Brock University

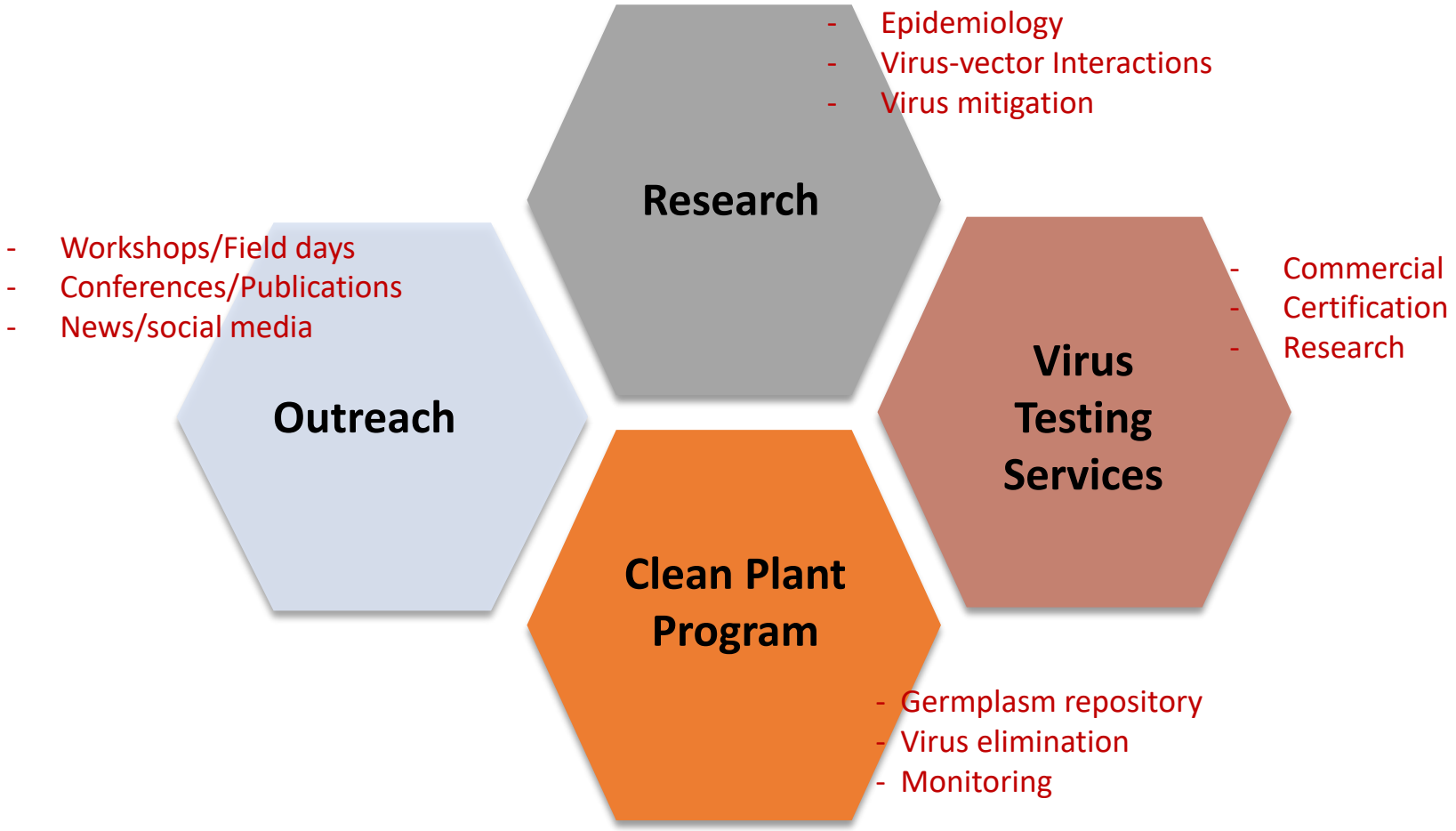
Cool Climate Oenology & Viticulture Institute

The Foundation of Canada's "CLEANSED" Project for developing genomic tools supporting clean plant program for grapevines

Sudarsana Poojari

January 14th, 1 -2 pm
H313, MCH, Brock University

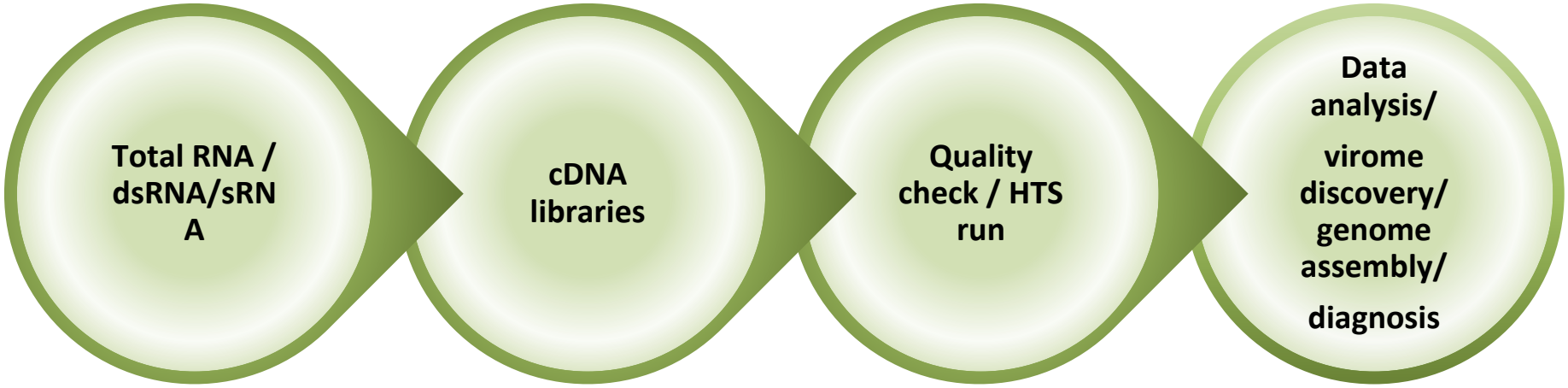
Sudarsana Poojari



PCR-based diagnostics

	Conventional PCR	Real-time PCR	Digital PCR
Detection	Yes	Yes	Yes
Quantitative	Semi	Relative	Absolute
Advantages	Sophisticated Downstream applications - Sequencing - Evolutionary analysis	Increased sensitivity Records amplification as it happens No post-PCR processing Require less template melting curve analysis	Not dependent on house-keeping genes / standards Highly accurate and finds 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)



- Detects all known and many novel viruses.
- Highly sensitive for mixed infections, low-titer viruses, and strain-level resolution

- Strongly depends on specialized bioinformatics expertise and infrastructure, and is expensive
- Targeted approaches improve sensitivity

CLEan plAnt extractioN and SEquencing Diagnostics (CLEANSED) for Clean Grapevines in Canada

1. Automation of HTS libraries and Viral RNA enrichment (Sud, Rott, Moffett)

- dsRNA enrichment
- Oligoprobes
- dsRNA binding protein

2. Optimization of the Virtool Web Application (Rott & Xuekui)

- Virtool development – upgrades

3. Technology Transfer of CLEANSED into a clean grapevine program (Úrbez Torres, Sud and Rott)

- National Survey – HTS-based
- Grapevine virome database from all four provinces (ON, BC, QC and NS)
- Policy changes for importing planting material (CFIA) – on regulated viruses

"Genomic Applications Partnership Program"

Brock University

Genome BritishColumbia
Leading > Investing > Connecting

Canadian Grapevine Certification Network
CGCN · RCCV
Réseau canadien de certification de la vigne

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

UNIVERSITÉ DE SHERBROOKE

University of Victoria

Conseil des vins du Québec

GenomeQuébec

computecanada

Ontario Genomics

illumina

CLEan plAnt extractioN and SEquencing Diagnostics (CLEANSED) for Clean Grapevines in Canada

1. Automation of HTS libraries and Viral RNA enrichment (Sud and Rott)

- dsRNA enrichment
- Oligoprobes
- dsRNA binding protein

2. Optimization of the Virtool Web Application (Rott & Xuekui)

- Virtool development – upgrades

3. Technology Transfer of CLEANSED into a clean grapevine program (Urbez Torres, Sud and Rott)

- National Survey – HTS based
- Grapevine virome database from all four provinces (ON, BC, QC and NS)
- Policy changes for importing planting material (CFIA) – on regulated viruses

Brock University

Genome British Columbia
Leading > Investing > Connecting

Canadian Grapevine Certification Network
CGCN · RCCV
Réseau canadien de certification de la vigne

