Defogging NGS and plant virus diagnostic

The role of a collaborative network as the COST Action FA 1407 - DIVAS

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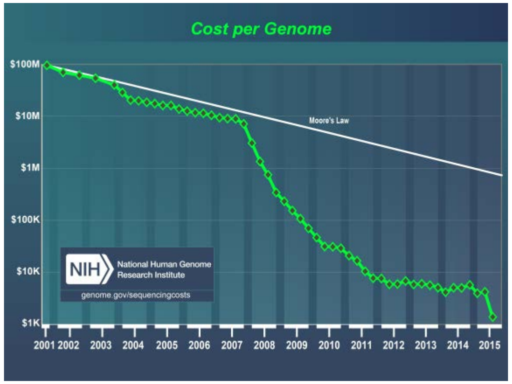
Introduction

NGS technologies



NGS Technologies

NGS technologies & plant virology

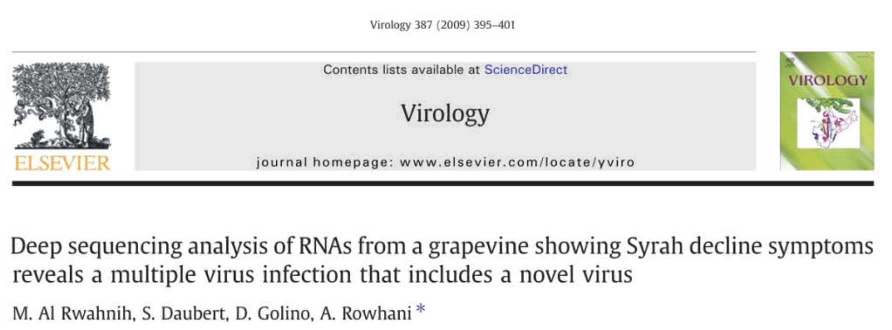
- ✓ Adopted from nearly a decade in R&D
- ✓ Wide and large adoption for Etiology:

Before NGS

With NGS

NGS technologies & plant virology

- ✓ Adopted from nearly a decade in R&D
- ✓ Wide and large adoption for Etiology: from 2009



Virology 387 (2009) 395–401

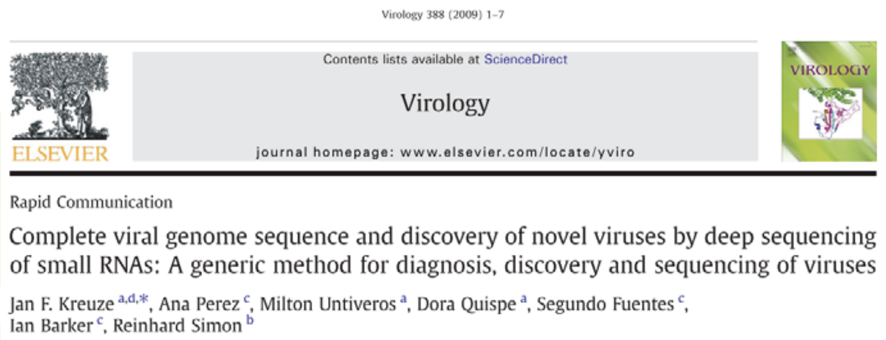
Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus

M. Al Rwahnih, S. Daubert, D. Golino, A. Rowhani *



Virology 388 (2009) 1–7

Contents lists available at ScienceDirect

Virology

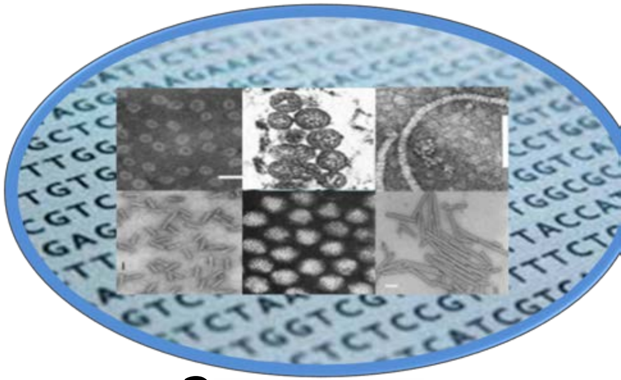
journal homepage: www.elsevier.com/locate/yviro

