Advances in the diagnosis of grapevine virus diseases

Cool Climate Oenology & Viticulture Institute

Brock University

Outline

- History of plant virus detection
- Recent Advancements in virus detection
- Grapevine viruses – detection methods
- Applications of plant virus disease diagnostics

History

1892: Dmitry Ivanovsky: Tobacco mosaic disease could pass through a porcelain filter

1898: Martinus Beijerinck: *contagium vivum fluidum*

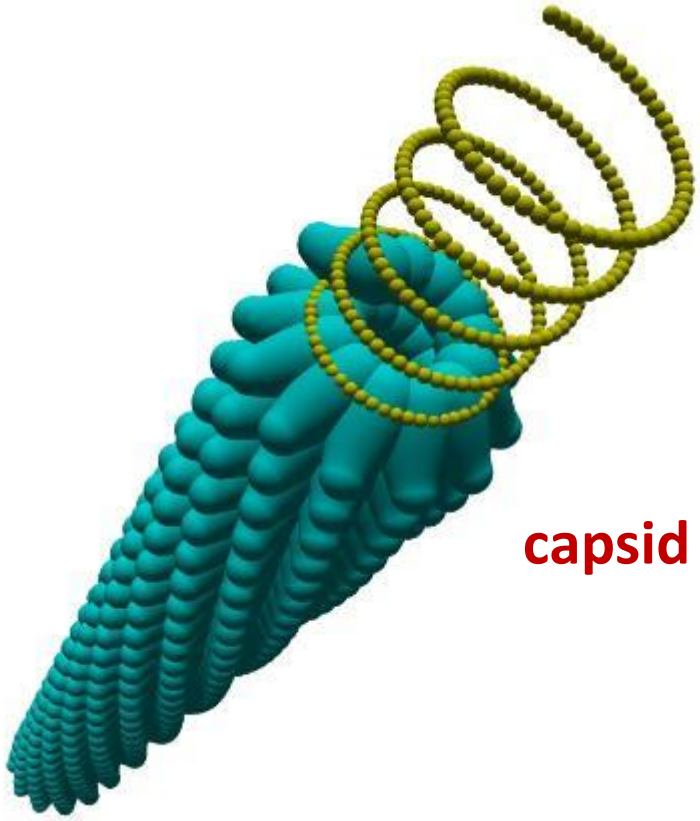
1931: Ernst Ruska: Electron microscope

"Plant viruses have obvious importance for food crops and ornamental plants, and a range of viruses are responsible for an estimated $60 billion in crop losses worldwide each year".

Lefeuvre et al. (2019). Evolution and ecology of plant viruses. *Nat Rev Microbiol* 17, 632–644

What is a virus

TMV (Tobacco Mosaic Virus)

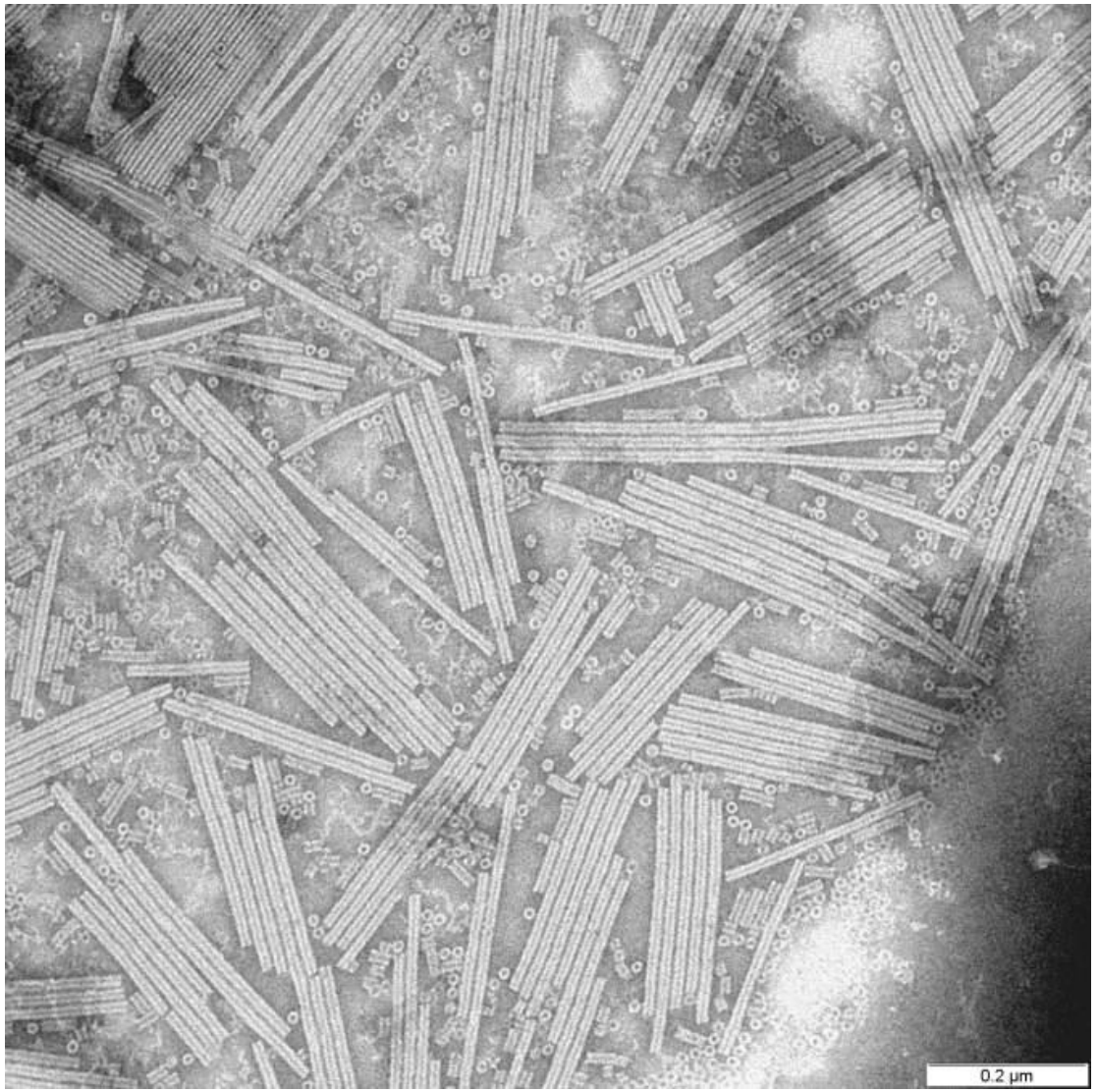


ss(+) RNA

capsid proteins

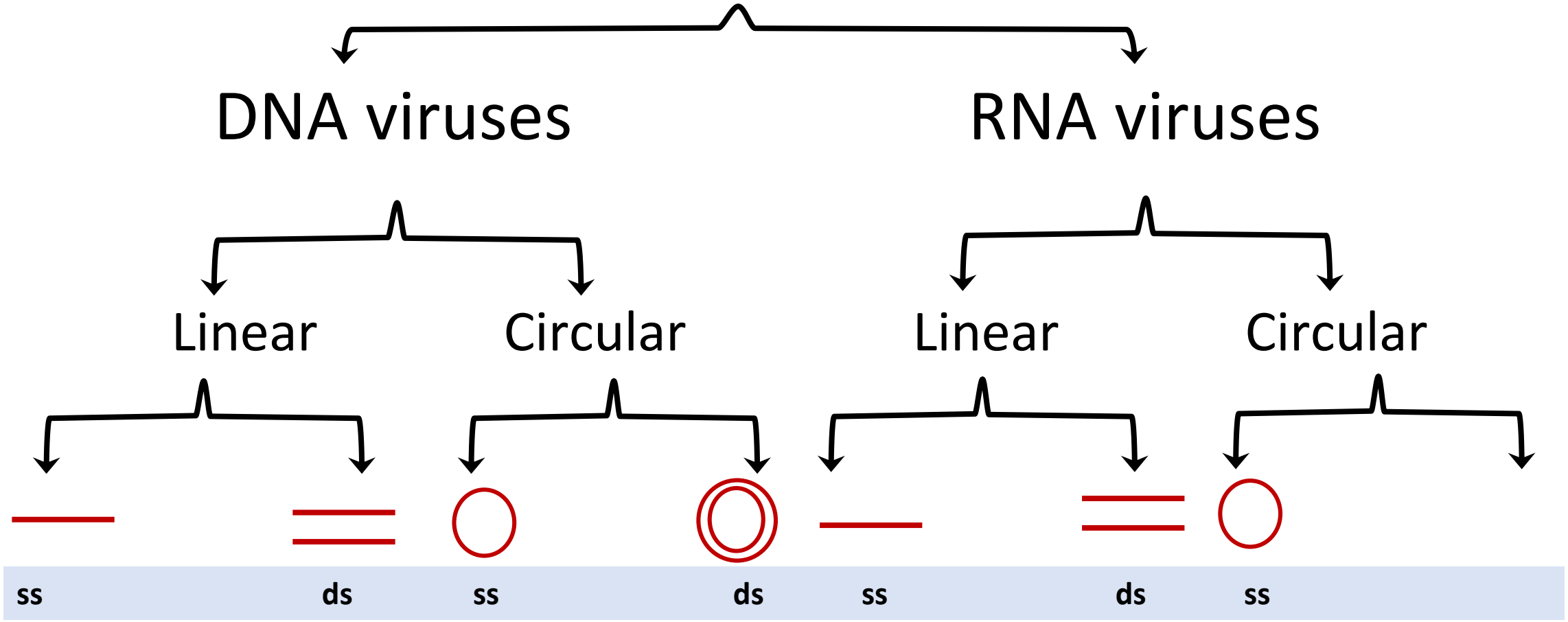
300 nm long by 18 nm in diameter

https://cronodon.com/BioTech/Virus_Tech.html



https://en.wikipedia.org/wiki/History_of_model_organisms

Virus Genome Diversity



Modified from: Boriana Marintcheva, in Harnessing the Power of Viruses, 2018

Importance of Diagnostics

- Not all viruses show symptoms
- Cause of a specific pattern in symptoms
- Source? Where did the virus come from?
- How the virus transmitted?
- Spatial and temporal disease spread
- Plant Protection and Quarantine
- Impact on End Product – Mitigation
- Disease Management

Importance of Diagnostics in Grapevine Production

- The sustainability of grapevine production system depends heavily on the health status of the propagating planting material being free of grapevine pathogens.
- Grapevine virus diseases have been known to cause significant economic loses by negatively impacting the yield and quality of the wine.
- The negative effects of grapevine virus diseases on rooting ability, graft in-take, vigour and berry quality have been demonstrated in commercial grapevine cultivars from distinct geo-climatic conditions.
- Disease diagnostics has become extremely important for the effective management of the virus spread and mitigation practices.