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

Université de Sherbrooke

University of Victoria

Conseil des vins du Québec

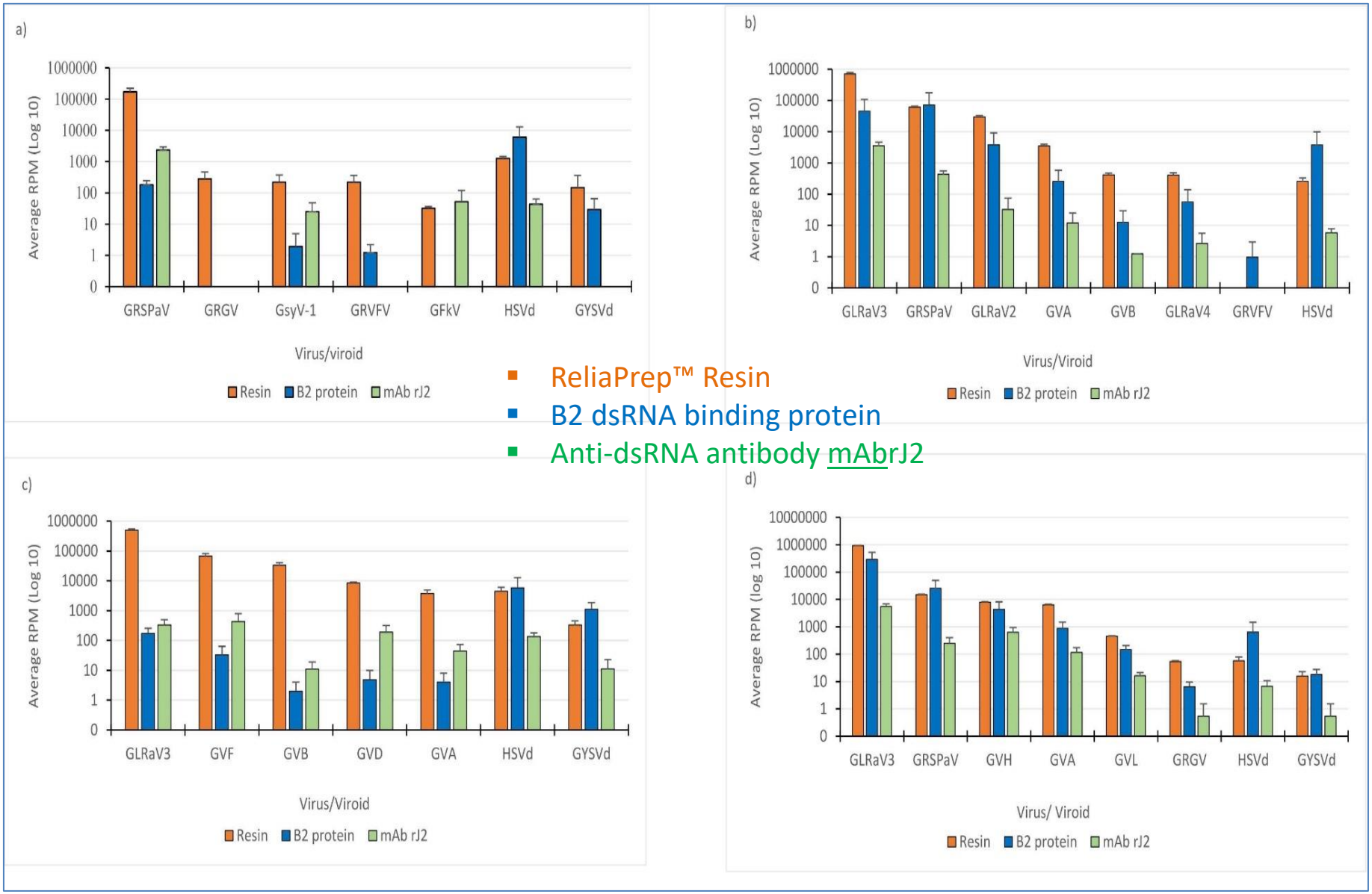
GenomeQuébec

illumina

computecanada

Ontario Genomics

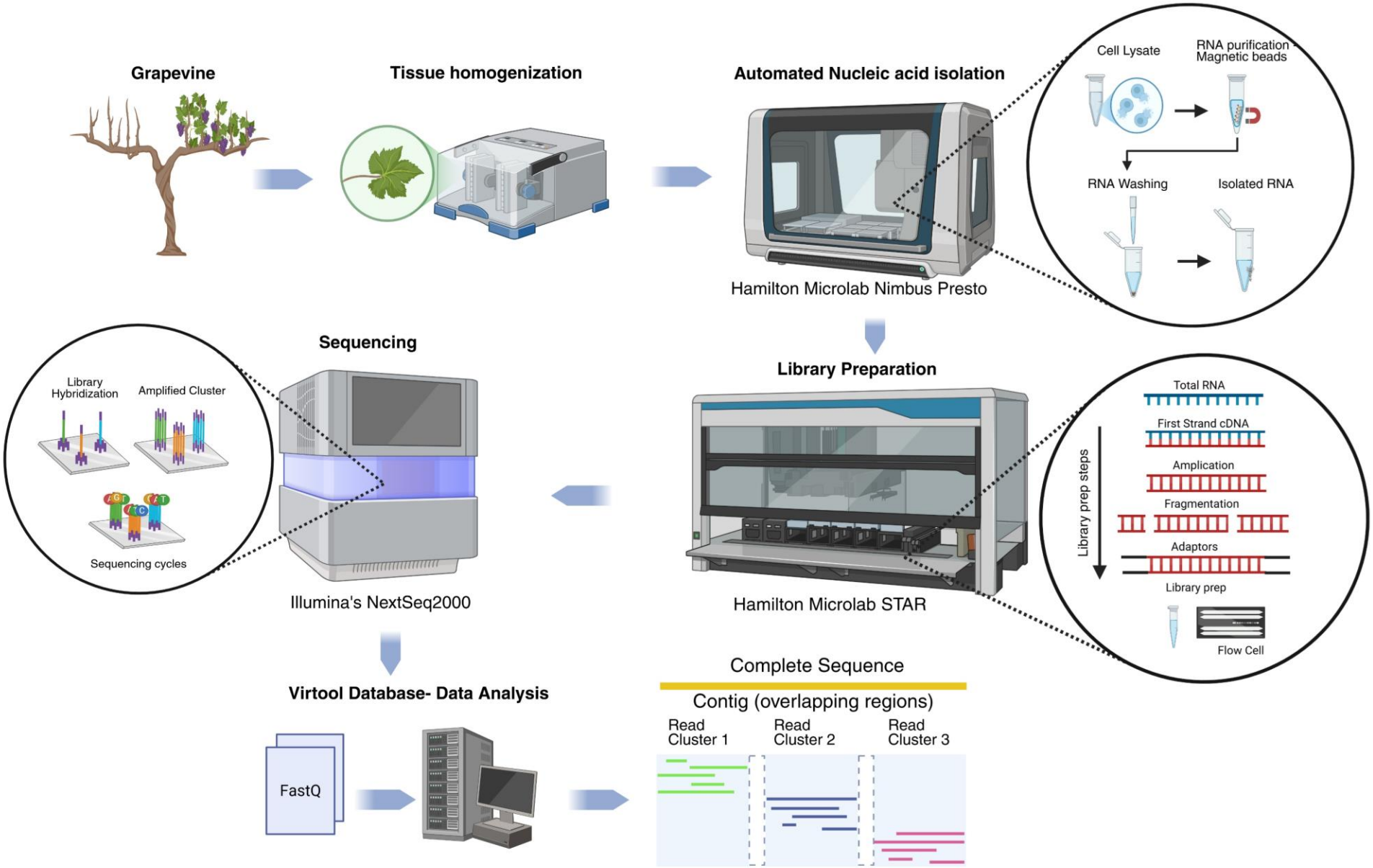
Comparative analysis of three dsRNA enrichment methods



- ReliaPrep™ Resin
- B2 dsRNA binding protein
- Anti-dsRNA antibody mAbrJ2

Rott M et al., https://doi.org/10.1016/j.jviromet.2024.114997

HTS Automation



CLEan plAnt extractioN and SEquencing Diagnostics (CLEANSED) for Clean Grapevines in Canada

1. Automation of HTS libraries and Viral RNA enrichment (Sud and Rott)

- dsRNA enrichment
- Oligoprobes
- dsRNA binding protein

2. Optimization of the Virtool Web Application (Rott & Xuekui)

- Virtool development – upgrades

3. Technology Transfer of CLEANSED into a clean grapevine program (Urbez Torres, Sud and Rott)

- National Survey – HTS based
- Grapevine virome database from all four provinces (ON, BC, QC and NS)
- Policy changes for importing planting material (CFIA) – on regulated viruses

Brock University

Genome British Columbia
Leading > Investing > Connecting

Canadian Grapevine Certification Network
CGCN · RCCV
Réseau canadien de certification de la vigne

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

Université de Sherbrooke

University of Victoria

Conseil des vins du Québec

GenomeQuébec

illumina

computecanada

Ontario Genomics

Virtool

Mike Rott and Xuekui Zhang

virtool.ca

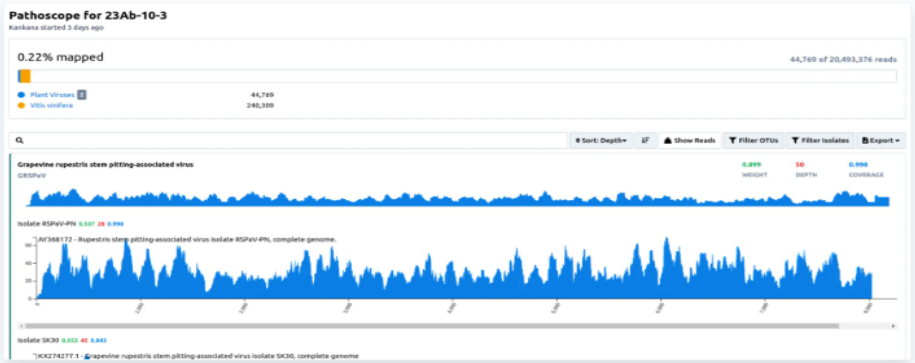
Virtool Docs

Viral infection diagnostics using high-throughput sequencing

Powerful and tested web-based bioinformatics tools for analyzing and managing your data—from samples to reference genomes.

Install Read Docs

Boyes, I., Hoffmann, R., and Rott, M. (2020). Virtool: Viral infection diagnostics using next-generation sequencing [Online]. Available online at: http://www.virtool.ca



Detect Known Pathogens

Use our fast detection workflow and simple result visualization.