Rapid Communication

Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses

Jan F. Kreuze [a,d,*], Ana Perez [c], Milton Untiveros [a], Dora Quisper [a], Segundo Fuentes [c], Ian Barker [c], Reinhard Simon [b]

>100 new viruses discovered

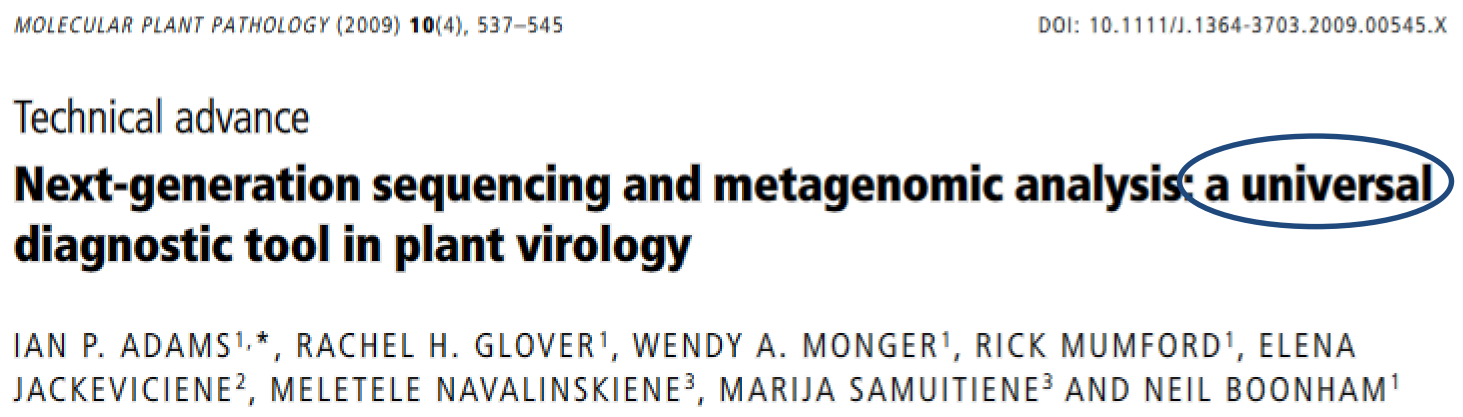


Halt material ? Reaction of a country ?

Do they pose a risk ?

NGS technologies & plant virology

- ✓ Adopted from nearly a decade in R&D
- ✓ Wide and large adoption for Etiology: from 2009, >100 new viruses
- ✓ Is there a potential for diagnostics ?



MOLECULAR PLANT PATHOLOGY (2009) **10**(4), 537–545 DOI: 10.1111/J.1364-3703.2009.00545.X

Technical advance

Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology

IAN P. ADAMS[1,*], RACHEL H. GLOVER[1], WENDY A. MONGER[1], RICK MUMFORD[1], ELENA JACKEVICIENE[2], MELETELE NAVALINSKIENE[3], MARIJA SAMUITIENE[3] AND NEIL BOONHAM[1]

