
1

## HTS and pest detection


2

## HTS and pest detection


ANY pest | ALL matters | Single read | MANY steps | KEY bioIT

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## HTS and pest detection

The big five !

ANY pest | ALL matters | Single read | MANY steps | KEY bioIT


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## How reliable is HTS detection ?

1. DNA shotgun sequencing for identification of a microbe (pure culture) or isolated nematode

| Item | # sequences |
|---|---|
| Generated reads | 5,000,000 |
| On target | 4,950,000 ? |

**DOUBT ?** Genomic databases (analytical specificity)

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## How reliable is HTS detection ?

2. RNA shotgun sequencing: plant sample (metagenome)

| Item | # sequences |
|---|---|
| Generated reads | 5,000,000 |
| Virus 1 | 200,000 |
| Virus 2 | 15 |
| Phytoplasma (16s) | 500 |

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## How reliable is HTS detection ?

ANY pest

2. RNA shotgun sequencing: plant sample (metagenome)

| Item | # sequences |
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| Generated reads | 5,000,000 |
| Virus 1 | 200,000 |
| Virus 2 | 15 |
| Phytoplasma (16s) | 500 |

Article

**Looking beyond Virus Detection in RNA Sequencing Data: Lessons Learned from a Community-Based Effort to Detect Cellular Plant Pathogens and Pests**

Annelies Haegeman [1,*], Yoika Foucart [1], Kris De Jonghe [1], Thomas Goedefroit [1], Maher Al Rwahnih [2], Neil Boonham [3], Thierry Candresse [4], Yahya Z. A. Gaafar [5], Oscar P. Hurtado-Gonzales [6], Zala Kogej Zwitter [7,8], Denis Kutnjak [7], Janja Lamovšek [9], Marie Lefebvre [4], Martha Malapi [10], Irena Mavrič Pleško [9], Serkan Önder [11], Jean-Sébastien Reynard [12], Ferran Salavert Pamblanco [5], Olivier Schumpp [13], Kristian Stevens [7], Chandan Pal [7], Lucie Tamisier [14], Çiğdem Ulubaş Serçe [15], Inge van Duivenbode [16], David W. Waite [17], Xiaojun Hu [6], Heiko Ziebell [18] and Sébastien Massart [19,*]

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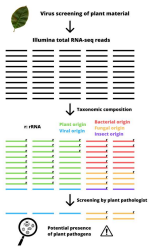
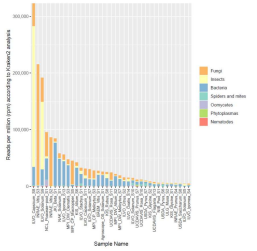
## How reliable is HTS detection ?

ANY pest




rRNA

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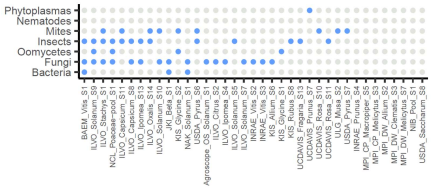
## How reliable is HTS detection ?

ANY pest




Considering the 37 selected datasets, in total 67 pathogens (39 different taxa) were detected (≥100 rpm) from 29 of the datasets (78%). Confirmation by PCR in >70% of the cases

Ok but really preliminary: many pending issues: what about sensitivity and selectivity ?

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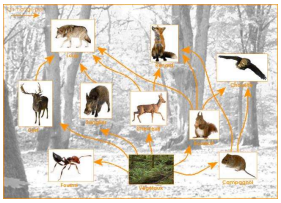
## The hunting trophy bias in research

ALL matters

**Virus discovery**


A new virus at high abundance (complete genome & high coverage)

**Diagnostics (or virus ecology)**



Other viruses/organisms ? If low abundance ?

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## How reliable is HTS detection ?

Single read

2. RNA shotgun sequencing: plant sample (metagenome)

| Item | # sequences |
|---|---|
| Generated reads | 5,000,000 |
| Virus 1 | 200,000 |
| Virus 2 | 15 ? |
| Phytoplasma (16s) | 500 ? |

**DOUBT ?** genomic databases (analytical specificity)

15 reads are enough ? (analytical sensitivity)

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## How reliable is HTS detection ?

