



European Cooperation in
Science and Technology



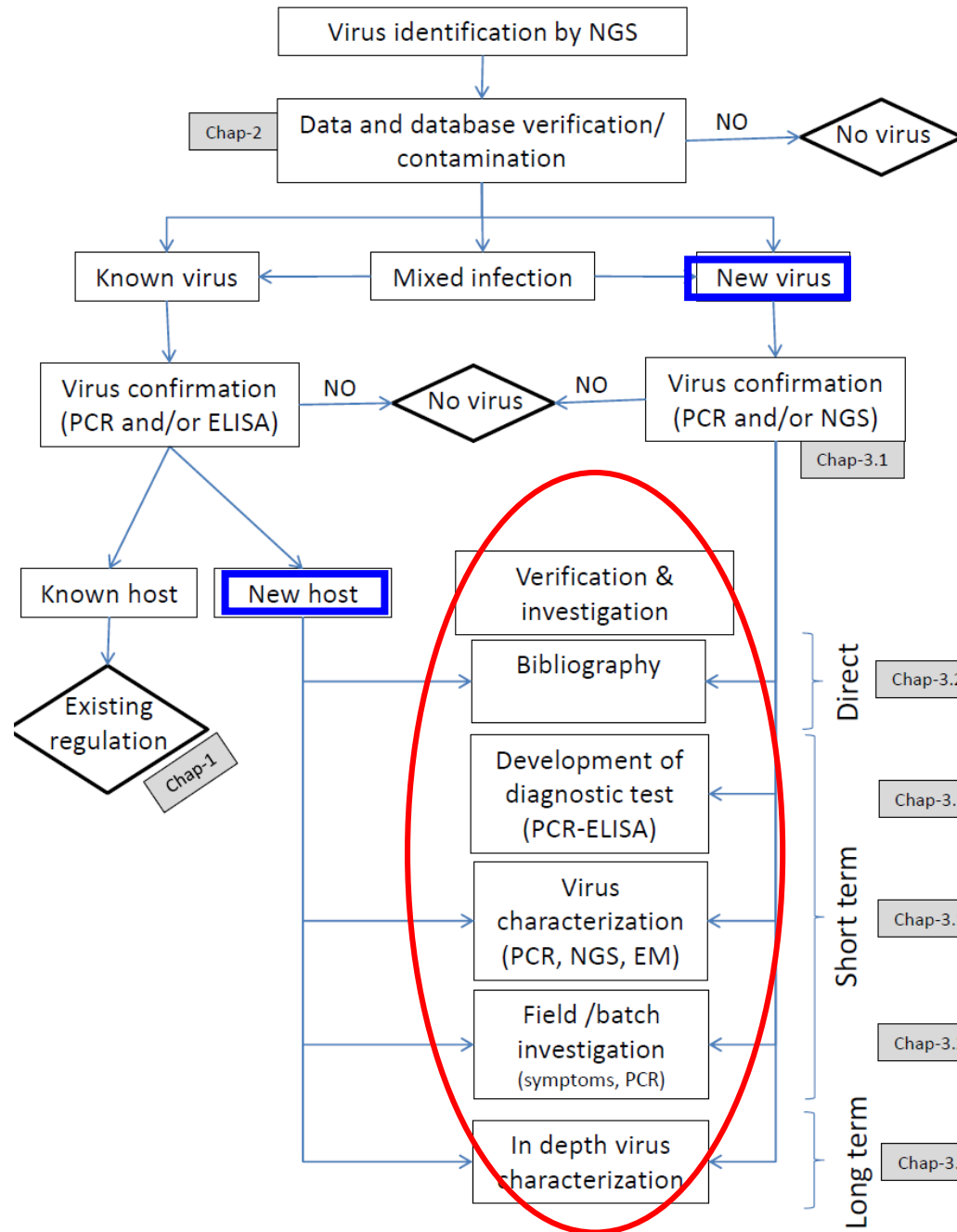
Framework for the evaluation of biosecurity, commercial, regulatory and scientific impacts of plant viruses and viroids identified by NGS technologies.

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- I. CURRENT SITUATION FOR EXISTING VIRUSES**
 - II. EMERGING CHALLENGES FOR SCIENTISTS AND AUTHORITIES**
 - III. EARLY STEPS OF BIOLOGICAL CHARACTERIZATION**
 - IV. IN-DEPTH BIOLOGICAL CHARACTERIZATION**
- CONCLUSION**

I. CURRENT SITUATION FOR EXISTING VIRUSES

In Europe: EPPO guideline



II. Emerging challenges for scientists and authorities

Previously:

Symptoms => investigations => detection of virus => EPPO

Now:

NGS: inverted approach on diagnostic:

Detection of virus (symptomatic or asymptomatic plants)

=> investigations ???

II. Emerging challenges for scientists and authorities

=> QUESTIONS

Is every virus a threat ?

Or natural symbionts in the ecosystems ?

Actions to take with new sequences in terms of plant health ?

