



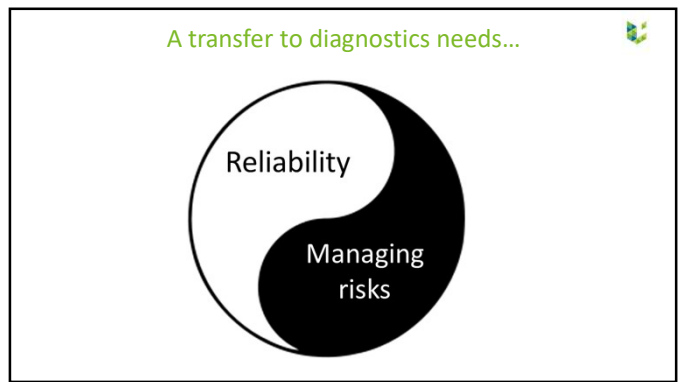
1



2



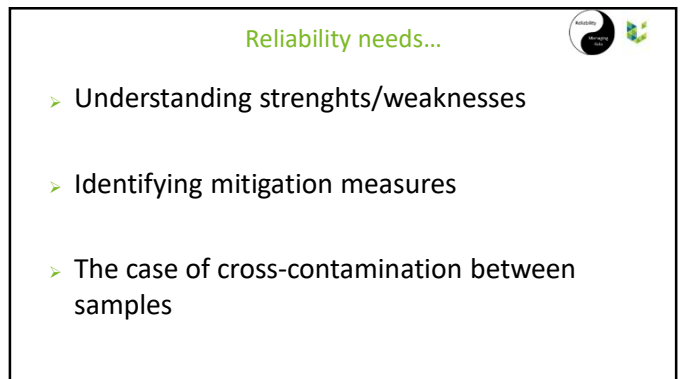
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5



6

### Under the carpet .... the cross-contamination & the analytical sensitivity

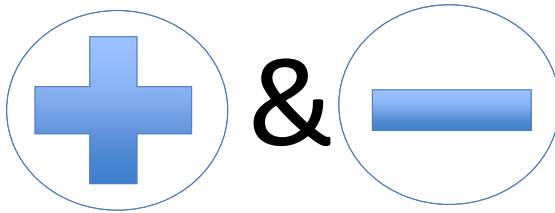


- Analytical sensitivity
  - 10-100M reads
  - 10- 100 reads on target (1 ppm)
- Low level infection or contamination ?

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### The cross-contamination & the controls

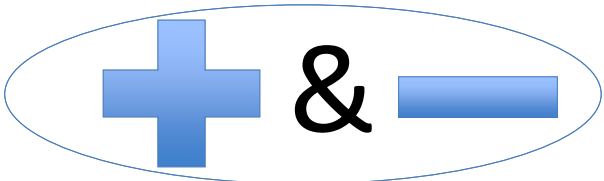
- About controls: before HTS



8

### The cross-contamination & the controls

- With HTS, a positive control for a target (pest) is also a negative control for any other target (pest)



9

### The new type of control: alien control



For virus detection by HTS, an alien control corresponds to a matrix containing one or several targets (called alien targets) that cannot be present in the samples to be tested.

It is processed with the samples as an external control



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### What is an alien control ?



... so the detection of reads from an alien target in a sample or another control can be unequivocally considered as a contamination from the alien control.

Alien control can also be used as a negative control



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### Using an alien to monitor contamination: VIRUS



Research  
Validation of High-Throughput Sequencing as Virus Indexing Test for *Musa* Germplasm: Performance Criteria Evaluation and Contamination Monitoring Using an Alien Control  
Wei Rong<sup>1</sup> | Johan Rollin<sup>1-2</sup> | Marwa Hanafi<sup>1</sup> | Nicolas Roux<sup>3</sup> | Sebastien Massart<sup>1,4</sup> |

- totalRNA on *Musa* samples
- Indexed previously (GHU)
- Wheat sample with BYDV as alien control





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### Using an alien to monitor contamination: VIRUS

- totalRNA on Musa samples
- Indexed previously (GHU)
- Wheat sample with BYDV as alien control