Family *Closteroviridae*

Ampelovirus

Grapevine leafroll-associated virus 1
Grapevine leafroll-associated virus 3
Grapevine leafroll-associated virus 5
Little cherry virus 2
Pineapple mealybug wilt-associated virus 1
Pineapple mealybug wilt-associated virus 2

Closterovirus

Beet yellow stunt virus
Beet yellow virus
Burdock yellows virus
Carnation necrotic fleck virus
Carrot yellow leaf virus
Citrus tristeza virus
Grapevine leafroll-associated virus 2
Wheat yellow leaf virus

Crinivirus

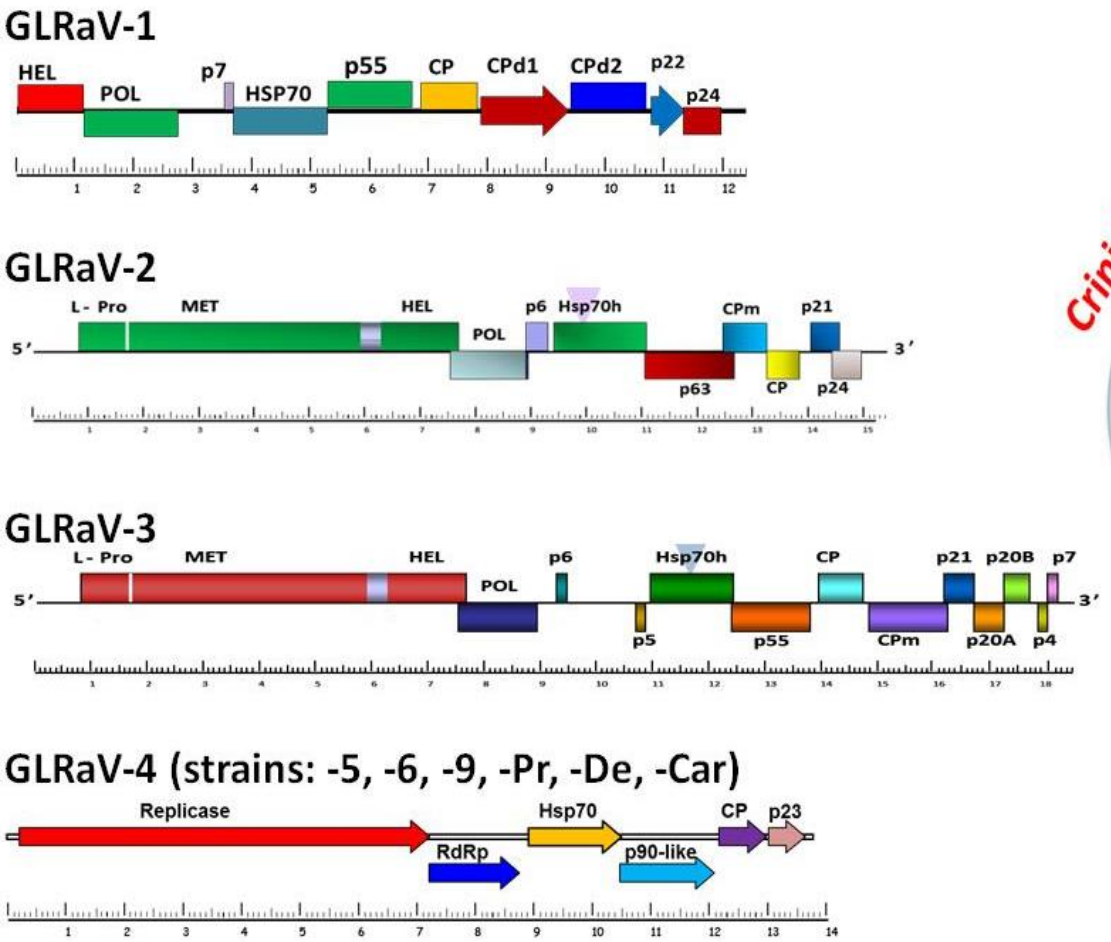
Abutilon yellows virus
Beet pseudoyellows virus
Cucurbit yellow stunting disorder virus
Lettuce infectious yellows virus
Sweet potato chlorotic stunt virus
Tomato chlorosis virus
Tomato infectious chlorosis virus
Potato yellow vein virus

Karasev, 2000 *Annu. Rev. Phytopathol.* 38: 293–324
Martelli et al.,2002 *Arch. of Virol.* 147: 2029-2044

