Nederlandse Voedsel- en
Warenautoriteit
*Ministerie van Landbouw,
Natuur en Voedselkwaliteit*

Accreditation of HTS tests in plant health diagnostics

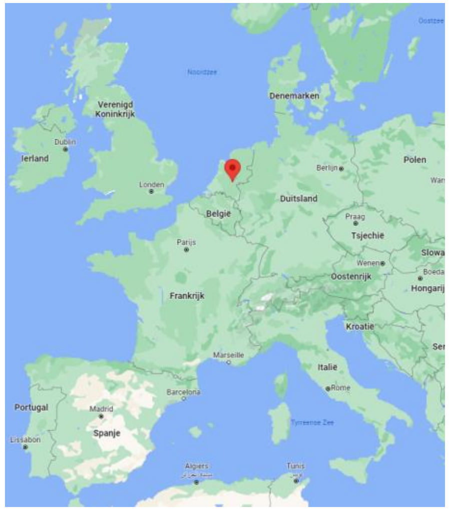
Bart van de Vossenberg, NIVIP, NL
EPPO workshop heads of labs
19-4-2023

Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP)

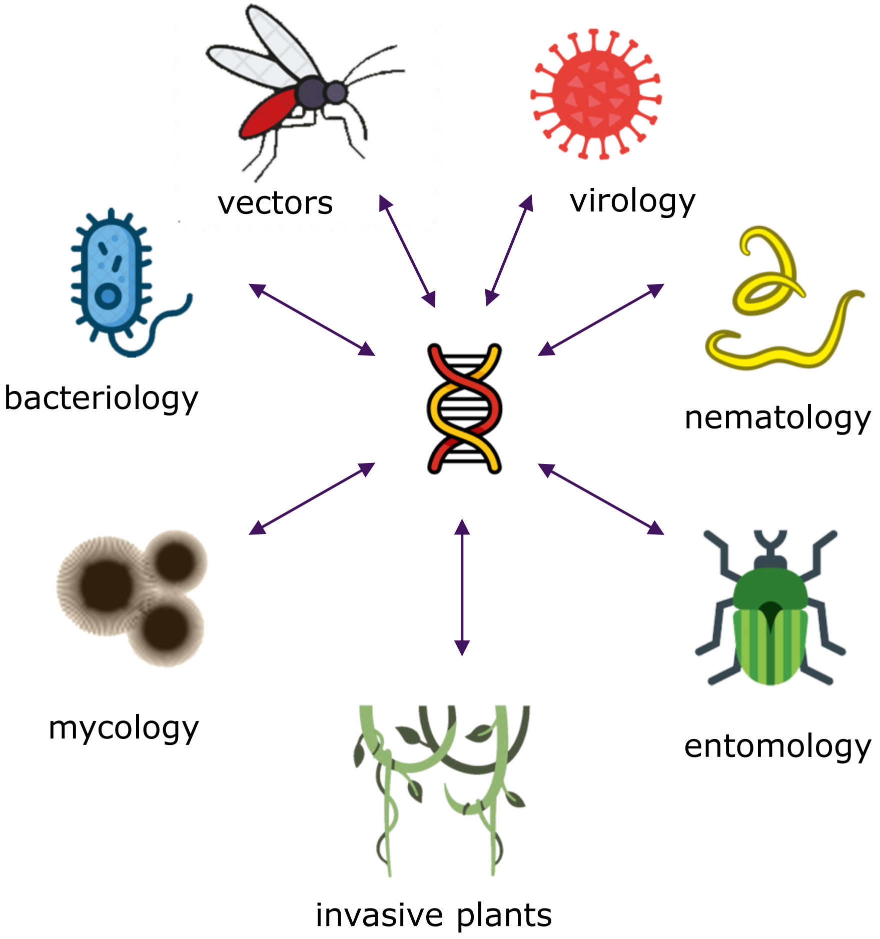
- International knowledge center and governmental authority on regulatory plant health, invasive plant species and vectors of human and veterinarian diseases
- Part of the NVWA, NPPO-NL, EURL Bacteriology and EURL virology

One department with many "faces"

- ~120 staff members
- 7 organism specific groups, 1 molecular group, supporting groups (greenhouses, kitchen)
- Diagnostics, applied research, risk analysis, policy advice, collections, training missions



The Molecular Biology group



- Supports the organism specific groups with diagnostics and applied research projects
- Different organism specific groups with their own molecular challenges