Library name	Batch	Number of reads mapped to RefSeq without detection threshold	CMV	BYDV
Alien control	1	3	1	2325
Alien control	1	1	2	2346
Alien control	1	1	11	885
Alien control	1	6	6	885
FC1543	1	2	4	2028
FC1298	1	1	1	1
FC1543	1	10	1	7
FC1543	1	1	3	7
FC1293	1	1	1	5
FC1793	1	1	4	4
FC1799	1	3	4	4
FC1827	1	1	4	4
FC1831	1	1	4	4
FC1833	1	2	4	4
FC1840	1	2	2	2
FC1843	1	2	2	2
FC1845	1	1	1	1
FC1845	1	1	1	1
FC1852	1	369	3	1368
FC1855	1	33691	3	1368
Positive control Mx1	1	22	3	1368
Positive control Mx1	1	2621	3	1368
Positive control Mx1	1	338	3	1368
Positive control Mx1	1	1847	3	1368
Positive control Mx1	1	17891	3	1368






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### Using an alien to monitor contamination

- totalRNA on Musa samples
- Indexed previously (GHU)
- 3 batches of sequencing
- Wheat sample with BYDV as alien control

Library name	Batch	Number of reads mapped to RefSeq without detection threshold	CMV	BYDV
Alien control	1	3	1	2325
Alien control	1	1	2	2346
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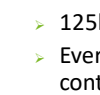
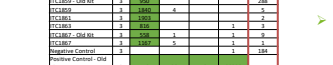



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### Using an alien to monitor contamination

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Positive control Mx1	1	17891	3	1368

- 125k reads in the alien control
- Every banana sample has alien contamination
- Max: 288 (another with 184)
- Sample EM4 has BBrMV ? New record on a continent...

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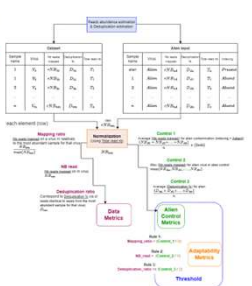
### Using an alien to monitor contamination

BMC Biology



Open Access

Cont-ID: detection of sample cross-contamination in viral metagenomic data

John Hollin<sup>1,2</sup>, Wei Rong<sup>1,2</sup> and Sébastien Mascart<sup>1,2</sup>



- Cont-ID pipeline

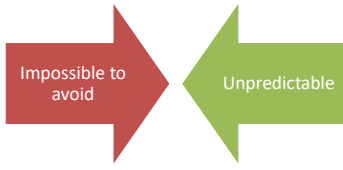

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### Conclusion on contamination

Impossible to avoid

Unpredictable

Monitoring is needed (preferably by alien control)

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### A transfer to diagnostics needs...


Reliability

Managing risks




18

Managing risks needs...



- Biological information for science-based decision
- Where are we ?
- The case of viruses

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20



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## 2. Exploration of the desert: fruit tree & tomato



**Is There a "Biological Desert" With the Discovery of New Plant Viruses? A Retrospective Analysis for New Fruit Tree Viruses**

Wenqing Han<sup>1,2</sup>, Shuang Li<sup>1,2</sup> and Sebastian Mansueti<sup>1,2</sup>

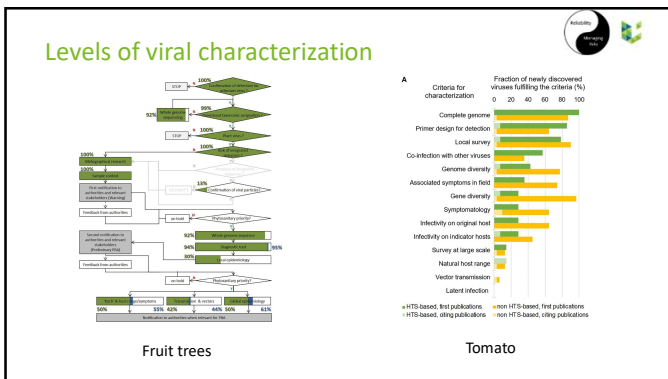
**Global Advances in Tomato Virome Research: Current Status and the Impact of High-Throughput Sequencing**

Mark Paul Sallie Rees<sup>1,2</sup>, Ana Mijangos<sup>1,2</sup>, Natalia MANN<sup>1,2</sup>, Maja Reuber<sup>1,2</sup> and Paolo Bonaldi<sup>1,2</sup>