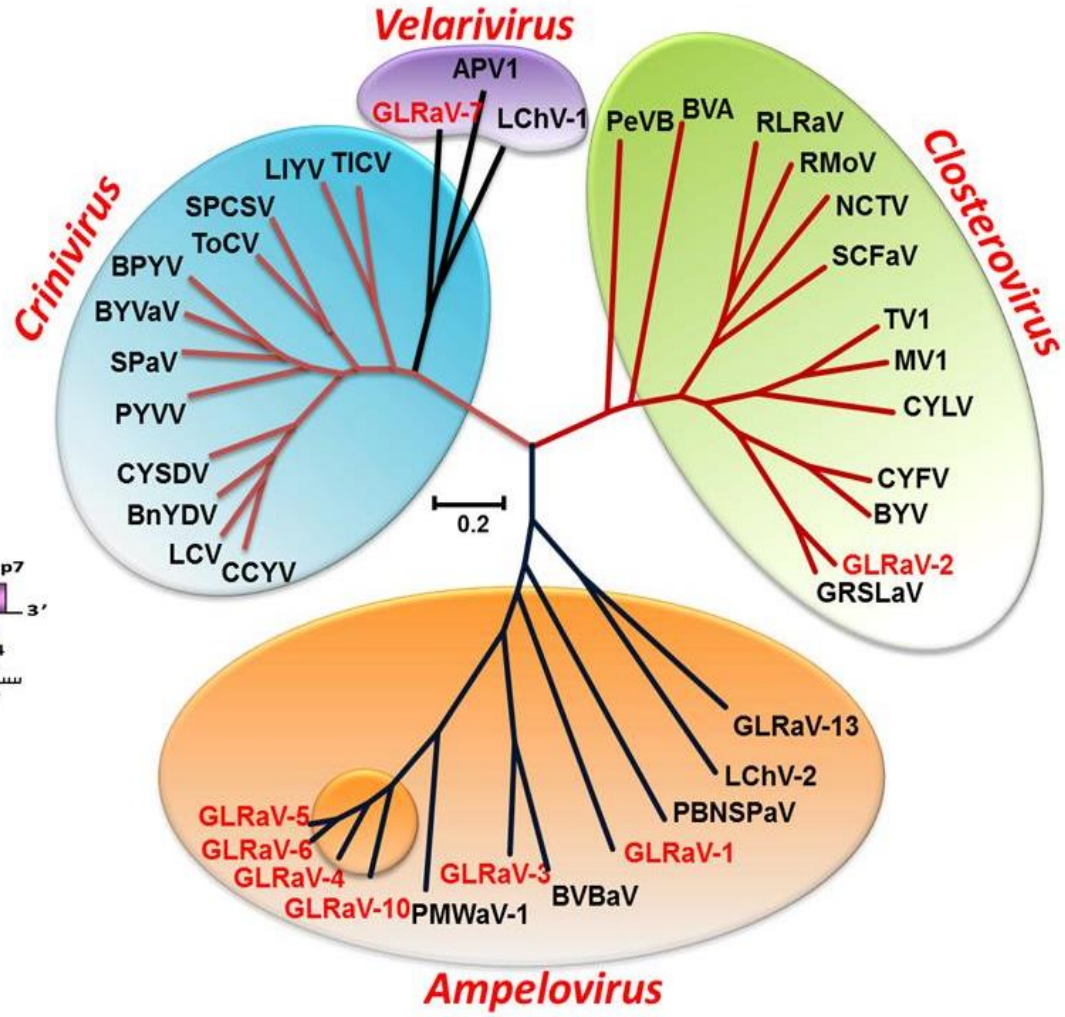
www. ICTV.org

Family *Closteroviridae*

Genome Complexity

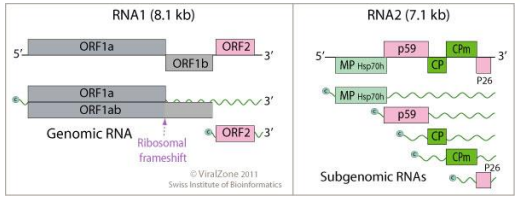


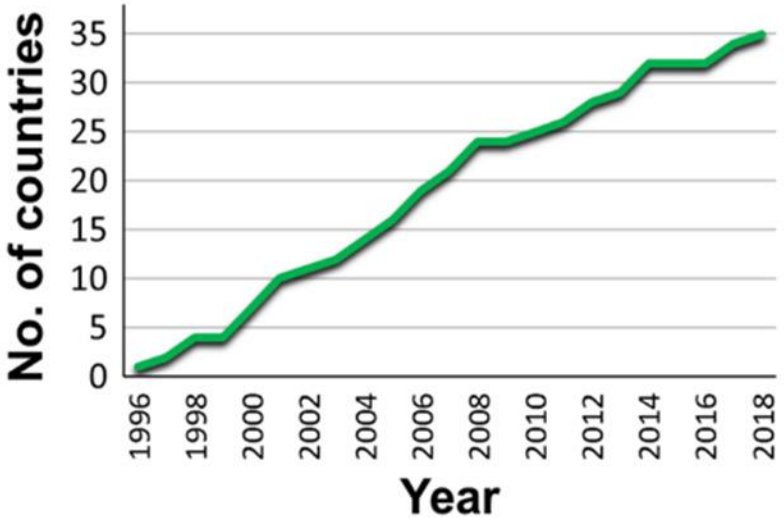
Phylogenetic Tree

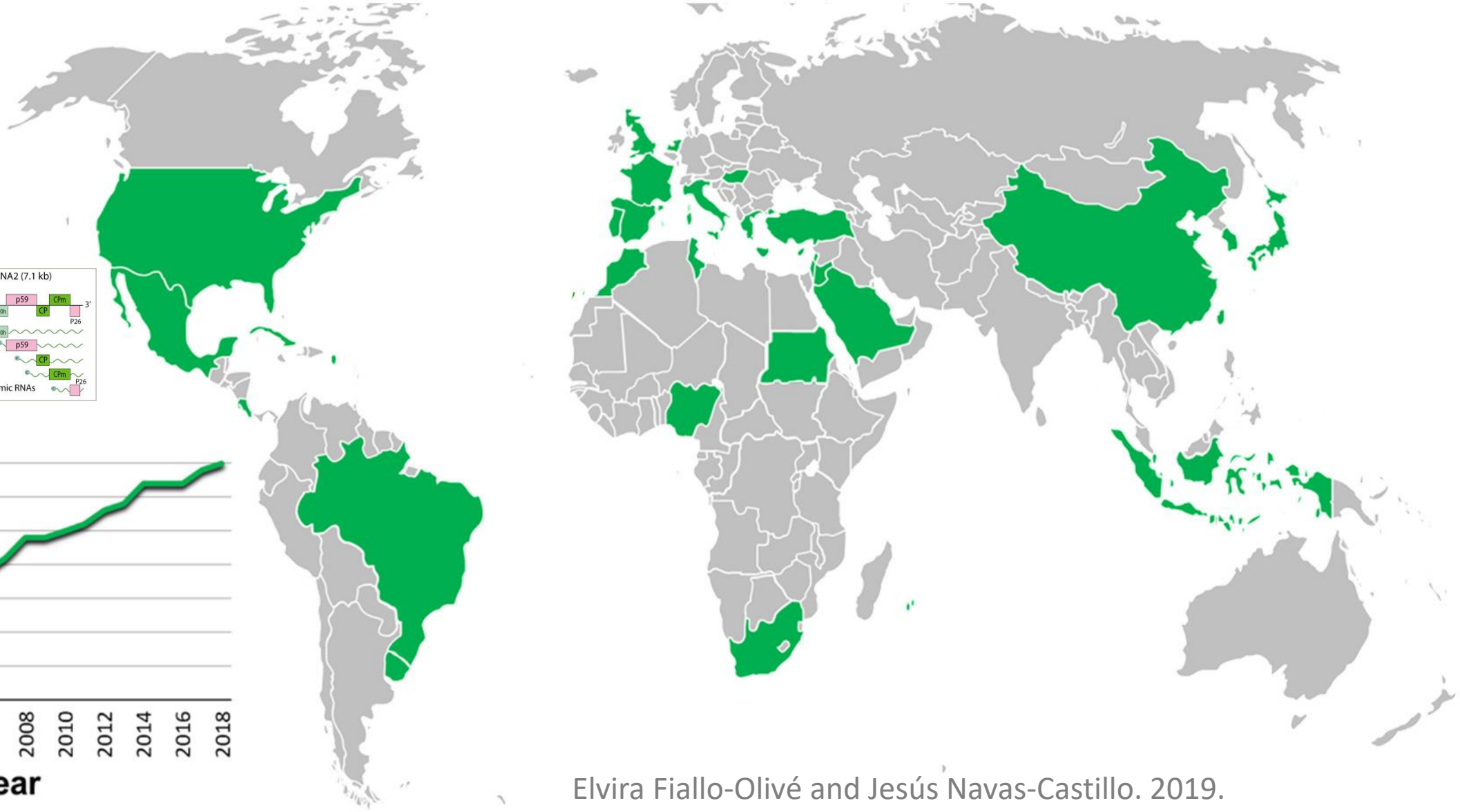


Geographical distribution of Tomato Chlorosis Virus

Genome

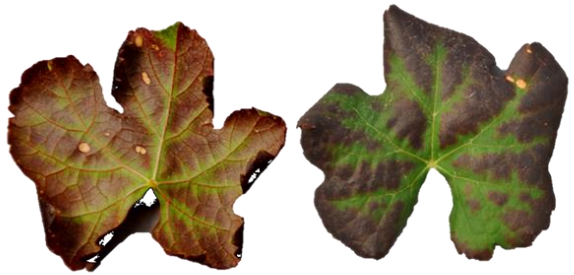
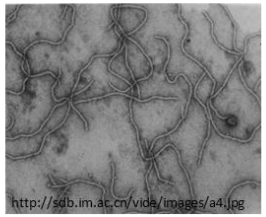
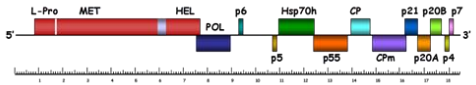
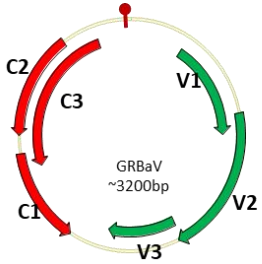




