

Portable advanced molecular technologies for detection of plant pathogens

EPPO Conference on Diagnostics
Vienna, 2026-04-22/24

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Who we are



EPPO Conference on Diagnostics
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Emergent Plant Disease Prevention and Management Team

NRL Virology - IVIA

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NRL Bacteriology - IVIA

Silvia Barbé
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Micology Unit - IVIA

Carlos Salas-Sierra
Ana Pérez-Sierra

Diagnostic expertise in viruses, bacteria and fungi with a common goal:

Validated tools that can move from the lab to real on-site use

Why rapid and portable devices

-  Rapid detection is essential to support surveillance programs, management strategies, and safeguard plant trade at border control posts.
-  Advanced molecular diagnostic technologies, are linked to laboratory facilities and specialized non portable equipment.
-  Early detection and rapid response against emerging threats require validated tools able to be applied in a real diagnostic on-site context

Portable molecular technologies

Combining laboratory robustness with on-site speed and versatility

1 Real-time RPA

Fast isothermal amplification for sensitive, specific, field-ready detection



2 Nanopore sequencing

Rapid detection and characterization with portable high-throughput sequencing



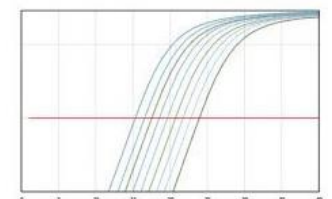
3 Magnetic induction real-time PCR

Compact PCR tool designed to bring robust molecular testing closer to the point of need



Fast. Accurate. Compact.

Speed Accuracy Size Connectivity



Real-time RPA: Huanglongbing

A high-impact citrus bacterial disease



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Huanglongbing (HLB) is a devastating disease that affects all commercial citrus species worldwide.

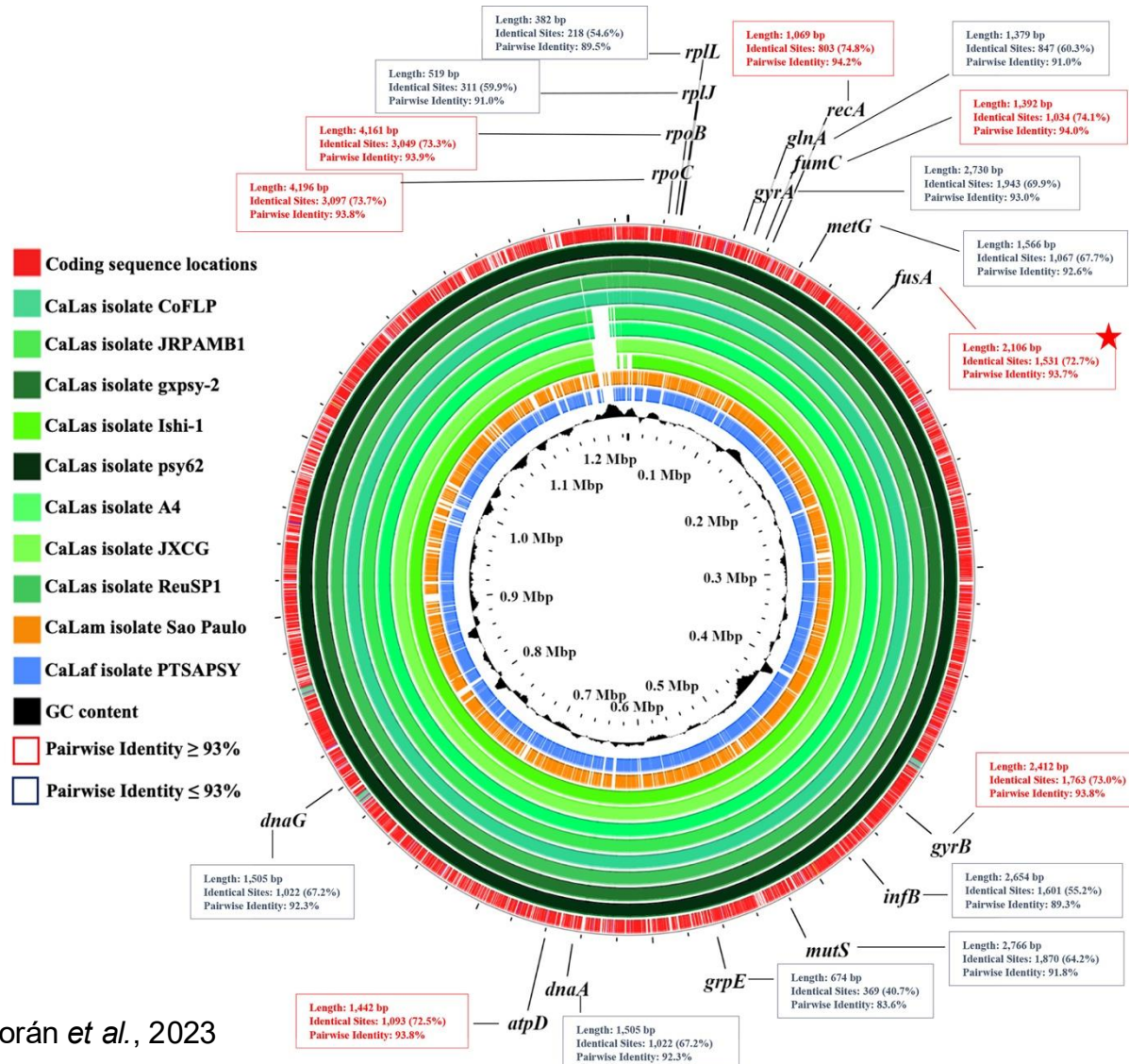
The disease is associated with bacteria of three species of the genus '*Candidatus Liberibacter*' transmitted by psyllid vectors.