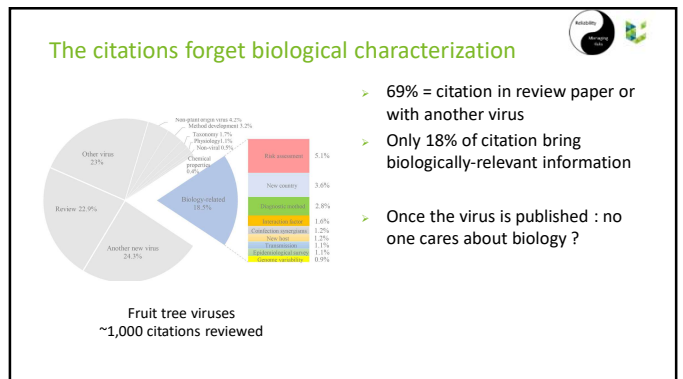
- A decade of virus discovery by HTS
- 91 putative new viral species
- How have they been characterized ?
- Reviewing 1,000 publications

- A decade of virus discovery by sanger and HTS
- 45 putative new viral species
- How have they been characterized ?

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23



24

### The Darwinian process of virus characterization

- Characterisation process = Darwinian
- Only the most threatening new viruses will be characterized and continue to live the characterization process



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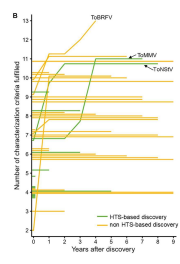

### The Darwinian process of virus characterization

- Framework = 14 steps
- « So quite » after publication
- Strong selection pressure:

45 new viruses on tomato

↓


13 for ToBRFV in 4 years  
11 for ToMMV & ToNStV in 5 years (1st HTS)

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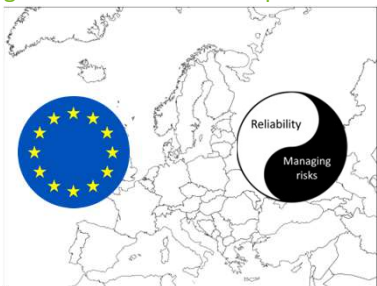


### Risk management of HTS results - conclusion

- Biological information is the Achille's Hell for risk management of « non heavily threatening » pests



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### The transfer to diagnostics... triggered a decade of European efforts

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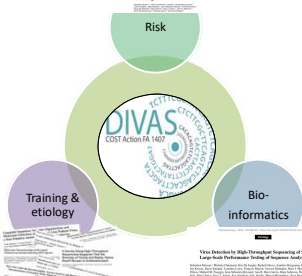

### When and how it started at large scale ?

- COST Action DIVAS: 2015-2019
  - Focus on HTS use for the detection of plant viruses and its consequences
  - Networking / training / think tank
  - 230 scientists having participated
    - Researchers
    - Diagnosticians
    - Plant health authorities (RPPO, NPPO)

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### When and how it started at large scale ?

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### When and how it started at large scale

The diagram shows a central circle labeled 'DIVAS COST Action 1410'. This central circle is surrounded by three larger circles: 'Risk' at the top, 'Bio-informatics' at the bottom right, and 'Training & etiology' at the bottom left. The background features a grid of small text boxes, likely representing project milestones or activities.

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### When and how it started at large scale ?

- COST Action DIVAS: 2014-2019: output 1

The screenshot shows the title and authors of a paper: 'A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies'. A green box labeled 'Risk' is overlaid on the right side of the image.

- First multi-stakeholder approach

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### When and how it started at large scale ?

- COST Action DIVAS: 2014-2019: output 2

The screenshot shows the title and authors of a paper: 'Virus Detection by High-Throughput Sequencing of Small RNAs: Large-Scale Performance Testing of Sequence Analysis Strategies'. A green box labeled 'Bio-informatics' is overlaid on the right side of the image.

- Same datasets to 21 laboratories
- Bioinformatics steps have a huge impact on the ability to detect viruses

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### When and how it started at large scale ?

- COST Action DIVAS: 2014-2019: output 3

The screenshot shows the title and authors of a paper: 'Complete Sequences for Long-Range Detection and Molecular Epidemiology of a New Plant Virus'. A green box labeled 'Training & etiology' is overlaid on the right side of the image.