Source: virtoll.ca; (Boyes et al., 2020; https://www.virtool.ca/)

CLEan plAnt extractioN and SEquencing Diagnostics (CLEANSED) for Clean Grapevines in Canada

1. Automation of HTS libraries and Viral RNA enrichment (Sud and Rott)

- dsRNA enrichment
- Oligoprobes
- dsRNA binding protein

2. Optimization of the Virtool Web Application (Rott & Xuekui)

- Virtool development – upgrades

3. Technology Transfer of CLEANSED into a clean grapevine program (Úrbez Torres, Sud and Rott)

- National Survey – HTS based
- Grapevine virome database from all four provinces (ON, BC, QC and NS)
- Policy changes for importing planting material (CFIA) – on regulated viruses

Brock University

Genome BritishColumbia
Leading > Investing > Connecting

Canadian Grapevine Certification Network
CGCN · RCCV
Réseau canadien de certification de la vigne

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments
Canada

UNIVERSITÉ DE SHERBROOKE

University of Victoria

Conseil des vins du Québec

GenomeQuébec

computecanada

Ontario Genomics

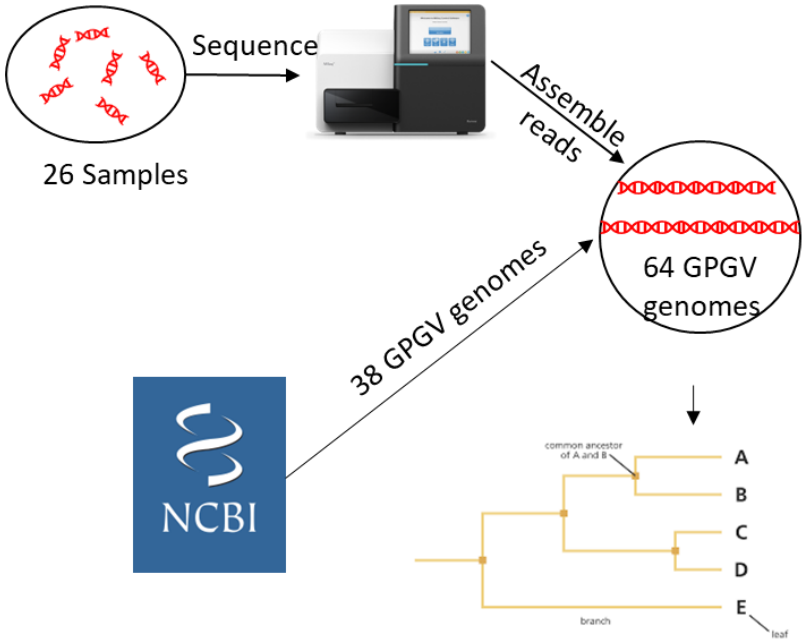
illumina

Grapevine Pinot Gris Virus – Case Study:1

Any link between symptoms and genotype?

- 26 GPGV samples (13 ON, 8 BC, 5 QC, 1 NS)
- Sequence them using Illumina – MiSeq (TruSeq total RNA)
- Add in publicly available sequences (38 sequences)
- Phylogenetic analysis – genome based

cv. Pinot Noir



Minh Vu et al. *Viruses 15*(3), 735; **https://doi.org/10.3390/v15030735**

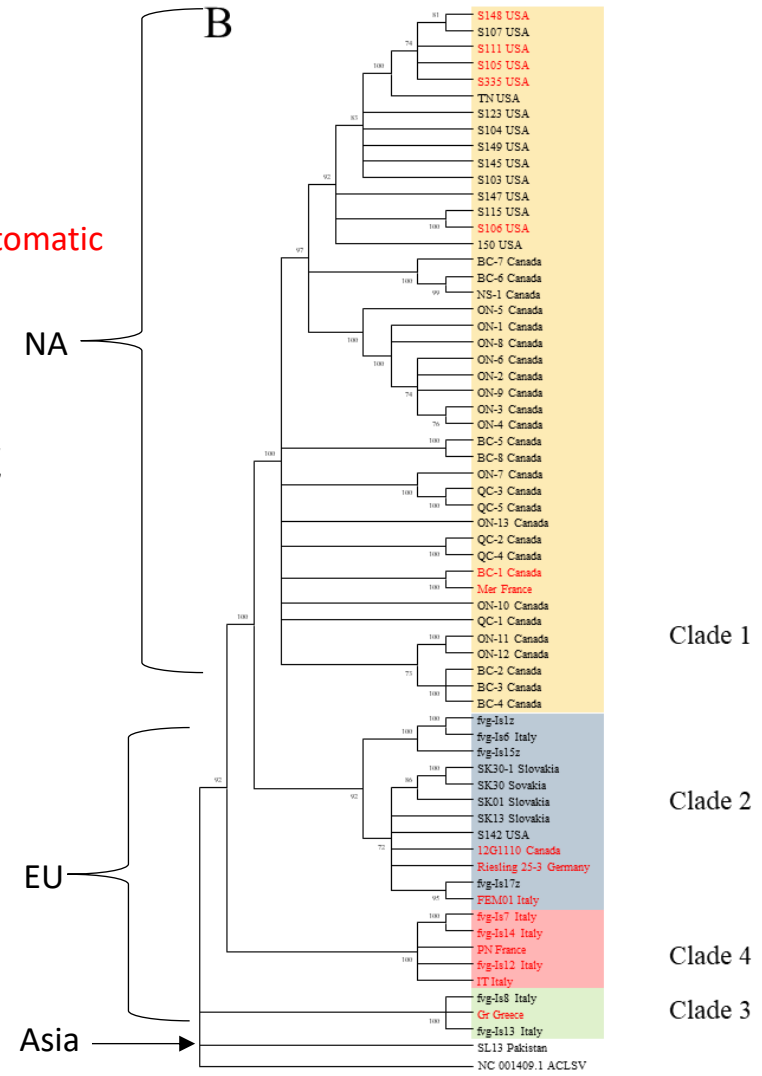
Grapevine Pinot Gris Virus – Case Study:1

Any link between symptoms and genotype?

Note: Red = symptomatic

What did we find?

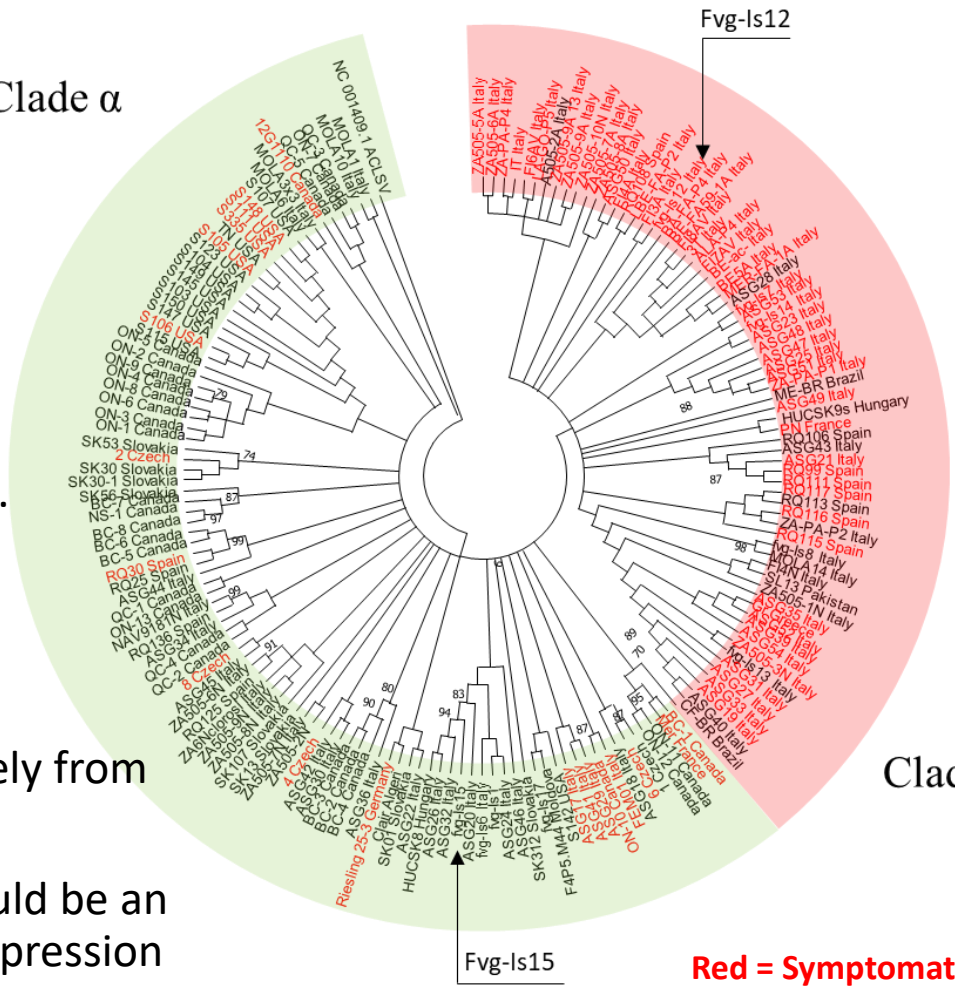
- Isolates are separated mostly based on origin
- All North American isolates grouped into ***Clade 1***
- There is no apparent separation of isolates from different regions of Canada
- The only closely related isolate is **GPGV-Mer** from France



Minh Vu et al. *Viruses 15*(3), 735; **https://doi.org/10.3390/v15030735**

Grapevine Pinot Gris Virus: MP-CP region

- 169 isolates segregate into two clades.
- Clade α is predominantly asymptomatic (80/99 isolates or 80%)
- Clade β is predominantly symptomatic (54/69 isolates, or 78.2%).
- All North American isolates are in clade α.