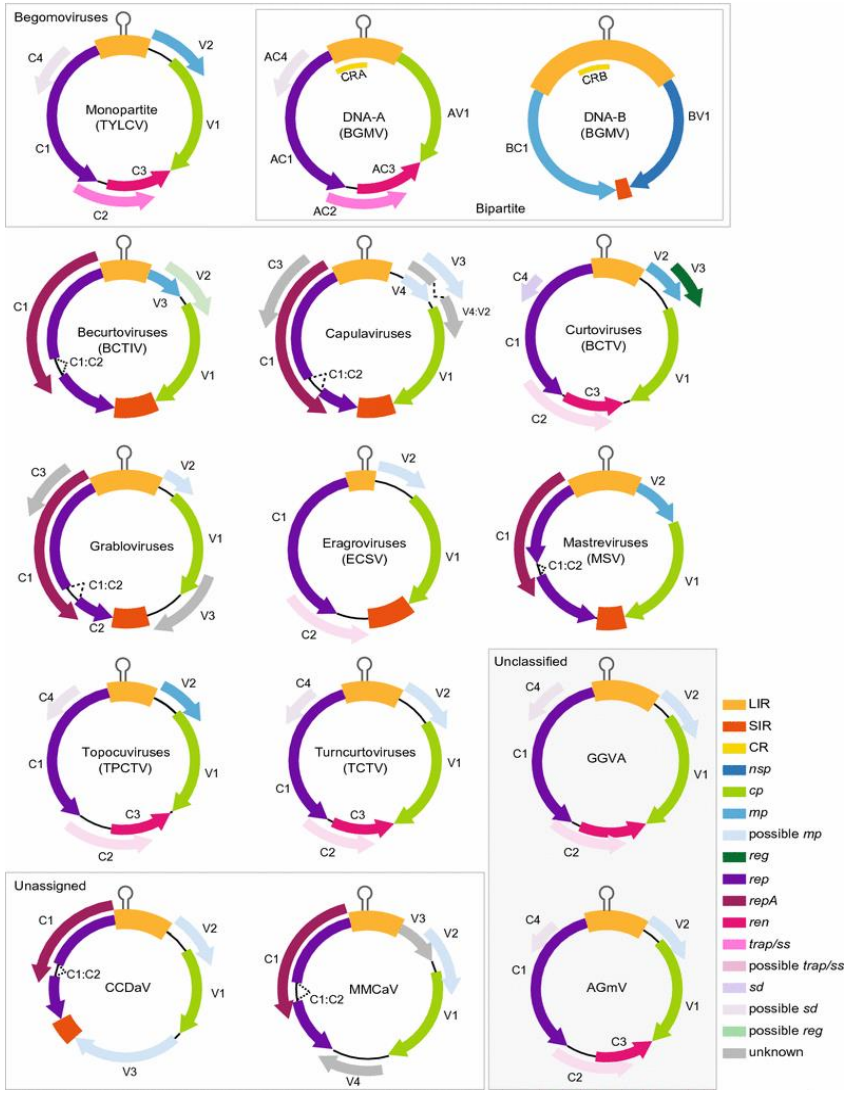


Elvira Fiallo-Olivé and Jesús Navas-Castillo. 2019. 10.1111/mpp.12847

Family *Geminiviridae*

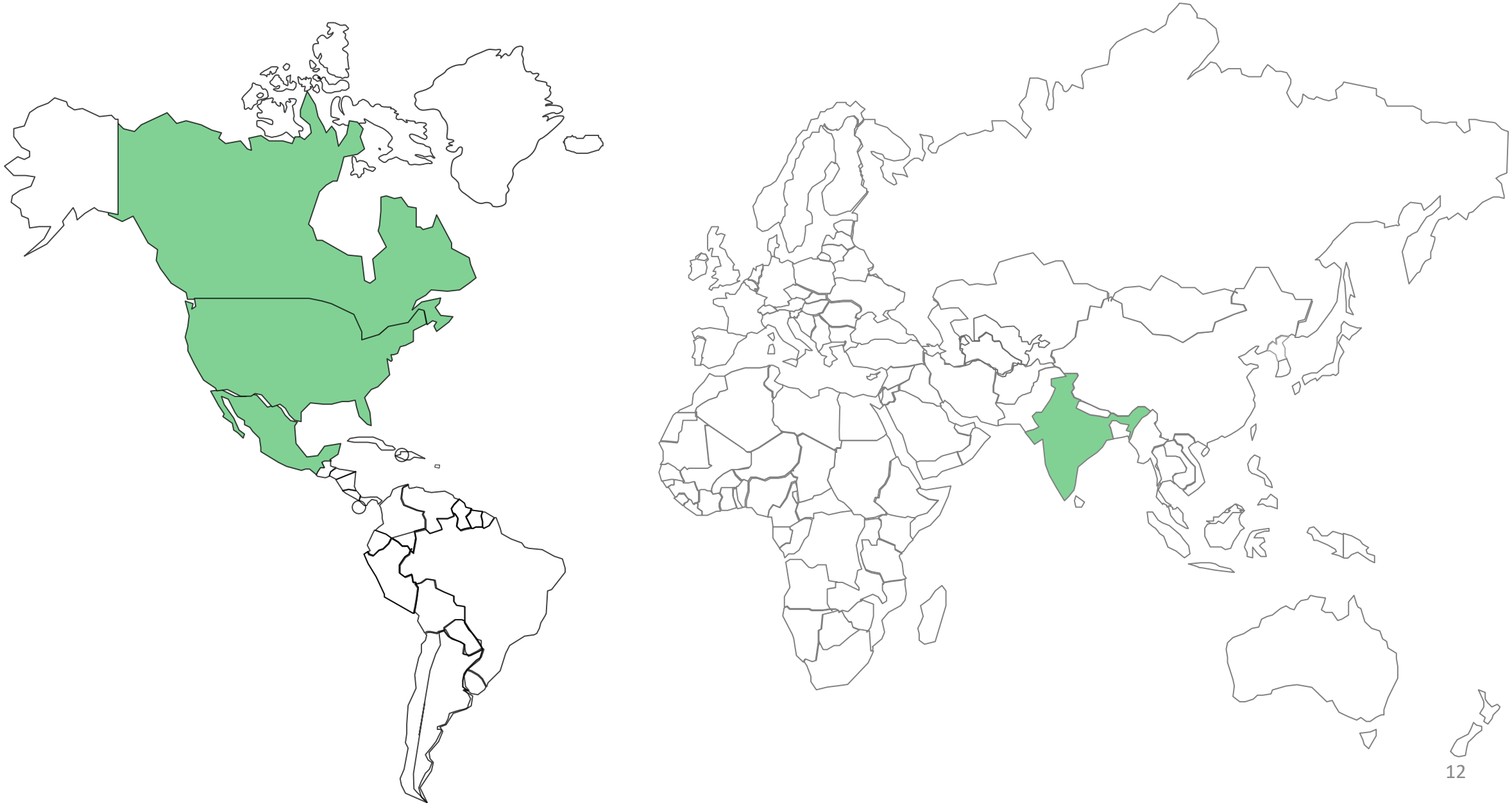
	Grapevine leafroll virus	Grapevine red blotch virus
Symptoms		
Virus particles		?
Genetic material	RNA	DNA
Genome Organization		

Geminiviridae



DOI: 10.1007/s00705-017-3268-6

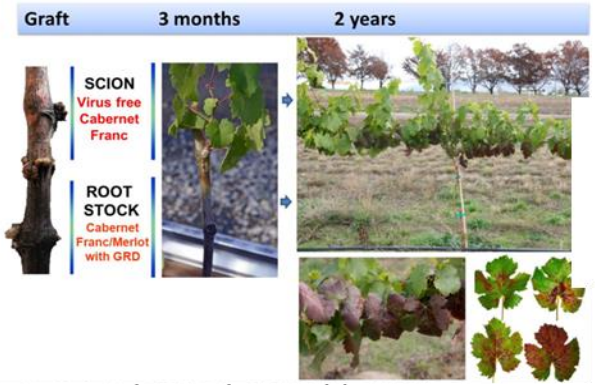
Geographical distribution of Grapevine Red Blotch Virus



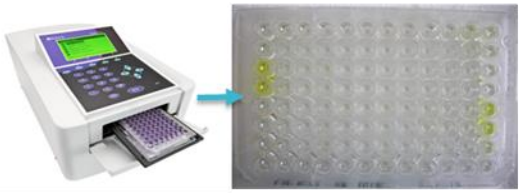
How to detect grapevine viruses?

Grapevine Virus Diagnostics

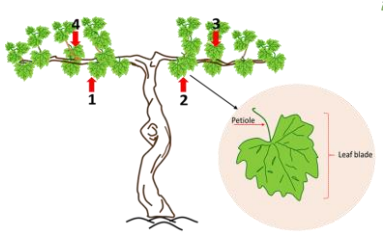
Symptom Based	Biological Indexing	Serological (ELISA)	Molecular (PCR, qPCR, ddPCR & NGS)
Non-specific Inaccurate	Symptom based Labor intensive Time consuming	Specific No Abs for all viruses Less sensitive than PCR	Highly specific Post-PCR process Multiplex End-Point Quantitative



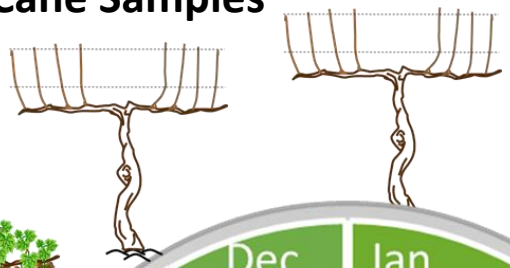
Poojari et al. 2013. PloS ONE 8(6): e64194

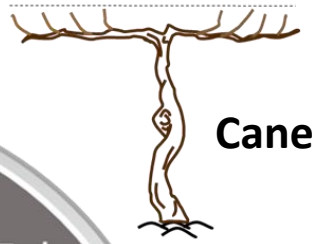


What is the preferred sampling time and tissue type for virus detection?