- Training for virus detection from HTS dataset
- >100 scientists trained

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### Valitest project: a follow-up for any pest

- VALITEST project (2018-2021)
- Focus on the validation and reliability of diagnostic tests
- Any technique and any pest
- One WP with a strong focus on HTS guidelines

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### Valitest project: a follow-up for any pest



- Writing guidelines for using HTS in diagnostics setting
- Two scientific publications and one EPPO standard

The infographic consists of several colored boxes with text: a green box with '55 co-authors', a green box with '>1,500 revisions', a blue box with 'Any pest', and a purple box with 'Any technology'. There are also small images of a person, a book, and a DNA helix.

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**Valitest project: a follow-up for any pest**

➤ **First publication: building the foundations**

**Bulletin OEPP  
Eppo Bulletin**

ORIGINAL ARTICLE | Free Access

**Facilitating the adoption of high-throughput sequencing technologies as a plant pest diagnostic test in laboratories: A step-by-step description**

Benedicte Leber, Ian Adams, Maher Al-Bashir, Steve Benoit, Guillaume Blossey, Arnaud G. Boulin, Neil Bouman, Thierry Candresse, Anne Chaperon, Vito De Longhe, Arthur Dou, Vito E. A. Gialini, Pascal Gauthier, Annalisa Inglese, Welfredo Ho, Oscar Hurtado-Gonzales, Wilfried Jucker, Jan Krizan, Denis Knapik, Bianca Landa, Mirgesha Liu, Francesco Mader, Marlene Mader-Vogel, Hans-Martin Reuter, Martin Rastbach, Marlene Reuter, Angelantonio Minerva, Dimitra Molok, Adriana Moreira, Mark N. Hultine, Françoise Pottier, Alexander M. Pottier, Julien Pottier, Rüdiger Raus, Rocco Renucci, Yasmine Renucci, Rocco Renucci, Johanna W. Roeschmann, Johan Rubin, Pasquale Salsani, Johanna Santalla, Rose Souze-Richards, David Spadaro, David, Stathinos, Sophie Subbarao, René van der Vlugt, Lucie Terrier, Charlotte Tronin, Iria Vazquez-Garcia, Claudia S. L. Vicente, Bart T. L. H. Vossenbergh, Thierry Wezel, Niko Zisler, Sébastien Mazzoni | See Author details

First published: 08 August 2022 | <https://doi.org/10.1111/epp.12963>

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**Valitest project: a follow-up for any pest**

➤ **Running it: from adoption to validation**



**Peer Community Journal**  
Section: Infections

**Guidelines for the reliable use of high throughput sequencing technologies to detect plant pathogens and pests**


Sébastien Mazzoni, Ian Adams, Maher Al-Bashir, Steve Benoit, Guillaume Blossey, Arnaud G. Boulin, Neil Bouman, Thierry Candresse, Anne Chaperon, Vito De Longhe, Arthur Dou, Vito E. A. Gialini, Pascal Gauthier, Annalisa Inglese, Welfredo Ho, Oscar Hurtado-Gonzales, Wilfried Jucker, Jan Krizan, Denis Knapik, Bianca Landa, Mirgesha Liu, Francesco Mader, Marlene Mader-Vogel, Hans-Martin Reuter, Martin Rastbach, Marlene Reuter, Angelantonio Minerva, Dimitra Molok, Adriana Moreira, Mark N. Hultine, Françoise Pottier, Alexander M. Pottier, Julien Pottier, Rüdiger Raus, Rocco Renucci, Yasmine Renucci, Rocco Renucci, Johanna W. Roeschmann, Johan Rubin, Pasquale Salsani, Johanna Santalla, Rose Souze-Richards, David Spadaro, David, Stathinos, Sophie Subbarao, René van der Vlugt, Lucie Terrier, Charlotte Tronin, Iria Vazquez-Garcia, Claudia S. L. Vicente, Bart T. L. H. Vossenbergh, Thierry Wezel, Niko Zisler, Sébastien Mazzoni | See Author details

Volume 3 (2023), article 402

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**Valitest project: a follow-up for any pest**

