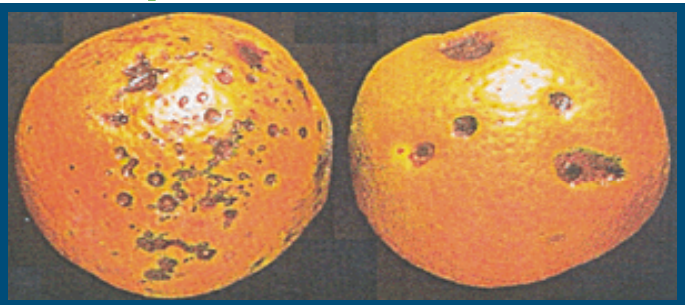
TaqMan PCR *Guignardia citricarpa*

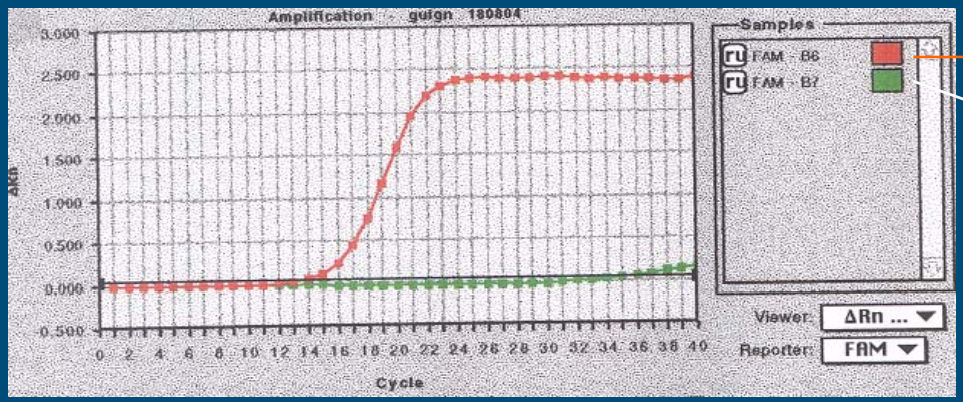


- Symptoms

```
 1  T G A A A T A C G T A A T C C T G A A A G G T G A T G G A A G G G A G G C C T T  G CITRICARPA
 1  . . T . . . . A C . T C . A T . . . . . . . . - - . C C . G A . T . . . . G C .  G_mangiferaeay277711

41  A A A A A A G C C G C C C G A C C T A C C T T C A C A C C C T T G T G T A T C T  G_CITRICARPA
39  . C . . - - - . - . . . G A . A T G . . . . . T . . . . . . . . . . . . C T C  G_mangiferaeay277711
```

- ITS sequence:



G. citricarpa

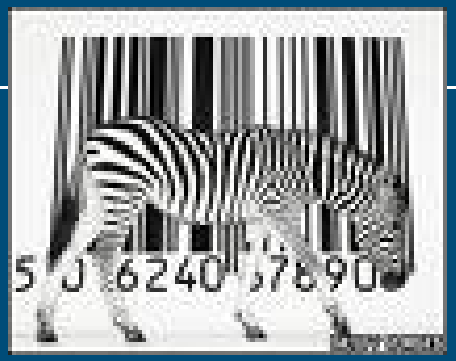
G. mangiferae

Specificity TaqMan PCR; non target is *G. mangiferae*

Examples

- *Guignardia citricarpa* (citrus black spot)
- *Phytophthora ramorum* (sudden oak death)
- *Thrips palmi* (melon thrips)
- *Tuta absoluta* (tomato leaf miner)
- *Bursaphelenchus xylophilis* (pinewood nematode)
- *Agrilus planipennis* (emerald ash borer)
- *Anoplophora chinensis* (citrus longhorn beetle)
- *Aedes albopictus* (asian tiger mosquito)
- ……

DNA barcodes



DNA barcoding is a new technique that uses short DNA sequence of a standard and agreed piece of DNA in the genome as a molecular diagnostic marker for species identification

QBOL:

Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health

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What is QBOL?

- > 20 organizations (universities, research institutes and phytosanitary organizations) in 15 countries
- Financed by EU 7th Framework Program
  - 3 M€ for 3 years
- Developing DNA barcoding to identify quarantine organisms in support of plant health

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SEVENTH FRAMEWORK PROGRAMME

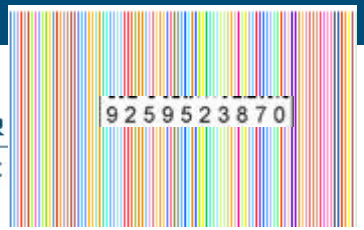


Why DNA barcoding?