Single read

2. RNA shotgun sequencing: plant sample

| Item | # sequences | # sequences - sample 2 |
|---|---|---|
| Generated reads | 5,000,000 | 5,000,000 |
| Virus 1 | 200,000 | 0 |
| Virus 2 | 15 ? | 500,000 |
| Phytoplasma (16s) | 500 | |

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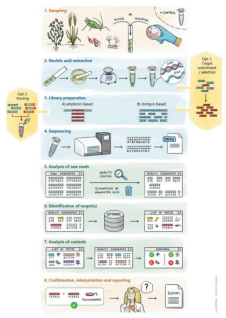
## How reliable is HTS detection ?



Bulletin OEPP
EPPO Bulletin

ORIGINAL ARTICLE

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

Benedicte Lebas, Ian Adams, Maher Al Rwahnih, Stevie Boeyen, Guillaume J. Bilodeau, Arnaud G. Blouin, Neil Boonham, Thierry Candresse, Anne Chandelier, Kris De Jonghe, Adrian Fox, Yahya Z. A. Gaafar, Pascal Gerrit, Annelies Haegeman, Wellcome Ho, Oscar Hurtado-Gonzales, Wilfried Jonkers, Jan Kreuze, Denis Kutnjak, Blanca Landa, Mingxin Liu, François Maclot, Martha Malapi-Wight, Hans J. Maree, Francesco Martoni, Natasha Mehle, Angelantonio Minafra, Dimitre Mollov, Adriana Moreira, Mark Nakhla, Françoise Petter, Alexander M. Piper, Julien Ponchart, Robbie Rae, Benoit Remenant, Yazmin Rivera, Brendan Rodoni, Johanna W. Roenhorst, Johan Rollin, Pasquale Saldarelli, Johanna Santala, Rose Souza-Richards, Davide Spadaro, David J. Studholme, Stefanie Sultmanis, René van der Vlugt, Lucie Tamisier, Charlotte Trontin, Ines Vázquez-Iglesias, Claudia S. L. Vicente, Bart T. L. H. Vossenberg, Thierry Wetzel, Heiko Ziebell , Sebastien Massart ... See fewer authors

First published: 08 August 2022 | https://doi.org/10.1111/epp.12863

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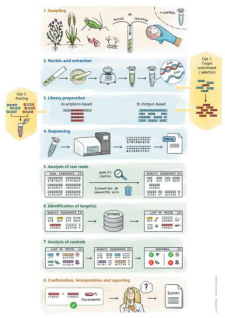
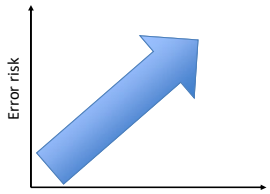
## How reliable is HTS detection ?



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## In results we trust: bioinformatics

- 12 viruses/viroids in 10 datasets
- Same data sent to 21 laboratories
- Each participant had to identify viruses present in the datasets

Virology

**Virus Detection by High-Throughput Sequencing of Small RNAs: Large-Scale Performance Testing of Sequence Analysis Strategies**

Sébastien Massart, Michela Chiumenti, Kris De Jonghe, Rachel Glover, Annelies Haegeman, Igor Koloniuk, Petr Komínek, Jan Kreuze, Denis Kutnjak, Lorraine Lotos, François Maclot, Varvara Maliogka, Hans J. Maree, Thibaut Olivier, Antonio Olmos, Mikhail M. Pooggin, Jean-Sébastien Reynard, Ana B. Ruiz-García, Dana Safarova, Pierre H. H. Schoenberger, Nea Sela, Silvio Turco, Reva J. Varsha, Eva Varkonyi, Eric Verdin, Marcel Westenberg, Yves Brostaux, and Thierry Candresse

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## In results we trust: bioinformatics