➤ **Running it: from adoption to validation**



**Peer Community Journal**  
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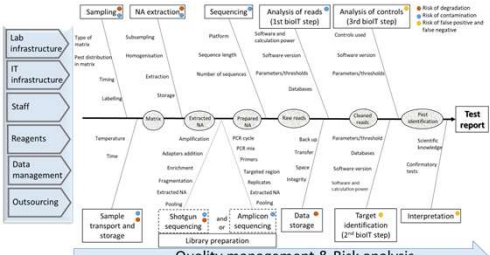
Sébastien Mazzoni, Ian Adams, Maher Al-Bashir, Steve Benoit, Guillaume Blossey, Arnaud G. Boulin, Neil Bouman, Thierry Candresse, Anne Chaperon, Vito De Longhe, Arthur Dou, Vito E. A. Gialini, Pascal Gauthier, Annalisa Inglese, Welfredo Ho, Oscar Hurtado-Gonzales, Wilfried Jucker, Jan Krizan, Denis Knapik, Bianca Landa, Mirgesha Liu, Francesco Mader, Marlene Mader-Vogel, Hans-Martin Reuter, Martin Rastbach, Marlene Reuter, Angelantonio Minerva, Dimitra Molok, Adriana Moreira, Mark N. Hultine, Françoise Pottier, Alexander M. Pottier, Julien Pottier, Rüdiger Raus, Rocco Renucci, Yasmine Renucci, Rocco Renucci, Johanna W. Roeschmann, Johan Rubin, Pasquale Salsani, Johanna Santalla, Rose Souze-Richards, David Spadaro, David, Stathinos, Sophie Subbarao, René van der Vlugt, Lucie Terrier, Charlotte Tronin, Iria Vazquez-Garcia, Claudia S. L. Vicente, Bart T. L. H. Vossenbergh, Thierry Wezel, Niko Zisler, Sébastien Mazzoni | See Author details

Volume 3 (2023), article 402

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**Valitest project: a follow-up for any pest**

➤ **The risk analysis (+ table on mitigation)**



**Quality management & Risk analysis**



The flowchart details the process from sample transport and storage to a test report. It includes stages like NA extraction, Sequencing, Analysis of reads, and Analysis of controls. A central box lists infrastructure categories: Lab infrastructure, IT infrastructure, Staff, Reagents, Data management, and Outsourcing. A final box highlights 'Quality management & Risk analysis' with a blue arrow pointing to the right.

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**Continuing research and development**

➤ **INEXTVIR project (2019-2023)**

➤ **Updating the framework:**

**Managing the deluge of newly discovered plant viruses and viroids: an optimized scientific and regulatory framework for their characterization and risk analysis**


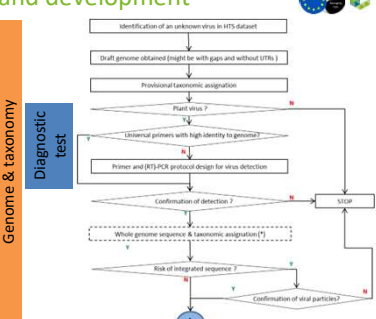
Nuria Fontevilla-Porceda, Margot Raboin, Apolline Baudouin, Marie Perle, Isabelle, Simon Raboin, Christelle Tronin, Carole Teyssie, Miguel A. Alvarez, Neil Bouman, Marlene Mader-Vogel, Thierry Candresse, Marlene Reuter, Marlene Reuter, Denis Krizan, Annalisa Inglese, Françoise Pottier, Alexander M. Pottier, Julien Pottier, Rüdiger Raus, Rocco Renucci, Yasmine Renucci, Rocco Renucci, Johanna W. Roeschmann, Johan Rubin, Pasquale Salsani, Johanna Santalla, Rose Souze-Richards, David Spadaro, David, Stathinos, Sophie Subbarao, René van der Vlugt, Lucie Terrier, Charlotte Tronin, Iria Vazquez-Garcia, Claudia S. L. Vicente, Bart T. L. H. Vossenbergh, Thierry Wezel, Niko Zisler, Sébastien Mazzoni | See Author details

Volume 3 (2023), article 402

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**Continuing research and development**

➤ **Update in 2022:**

**Genome & taxonomy Diagnostic test**

The flowchart shows the process from identification of an unknown virus in an HTS dataset to confirmation of viral particles. It includes steps like Draft genome obtained, Provisional taxonomic assignment, and Confirmation of detection. A vertical bar on the left indicates 'Genome & taxonomy' and 'Diagnostic test'.