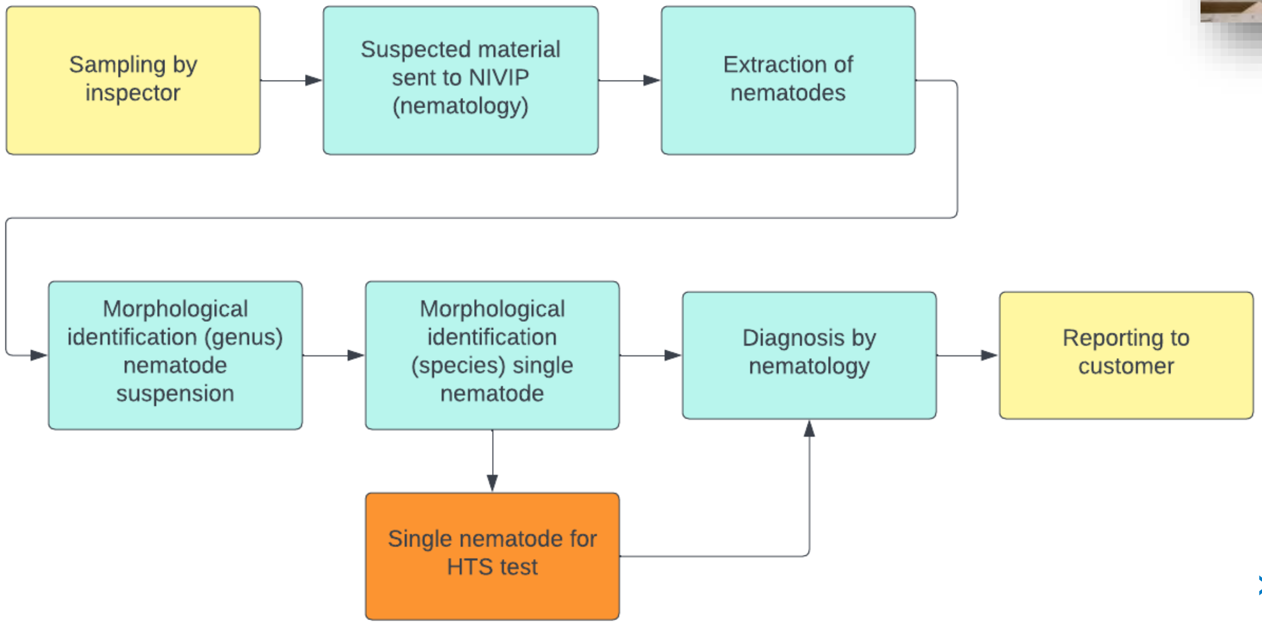
- 8 Researchers (incl. 1 bio-informatician)
- 11 (research) technicians
- 3 bio-informaticians

- Specific knowledge on molecular biological diagnostics, phylogeny, and (population) genomics per organism group

A group driven by technical advancements

- Four molecular laboratories to separate essential procedural steps
- 144 molecular tests embedded in the quality assurance system for “routine” use
- Tests grouped in four methods
  - conventional PCR,
  - real-time PCR,
  - PCR-Sequencing (Sanger),
  - HTS (Illumina sequencing)

Molecular biological tests: part of the diagnostic process



Sampling by inspector

Suspected material sent to NIVIP (nematology)