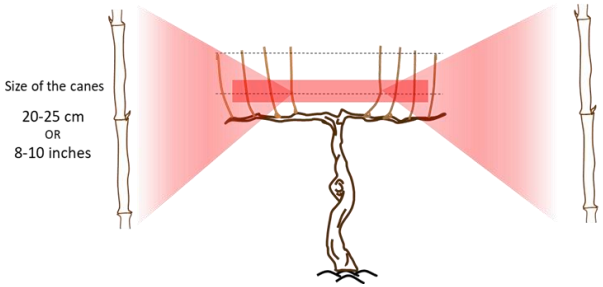


Cane Samples

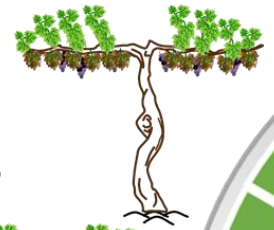


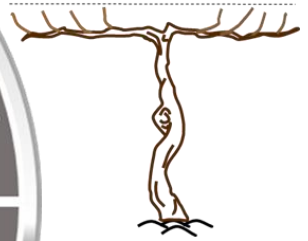


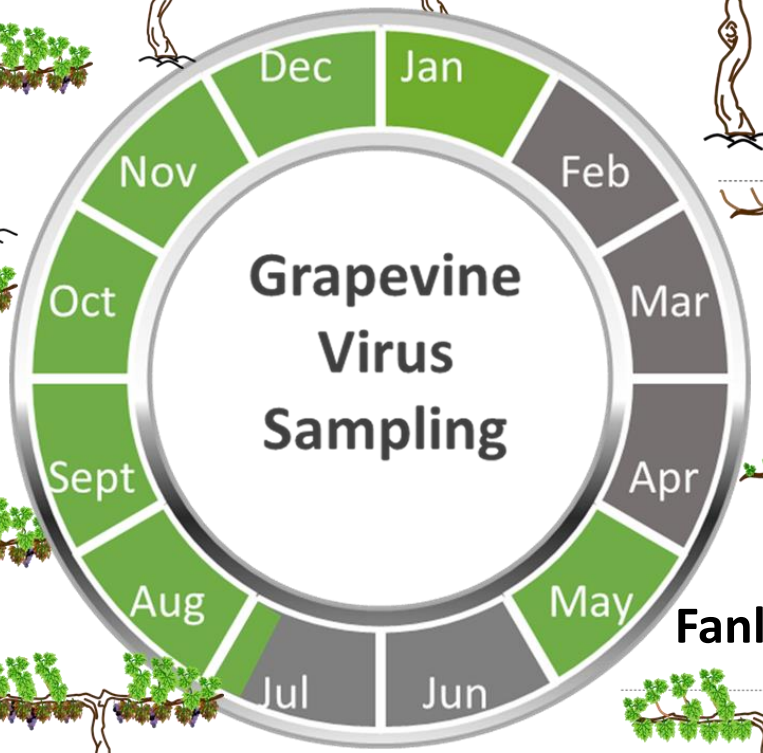
Cane Samples



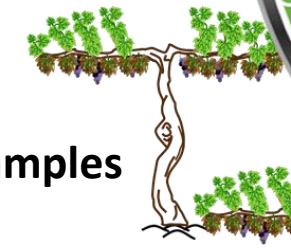
Leaf Samples

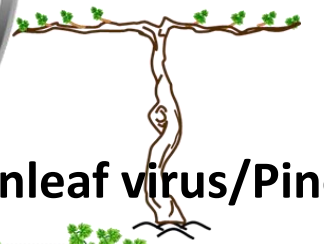




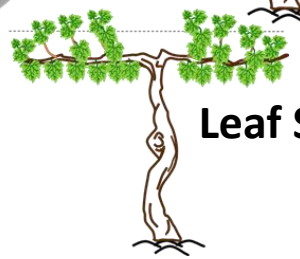


Leaf Samples





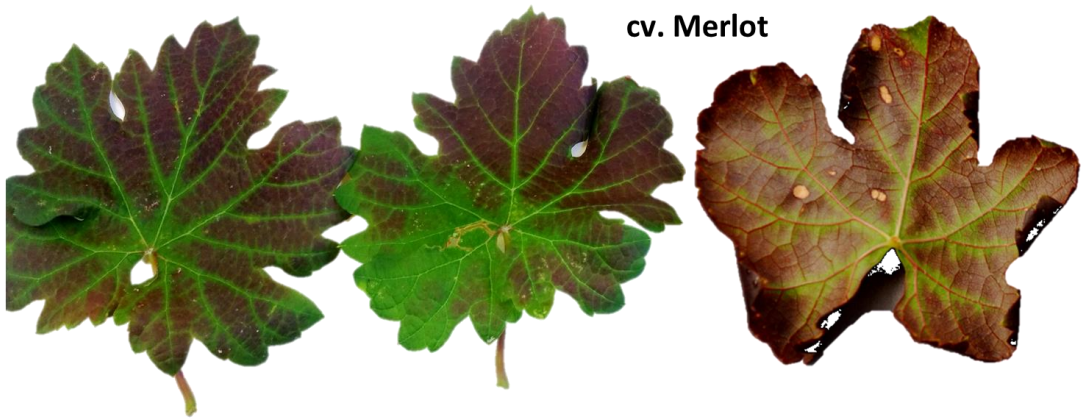
Fanleaf virus/Pinot gris virus



Leaf Samples

Symptom based detection

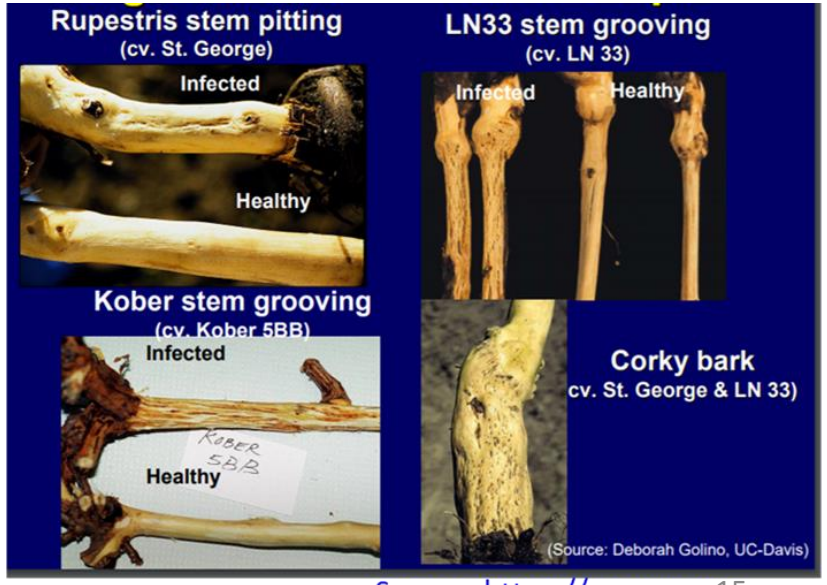
Grapevine leafroll-associated viruses



Grapevine red blotch virus

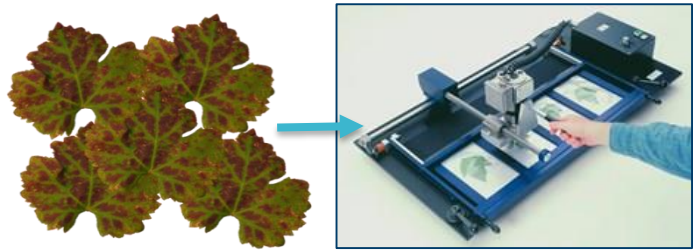
Grapevine fanleaf virus

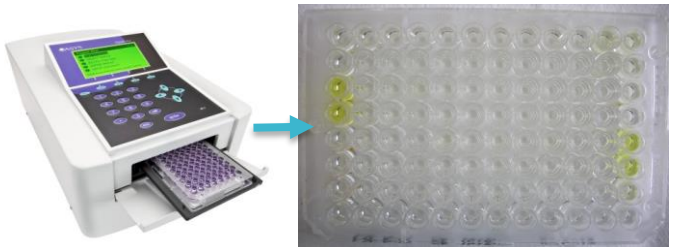
Grapevine Pinot Gris Virus



Source: https://wawgg.org

ELISA



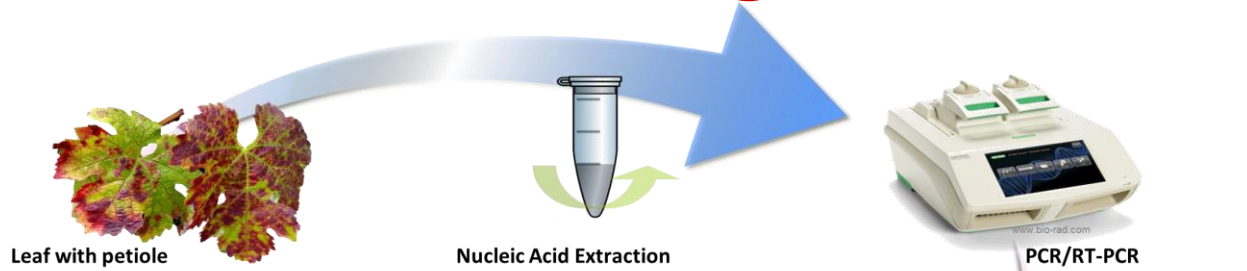


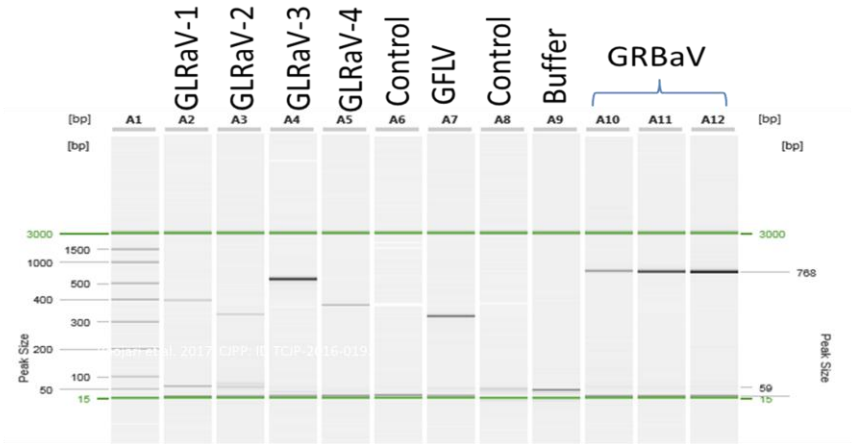
Advantages	Limitations
Less technical knowledge Large scale detection	Extraction of large number of samples in short time Time consuming Antibody availability is limited Nonspecific reactions Quality of antibodies Detection limitations with virus variants/strains

Arabis mosaic virus
Grapevine fanleaf virus
Raspberry ringspot virus-ch
Raspberry ringspot virus-g
Tomato black ring virus
Tobacco ringspot virus
Tomato ringspot virus
Tomato ringspot virus-Ch
Strawberry latent ringspot virus

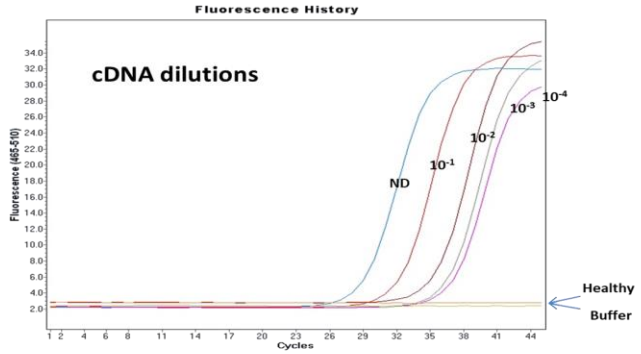
Grapevine leafroll-assoc. virus 1
Grapevine leafroll-assoc. virus 2
Grapevine leafroll-assoc. virus 3
Grapevine leafroll-assoc. virus 4 and strain 6
Grapevine virus A
Grapevine fleck virus
Grapevine pinot gris virus