Impact on Regulations ? Quarantine ? Import/export ?

=> Guidelines proposals needed

if we challenge the dogma that all viruses are pathogens

Important to understand the biology of any new virus identified

>> basis to the evaluation of the risk and to scientifically-based decisions

6 categories of information needed

1. Identity of a pest
2. Data on its distribution
3. Host range
4. Modes of spread
5. the local agro-environmental conditions
6. the ability to cause a disease

PROBLEMS / LIMITATIONS OF NGS

The main biases in NGS-based virus diagnostics are:

- * biological
- * technical
- * bioinformatic

- **Biological issues:**

- contaminations (mycoviruses, etc...)
 - integrated sequences in the genome of the host

- **Technical issues:**

- contaminations in the lab (nucleic acid extractions, amplified DNA etc...)
 - contaminations through the previous run...

- **Informatic issues:**

- close viruses > obtention of chimeric sequences
 - conserved sequences > specificity of blast detection
 - new virus > no such viral sequence in databases > no results

- Informatic issues:

NGS results

First multi BLAST : matches found > 900 sequences



Query	Number of hits	Lowest E-value	Accession (E-value)	Description (E-value)	Greatest identity %	Accession (identity %)	Description (identity %)	Greatest positive %	Accession (positive %)	Description (positive %)	Greatest hit length	Accession (hit length)	Description (hit length)	Greatest bit score	Accession (bit score)	Description (bit score)
Small RNAs contig 1	421	2,73953E-59	HQ834962	Arabis mosaic virus isolate DU13 segment RNA2 polyprotein P2 gene, complete cds	98,9247312	HQ834962	Arabis mosaic virus isolate DU13 segment RNA2 polyprotein P2 gene, complete cds	100	HQ834962	Arabis mosaic virus isolate DU13 segment RNA2 polyprotein P2 gene, complete cds	279	HQ834962	Arabis mosaic virus isolate DU13 segment RNA2 polyprotein P2 gene, complete cds	230,172	HQ834962	Arabis mosaic virus isolate DU13 segment RNA2 polyprotein P2 gene, complete cds
Small RNAs contig 2	122	2,32036	FJ977568	Avian metapneumovirus strain aMPV/MN/turkey/2a/97, complete genome	77,7777778	JN941974	Sugarcane streak mosaic virus isolate CBV2000V59 HC-Pro protein gene, partial cds	88,8888889	JN941974	Sugarcane streak mosaic virus isolate CBV2000V59 HC-Pro protein gene, partial cds	135	KF208813	HIV-1 isolate 100378_200 from South Africa gag protein (gag) gene, complete cds	32,2255	FJ977568	Avian metapneumovirus strain aMPV/MN/turkey/2a/97, complete genome
Small RNAs contig 3	89	0,126358	DQ198083	Ovine herpesvirus 2, complete genome	71,4285714	AM420293	Saccharopolyspora erythraea NRRL2338 complete genome	78,5714286	JF797217	Suid herpesvirus 1 strain Bartha, complete genome	183	JF937105	Mycobacterium phage Rey, complete genome	39,5569	DQ198083	Ovine herpesvirus 2, complete genome
Small RNAs contig 4	1	7,8596	KF025499	Porcine bocavirus 3 isolate IA159-4 VP1/VP2 gene, complete cds	50	KF025499	Porcine bocavirus 3 isolate IA159-4 VP1/VP2 gene, complete cds	88,8888889	KF025499	Porcine bocavirus 3 isolate IA159-4 VP1/VP2 gene, complete cds	54	KF025499	Porcine bocavirus 3 isolate IA159-4 VP1/VP2 gene, complete cds	30,3927	KF025499	Porcine bocavirus 3 isolate IA159-4 VP1/VP2 gene, complete cds
Small RNAs contig 6	3	0,457348	EF710637	Cotesia sesamiae Mombasa bracovirus clone BAC 114, complete sequence	51,6129032	EF710637	Cotesia sesamiae Mombasa bracovirus clone BAC 114, complete sequence	67,7419355	EF710637	Cotesia sesamiae Mombasa bracovirus clone BAC 114, complete sequence	93	EF710637	Cotesia sesamiae Mombasa bracovirus clone BAC 114, complete sequence	34,6276	EF710637	Cotesia sesamiae Mombasa bracovirus clone BAC 114, complete sequence
Small RNAs contig 7	295	3,61892E-27	KC138733	Arabis mosaic virus isolate NW segment RNA2 polyprotein 2 gene, complete cds	100	KC138733	Arabis mosaic virus isolate NW segment RNA2 polyprotein 2 gene, complete cds	100	KC138733	Arabis mosaic virus isolate NW segment RNA2 polyprotein 2 gene, complete cds	150	KC138733	Arabis mosaic virus isolate NW segment RNA2 polyprotein 2 gene, complete cds	121,28	KC138733	Arabis mosaic virus isolate NW segment RNA2 polyprotein 2 gene, complete cds
Small RNAs contig 8	7	0,360452	EU782025	Avian paramyxovirus 3 strain turkey/Wisconsin/68, complete genome	54,1666667	AJ630128	Bacteriophage S-PM2 complete genome	69,2307692	EU782025	Avian paramyxovirus 3 strain turkey/Wisconsin/68, complete genome	105	AM420293	Saccharopolyspora erythraea NRRL2338 complete genome	37,2662	EU782025	Avian paramyxovirus 3 strain turkey/Wisconsin/68, complete genome