HLB has no cure, so preventing its introduction into HLB-free areas is the best strategy to control its spread.

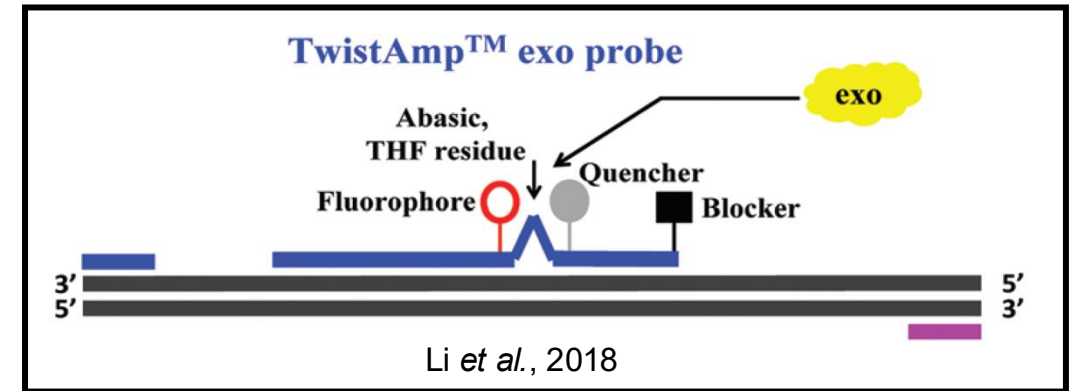
The use of accurate, sensitive, specific, and reliable detection methods is critical for good integrated management of this serious disease.



Design of a real-time RPA assay for HLB associated '*Candidatus Liberibacter*' spp. detection



Alignment of 11 complete genomes and evaluation of 17 housekeeping genes

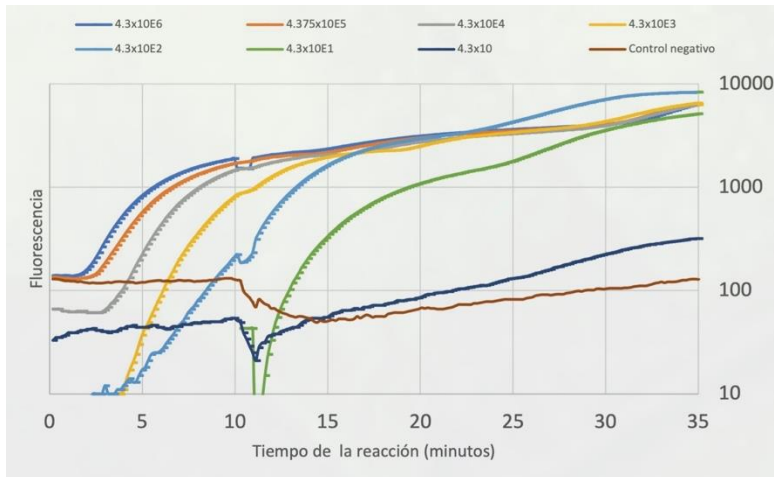


Detection of the three HLB-associated species: CaLas, CaLaf, and CaLam.