NGS technologies & plant virology

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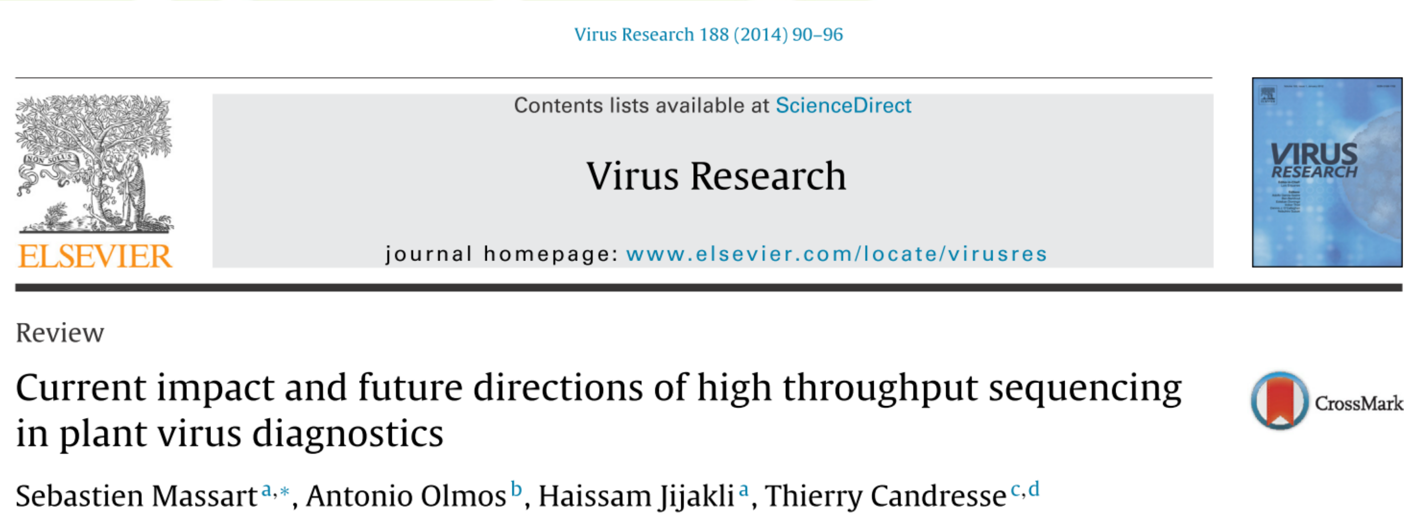

Virus Research 188 (2014) 90–96

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

ELSEVIER

Review

Current impact and future directions of high throughput sequencing in plant virus diagnostics

Sebastien Massart[a,*], Antonio Olmos[b], Haissam Jijakli[a], Thierry Candresse[c,d]

NGS technologies & virus diagnostic

❖ Technical challenges

- Laboratory protocol ?
- Bioinformatic algorithms ?

❖ Performance evaluation

- Sensitivity variability ?
- Specificity ?
- Reproducibility ?
- Repeatability ?

❖ Routine analysis

- Contamination ?

For all you seq...

RNA

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NGS technologies & plant virology

Risk?

Diagnostic?

Taxonomy ?

Population genetics ?

Need for an international collaboration to handle the challenges of integrating NGS into plant virus study and diagnostic

The COST Action

What is a COST Action ?

✓ Funded by **European Union**

✓ COST is an EU-funded program that enables researchers to set up their **interdisciplinary research networks** in Europe and beyond.

✓ For conferences, meetings, training schools, short scientific exchanges or other networking activities.

✓ By creating open spaces where people and ideas can grow, we **unlock the full potential of science**.

COST Action for NGS & plant viruses

✓ COST Action FA 1407

“Coordinate and raise the European capacity to apply NGS technologies for the **study and diagnosis of viral diseases** of vegetatively propagated plants, seeds and seedlings”

✓ Acronym: DIVAS

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What is DIVAS ?

Deep

Investigation of

Viral

Associated

Sequences

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DIVAS’ Countries and participants