Extraction of nematodes

Morphological identification (genus) nematode suspension

Morphological identification (species) single nematode

Diagnosis by nematology

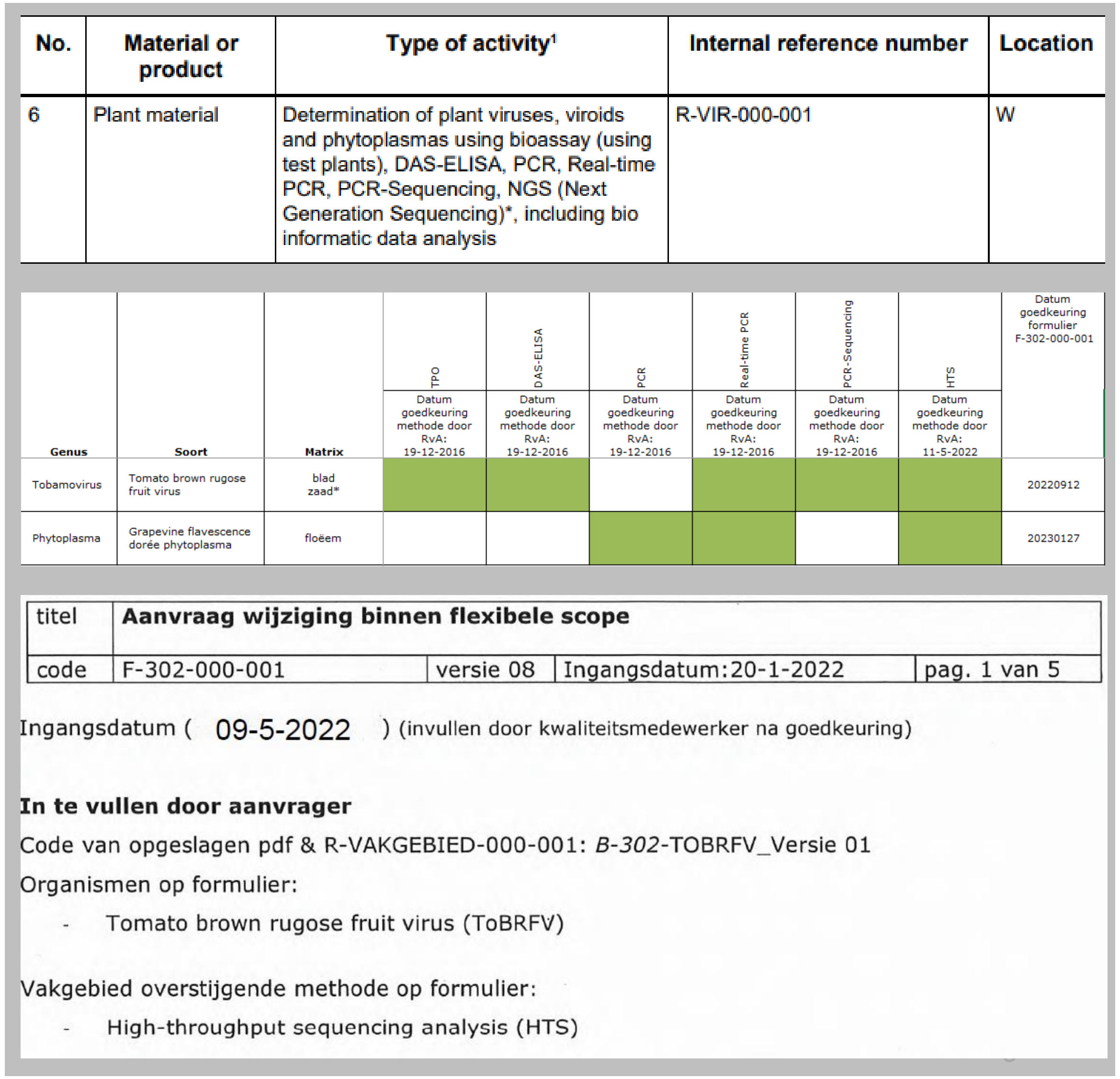
Reporting to customer

Single nematode for HTS test

- ISO17025 accredited for all molecular methods under flexscope

Phytosanitary flexible scope

- Not individual tests or methods are focus of the accreditation scope but the diagnostic process for a given pest
- Scope generically lists methods for which tests are validated and available
- An internal quality document lists specific pest + method combinations that can be used under accreditation
- Links are provided to individual tests and validation data



No.	Material or product	Type of activity[1]	Internal reference number	Location
6	Plant material	Determination of plant viruses, viroids and phytoplasmas using bioassay (using test plants), DAS-ELISA, PCR, Real-time PCR, PCR-Sequencing, NGS (Next Generation Sequencing)*, including bio informatic data analysis	R-VIR-000-001	W

Genus	Soort	Matrix	TPO Datum goedkeuring methode door RvA: 19-12-2016	DAS-ELISA Datum goedkeuring methode door RvA: 19-12-2016	PCR Datum goedkeuring methode door RvA: 19-12-2016	Real-time PCR Datum goedkeuring methode door RvA: 19-12-2016	PCR-Sequencing Datum goedkeuring methode door RvA: 19-12-2016	HTS Datum goedkeuring methode door RvA: 11-5-2022	Datum goedkeuring formulier F-302-000-001
Tobamovirus	Tomato brown rugose fruit virus	blad zaad*	🟩	🟩		🟩	🟩	🟩	20220912
Phytoplasma	Grapevine flavescence dorée phytoplasma	floëem			🟩	🟩		🟩	20230127

titel	**Aanvraag wijziging binnen flexibele scope**			
code	F-302-000-001	versie 08	Ingangsdatum:20-1-2022	pag. 1 van 5

Ingangsdatum (09-5-2022) (invullen door kwaliteitsmedewerker na goedkeuring)

In te vullen door aanvrager

Code van opgeslagen pdf & R-VAKGEBIED-000-001: *B-302*-TOBRFV_Versie 01

Organismen op formulier:

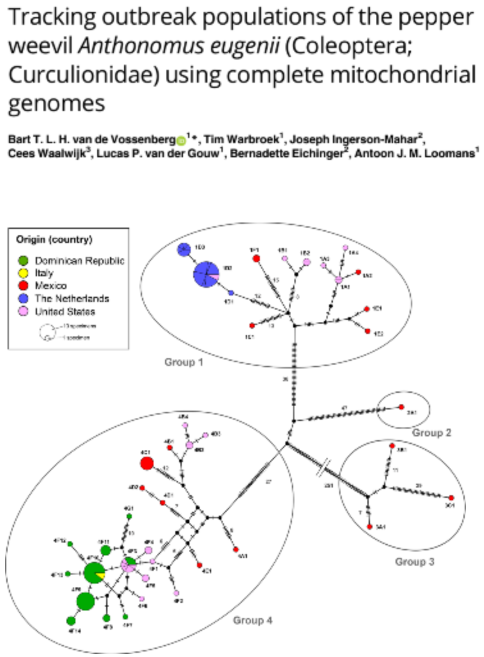
- Tomato brown rugose fruit virus (ToBRFV)