- Diagnostic sensitivity
  - 70%
  - Variable between labs
  - Depending on sequencing depth
- Bioinformatics really matters

| labID | Sensitivity | | | |
|---|---|---|---|---|
| | 50,000 | 250,000 | 2,500,000 | Average |
| A | 10% | 53% | 90% | 51% |
| B | 30% | 35% | 80% | 46% |
| C | 60% | 71% | 80% | 70% |
| D | 50% | 82% | 100% | 78% |
| E | 30% | 82% | 80% | 68% |
| F | 80% | 88% | 100% | 89% |
| G | 20% | 53% | 100% | 57% |
| H | 30% | 65% | 70% | 57% |
| J | 70% | 94% | 100% | 89% |
| K | 40% | 71% | 90% | 68% |
| M | 50% | 94% | 90% | 81% |
| N | 30% | 82% | 90% | 70% |
| O | 20% | 41% | 40% | 35% |
| P | 20% | 59% | 70% | 51% |
| R | 100% | 100% | 100% | 100% |
| S | 50% | 100% | 100% | 86% |
| T | 90% | 100% | 100% | 97% |
| V | 60% | 88% | 80% | 78% |
| W1 | 40% | 82% | 90% | 73% |
| W2 | 60% | 82% | 90% | 78% |
| X | 30% | 71% | 80% | 62% |
| AVERAGE | 46% | 75% | 86% | 70% |

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## In results we trust: bioinformatics

Bioinformatic analysis was carried out « according to default parameters »

Will you do it in the laboratory ?

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## In results we trust: bioinformatics

- « The holy trinity » from Roublev (XV century)

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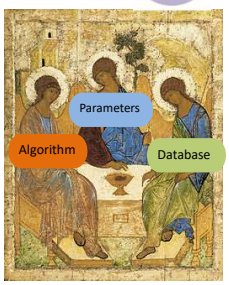
## In results we trust: bioinformatics

KEY bioIT

- The « bioinformatics trinity »
- Algorithms, their parameters and the database can create bias

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## Summary of key elements

ANY pest | ALL matters | Single read | MANY steps | KEY bioIT

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LIÈGE université Gembloux Agro-Bio Tech

## The contamination swamp

« Under the carpet » story

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## 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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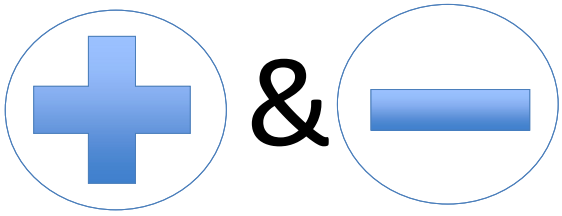
4. Alien invasion

- About controls: before HTS

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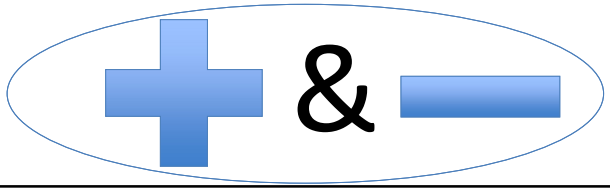
4. Alien invasion

- With HTS, a positive control for a virus is also a negative control for any other virus

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## The new type of control: alien control

For pest detection by HTS, an alien control corresponds to a matrix containing one or several pests (called alien pests) 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 pests 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

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

Research

Validation of High-Throughput Sequencing as Virus Indexing Test for *Musa* Germplasm: Performance Criteria Evaluation and Contamination Monitoring Using an Alien Control

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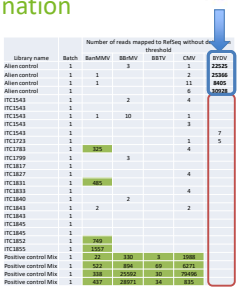
## Using an alien to monitor contamination