The assay targets a 201 bp region of the *fusA* gene chosen based on its high conservation (average identity 93.7%).

Analytical sensitivity:

- 4-8 copies/ μ l synthetic DNA
- 40 copies/ μ l in infected plant material



Selectivity:

- *Citrus sinensis*, *Citrus hystrix*, *Citrus reshni*
- Rusk citrange
- *Diaphorina citri*



Morán *et al.*, 2023

Analytical specificity:

Inclusivity

- Able to detect CaLas, CaLaf, and CaLam
- Different geographic origins (Cuba, Brazil, Costa Rica, USA, South Africa)



Exclusivity

- No cross-reactions with non-target bacteria or negative samples

Repeatability and Reproducibility:

Relatively low titer plant samples analyzed in triplicate by different operators, different testing days using two portable fluorimeters (Genie II, AmplifyRP XRT)



Performance in field conditions



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226 samples

- Brazil
- Costa Rica
- Spain



		Real-time PCR		
		Positive	Negative	Total
Real-time RPA	Positive	99	0	99
	Negative	19	108	127
	Total	118	108	226
Diagnostic sensitivity		83.89 %		
Diagnostic specificity		100 %		

Nanopore sequencing: Citrus black spot

A major citrus fungal disease



Citrus black spot (CBS) is caused by the fungus *Phyllosticta citricarpa*

CBS is a major citrus disease with strong impact on fruit quality, external appearance, and marketability

Early and reliable detection is essential to support rapid containment and particularly relevant at border inspection

A strong target for portable, fast, and onsite diagnostic tools

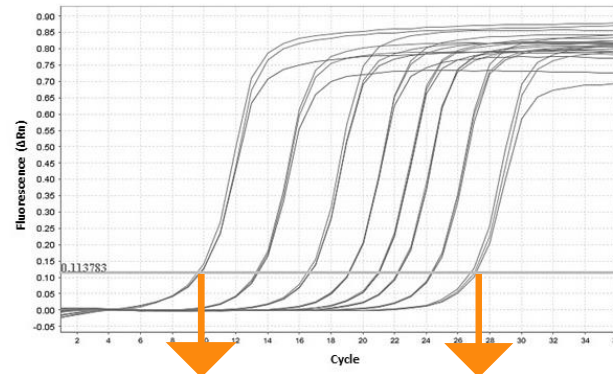


Nanopore sequencing for *P. citricarpa* detection

Artificial inoculations and selection of samples



Selection of high and low fungal titer samples to determine detection limits of the Nanopore sequencing method



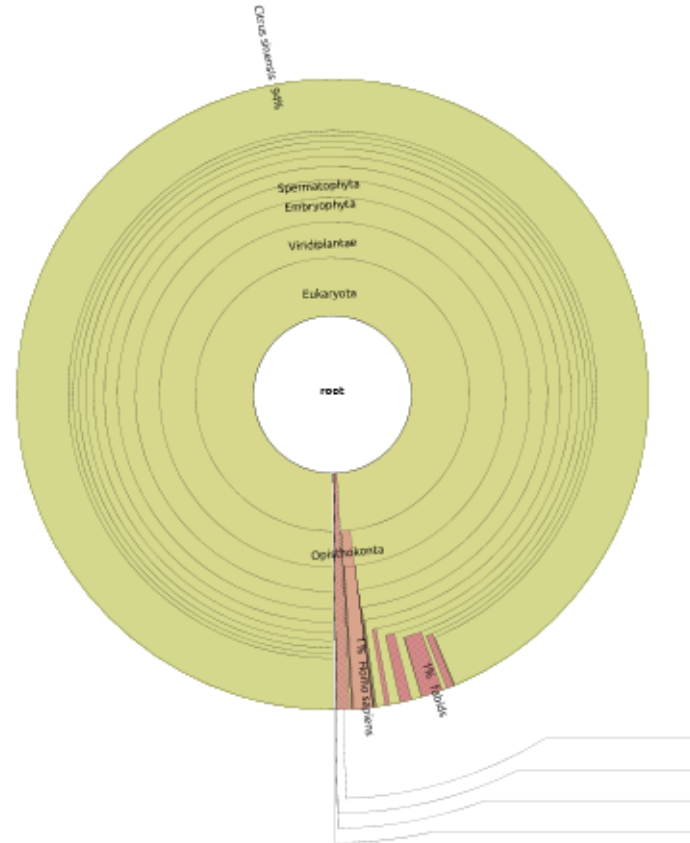
**Real time qPCR
Van Gent-Pelzer
et al., 2007.**