Vakgebied overstijgende methode op formulier:

- High-throughput sequencing analysis (HTS)

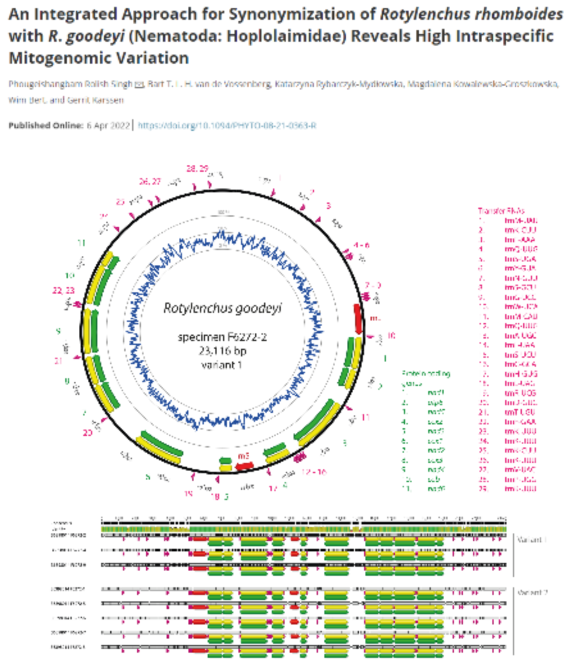
High Throughput Sequencing (HTS)

- Since 2014, HTS (Illumina sequencing) is used in applied research at NIVIP
- *ad hoc* analysis pipelines were constructed for specific research questions
- Many manual analysis steps with little standardization
- Since 2019 from *ad hoc* research applications to standardized diagnostics



Tracking outbreak populations of the pepper weevil *Anthonomus eugenii* (Coleoptera; Curculionidae) using complete mitochondrial genomes

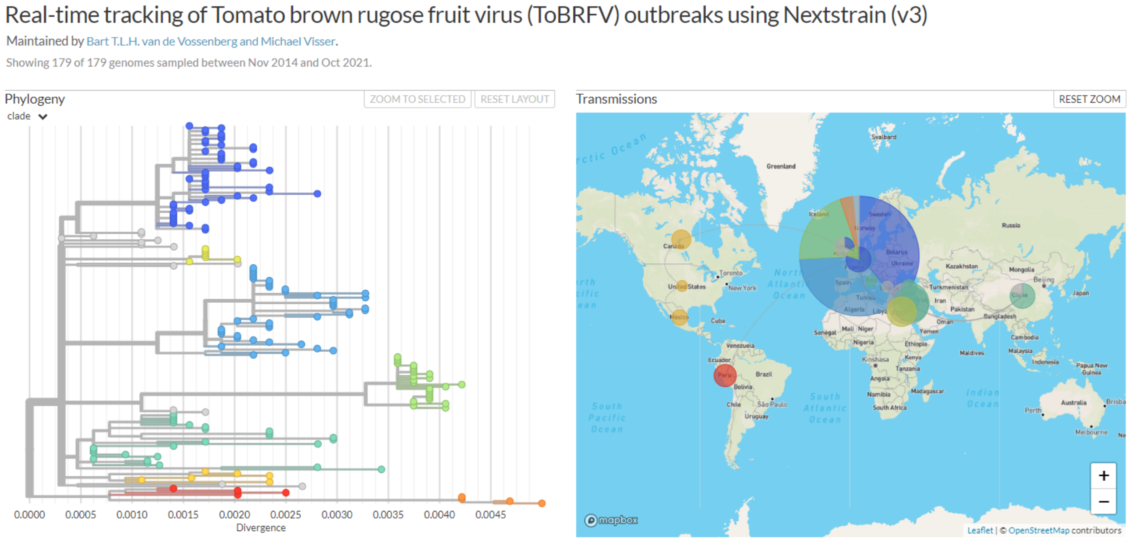
Bart T. L. H. van de Vossenberg[1]*, Tim Warbroek[1], Joseph Ingerson-Mahar[2], Cees Waalwijk[3], Lucas P. van der Gouw[1], Bernadette Eichinger[2], Antoon J. M. Loomans[1]



An Integrated Approach for Synonymization of *Rotylenchus rhomboides* with *R. goodeyi* (Nematoda: Hoplolaimidae) Reveals High Intraspecific Mitogenomic Variation

Phougeishangbam Rolish Singh, Bart T. L. H. van de Vossenberg, Katarzyna Rybarczyk-Mydłowska, Magdalena Kowalewska-Groszkowska, Wim Bert, and Gerrit Karssen

Published Online: 6 Apr 2022 | https://doi.org/10.1094/PHYTO-08-21-0363-R



Real-time tracking of Tomato brown rugose fruit virus (ToBRFV) outbreaks using Nextstrain (v3)

Maintained by Bart T.L.H. van de Vossenberg and Michael Visser.