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



| Library name | Batch | BanMMV | BBrMV | BBTV | CMV | BYDV |
|---|---|---|---|---|---|---|
| Alien control | 1 | | 3 | | 1 | 22525 |
| Alien control | 1 | 1 | | | 2 | 25366 |
| Alien control | 1 | 1 | | | 11 | 8405 |
| Alien control | 1 | | | | 6 | 30928 |
| ITC1543 | 1 | | | 2 | 4 | |
| ITC1543 | 1 | | | | | |
| ITC1543 | 1 | 1 | 10 | | 1 | |
| ITC1543 | 1 | | | | 3 | |
| ITC1543 | 1 | | | | | 7 |
| ITC1723 | 1 | | | | 1 | 5 |
| ITC1783 | 1 | 325 | | | 4 | |
| ITC1799 | 1 | | 3 | | | |
| ITC1817 | 1 | | | | | |
| ITC1827 | 1 | | | | 4 | |
| ITC1831 | 1 | 485 | | | | |
| ITC1833 | 1 | | | | 4 | |
| ITC1840 | 1 | | 2 | | | |
| ITC1843 | 1 | 2 | | | 2 | |
| ITC1843 | 1 | | | | | |
| ITC1845 | 1 | | | | | |
| ITC1845 | 1 | | | | | |
| ITC1852 | 1 | 769 | | | | |
| ITC1855 | 1 | 1557 | | | | |
| Positive control Mix | 1 | 22 | 330 | 3 | 1988 | |
| Positive control Mix | 1 | 522 | 894 | 69 | 6271 | |
| Positive control Mix | 1 | 338 | 25502 | 30 | 79496 | |
| Positive control Mix | 1 | 417 | 28971 | 14 | 835 | |

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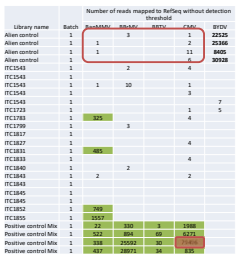
## Using an alien to monitor contamination

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



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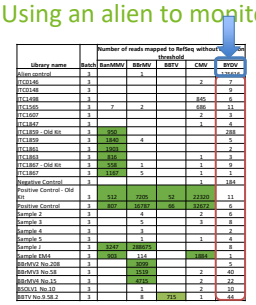
## Using an alien to monitor contamination



- 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

| Library name | Batch | Number of reads mapped to RefSeq without detection threshold | | | | |
|---|---|---|---|---|---|---|
| | | BanMMV | BBrMV | BBTV | CMV | BYDV |
| Alien control | 3 | | 1 | | | 125816 |
| TC0146 | 3 | | | | 2 | 7 |
| TC0148 | 3 | | | | | 9 |
| TC1498 | 3 | | | | 845 | 6 |
| TC1565 | 3 | 7 | 2 | | 686 | 11 |
| TC1607 | 3 | | | | 2 | 3 |
| TC1847 | 3 | | | | 1 | 4 |
| TC1859 - Old Kit | 3 | 950 | | | | 288 |
| TC1859 | 3 | 1840 | 4 | | | 5 |
| TC1861 | 3 | 1903 | | | | 2 |
| TC1863 | 3 | 836 | | | 1 | 3 |
| TC1867 - Old Kit | 3 | 558 | 1 | | 1 | 9 |
| TC1867 | 3 | 1357 | 5 | | 1 | 1 |
| Negative Control | 3 | | | | 1 | 184 |
| Positive Control - Old Kit | 3 | 512 | 7205 | 52 | 22320 | 11 |
| Positive Control | 3 | 807 | 16787 | 66 | 32672 | 6 |
| Sample 2 | 3 | | 4 | | 2 | 6 |
| Sample 3 | 3 | | 5 | | 3 | 8 |
| Sample 4 | 3 | | 3 | | | 2 |
| Sample 5 | 3 | | 1 | | 1 | 4 |
| Sample J | 3 | 3247 | 28825 | | | 8 |
| Sample EM4 | 3 | 903 | 114 | | 1864 | 1 |
| BBrMV2 No.208 | 3 | | 3099 | | | 5 |
| BBrMV3 No.58 | 3 | | 3519 | | 2 | 60 |
| BBrMV4 No.15 | 3 | | 4715 | | 2 | 22 |
| BSDLV1 No.10 | 3 | | 1 | | 2 | 10 |
| BBTV No.9.58.2 | 3 | | 8 | 755 | 1 | 44 |

- CMV : infected or false positive ?
- RT-PCR & new HTS

- Check on other projects
- CMV+ samples (>500k reads)
- 100% identity

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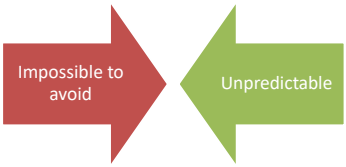
## So, contaminations are ….