Application of nanopore sequencing for CBS detection

Sequencing → Taxonomic classification

P. citricarpa detection



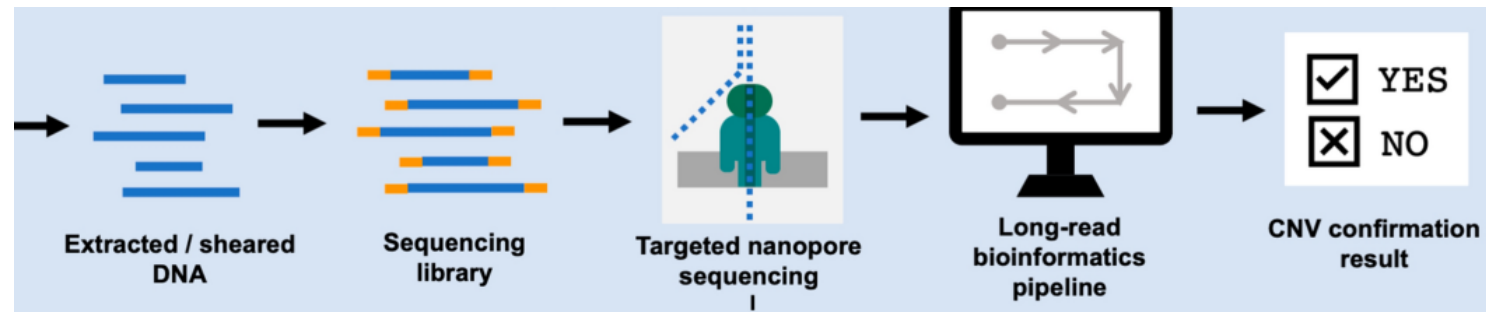
Dilution	Ct	Mapped reads
10 ⁻¹	17.231	243
10 ⁻²	21.208	378
10 ⁻³	25.609	254
10 ⁻⁴	28.261	150
10 ⁻⁵	32.117	74
10 ⁻⁶	36.468	11
10 ⁻⁷	39.864	1

Rapid DNA Extraction adapted to Nanopore sequencing



Rapid DNA extraction methods to be adapted to the HTS workflow are being evaluated

Automatic Pipeline designed in our Lab



Magnetic induction real-time RT-PCR: yellow vein clearing

An emerging citrus viral disease



Citrus yellow vein clearing virus (CYVCV) is an emerging virus included in EPPO alert list (2022)

CYVCV is associated with yellow vein clearing, leaf curling, and shoot symptoms, especially in susceptible hosts

First detections in EU (Italy, 2024; Spain 2025)