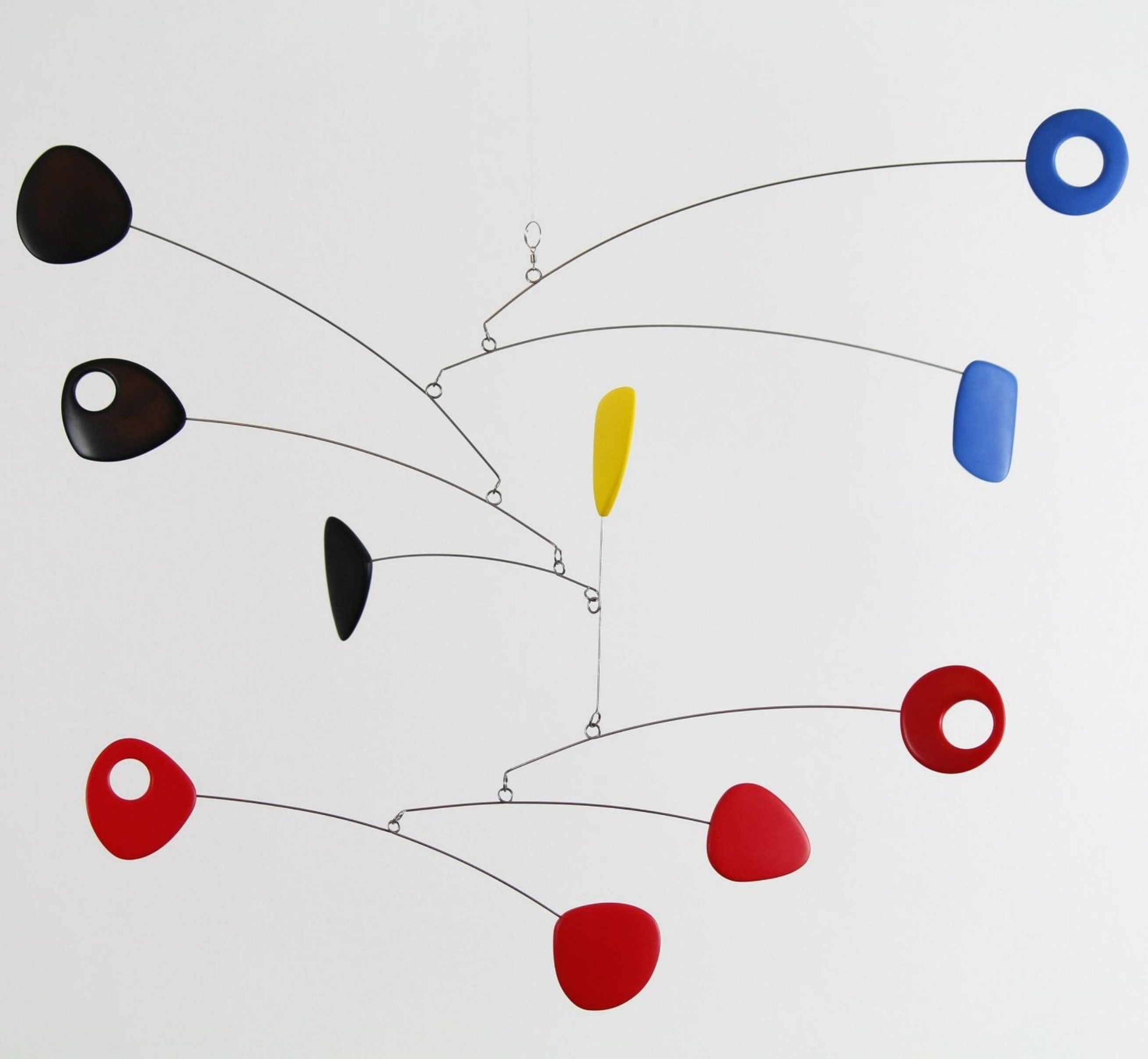
Showing 179 of 179 genomes sampled between Nov 2014 and Oct 2021.

Implementing Illumina sequencing in standardized diagnostic setting

- The power of HTS needed in a diagnostic setting

- Many different aspects to cover
- Constant refining needed with growing demand

- Increase in one factor results in disbalance and triggers the need to adjust other factors
  - kinetic mobile



Analysis on stand-alone PC
CLC genomics workbench
Windows environment
Single user

Multiple Users

Linux

Multiple PCs in local network
License server
Windows and Linux

More samples

Server too slow

Microsoft Azure

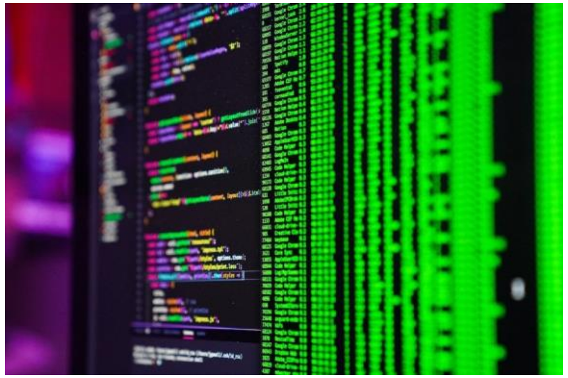
Multiple PCs in virtual network
Windows and Linux PCs
Databases
Transfer of data from lab to cloud