Monitoring is needed (preferably by alien control)

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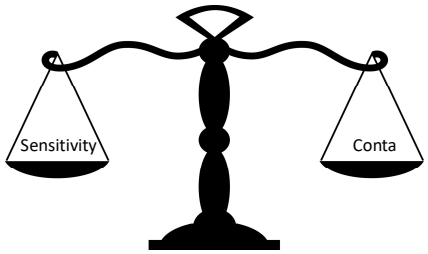
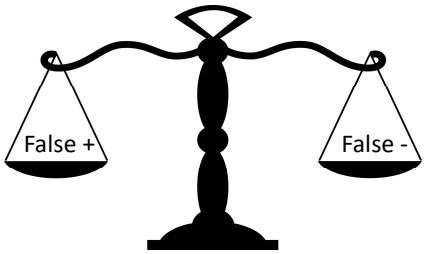
## Fine, I monitor it but… which threshold to apply ?




33

## Fine, I monitor it but… which threshold to apply ?

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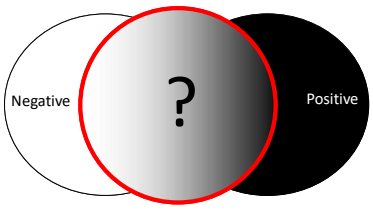
## Fine, I monitor it but… which threshold to apply ?




The grey zone

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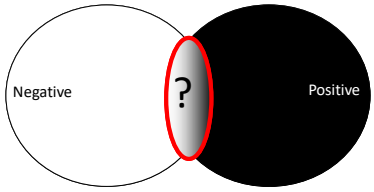
## Fine, I monitor it but… which threshold to apply ?




How can I reduce my grey zone while maintaining reliability ?

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## Fine, I monitor it but… which threshold to apply ?

# 10

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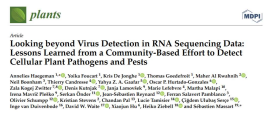
## Fine, I monitor it but… which threshold to apply ?

# rpm

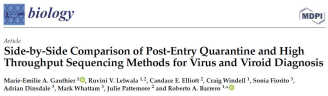
1. A fixed absolute value


plants

Article

Looking beyond Virus Detection in RNA Sequencing Data: Lessons Learned from a Community-Based Effort to Detect Cellular Plant Pathogens and Pests

2. A fixed relative value (%) to the maximum in a sample


biology

Article

Side-by-Side Comparison of Post-Entry Quarantine and High Throughput Sequencing Methods for Virus and Viroid Diagnosis

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## Fine, I monitor it but… which threshold to apply ?

- Adaptative depending on run's contamination monitored by Alien control
- 3 levels evaluated :
  - no filter (fixed)
  - 10 reads filter (fixed)
  - Maximum of alien contamination in a sample: adaptative between batches: from 7 to 288

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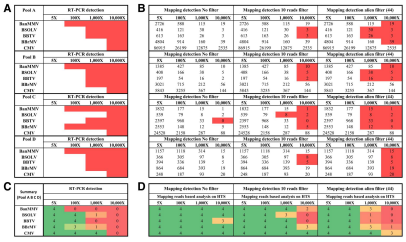
## Fine, I monitor it but… which threshold to apply ?

- Analytical sensitivity



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## Fine, I monitor it but… which threshold to apply ?

- Analytical sensitivity -> accuracy

| Sample | Filter | Accuracy | False positive | False negative |
|---|---|---|---|---|
| Batch 1 | No filter | 49% | 51% | 0% |
| | 10 reads | 93% | 5% | 2% |
| | Alien control | 93% | 5% | 2% |
| Batch 2 | No filter | 29% | 71% | 0% |
| | 10 reads | 84% | 16% | 0% |
| | Alien control | 94% | 3% | 3% |
| Batch 3 | No filter | 43% | 57% | 0% |
| | 10 reads | 94% | 6% | 0% |
| | Alien control | 92% | 4% | 4% |
| Batch 4 (with dilution) | No filter | 86% | 11% | 2% |
| | 10 reads | 88% | 3% | 9% |
| | Alien control | 77% | 2% | 20% |
| Batch 5 | No filter | 80% | 20% | 0% |
| | 10 reads | 90% | 10% | 0% |
| | Alien control | 100% | 0% | 0% |

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## Fine, I monitor it but… which threshold to apply ?