- ✓ Cost countries: 28
- ✓ 150 virologists supported

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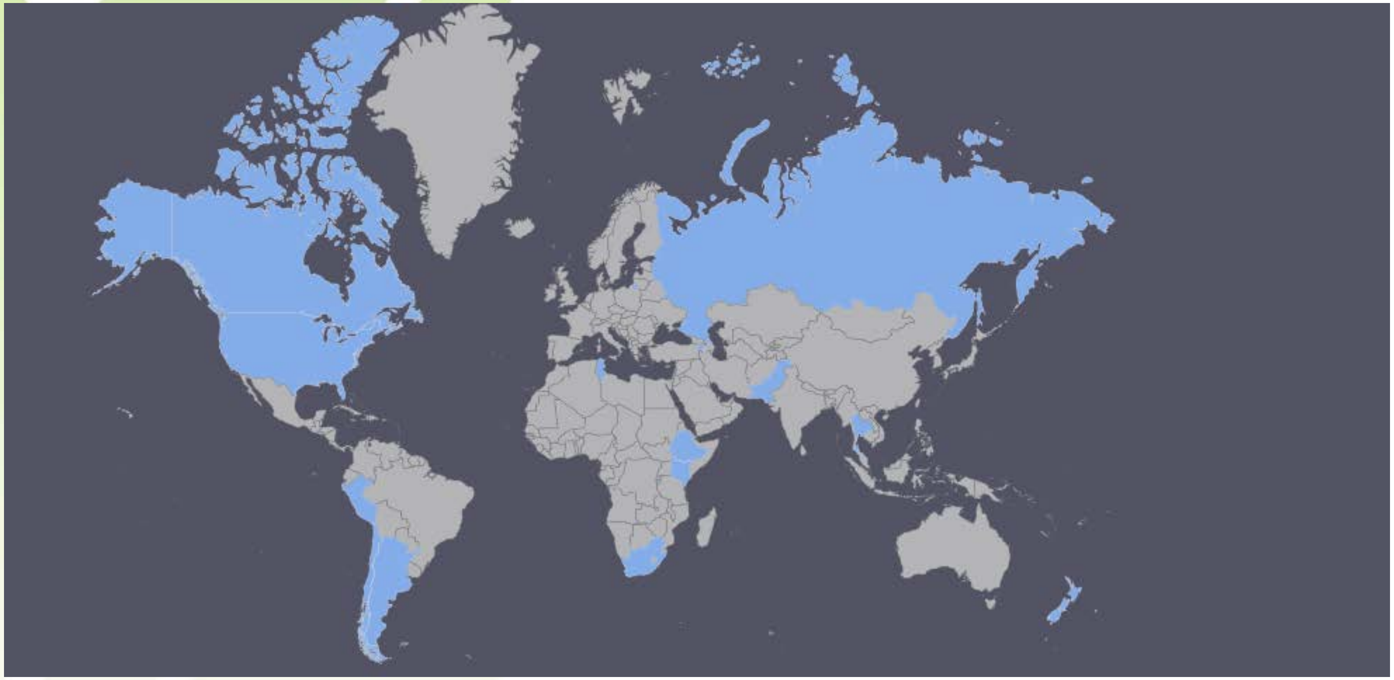
DIVAS' Countries and participants

Connections with other countries :



- ✓ 8 other countries with formal agreement
- ✓ 4 other participating countries

DIVAS numbers

6
International meetings

5
Training schools

28
Short Term Scientific Missions

255 days
of training

43
Months of STSM

102
Trainees

Objectives of the COST Action

COST Action for leveraging plant virus control through NGS

1. Provide a **research framework** to reach a comprehensive characterization of the plant virome and to understand its impact on plants

Objectives of the COST Action

COST Action for leveraging plant virus control through NGS

1. Research framework for characterization & impact
2. Coordinate and harmonize European knowledge-base for **technological standards and validation** of reliable NGS protocols

Objectives of the COST Action

COST Action for leveraging plant virus control through NGS

1. Research framework for characterization & impact
2. NGS technological standards and validation
3. Discuss, agree and disseminate **decision schemes on plant virus diagnostic** for policy makers, NPPO, EPPO, diagnostic lab