- Informatic issues:

Set parameters: number of hits / E-value / viral sequences

Arabis mosaic virus isolate NW segment RNA2 polyprotein 2 gene
Arabis mosaic virus isolate DU13 segment RNA2 polyprotein P2 gene
Grapevine fanleaf virus
Grapevine deformation virus
Grapevine Pinot gris virus
Grapevine rupestris stem pitting virus
Grapevine Bulgarian latent virus segment 1, isolate Serb1
Chilli veinal mottle virus isolate YN-tobacco
Petunia x hybrida clone 3 integrated petunia clearing vein virus
... ..

Number of hits >100

E-value < 3 (statistical value that gives an idea of the similarity of the sequence to the reference)

Viruses only

- Informatic issues:

Set parameters: number of hits / E-value / viral sequences

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Grapevine Bulgarian latent virus segment 1, isolate Serb1
Chilli veinal mottle virus isolate YN-tobacco
Petunia x hybrida clone 3 integrated petunia clearing vein virus
... ..
Ovine herpesvirus 2
Porcine cytomegalovirus strain BJ09
HIV-1
... ..

Number of hits >100

E-value < 3 (statistical value that gives an idea of the similarity of the sequence to the reference)

Viruses only



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III. EARLY STEPS OF BIOLOGICAL CHARACTERIZATION OF NEW VIRUSES

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4.1 Confirmation of virus detection

4.2 Provisional taxonomic assignation

Virus family / genus => preliminary information => Plant virus

Risk of integrated sequence ? Confirmation of particles

4.3 Bibliography

4.4 Sample documentation

Symptoms, location, surrounding crops, ...



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4.5 Full genome sequencing and annotation

4.6 Development of a diagnostic protocol

4.7 Field/batch observation



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IV. IN-DEPTH BIOLOGICAL CHARACTERIZATION

Classical / molecular virology

TO IDENTIFY THE CAUSATIVE AGENT OF A PARTICULAR DISEASE:

Robert Koch >> **Four criteria (Koch postulate):**

- the microorganism or other pathogen must be **present in all cases of the disease**
- the pathogen can be isolated from the diseased host and **grown in pure culture (purified from its host)**
- the pathogen from the pure culture must **cause the disease when inoculated into a healthy, susceptible laboratory animal (plant)**
- the pathogen must be **reisolated** from the new host and **shown to be the same** as the originally inoculated pathogen

IV. IN-DEPTH BIOLOGICAL CHARACTERIZATION

Koch postulate => Infectious clones

Infectious clones



Symptoms / systemic infection

IV. IN-DEPTH BIOLOGICAL CHARACTERIZATION

Cracking down mixed infection

Infectious clones



Interactions between viruses (synergy / antagonism)

IV. IN-DEPTH BIOLOGICAL CHARACTERIZATION

Mode of transmission (horizontal and vertical)

- vectors ?
- Seeds, pollen ?
- grafting, mechanical transmission?



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IV. IN-DEPTH BIOLOGICAL CHARACTERIZATION

- Host range > woody / herbaceous plants
- Survey at larger scales (global epidemiology)
- Antibody / detection tests development



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CONCLUSION

- IMPACT ON REGULATION

=> HELP SCIENTISTS / AUTHORITIES

- ESTABLISHING PRIORITIES

- DECISION MAKING

LOT OF OPEN QUESTIONS.....

ACKNOWLEDGMENTS

The screenshot shows a web browser window with the URL <https://www.frontiersin.org/articles/10.3389/fmicb.2017.00045/full>. The page is titled "A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies". The article is categorized as a "PERSPECTIVE ARTICLE" and was published in "Front. Microbiol." on 24 January 2017. The authors listed are Sebastien Massart^{1*}, Thierry Candresse², José Gil³, Christophe Lacomme⁴, Lukas Predajna⁵, Maja Ravnikar⁶, Jean-Sébastien Reynard⁷, Artemis Rumbou⁸, Pasquale Saldarelli⁹, Dijana Škorić¹⁰, Eeva J. Vainio¹¹, Jari P. T. Valkonen¹², Hervé Vanderschuren¹³, Christina Varveri¹⁴, and Thierry Wetzel¹⁵. The article has received 2,895 total views and an average score of 4. The page also features a "Check for updates" button, "Download Article" and "Export citation" options, and a "View Article Impact" button. The Frontiers logo is visible in the bottom right corner of the article page.

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