- Increasing world wide trading of plants enhances risk of spreading harmful organisms
- Decreasing taxonomic knowledge to identify Q-organisms
- Result in significant possible economic damage
- DNA barcoding offers accurate identification and focuses on strengthening the link between traditional and molecular taxonomy

Three principle QBOL Objectives

- *to DNA barcode relevant Q-organisms + morphologically and/or taxonomically related organisms*
- *to develop a database of DNA barcode sequences plus relevant taxonomic/geographic/host data*
- *to develop a DNA bank for the selected set of Q-organisms + morphologically and/or taxonomically related organisms*



Targets Quarantine

- Which?
  - Fungi
  - Arthropods
  - Bacteria
  - Nematodes
  - Viruses
  - Phytoplasmas
- Council Directive 2000/29/EC


Phytophthora ramorum


Anoplophora chinensis


Xanthomonas fragariae


Pinewood nematode


Polerovirus


Phytoplasm

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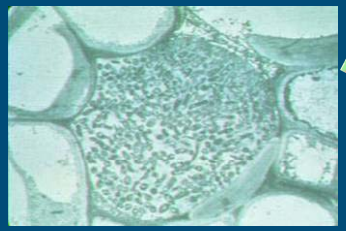
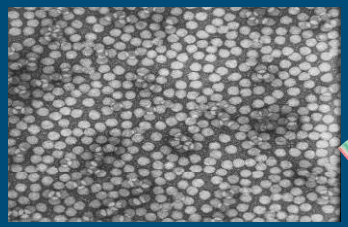
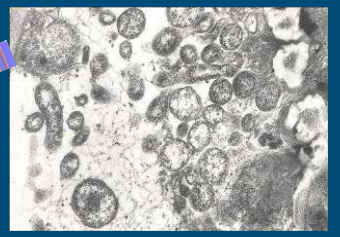
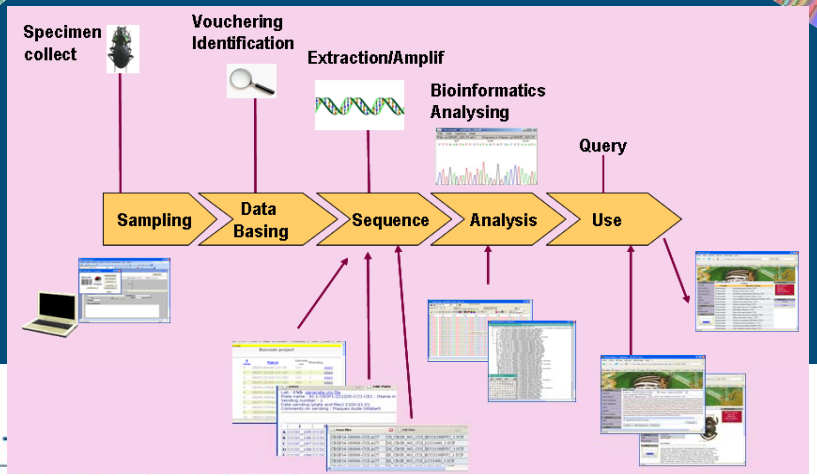
DNA barcode identification









Partners QBOL

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www.qbol.org

QBOL

QBOL: Barcoding for Plant Health

- Genes with sequence difference between Q-organisms and closely related organisms
- Easy to amplify with generic primers
- Culture collections
- Taxonomic experience
- Accessible Database
- Development of ID and DET methods
- Validation – EPPO/IPPC
- Implementation – NPPO's

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QBOL

Future: DNA barcoders

Conclusions

- International trade is increasing
- Importance of an good EU system for Identification and Detection
- Importance of good collections
- International collaboration (QBOL)
- Start up of international Q-bank with NL as coordinator

Acknowledgements

- Cluster Molecular Phytopathology lead by Carolien Zijlstra:

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- Plant Protection Service, Inspection Services
- Analysis laboratoria
- Partners QBOL project
- Companies: ABI, Cepheid, eBiosense, Isogen, BioTrove
- Government: National, European Union
- Product Commodities

Thank you very muc

for your attention

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