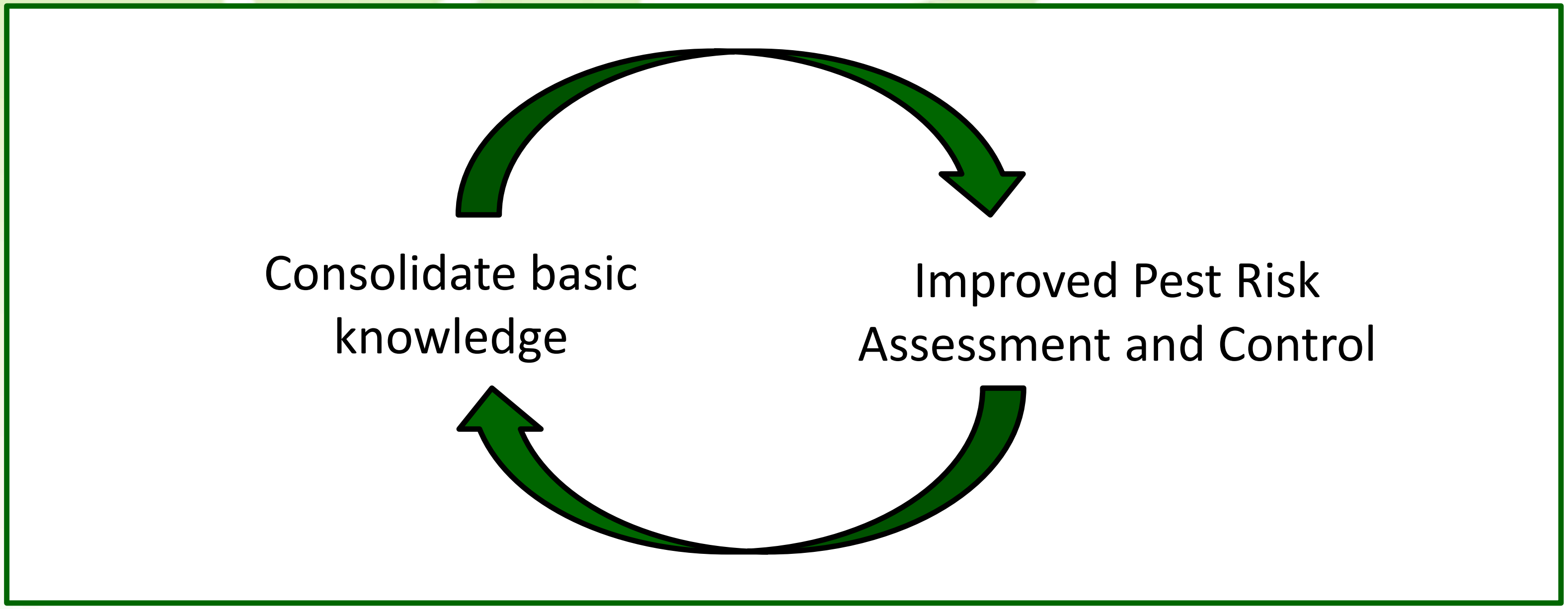
Objectives of the COST Action

COST Action for leveraging plant virus control through NGS

1. Research framework for characterization & impact
2. NGS technological standards and validation
3. Decision schemes on plant virus diagnostic
4. Discuss, agree and disseminate a scientific position and expertise on the **impact of NGS on virus taxonomy and on the plant-virus interactions**

Objectives of the COST Action

COST Action for leveraging plant virus control through NGS



Consolidate basic knowledge

Improved Pest Risk Assessment and Control

Transversal Action bridging various stakeholders

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Obj. 1: Research framework

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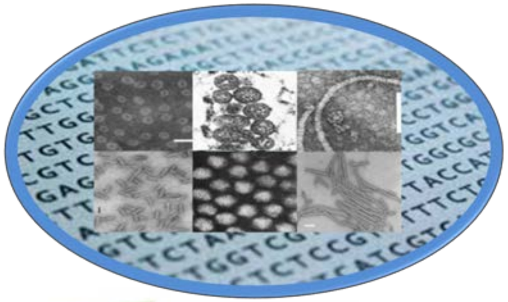
Research framework



>100 new viruses discovered

“naked” genome sequences !!!