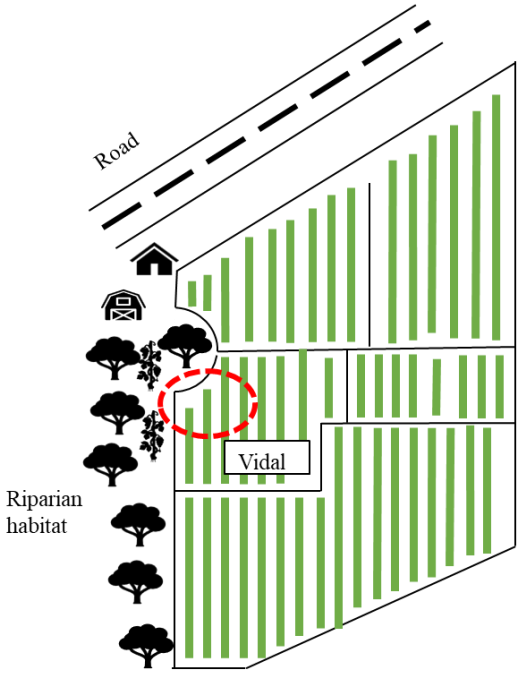


- The source of GPGV in Canada is most likely from France
- Genetic variation in the **MP-CP region** could be an important component of the symptom expression

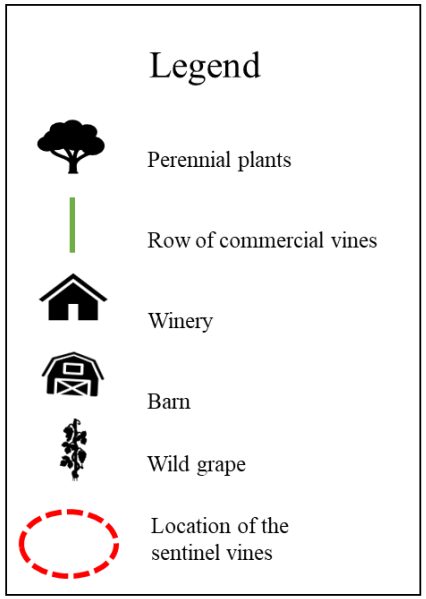
Minh Vu et al. *Viruses*, *15*(3), 735; https://doi.org/10.3390/v15030735

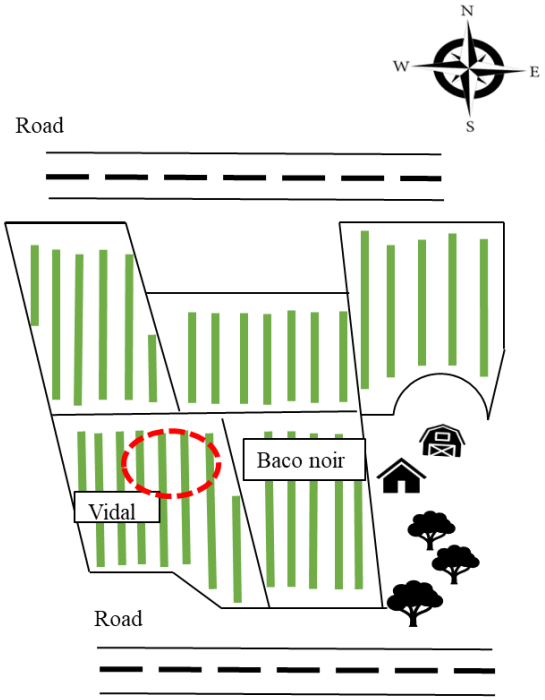
Spread of GPGV and GRBV in Ontario vineyards?

Case Study-2



The organic vineyard A





The traditional vineyard B

- **20** vines from each vineyard were tested **with PCR and HTS**
- **PCR result for vineyard A: 20/20 for GPGV and 19/20 for GRBV**
- **PCR result for vineyard B: 14/20 for GPGV and 13/20 for GRBV**
- HTS result: detected other viruses and viroids: GRSPaV, HSVd, GaTLV, GRLaV-2 and 3, GRGV, GSyV and GBV.

https://doi.org/10.3390/biology12101279

Sentinel vine preparation

- **40** *Vitis vinifera* cv. Chardonnay. Maintained at Brock's Phytotron for **2 years**
- Rootstock and scion material: Tested for GPGV & GRBV using PCR and HTS.
- **PCR and HTS result:** 0/40 for both GPGV and GRBV

Grapevine rupestris stem pitting-associated virus

Hop stunt viroid

Grapevine-associated Tymo-like virus

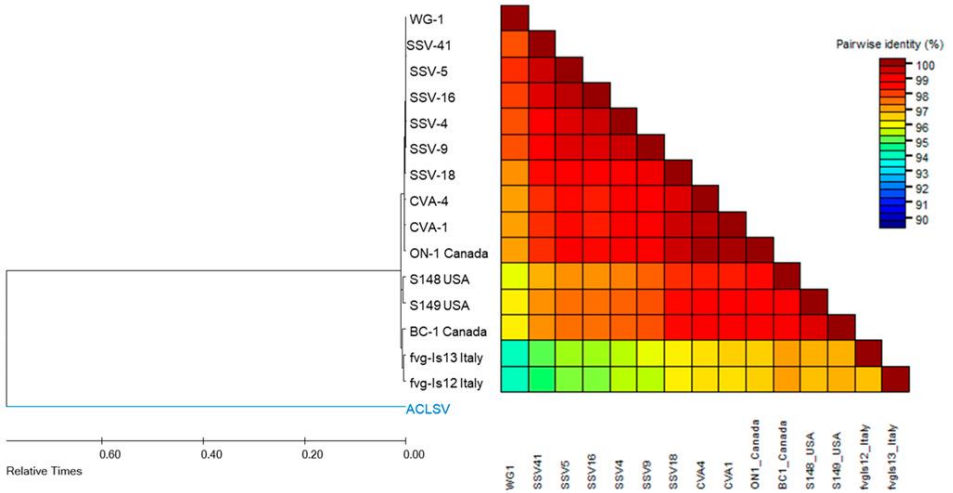
Spread of GPGV and GRBV in Ontario vineyards?

Sentinel vines	PCR		HTS	
	Pre-exposure	15 months post exposure	Pre-exposure	15 months post exposure
Vineyard A (Organic)	GPGV: 0/40 GRBV: 0/40	GPGV: 10/40 GRBV: 0/40	GPGV: 0/40 GRBV: 0/40	GPGV: 13/40 GRBV: 0/40
Vineyard B (Conventional)	GPGV: 0/40 GRBV: 0/40	GPGV: 1/40 GRBV: 0/40	GPGV: 0/40 GRBV: 0/40	GPGV: 1/40 GRBV: 0/40

- No sign of GRBV spreading
- GPGV spread in the organic vineyard is higher

- Very high sequence similarity
- Short evolutionary distance

  => This is a direct infection



https://doi.org/10.3390/biology12101279

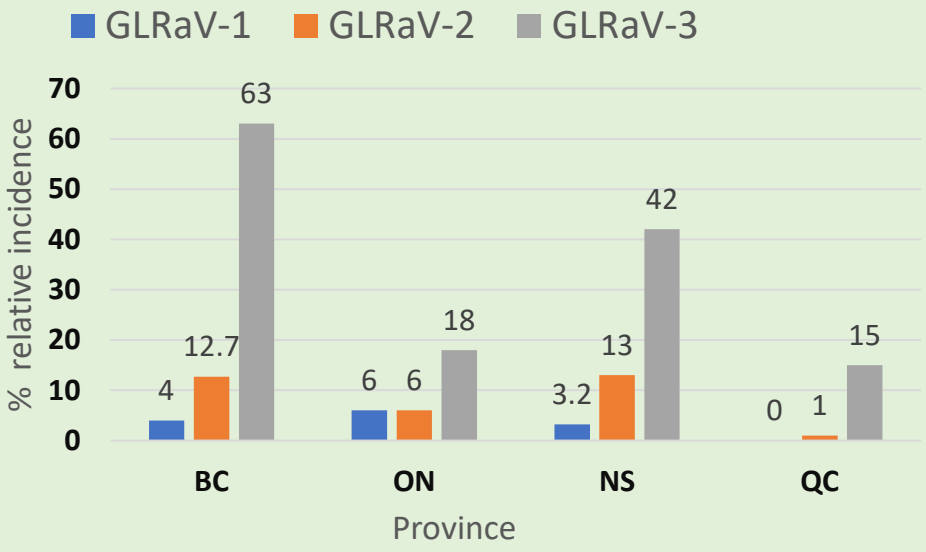
Metagenomic Survey

Grapevine samples:

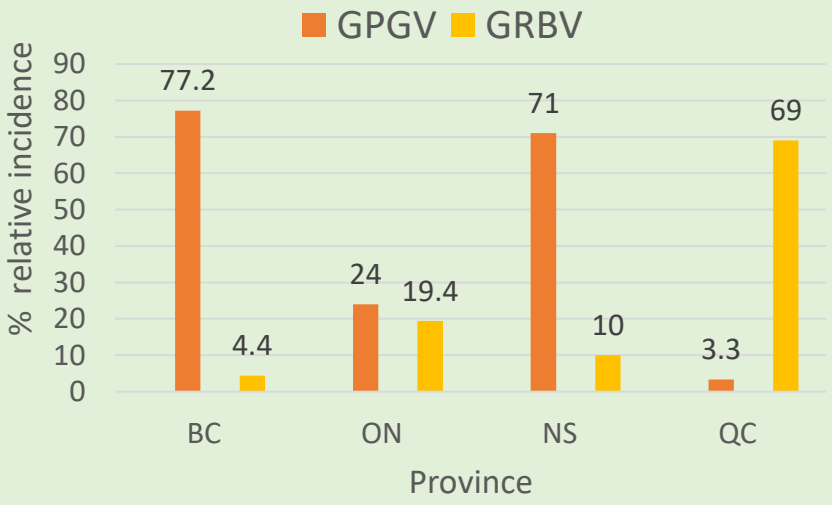
A total of 310 samples representing composite grapevine samples from 26 cultivars including red and white *V. vinifera* and American-French hybrids, were sampled