- Absolute number of reads or rpm ?
- BBTV: low abundance virus (380 reads on average and maximum 2,397 reads per sample vs. 25,000 to 120,000 reads for alien virus)
- Batch with 282 alien contaminating reads -> many false negative for BBTV
- Relative value is needed

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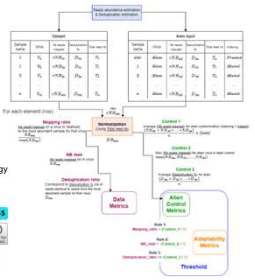
## Using an alien to monitor contamination

- Cont-ID pipeline: automatic analysis with expert interpretation



Rollin et al. BMC Biology (2023) 21:217
https://doi.org/10.1186/s12915-023-01708-w

BMC Biology

SOFTWARE Open Access

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

Johan Rollin[1,2†], Wei Rong[1†] and Sébastien Massart[1*]

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## Using an alien to monitor contamination

Parameter for Alien – background quantification (ELISA-like)

**Normalization** (Using Total read nb)

$NB_{nm}$

**Control 1**

Average (Nb reads mapped) for alien contamination (indexing = Asbent)

$$\frac{(NB_{A1}+NB_{A2}+\ldots+NB_{An})}{n} + (3sdo)$$

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## Using an alien to monitor contamination



Parameter for Alien – number in Alien control

**Control 2**

Max (Nb reads mapped) for alien virus in alien control

$$max(NB_{An}, NB_{A1}, \ldots, NB_{An})$$

45

## Using an alien to monitor contamination



Parameter for Alien – number in Alien control

**Control 3**

Average (Deduplication %) for alien

$$\frac{(D_{An}+D_{A1}+\ldots+D_{An})}{n}$$

46

## Using an alien to monitor contamination



Reads mapped on each target (virus) in each sample:

RATIO

**Mapping ratio**

Nb reads mapped on a virus m relatively to the most abundant sample for that virus

$$\frac{NB_{nm}}{max(NB_{nm})}$$

**Normalization** (Using Total read nb)

$NB_{nm}$

**Control 1**

Average (Nb reads mapped) for alien contamination

$$\frac{(NB_{A1}+NB_{A2}+\ldots+NB_{An})}{n} + (3sdo)$$

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## Using an alien to monitor contamination



Reads mapped on each target (virus) in each sample:

NUMBER

**NB read**

Nb reads mapped on m virus.

$NB_{nm}$

**Control 2**

Max (Nb reads mapped) for alien virus in alien control

$$max(NB_{An}, NB_{A1}, \ldots, NB_{An})$$

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## Using an alien to monitor contamination


Reads mapped on each target (virus) in each sample:
DEDUPLICATION

**Deduplication ratio**
Correspond to Deduplication % (ratio of reads identical to reads from the most abundant sample for that virus)
$D_{nm}$

**Data Metrics**

For virus n in sample m

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## Using an alien to monitor contamination


**Rule 1:**
Mapping_ratio > (Control_1 / X)

**Rule 2:**
NB_read > (Control_2 / Y)

**Rule 3:**
Deduplication_ratio <= (Control_3 / Z)

**Adaptability Metrics**

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## But still a long way to go… April 2022

nature COMMUNICATIONS

ARTICLE

OPEN

The pollen virome of wild plants and its association with variation in floral traits and land use

Supplementary material

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## Investigating contamination from Impatiens

Genome identity between samples ?

| RNA 1 | KX834010_Impatiens | KX834010_Oenothera | KX834010_Lotus | Consensus | KX834010_Solidago |
|---|---|---|---|---|---|
| KX834010_BCRV_contig_6_Impatiens (reversed) | | 0 | 100 | 100 | 99 |
| KX834010_BCRV_contig_722_Oenothera (reversed) | 0 | | 100 | 100 | 0 |
| KX834010_BCRV_contig_154_Lotus (reversed) | 100 | 100 | | 100 | 100 |
| Consensus | 100 | 100 | 100 | | 100 |
| KX834010_BCRV_contig_1293_Solidago (reversed) | 99 | 0 | 100 | 100 | |

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## Investigating contamination from Impatiens

SNP analysis ?