Biological characterisation to evaluate the risk

Research framework

frontiers in Microbiology

PERSPECTIVE
published: 24 January 2017
doi: 10.3389/fmicb.2017.00045

Check for updates

A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies

Sebastien Massart[1], Thierry Candresse[2], José Gil[3], Christophe Lacomme[4], Lukas Predajna[5], Maja Ravnikar[6], Jean-Sébastien Reynard[7], Artemis Rumbou[8], Pasquale Saldarelli[9], Dijana Škorić[10], Eeva J. Vainio[11], Jari P. T. Valkonen[12], Hervé Vanderschuren[13], Christina Varveri[14] and Thierry Wetzel[15]*

Thierry Wetzel – Thursday afternoon

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Obj. 1: Characterization of plant virome

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Characterization of plant virome-1

- Classical training school based on theoritical examples or on reference samples
  - Consequences: come back to office, no time to validate learning outcomes
- Training school for virus discovery on relevant samples (including lab, sequencing and bioinformatics)
  - Leveraging learnings outcome by continuous analyses and focus after the training school

Characterization of plant virome-1

→ Bridging training and production of scientific knowledge

- ✓ **2 training schools with 30 trainees in 2017**
- ✓ **New training schools next year: for more information:**

sebastien.massart@uliege.be

Characterization of plant virome-2

Variant detection in NGS data ?

Characterization of plant virome-2

Characterization of plant virome-2

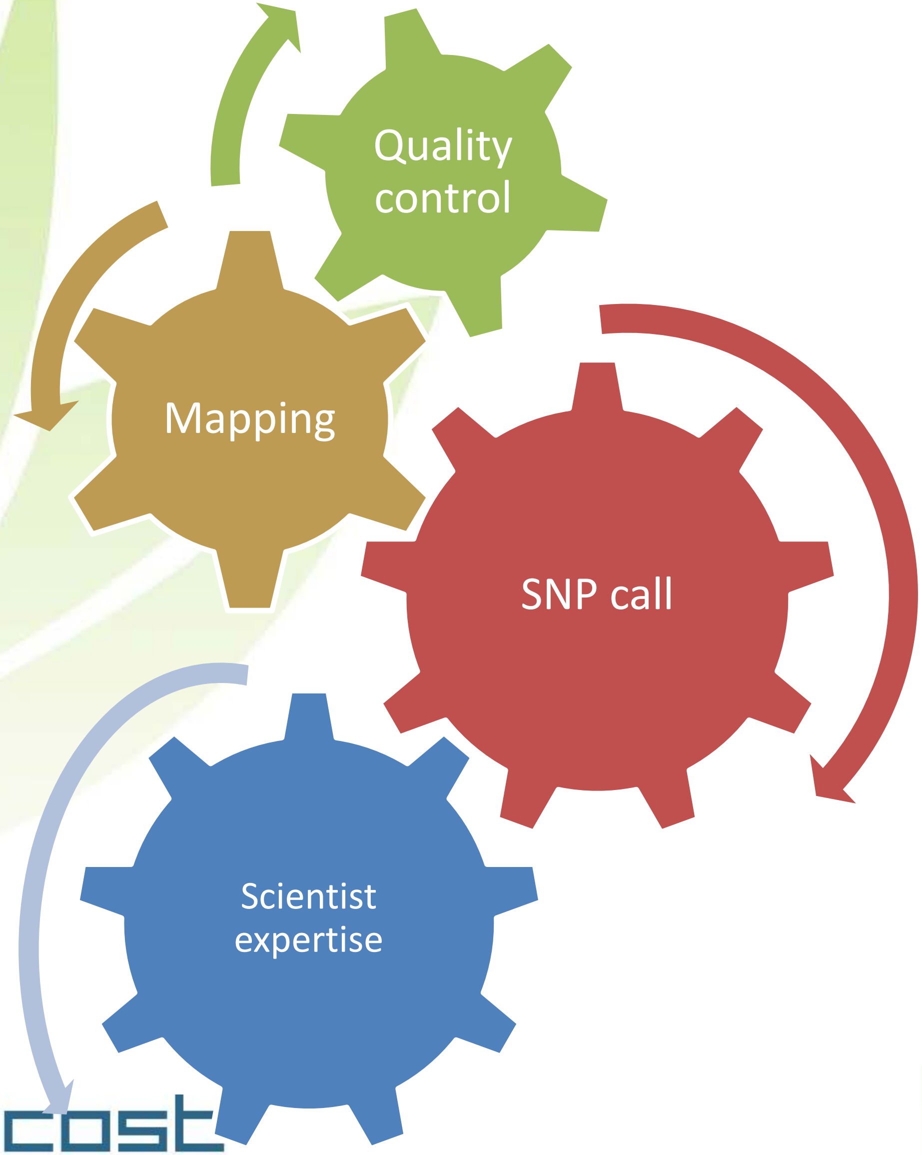
Variant detection in NGS data ?