- 90 composite samples from nine cultivars from BC
- 67 composite samples from 14 cultivars from ON
- 31 composite samples from eight cultivars from NS
- 122 composite samples from 15 cultivars from QC

Leafroll Disease Complex



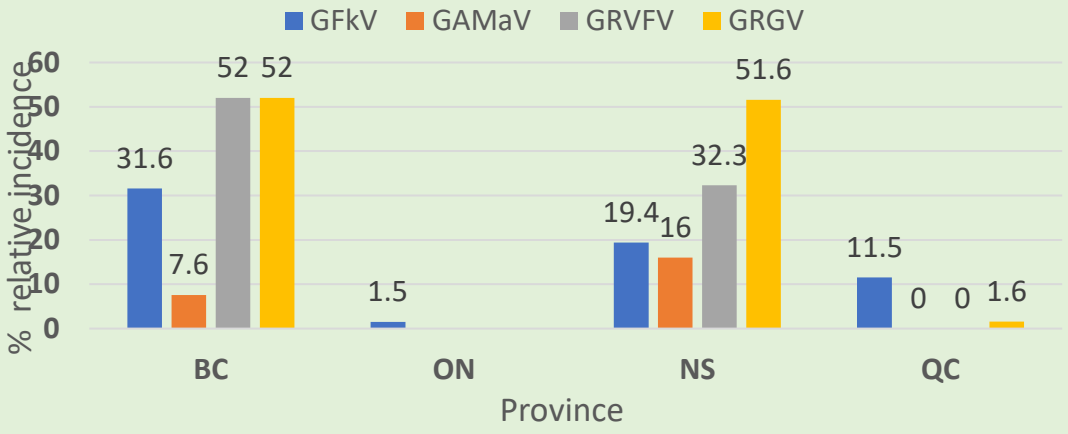
GPGV and GRBV



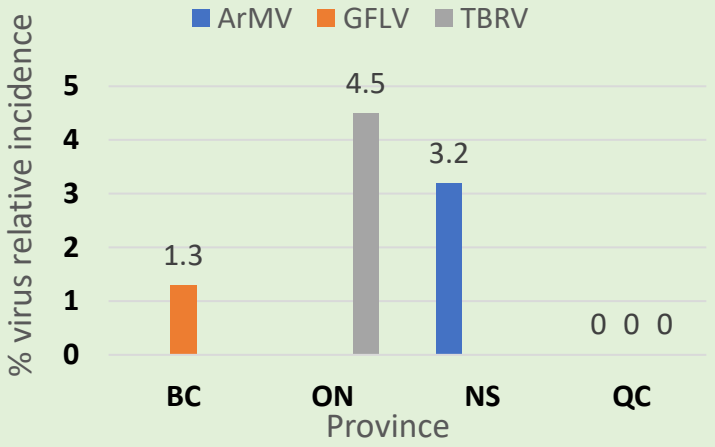
Vemulapati *et al. 2025. Agriculture*-3687620

Metagenomic Survey

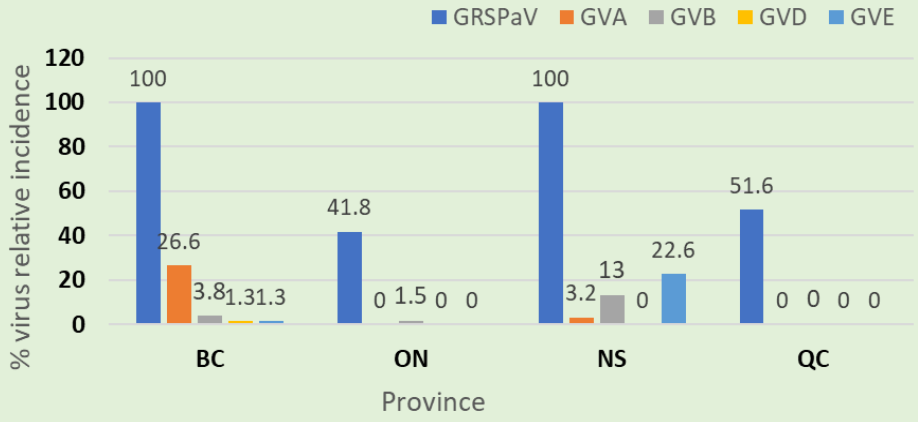
Fleck disease complex



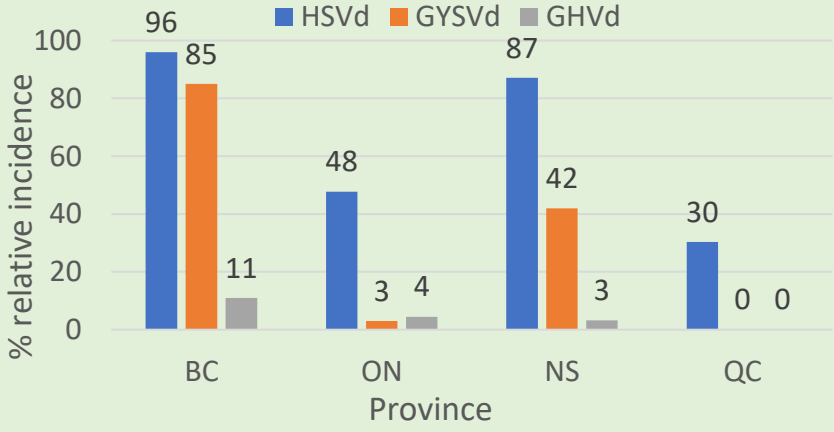
Infectious Degeneration/Decline



Rugose Wood Complex



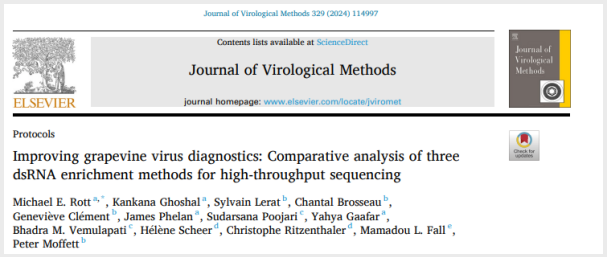
Viroids



Vemulapati *et al. 2025. Agriculture*-3687620

Project Achievements

6 High-Impact Publications


Journal of Virological Methods 329 (2024) 114997

Contents lists available at ScienceDirect

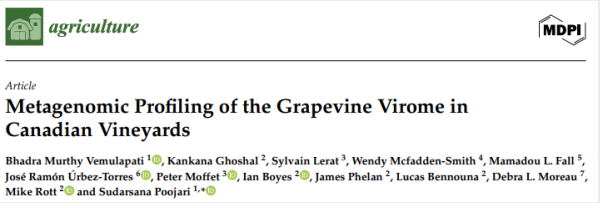
Journal of Virological Methods

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

Protocols

Improving grapevine virus diagnostics: Comparative analysis of three dsRNA enrichment methods for high-throughput sequencing

Michael E. Rott [a,*], Kankana Ghoshal [a], Sylvain Lerat [b], Chantal Brosseau [b], Geneviève Clément [b], James Phelan [a], Sudarsana Poojari [c], Yahya Gaafar [a], Bhadra M. Vemulapati [c], Hélène Scheer [d], Christophe Ritzenthaler [d], Mamadou L. Fall [a], Peter Moffett [b]


agriculture

MDPI

Article

Metagenomic Profiling of the Grapevine Virome in Canadian Vineyards

Bhadra Murthy Vemulapati [1], Kankana Ghoshal [2], Sylvain Lerat [3], Wendy Mcfadden-Smith [4], Mamadou L. Fall [5], José Ramón Úrbez-Torres [6], Peter Moffet [3], Ian Boyes [2], James Phelan [2], Lucas Bennouna [2], Debra L. Moreau [7], Mike Rott [2] and Sudarsana Poojari [1,*]


OXFORD

Briefings in Bioinformatics, 2024, 25(6), bbae501

https://doi.org/10.1093/bib/bbae501

Problem Solving Protocol

Diagnostics of viral infections using high-throughput genome sequencing data

Haochen Ning[1], Ian Boyes[2], Ibrahim Numanagić[3], Michael Rott[2,*], Li Xing[4,*], Xuekui Zhang[1,*]


Journal of Biomedical Informatics 146 (2023) 104501

Contents lists available at ScienceDirect

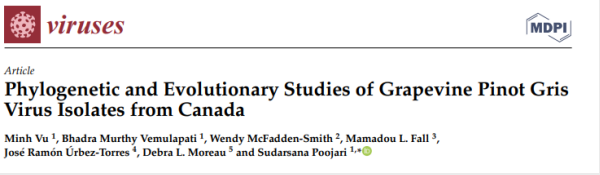
Journal of Biomedical Informatics

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

Original Research

ADSP: An adaptive sample pooling strategy for diagnostic testing

Xuekui Zhang [a,*], Xiaolin Huang [a], Li Xing [b]

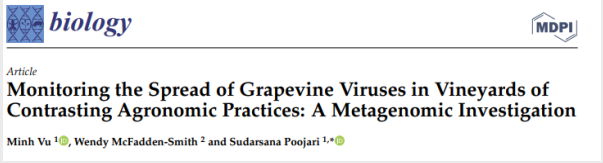

viruses

MDPI

Article

Phylogenetic and Evolutionary Studies of Grapevine Pinot Gris Virus Isolates from Canada

Minh Vu [1], Bhadra Murthy Vemulapati [1], Wendy McFadden-Smith [2], Mamadou L. Fall [3], José Ramón Úrbez-Torres [4], Debra L. Moreau [5] and Sudarsana Poojari [1,*]


biology

MDPI

Article

Monitoring the Spread of Grapevine Viruses in Vineyards of Contrasting Agronomic Practices: A Metagenomic Investigation

Minh Vu [1], Wendy McFadden-Smith [2] and Sudarsana Poojari [1,*]

Publishing Soon: Journal: VIROLOGY . Title: Targeted High-Throughput Sequencing Improves Sensitivity of Grapevine Virus Detection Using Custom Oligonucleotide Panels
Submitted date: Jan 2026

72

Personnel

Project involved a diverse team of researchers, students and technicians which drove the project.
Lab members and students were trained in state-of-the-art HTS facilities.