PCR-based diagnostics

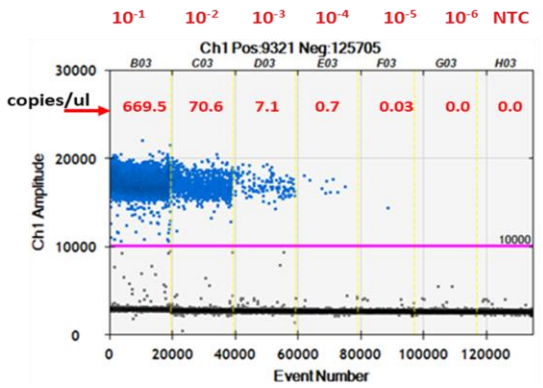




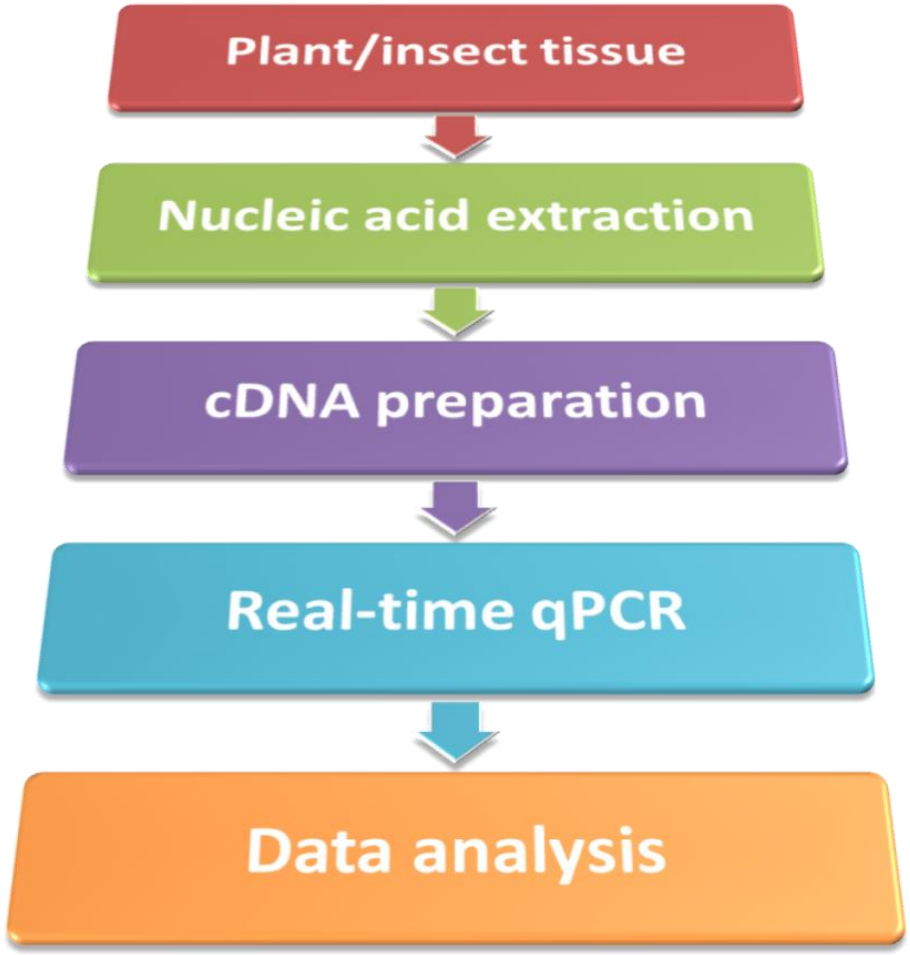
SYBR-based qPCR

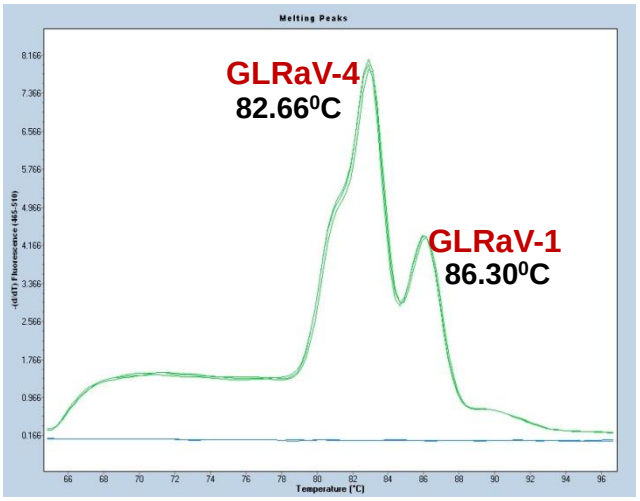


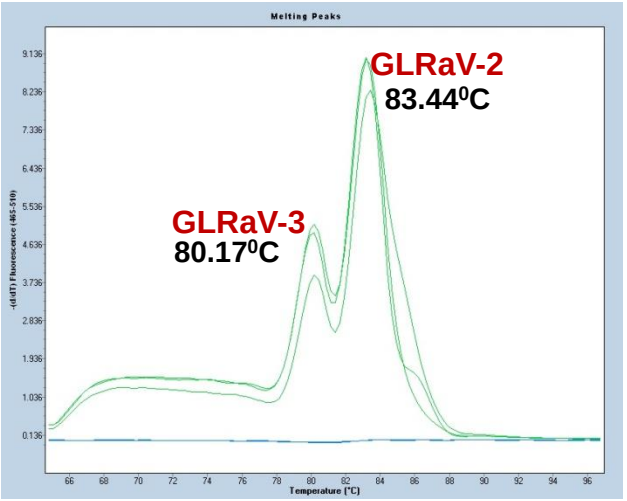
Droplet Digital PCR

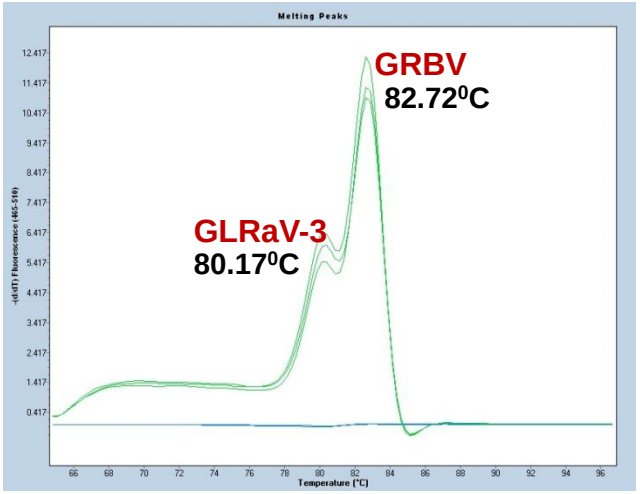


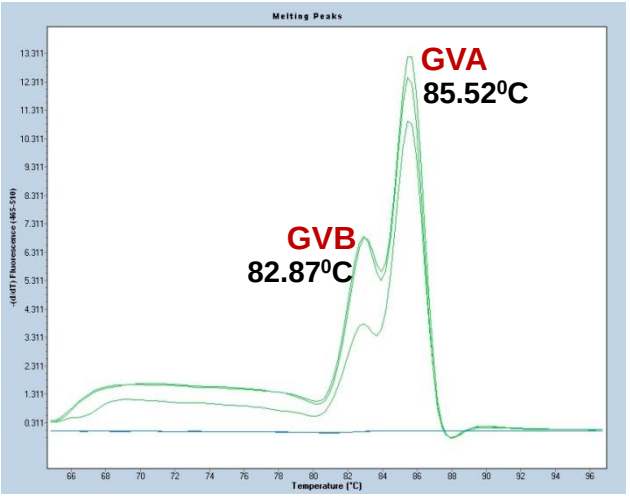
Validation of Duplex RT-qPCR





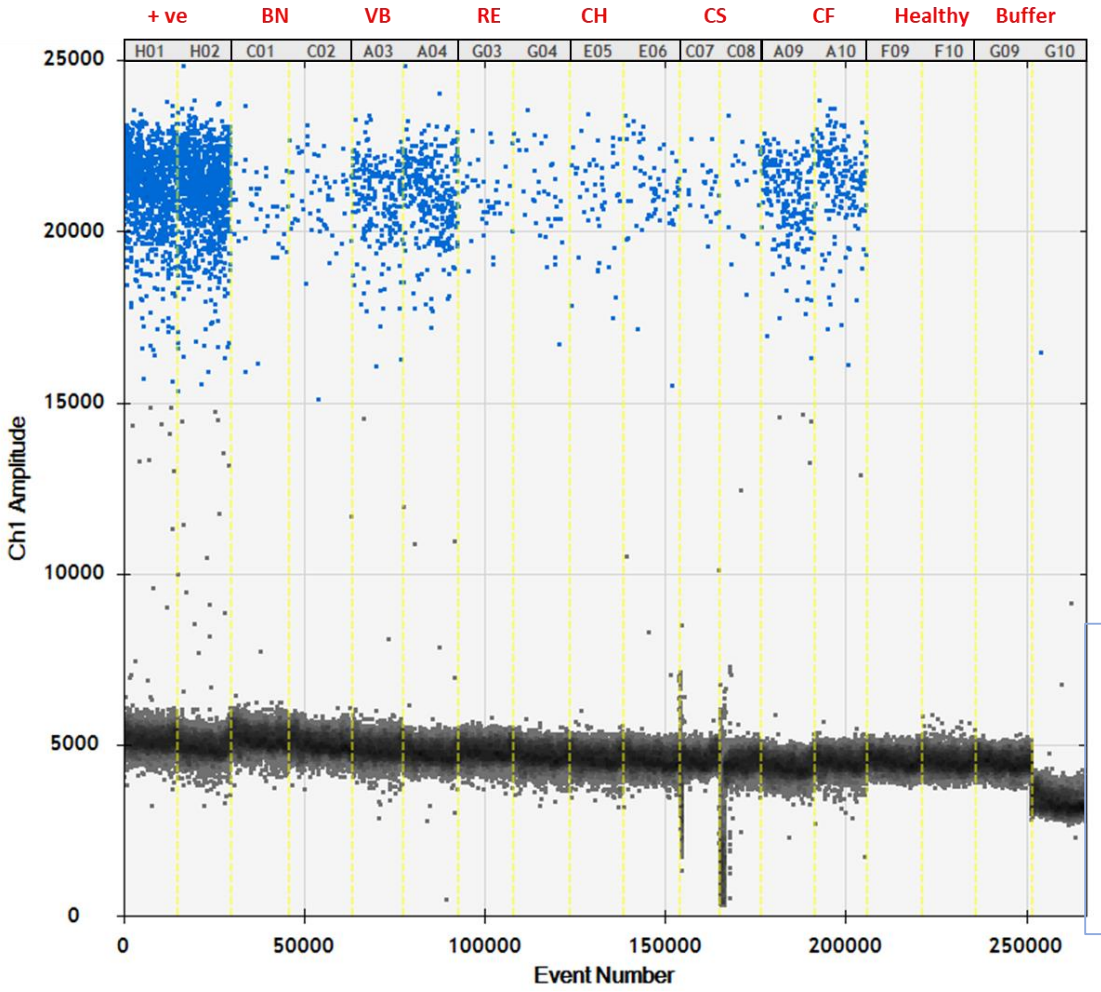


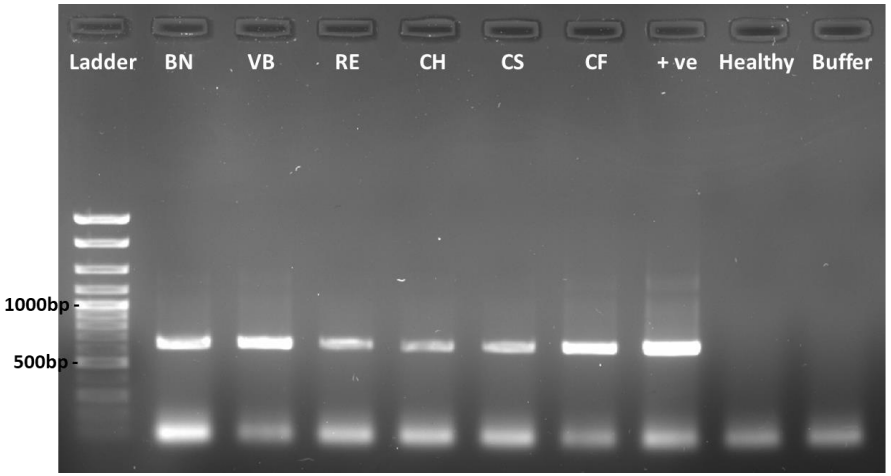




Poojari et al.,2016. J Virol Methods. doi: 10.1016/j.jviromet.2016.05.013.

Detection of GRBV by Droplet Digital™ PCR (ddPCR™)



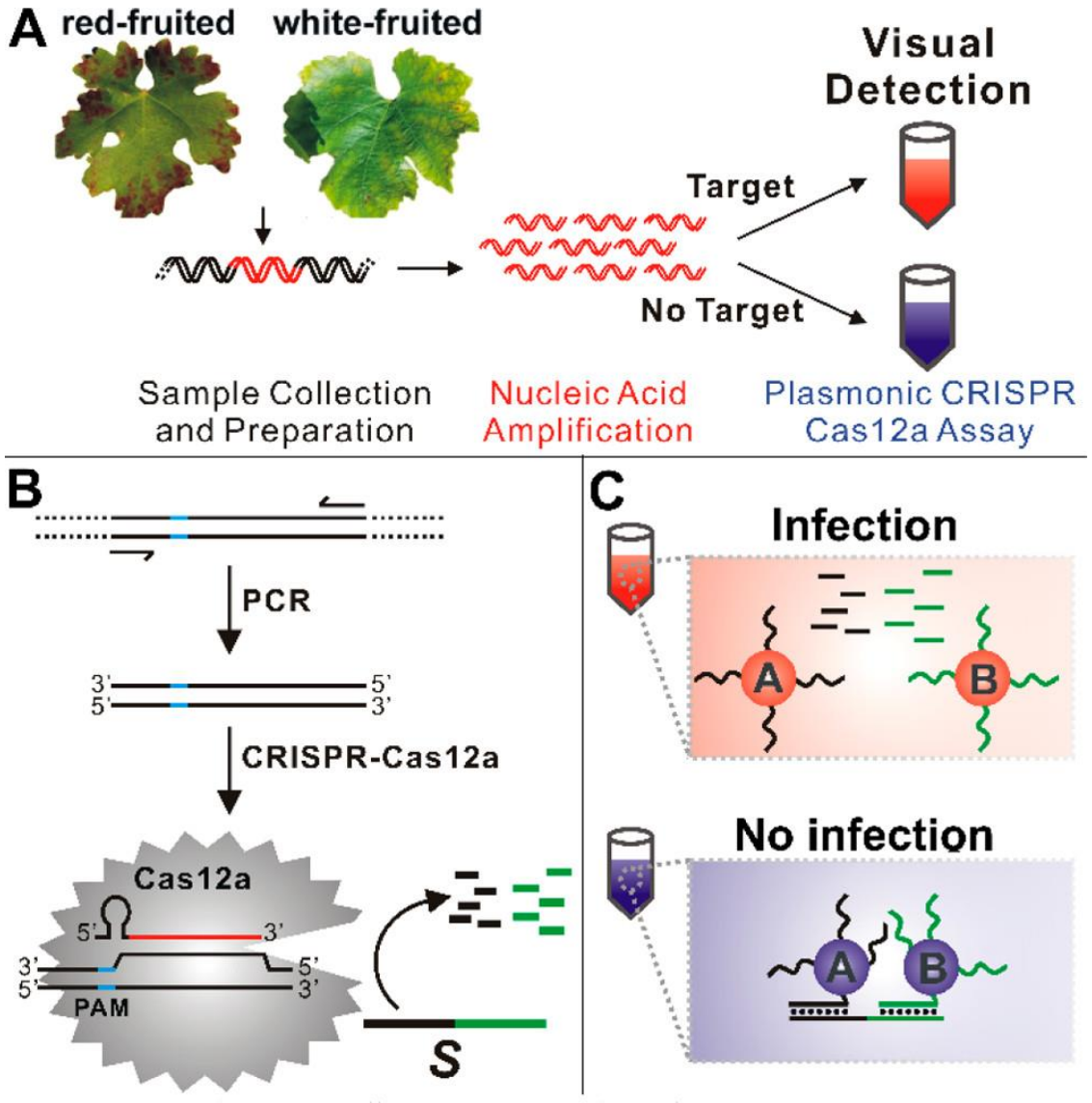


End-point PCR

