Netherlands Food and Consumer
Product Safety Authority
Ministry of Economic Affairs

Obtaining an ISO17025 accreditation for PCR-sequencing

Experiences of the Dutch NPPO

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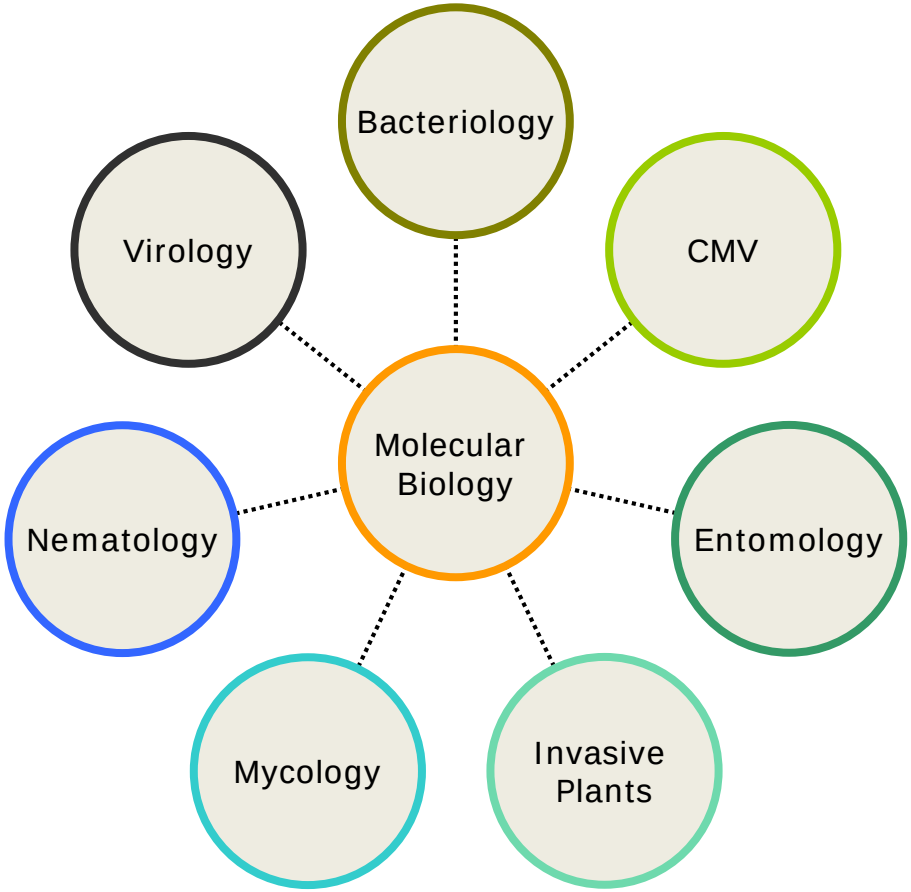
Molecular Biology at NPPO-NL



- MolBio is a separate group of NRC
- Diagnostics and applied research

- Conventional (RT-)PCR (RFLP)
- Real-time (RT-)PCR
- PCR-sequencing (Sanger)

- At present ISO17025 accredited for several real-time PCR tests under a fixed scope

Ambitions of the NPPO-NL

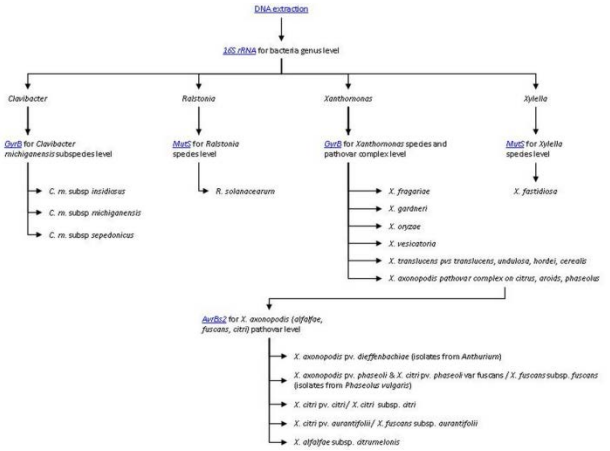
- Accreditation of phytosanitary diagnostics under flexible scope
- Emphasis on overall species identification instead of individual tests
- Flexibility the detection and identification of organism A in matrix B using tests X, Y, Z