Data from lab

Connection too slow

Own dedicated data line

Commercial tools not fit for purpose



Create custom scripts
New bioinformatic expertise needed

More samples

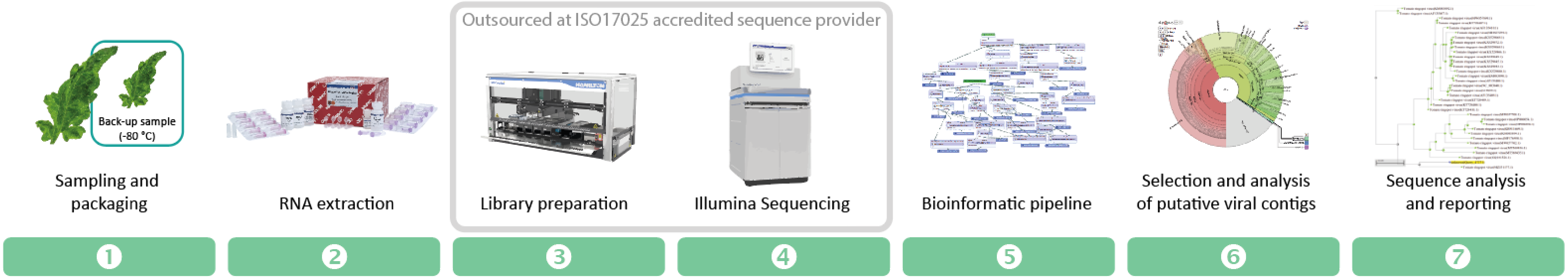
Increase data storage
Train new staff to analyze samples

Computational intensive pipelines

shorter turnover time needed

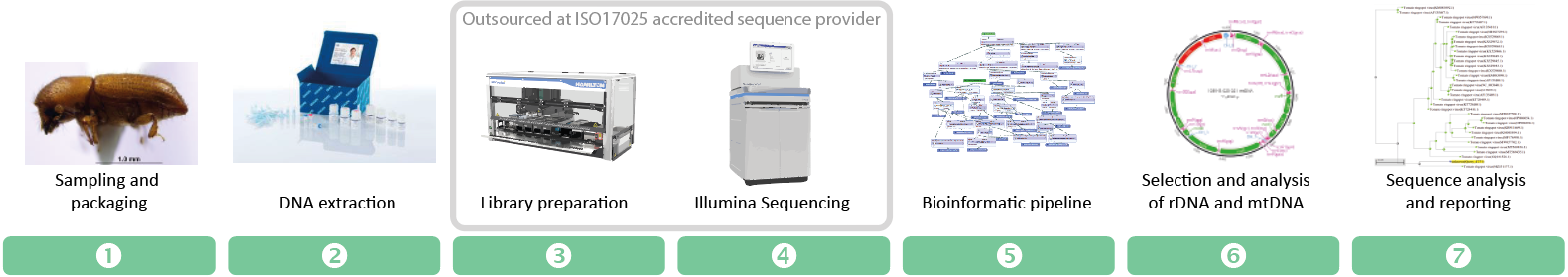
Defining the tests

- A test is a specific application of a method (EPPO PM7/76)
- Includes everything from nucleic acid extraction to data interpretation (EPPO PM7/98)



Outsourced at ISO17025 accredited sequence provider

1. Sampling and packaging
2. RNA extraction
3. Library preparation
4. Illumina Sequencing
5. Bioinformatic pipeline
6. Selection and analysis of putative viral contigs
7. Sequence analysis and reporting



Outsourced at ISO17025 accredited sequence provider

1. Sampling and packaging
2. DNA extraction
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7. Sequence analysis and reporting

Describing tests in standard operating procedures

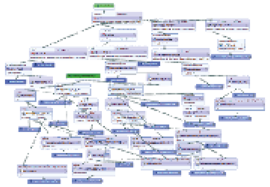
Sampling and packaging

DNA extraction

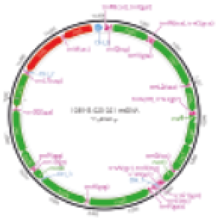
Outsourced at ISO17025 accredited sequence provider