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### Continuing research and development

Update in 2023:

- Bibliography
- DECISION
- Association
- DECISION
- Filling data gaps

Frequency of evaluation depending on information provided

Plant Pathology  
Reconsidering causal association in plant virology  
Adapted from: [QR code]  
Free published 27 April 2020 | <https://doi.org/10.1111/ppa.12198> | Citations: 4

INEXTVIR

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### Biological experiments are expensive !

GenoPREDICT  
An Open and Collaborative e-Infra-Structure Approach for Viral Characterisation and Epidemic Prediction

Prediction of biological properties from genomics and proteomics data using artificial intelligence ?

(note from the presenter: AI is a fancy word but, essentially, it is about basic statistics applied at large scale)

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### Biological experiments are expensive !

GenoPREDICT  
An Open and Collaborative e-Infra-Structure Approach for Viral Characterisation and Epidemic Prediction

AQA1DBFVH5  
Alphacoronavirus  
physostegiae

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### Continuing research and development

Euphresco network & HTS

- Ring testing
- Bioinformatics network
- Data sharing for better biosecurity
- ...

Yearly call often integrating HTS-related work

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LIÈGE université  
Gembloux  
Agro-Bio Tech

### Conclusion: toward use of HTS in diagnostics

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### EFSA recommendation

SCIENTIFIC OPINION

ADOPTED: 21 November 2019  
doi: 10.2903/j.efsa.2020.5928

**Pest categorisation of non-EU viruses of *Rubus L.***

EFSA Panel on Plant Health (PLH),  
Claude Bragard, Katharina Dehnen-Schmitz, Pawlo Gonthier, Marie-Agnès Jacques, Josep Anton Jaques Miret, Annemarie Fejer Justesen, Alan MacLeod, Christer Sven Magnusson, Panagiotis Milonas, Juan A. Navas-Cortes, Stephen Parnell, Roel Potting, Philippe Lucien Reignault, Hans-Hermann Thulke, Wopke Van der Werf, Antonio Vicent Civero, Jonathan Yuen, Lucia Zappalà, Thierry Candresse, Elisavet Chatzivassiliou, Franco Finelli, Stephan Winter, Domenico Bosco, Michela Chiumenti, Francesco Di Serio, Franco Ferrilli, Tomasz Kaluski, Angelantonio Minafra and Luisa Rubino

**Abstract**  
The Panel on Plant Health of EFSA conducted a pest categorisation of 17 viruses of *Rubus L.* that were previously classified as either non-EU or of undetermined standing in a previous opinion. These infectious agents belong to different genera and are heterogeneous in their biology. Blackberry virus X, blackberry virus Z and wineberry latent virus were not categorised because of lack of information while grapevine red blotch virus was excluded because it does not infect *Rubus*. All 17 viruses are efficiently

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**EPPO official standard**

DOI: 10.1111/epp.12844

**EPPO STANDARD ON DIAGNOSTICS**

**PM 7/151 (1) Considerations for the use of high throughput sequencing in plant health diagnostics<sup>1</sup>**

Specific scope: This Standard describes elements to take into consideration for the use of high throughput sequencing (HTS) tests, including validation, quality control measures and interpretation and reporting of results to ensure HTS test results are robust and accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way. This Standard applies to all plant pest groups and HTS technologies. This Standard should be used in conjunction with PM 7/76 Use of EPPO diagnostic protocols. Specific approval and amendment: Approved in 2022-09. Authors and contributors are given in the Acknowledgements section.

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**Reference laboratories**

- EURL & NRL
- Proficiency testing using HTS to detect viruses (biological material and datasets)
- ISO 17025

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**Thanks for the support**

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**A team work in the lab**

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**Special thanks to hundreds of plant pathologists collaborating on disseminating HTS and making it more reliable**

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**LIÈGE université Gembloux Agro-Bio Tech**

**Thanks for the invitation & for your attention**

Prof. Sebastien Massart  
[sebastien.massart@uliege.be](mailto:sebastien.massart@uliege.be)  
 @Be\_Phytopath

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