- Covering all methods (not tests) performed in-house

- For MolBio, this includes molecular identification using PCR-sequencing according to Sanger

PCR-Sequencing (DNA Barcoding)

- Diagnostic method that uses a short standardised genetic marker in an organism's DNA to aid species identification
- Plant pest DNA Barcoding protocols from the QBOL project, Q-bank and EUPHRESCO DNA Barcoding project and selected virological tests
- EPPO standard on DNA barcoding for plant pests (viruses excluded) under preparation
- Tests are not used stand-alone, but always in support of the diagnosis,



PCR-sequencing at NPPO-NL

Aromia bungii

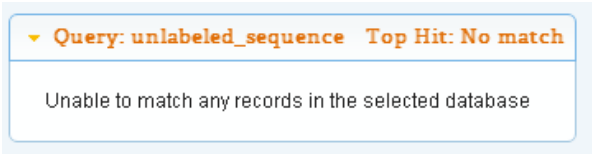
Validation: a requirement for accreditation

- One major challenge for accreditation of PCR-sequencing
- Validation: is the test fit for purpose?
- Determination of performance criteria (EPPO PM 7/98)
  - Analytical sensitivity, Analytical specificity, Selectivity, Repeatability, Reproducibility, Robustness
- Well described standard operating procedures (A-SOP) are the object of validation
  - Not available for consensus sequence preparation and analysis of consensus sequence(s)

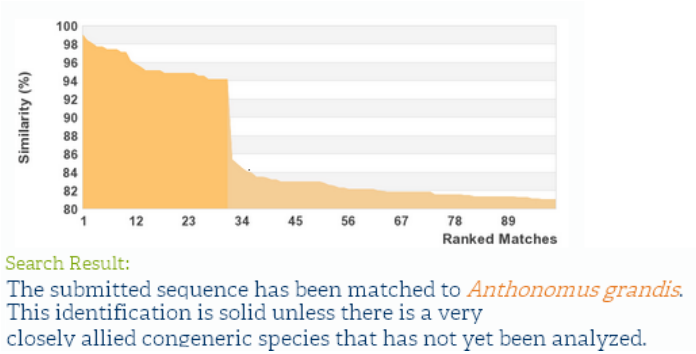
Regular review of validation status

- ISO17025 requirement: Regular review of performance criteria should be carried out to verify that a test is still fit for purpose

- Challenging when using online databases with changing content:



2009 - 2012



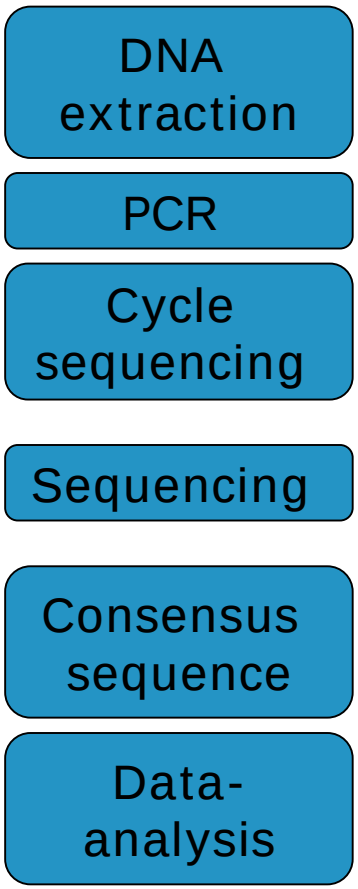
2013 - present

Remarks made during the ISO17025 audit underline the challenges of PCR-sequencing validation and verification

How to tackle the problem?

- Separation of PCR-test and sequence analysis
- Determine performance criteria for the two parts separately
- PCR test
  - Standardised procedures in A-SOP
  - Determine relevant performance criteria once, and update at regular intervals (5 years) following PM7/98
- Sequence analysis
  - General guidance: depends on expertise technician/researcher
  - Usability of the generated sequence for molecular identification on a certain taxonomical level is determined on *ad hoc* basis
  - Last analysis report is the up-to-date validation status for an “organism-matrix-locus (loci)” combination