*Lotus* RNA1 of BCRV has 20 mutations absent in *Impatiens*

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## Investigating contamination from Impatiens

SNP analysis ?

All the SNPs in Oenothera (73), Convulvus (9) and Vernonia (7) are present in Impatiens

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## Impact on regulation ?

SCIENTIFIC OPINION

EFSA Journal

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-Schmutz, Paolo 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 Civera, Jonathan Yuen, Lucia Zappalà, Thierry Candresse, Elisavet Chatzivassiliou, Franco Finelli, Stephan Winter, Domenico Bosco, Michela Chiumenti, Francesco Di Serio, Franco Ferilli, Tomasz Kaluski, Angelantonio Minafra and Luisa Rubino

For BCRV, ……., due to the insufficient information available the Panel was unable to conclude on the potential consequences in the EU territory.

However, **….. meet all the other criteria evaluated by EFSA to qualify as Union quarantine pests**.

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## The path toward diagnostics use

Guidelines for reliable HTS diagnostics

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## Valitest project: guidelines for any pest

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


55 co-authors

>1,500 revisions


Any pest

Any technology

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

- First publication: building the foundations

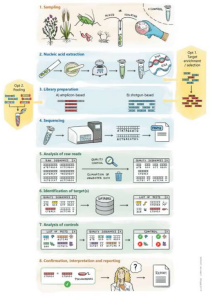
Bulletin OEPP EPPO Bulletin

ORIGINAL ARTICLE

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

Benedicte Lebas, Ian Adams, Maher Al Rwahnih, Steve Baeyen, Guillaume J. Bilodeau, Arnaud G. Blouin, Neil Boonham, Thierry Candresse, Anne Chandelier, Kris De Jonghe, Adrian Fox, Yahya Z. A. Gaafar, Pascal Gentit, Annelies Haegeman, Wellcome Ho, Oscar Hurtado-Gonzales, Wilfried Jonkers, Jan Kreuze, Denis Kutjnak, Blanca Landa, Mingxin Liu, François Maclot, Martha Malapi-Wight, Hano J. Maree, Francesco Martoni, Natasha Mehle, Angelantonio Minafra, Dimitre Mollov, Adriana Moreira, Mark Nakhla, Françoise Petter, Alexander M. Piper, Julien Ponchart, Robbie Rae, Benoit Remenant, Yazmin Rivera, Brendan Rodoni, Johanna W. Roenhorst, Johan Rollin, Pasquale Saldarelli, Johanna Santala, Rose Souza-Richards, Davide Spadaro, David J. Studholme, Stefanie Sultmanis, René van der Vlugt, Lucie Tamisier, Charlotte Trontin, Ines Vazquez-Iglesias, Claudia S. L. Vicente, Bart T. L. H. Vossenberg, Thierry Wetzel, Heiko Ziebell, Sebastien Massart ✉ … See fewer authors

First published: 08 August 2022 | https://doi.org/10.1111/epp.12863



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

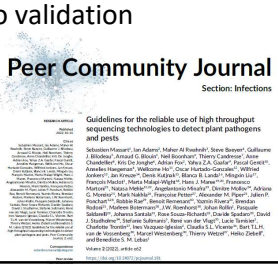
- Running it: from adoption to validation