34

Knowledge Translation & Transfer Activities

The project resulted in data and knowledge to share and transfer for the benefit of public research.
The KTT activities included seminars, conference presentation and posters.

28

External Collaborators

The project involved partnering with industry and academic partners from research institutes, federal agencies and private sector.

2

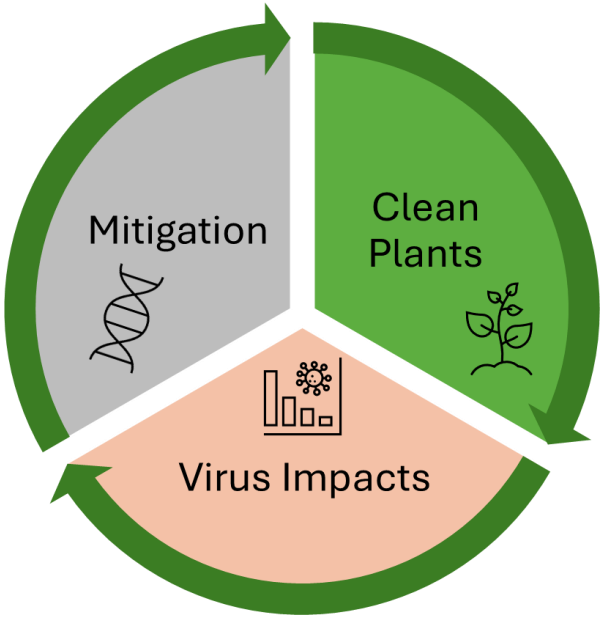
Software Tools

The project resulted in the development/improvement of wo HTS analysis tools.
Virtool Algorithm improvements & implementation of IIMI (**Identifying Infection with Machine Intelligence**)

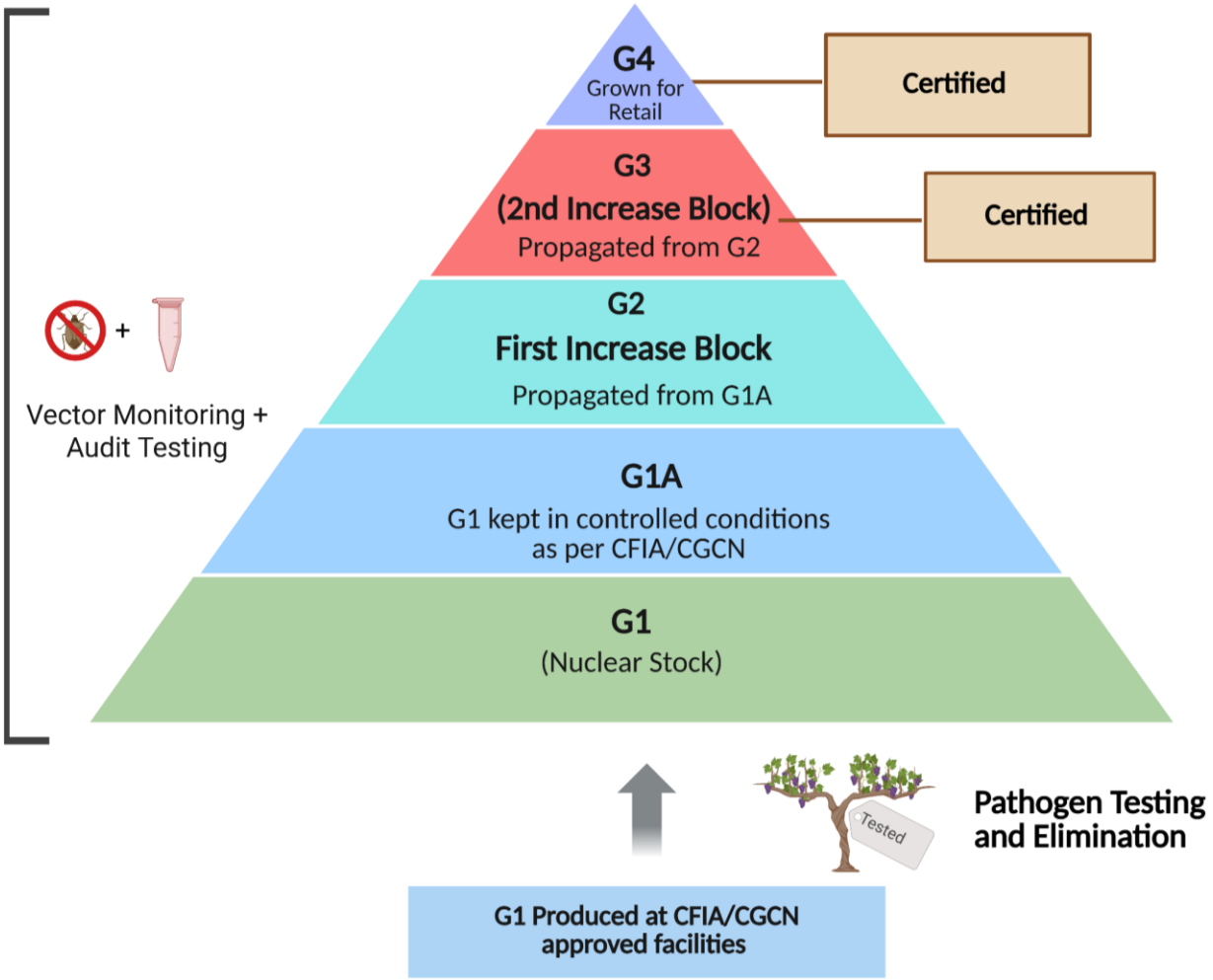
Clean Plant Program for Grapevines

What we have learned…

- Policy Changes: Federal and Provincial import/export regulations
- Canadian Grapevine Certification Network: Standards developed/improved
- Grapevine germplasm repository established: Focus on sustainable varieties
- Invested in the Canadian Grapevine Clean Plant Program at Brock University: Facilities and Workforce