PCR test versus sequence analysis



Determination or verification of Performance criteria

Analytical sensitivity – mainly relevant for viruses, viroids and phytoplasmas

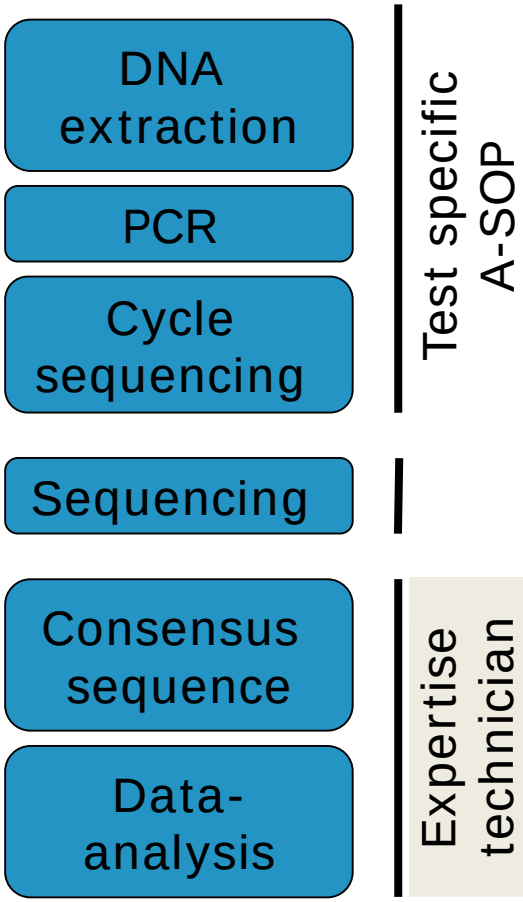
Analytical specificity – A (small) subset of target organisms (the full range of target organisms is not known)

Selectivity – relevant for viruses, viroids and phytoplasmas

Repeatability and Reproducibility

Robustness – relevant when introducing small changes to the test

PCR test versus sequence analysis



Analytical specificity - can change from day to day depending on the content of the (online) sources consulted.

Determined during each analyses
- Inter- versus intraspecies variation
- Clustering in taxon specific clades
- Using one or more loci

All results and observations have to be documented to ensure traceability

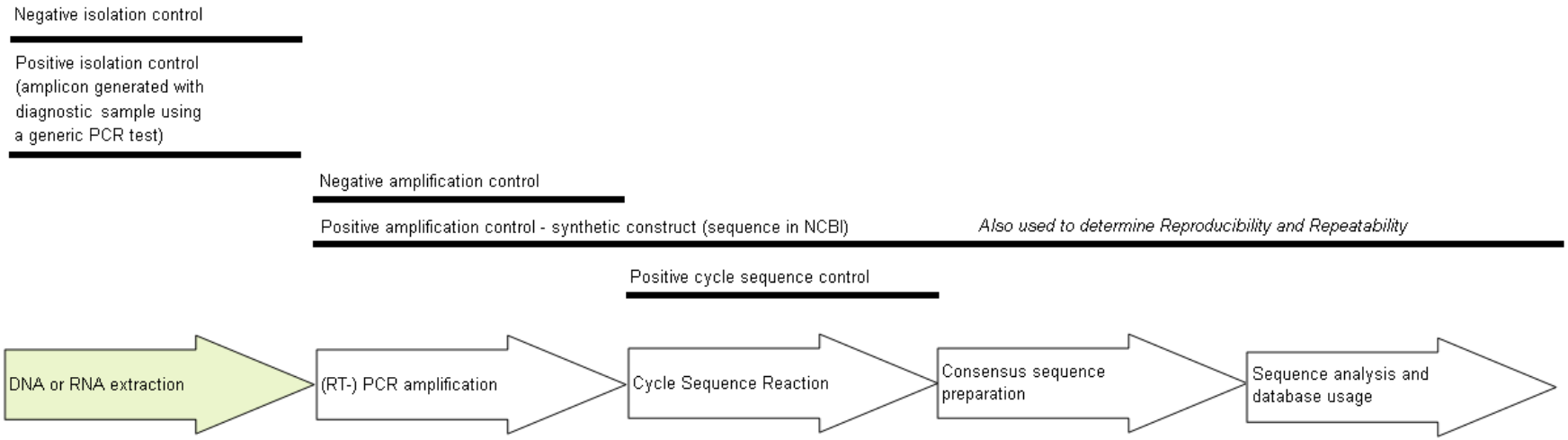
Conclusions are drawn using conservative terms