Peer Community Journal

Section: Infections

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

Sebastien Massart[1], Ian Adams[2], Maher Al Rwahnih[3], Steve Baeyen[4], Guillaume J. Bilodeau[5], Arnaud G. Blouin[6], Neil Boonham[7], Thierry Candresse[8], Anne Chandelier[9], Kris De Jonghe[9], Adrian Fox[2], Yahya Z.A. Gaafar[10], Pascal Gentit[11], Annelies Haegeman[4], Wellcome Ho[12], Oscar Hurtado-Gonzales[13], Wilfried Jonkers[12], Jan Kreuze[14], Denis Kutjnak[15], Blanca B. Landa[16], Mingxin Liu[17], François Maclot[9], Marta Malapi-Wight[18], Hans J. Maree[19,20], Francesco Martoni[21], Natasha Mehle[15,22], Angelantonio Minafra[23], Dimitre Mollov[24], Adriana G. Moreira[25], Mark Nakhla[26], Françoise Petter[27], Alexander M. Piper[28], Julien P. Ponchart[29], Robbie Rae[30], Benoit Remenant[31], Yazmin Rivera[32], Brendan Rodoni[33], Mathews Botermans[34], J.W. Roenhorst[34], Johan Rollin[4], Pasquale Saldarelli[35], Johanna Santala[36], Rose Souza-Richards[37], Davide Spadaro[38], David J. Studholme[39], Stefanie Sultmanis[40], René van der Vlugt[34], Lucie Tamisier[9], Charlotte Trontin[41], Ines Vazquez-Iglesias[42], Claudia S.L. Vicente[43], Bart T.L.H. van de Vossenberg[34], Marcel Westenberg[44], Thierry Wetzel[45], Heiko Ziebell[46], and Benedicte S. M. Lebas[1]



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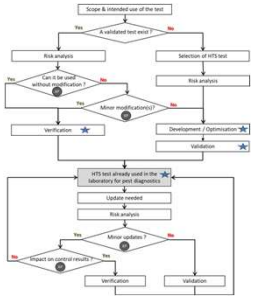
## Valitest project: guidelines for any pest

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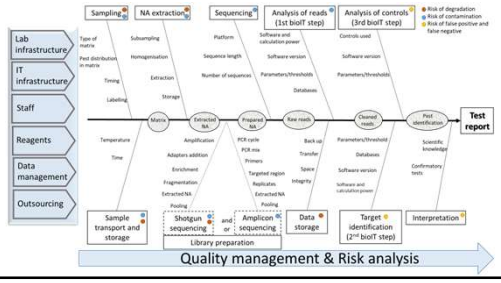
## Valitest project: guidelines for any pest

- The risk analysis (+ table on mitigation)

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## Adaptation toward official standard

DOI: 10.1111/epp.12884

EPPO STANDARD ON DIAGNOSTICS

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

**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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## Conclusion

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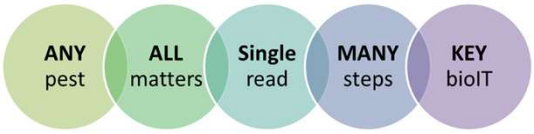
## Conclusion: technical challenges

- Super hype and high potential but …

« All that glitters is not gold »

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## Conclusion: technical challenges

- Each step can create bias

- Double check results of any publication

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## Conclusion: my experience


… the supplementary material of any publication !

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## Conclusion: toward larger scale application

- More complex process than previous tests
- Guidelines and official standard

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## Conclusion : toward larger scale application

- New pests (viruses) uncharacterized
- New hosts (and pathways)
- New countries
- Biology and risk management as rising epicenters of HTS earthquake…

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Valitest
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FÉDÉRATION WALLONIE-BRUXELLES
federal public service HEALTH, FOOD CHAIN SAFETY AND ENVIRONMENT

Thanks for the support

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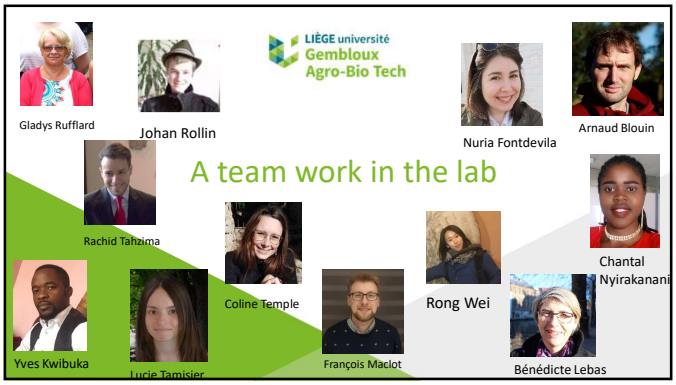


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Special thanks to hundreds of plant pathologists collaborating on disseminating HTS and making it more reliable

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## Thanks for the invitation & for your attention

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