Fluorescence amplitude plot representing the absolute quantification of grapevine red blotch virus (GRBV) DNA from 6 different isolates. BN-BacoNaoir; VB-Vidal Blanc; RE-Riesling; CH-Chardonnay; CS-Cabernet Sauvignon; CF-Cabernet Franc

Yongya Li et al., *Analytical Chemistry.* 2019. 91. 18. 11510-11513

Plasmonic CRISPR Cas12a assay for GRBV



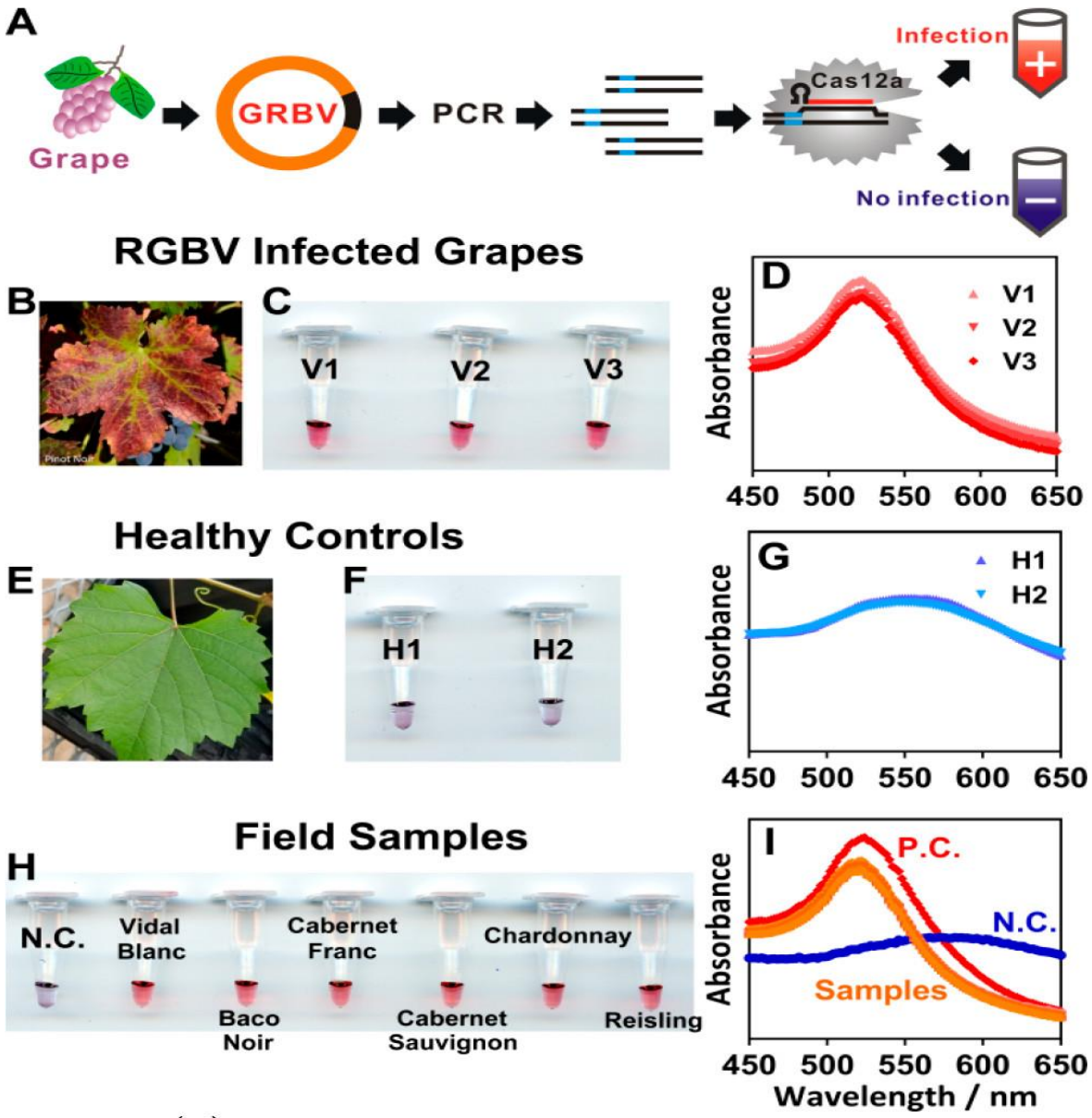
(A) Workflow for visual detection of GRBV infections in red-fruited and white-fruited grapevine samples.
(B) Target recognition using CRISPR-Cas12a and the activation of incriminate ssDNase activity.
(C) Color development using plasmonic DNA functionalized AuNPs.