Repeatability and reproducibility – first and second line controls

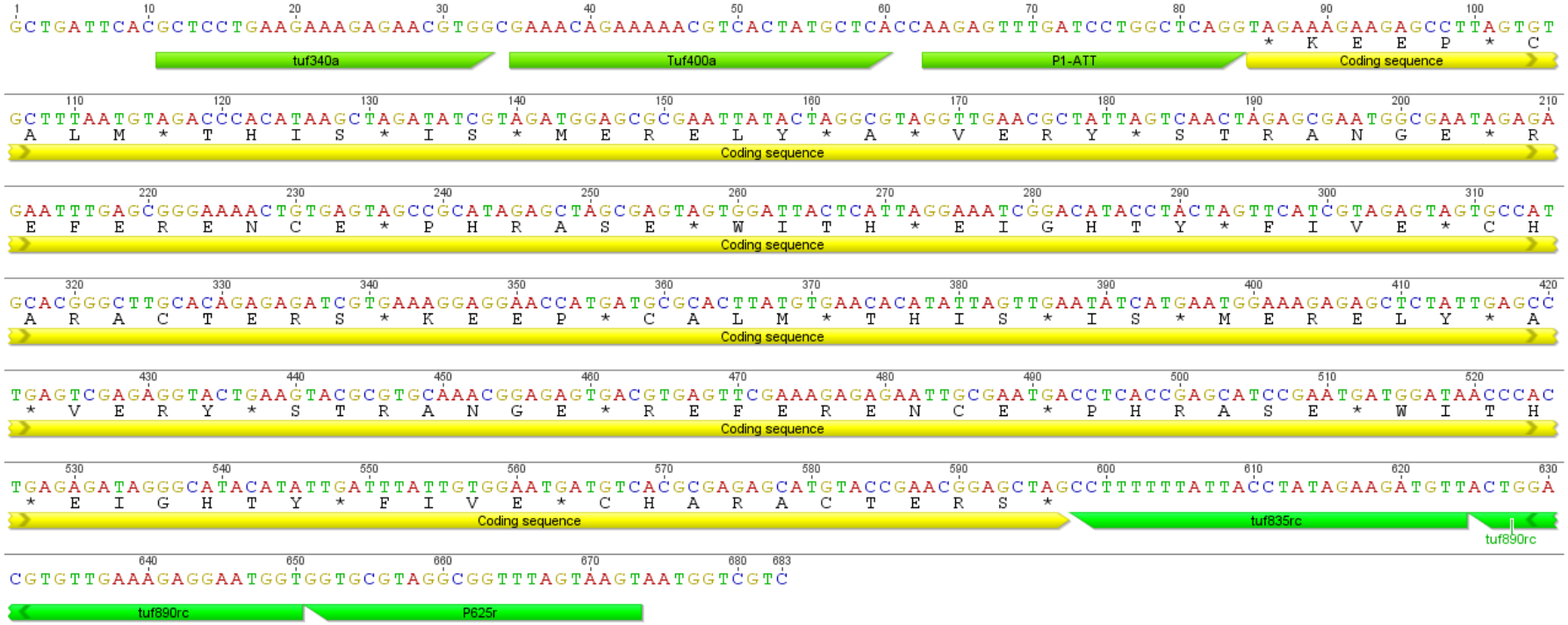
First line controls

- PIC, NIC, PAC, NAC and a positive cycle sequence control

- PAC is a synthetic construct per organism group
- PAC is used as a process control to monitor all steps from amplification to sequence analysis (also used for reproducibility and repeatability)



Finding the Easter egg



Internal Control Program

- How to make sure that technicians are proficient?
  - Internal training sessions
  - Internal workshops discussing “though” cases
  - Second assessor in particular cases (new organism-matrix-locus (loci) combinations)
  - 2nd line of controls (blind samples)
  - 3rd line of controls (proficiency tests) (e.g. Pospiviroids)

- Discussing results with taxonomical specialists increases the level of expertise
  - Being able to estimate the coverage of taxa in databases
  - Knowledge on organism-matrix-locus (loci) combinations
  - Knowledge on synonyms used

Conclusions

- We believe it is possible to acquire an accreditation for PCR-sequencing
- The Dutch accreditation board (RvA) is open to accreditation of methods in which expertise plays an important role
- We need to:
  - add extra validation data on the PCR test part of the method (mainly sensitivity and selectivity where relevant)
  - Keep track of repeatability and reproducibility of the sequence analysis part of the method over time (synthetic PACs)
- Report back to RvA in the coming eight months with our solutions for shortcomings found during the audit

Thank you very much for your attention

Are there any questions?

Acknowledgements

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