The Canadian Grapevine Certification Network - Standard



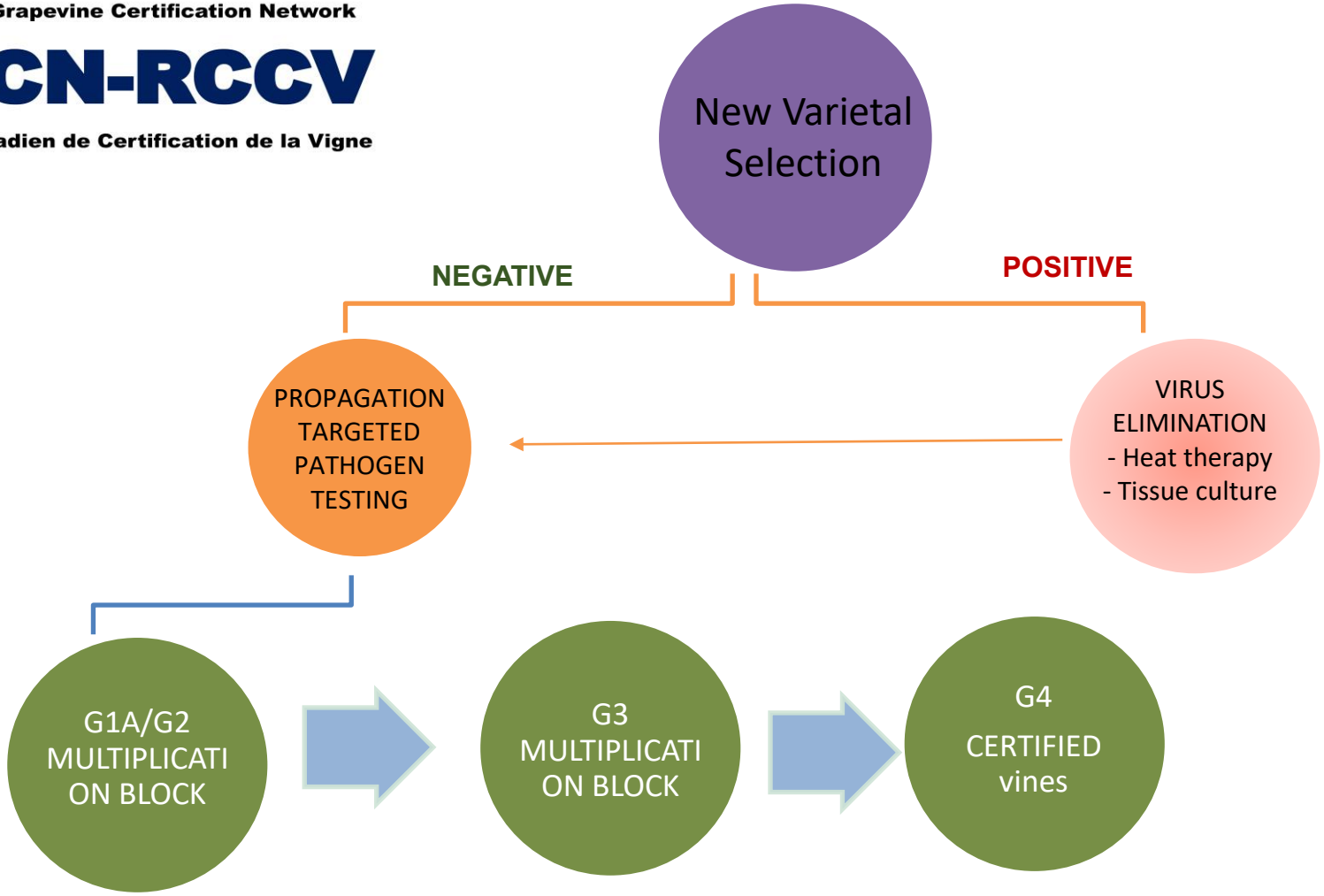
Created in https://BioRender.com

CGCN Certification Standard

Canadian Grapevine Certification Network

CGCN-RCCV

Réseau Canadien de Certification de la Vigne



- ✓ Isolated, virus-tested block
- ✓ Inspected regularly for disease symptoms
- ✓ Retested on a regular schedule

Virus Elimination Process for Grapevines

Scion

Rootstock

Tested +ve

meristems (0.1–0.5 mm)

Nuclear Stock

Scion Variety

Rootstock Variety

HTS testing

Propagation Stock

Grafted

Scion Variety

Rootstock Variety

Certified Rootstock Cutting

Certified Stock

Certified Plant

Certified Scion Cutting

HTS based screening saves 2 years of time

High Throughput Sequencing: Clean Plant Program

Traditional

Health Status Unknown

HTS based

Diagnostic tests for 30+ pathogens (ELISA, DNA)

Positive

Virus elimination (meristem culture: 1 yr.)
Woody + Herbaceous Indexing (2 yrs.)
Retesting (30+ pathogens) (weeks to months)

Negative **Negative**

HTS testing (days to weeks)

Positive

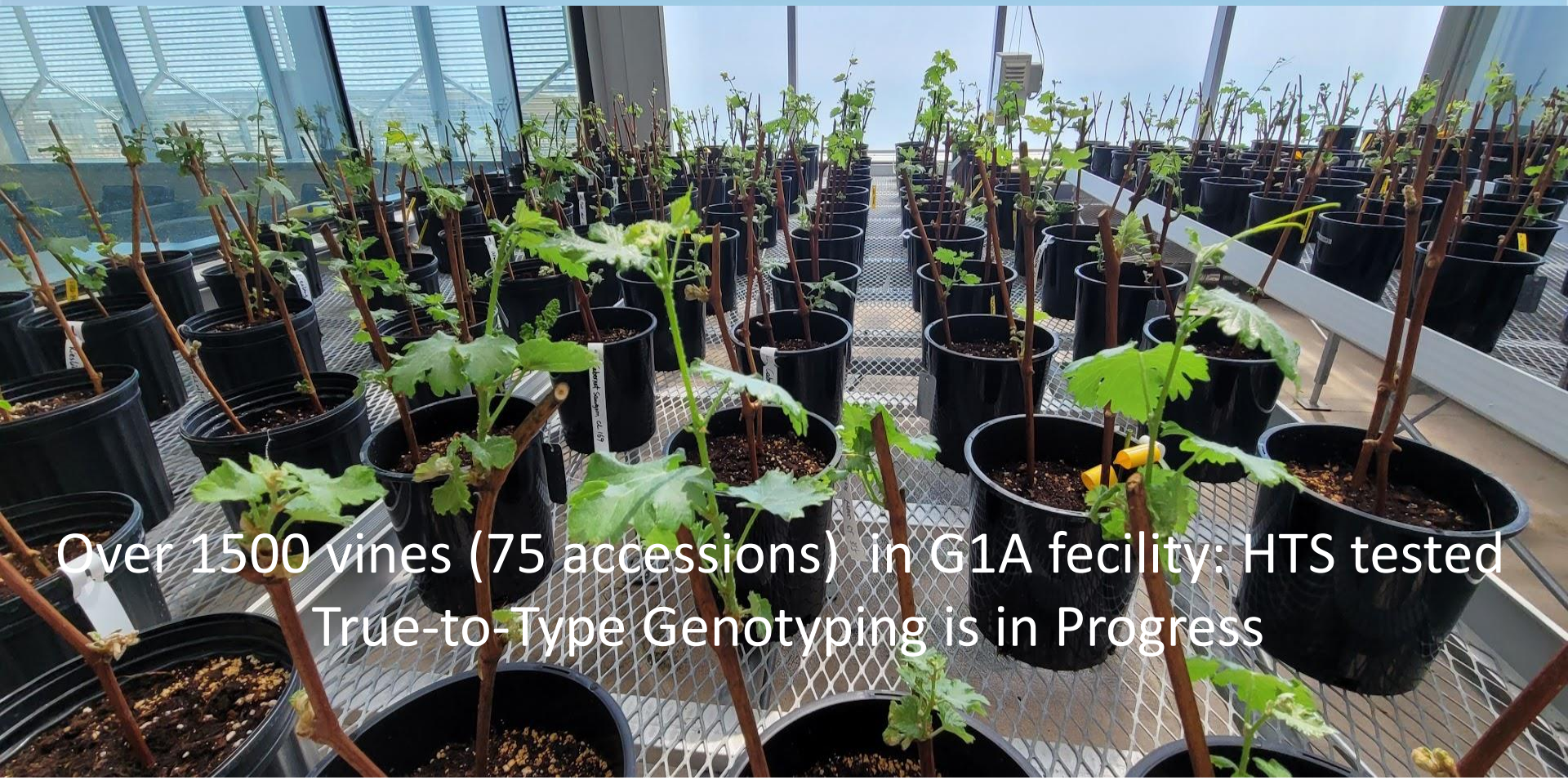
Virus elimination (meristem culture: 1 yr.)
Retesting with HTS (days to weeks)

HTS + Virtool

Clean Plant Program for Grapevines

TC Lab: Sud Poojari, CCOVI, Brock University

National Grapevine Germplasm Facility at Brock University



Over 1500 vines (75 accessions) in G1A fecility: HTS tested
True-to-Type Genotyping is in Progress

Ontario GRAPE & WINE RESEARCH Incorporated

Brock University

Canadian Grapevine Certification Network
CGCN · RCCV
Réseau canadien de certification de la vigne