Yongya Li et al., Naked-Eye Detection of Grapevine Red-Blotch Viral Infection Using a Plasmonic CRISPR Cas12a Assay
***Analytical Chemistry.* 2019. 91. 18. 11510-11513**

Cool Climate Oenology and Viticulture Institute, Brock University

Department of Chemistry, Centre for Biotechnology, Brock University

Plasmonic CRISPR Cas12a assay for GRBV



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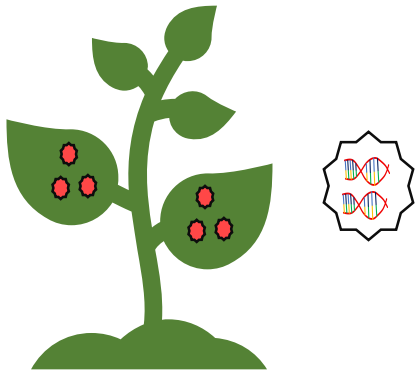
Department of Chemistry, Centre for Biotechnology, Brock University

PCR-based diagnostics

	Conventional PCR	Real-time PCR	Digital PCR
Detection	Yes	Yes	Yes
Quantitative	Semi	Relative	Absolute
Advantages	Sophisticated Rapid (in hours) Downstream applications - Sequencing - Evolutionary analysis	Increased sensitivity Records amplification as it happens No post-PCR processing Rapid (in minutes) Require less template melting curve analysis	Not dependent on house keeping genes / standards Highly accurate and useful to differentiate minor fold changes No post-PCR processing Require less template
Limitations	Size based results Post-PCR processing	Depend on reference genes Non-specific binding? Extensive optimization? Long amplicons? Multiplexing needs skills and knowledge	Large scale? Long amplicons? Multiplexing needs skills and knowledge

High Throughput Sequencing (HTS) based virus detection

High Throughput Sequencing (HTS) for virus detection



Virus suspected plant sample

Quality Check

Total RNA → Ribo depletion
Total RNA → Ribo + poly A depletion

Total Nucleic acids

Small RNA

Viral NA enrichment → Oligo probes
Viral NA enrichment → dsRNA

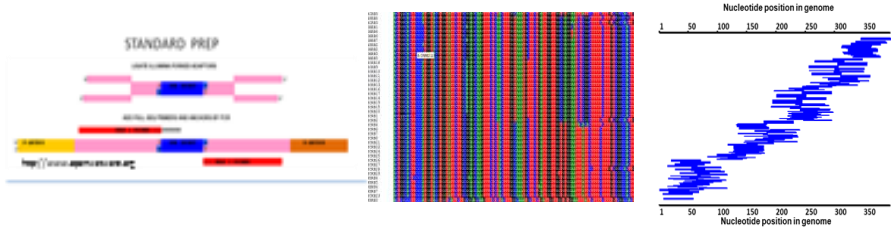
NGS sequence reads

Quality Check \ Adaptor sequence filter

De novo assembly of sequence reads

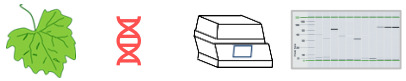
Blastn: host genome \ plant virus database

Virome discovery \ diagnosis



Virtool
Viral infection diagnostics using next-generation sequencing.

Virus Detection Methods and Applications

METHOD	HQP	EQP+DATA	TIME	APPLICATION
VISUAL				
BIOLOGICAL				
SEROLOGICAL				
-ELISA				
-LFIA				
PCR				
-End-point				
-qPCR/ddPCR				
HTS				

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Educational Resources

- Improvements in Grapevine virus diagnostics: Sudarsana Poojari
- Epidemiology and management of grapevine virus diseases: Tom Lowery
- Demystifying the status of grapevine viruses in British Columbia: José Ramón Úrbez Torres
- OMAFRA Grapevine leafroll virus
- OMAFRA: Grapevine red blotch

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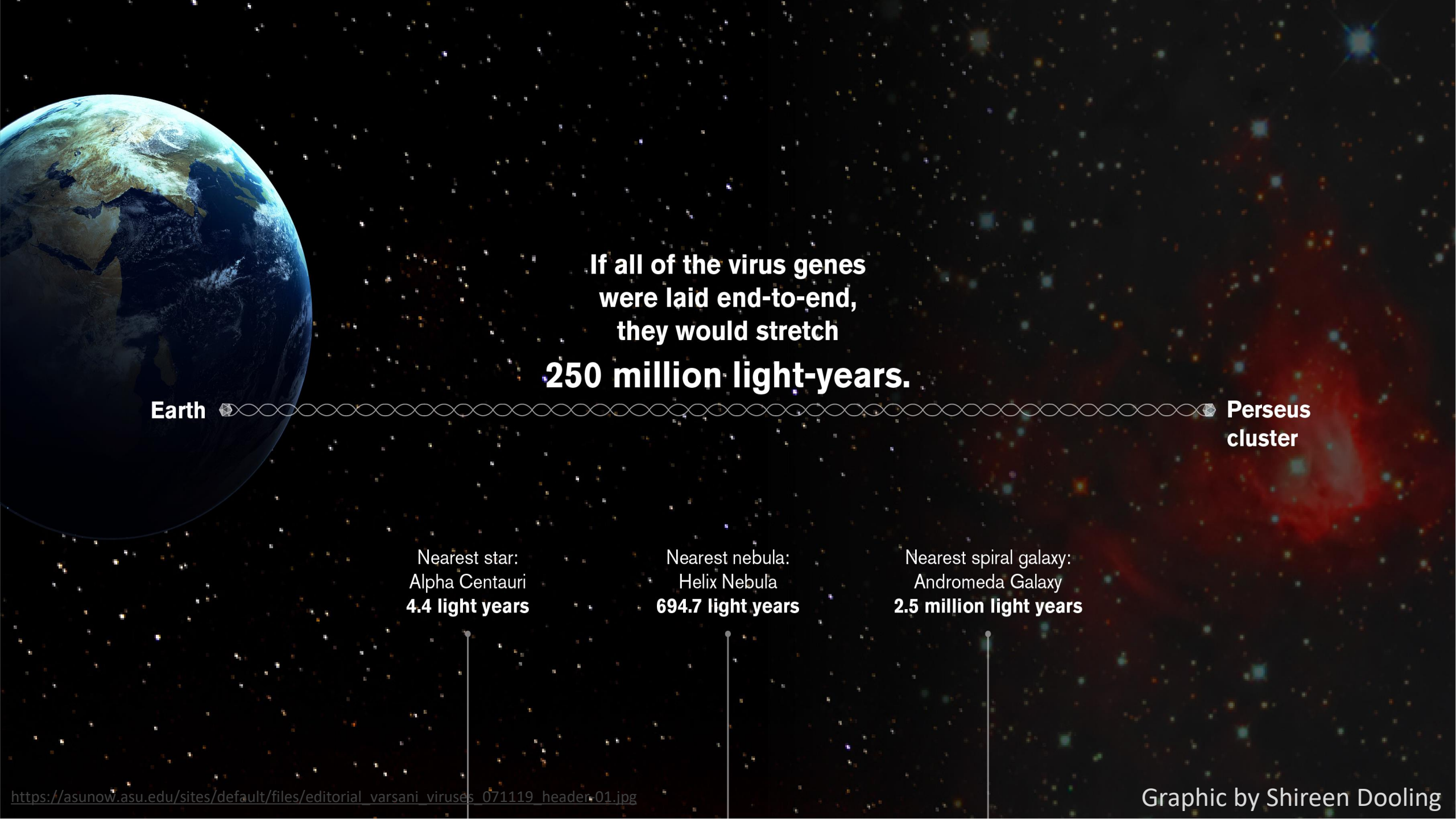

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