Library preparation

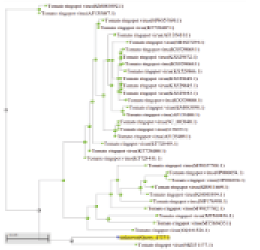
Illumina Sequencing



Bioinformatic pipeline

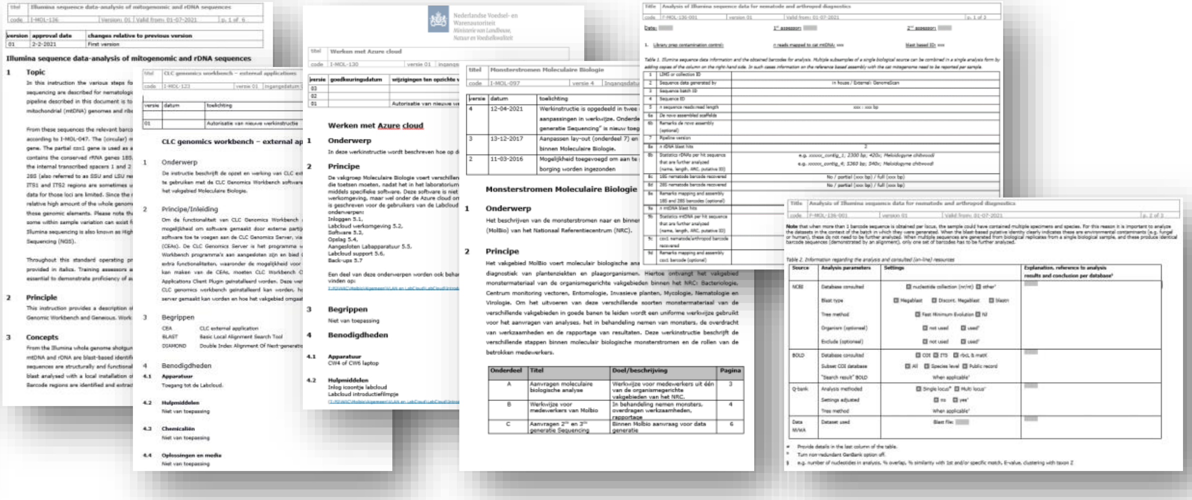


Selection and analysis of rDNA and mtDNA



Sequence analysis and reporting

① ② ③ ④ ⑤ ⑥ ⑦



Validating tests

Why should we validate or verify?

- To assess if a test is fit for purpose (scope)

How to validate or verify?

- Determination or verification of performance criteria (PM 7/98)
- Analytical sensitivity, Analytical specificity, Selectivity, Repeatability, Reproducibility
- Well characterized biological material

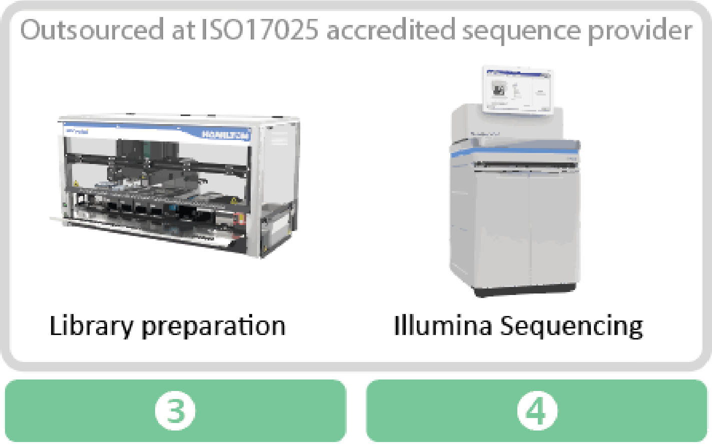
What to validate or verify?

- Well described analytical standard operating procedures (A-SOP)

Aromia bungii

What about subcontracting?

- Illumina DNAseq and RNAseq data is outsourced to an ISO17025 accredited sequence provider
- Library prep and sequencing according to standardized procedure that is fit for purpose
- Agreements on data yield and quality
- No multiplexing with plant samples other customers
- Sequence data and quality control data provided to allow assessment of sequence run success
- One standardized control is included in each sequence batch which is processed like a regular sample from library prep to analysis report



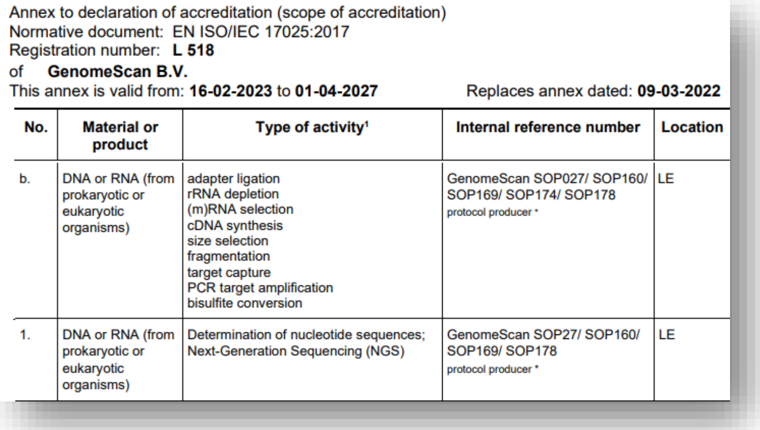
Outsourced at ISO17025 accredited sequence provider