National Grapevine Germplasm Facility at Brock University

Scion Varieties/Clones

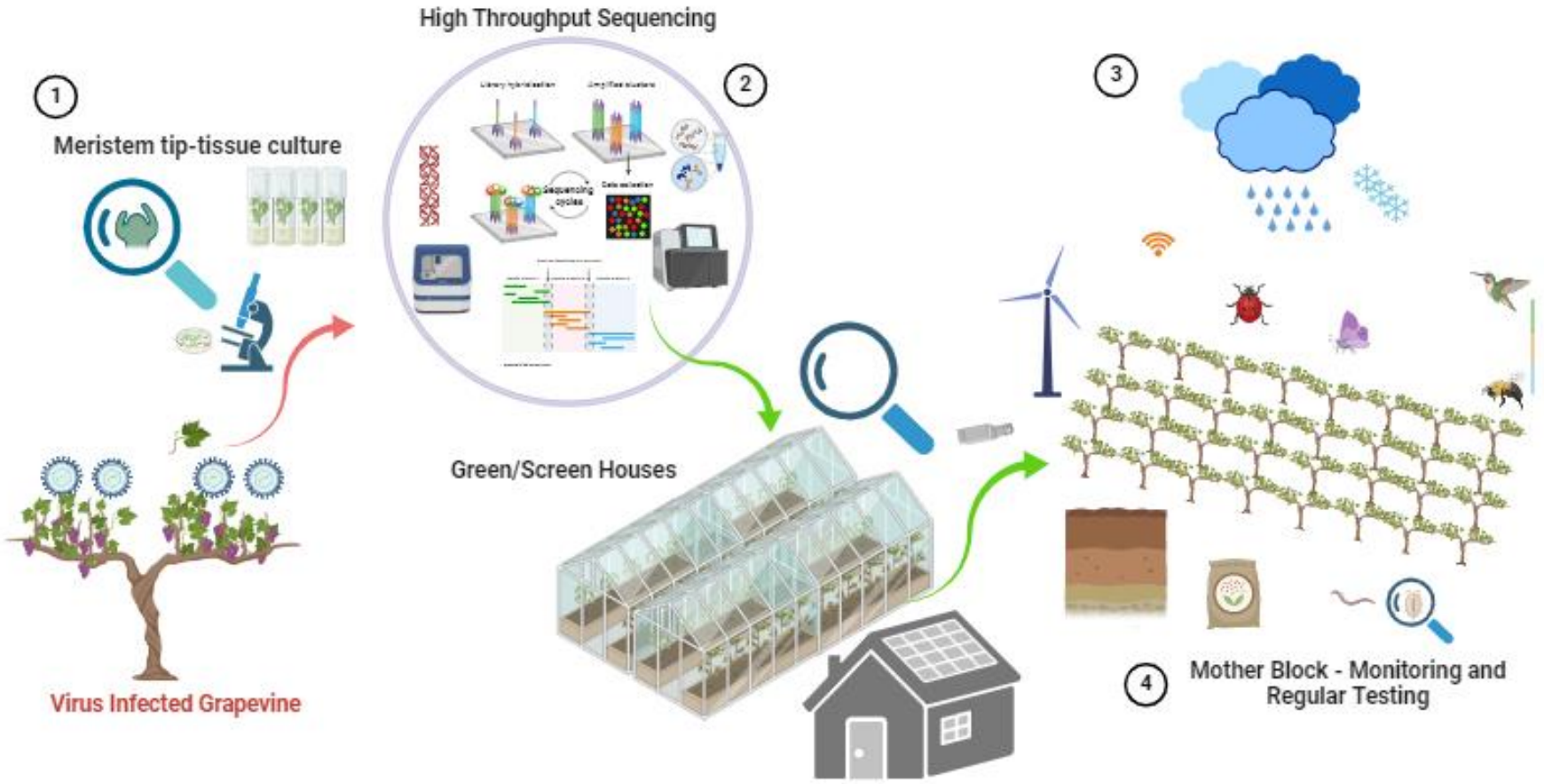
Cultivar Name	Number of Vines		Cultivar Name	Number of Vines	
	Phytotron	Tissue Culture Lab		Phytotron	Tissue Culture Lab
Syrah CL 7(877)	18	15	Pinot Noir 91	20	15
Cabernet Sauvignon CL 191	20	15	Merlot 15	20	30
Frontenac	29	20	Sauvignon Blanc 14	20	20
Frontenac Blanc	30	15	Sauvignon Blanc 20	20	20
Leon Millot	20	15	Sauvignon Kretos	15	
Foch	29	15	Sauvigon Rytos	15	
L 'Acadie	20	15	Fleurtai	15	
Riesling CL 21B	20	20	Soreli	15	
Cabernet Sauvignon CL 169	29	15	Cabernet Volos	15	
New York Muscat	20	20	Viognier CL 642	20	15
Vidal Blanc	20	30	Lucie Kuhlman	20	30
Pinot Gris CL 53	20	20	GM 318-57	18	15
Riesling CL 239-13 GM	20	20	Chardonnay 69	20	20
Riesling CL 49	19	30	Pinot Gris 09	31	15
Gewurztraminer CL 47	20	25	Riesling 01	30	30
Pinot Noir CL 777	20	15	Pinot Meunier 05	20	15
Sauvignon Blanc CL 317	20	20	Pinot Noir 70	20	20
Merlot CL 347	20	10	Pinot Noir 72	20	15
Cabernet Franc CL 214	25	20	Pinot Noir 73	20	15

Rootstocks

Cultivar Name	Number of Vines	
	Phytotron	Tissue Culture Lab
3309 C	29	20
Riparia Gloire	28	20
101-14	20	15
SO4	28	15
Schwarzmann 01	21	20

Total number of varieties/clones: 60+
Total number of vines in Phytotron: 1500+
Total number of explants in the lab: 800+

Clean Plant Program - Structure



Clean Plant Program for Grapevines

The Clean Agriculture for Sustainable Production (CASP) : $8.9million

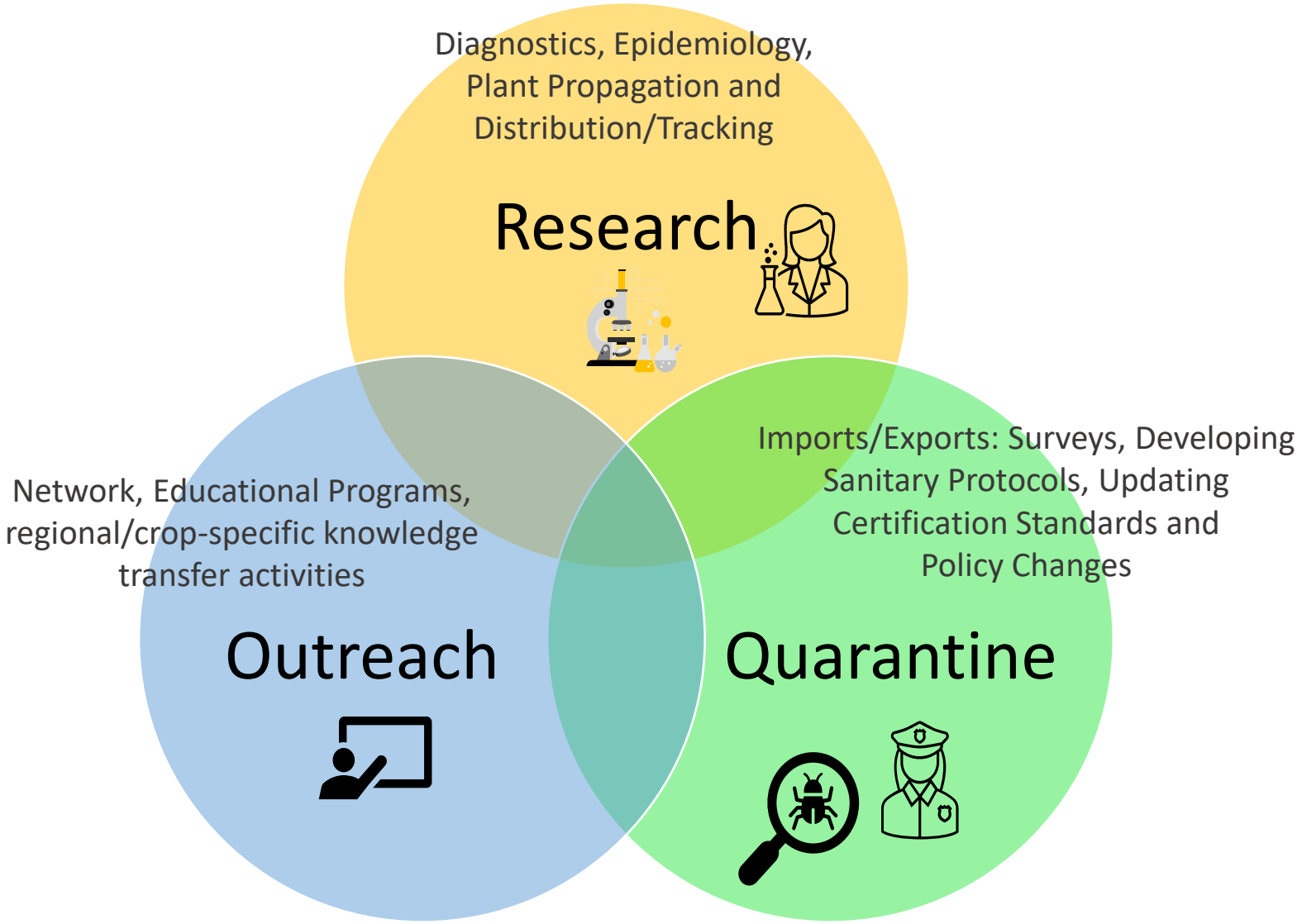
Poojari, Sudarsana. CCOVI, Biological Sciences, Brock University
Willwerth, Jim. Biological Sciences, Brock University

- 9000 sq. ft. Screenhouses
- 6000 sq. ft. Greenhouses
- Foundation vineyard
- Support facilities
- PPC-Leve2: Standards (CFIA)

INNOVATION.CA
CANADA FOUNDATION FOR INNOVATION | FONDATION CANADIENNE POUR L'INNOVATION

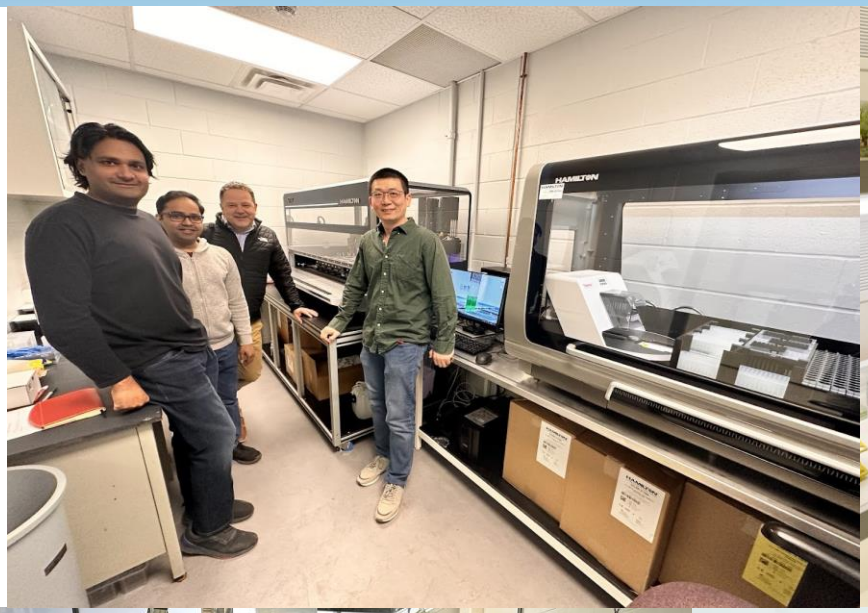
Ontario Research Fund

Clean Plant Program - Vision



Team

- Tony Wang
- Bhadra Murthy
- Adeel Asghar
- Balaji Devata
- Kevindi Gunasekara
- Junyi Xia
- Jenna Venneri
- Suzan Oduniyi
- Lucy Tyminski
- Linxue Zhang
- Minh Vu
- Chandana Suma
- Vinay Chundi



Acknowledgments