- Very significant differences between participants
- Only a limited number of SNPs detected by all participants
- Possible causes?
  - Differences between CLC and Genious?
  - Differences in software parameters?
- Mapping will likely have an impact
  - BAM file analyses comparisons

Characterization of plant virome-2

Provisional conclusion ?

Specific sessions on Friday



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Obj 3: Decision Scheme – recommendation for diagnostic

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DIVAS and virus diagnostics

- ✓ Olomouc meeting (March 2017) -> need for QA/QC for wider adoption of NGS in diagnostics
- ✓ Thursday morning and afternoon
- ✓ Small meeting (25 participants) in Brussels in 2018 to start drafting an EPPO standard for NGS in diagnostics

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Obj 4: Taxonomy

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DIVAS and Taxonomy

CONSENSUS
STATEMENT

OPEN

CONSENSUS STATEMENT

Virus taxonomy in the age of metagenomics

Peter Simmonds[1], Mike J. Adams[2], Mária Benkő[3], Mya Breitbart[4], J. Rodney Brister[5], Eric B. Carstens[6], Andrew J. Davison[7], Eric Delwart[8,9], Alexander E. Gorbalenya[10,11], Balázs Harrach[3], Roger Hull[12], Andrew M.Q. King[13], Eugene V. Koonin[5], Mart Krupovic[14], Jens H. Kuhn[15], Elliot J. Lefkowitz[16], Max L. Nibert[17], Richard Orton[7], Marilyn J. Roossinck[18], Sead Sabanadzovic[19], Matthew B. Sullivan[20], Curtis A. Suttle[21,22], Robert B. Tesh[23], René A. van der Vlugt[24], Arvind Varsani[25] and F. Murilo Zerbini[26]*

Abstract | The number and diversity of viral sequences that are identified in metagenomic data far exceeds that of experimentally characterized virus isolates. In a recent workshop, a panel of experts discussed the proposal that, with appropriate quality control, viruses that are known only from metagenomic data can, and should be, incorporated into the official classification scheme of the International Committee on Taxonomy of Viruses (ICTV). Although a taxonomy that is based on metagenomic sequence data alone represents a substantial departure from the traditional reliance on phenotypic properties, the development of a robust framework for sequence-based virus taxonomy is indispensable for the comprehensive characterization of the global virome. In this Consensus Statement article, we consider the rationale for why metagenomic sequence data should, and how it can, be incorporated into the ICTV taxonomy, and present proposals that have been endorsed by the Executive Committee of the ICTV.

Incorporation of metagenomics data (e.g. genome sequences only) in ICTV

The COST Action

What’s next ?

(until March 2019)

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Next activities of the COST Action

- ✓ Performance testing on bioIT fro virus diagnostics (EPPO guidelines – Friday pm)
- ✓ Exploring SNP analysis (Friday am & pm) -> PT ?
- ✓ Training school for NGS and virus discovery/detection
- ✓ Training school in Bari for diagnostic laboratories
- ✓ Meetings for Science and also Quality management

Keep posted of COST activities

COST Action Divas (FA 1407)

@ngs_virus

COST Action Divas

Thanks to all the COST participants building the results

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Special thanks to the Core Group

Angelantonio Minafra
Vice-Chair

Christina Varveri
STSM manager

Antonio Olmos
WG1 Leader

Thierry Wetzel
WG2 Leader

René van der Vlugt
WG3 Leader

Maja Ravnikar
WG4 Leader

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Thank you for your attention

For more information:

sebastien.massart@uliege.be

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