Library preparation

Illumina Sequencing

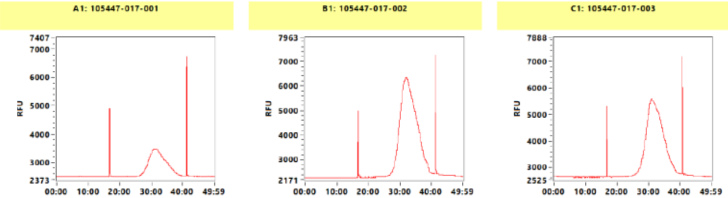
③

④

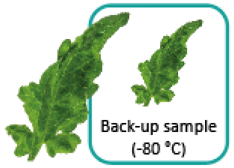


Annex to declaration of accreditation (scope of accreditation)
Normative document: EN ISO/IEC 17025:2017
Registration number: **L 518**
of **GenomeScan B.V.**
This annex is valid from: **16-02-2023** to **01-04-2027** Replaces annex dated: **09-03-2022**

No.	Material or product	Type of activity[1]	Internal reference number	Location
b.	DNA or RNA (from prokaryotic or eukaryotic organisms)	adapter ligation rRNA depletion (m)RNA selection cDNA synthesis size selection fragmentation target capture PCR target amplification bisulfite conversion	GenomeScan SOP027/ SOP160/ SOP169/ SOP174/ SOP178 protocol producer *	LE
1.	DNA or RNA (from prokaryotic or eukaryotic organisms)	Determination of nucleotide sequences; Next-Generation Sequencing (NGS)	GenomeScan SOP27/ SOP160/ SOP169/ SOP178 protocol producer *	LE



One control to serve many purposes (example virology)



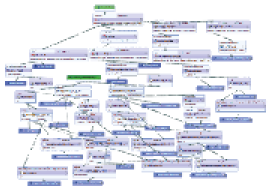
Sampling and packaging

RNA extraction

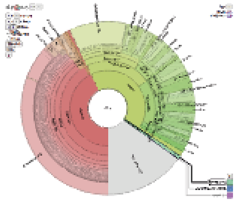
Outsourced at ISO17025 accredited sequence provider

Library preparation

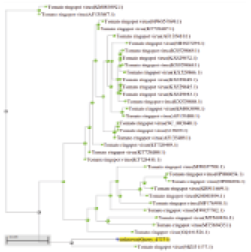
Illumina Sequencing



Bioinformatic pipeline



Selection and analysis of putative viral contigs



Sequence analysis and reporting

① ② ③ ④ ⑤ ⑥ ⑦

Healthy
N. benthamiana

Spiked with ERCC
RNA constructs

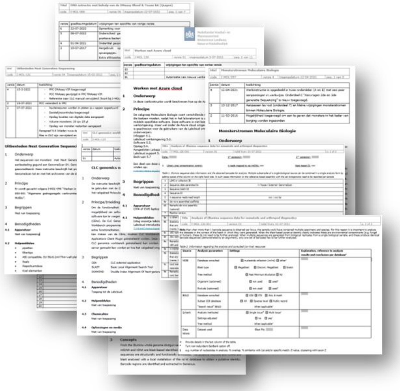
Positive Process Control batch

- Should pass entry QC and library QC

- Should produce 2Gb of data at >80% PHRED30

- Should complete the bioinformatic pipeline with all output (check on bioinformatic pipeline)
- Identification of plant species (only *N. benthamiana* should be identified, serves as positive control and contamination control)
- Recovery of ERCC constructs from positive process control sample (positive control)
- No ERCC constructs should be identified in diagnostic samples in the same batch (contamination control)
- Assessment of rRNA depletion success (indicative)

External audit and accreditation



Facilities and analysis software	Test description	Validation data	Proficient staff	Diagnostic samples

- Eight years later all elements were present for two HTS tests: external audit requested
- As of august 2022 NIVIP is able to perform two HTS tests under accreditation
- New tests can be added using regular methodology of our flexible scope

Summary

- High Throughput Sequencing is a powerful tool in plant health diagnostics
- Implementing the method is similar to implementing other methods but many aspects need to be covered
- The concepts for method, test and validation under a validation scope apply to HTS tests as well as any other molecular test
- Outsourcing data production to ISO17025 accredited sequence provider was accepted by Dutch accreditation board
- Discussions with our national accreditation board were very helpful in setting the stage for HTS in plant health diagnostics

Thank you for your attention

Entomology

Tibor Bukovinszki

Jan Mertens

Cynthia Pronk

Nematology

Gerrit Karssen

Evelyn van Heese

Anne Sophie van Bruggen

Virology

Annelien Roenhorst

Marleen Botermans

Pier de Koning

Molecular Biology

Aron van Duijnhoven

Thijn van Kempen

Tim Warbroek

Lucas van der Gouw

Michael Visser

Marcel Westenberg