A case for rapid, sensitive, and field-applicable diagnostic tools



Obtaining complete CYVCV Spanish genomes



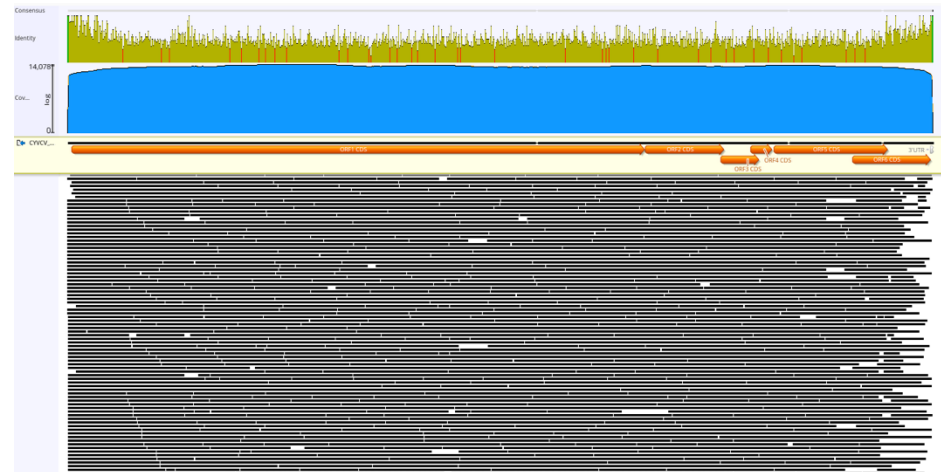
CYVCV infected plants



Total RNA Purification



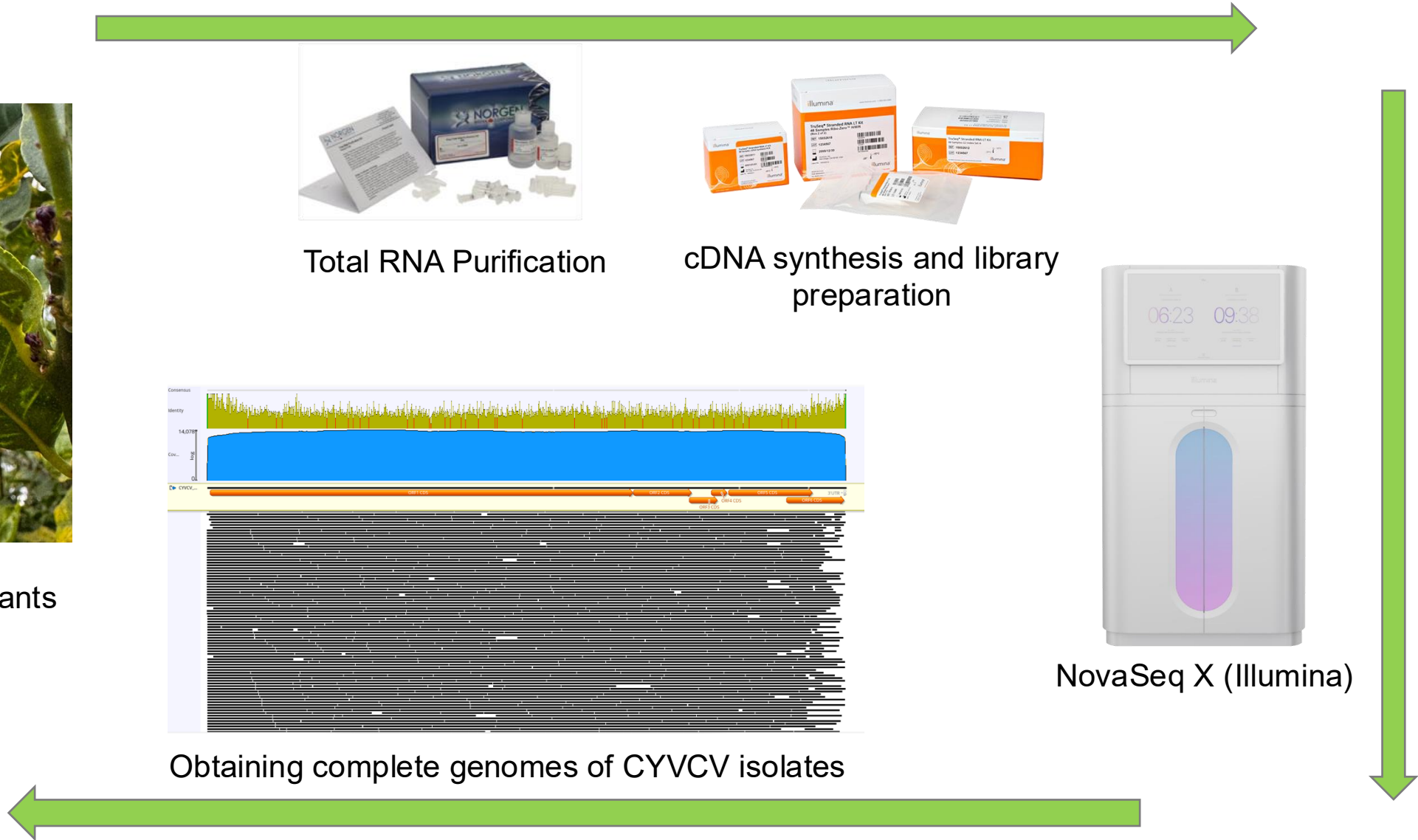
cDNA synthesis and library preparation



Obtaining complete genomes of CYVCV isolates

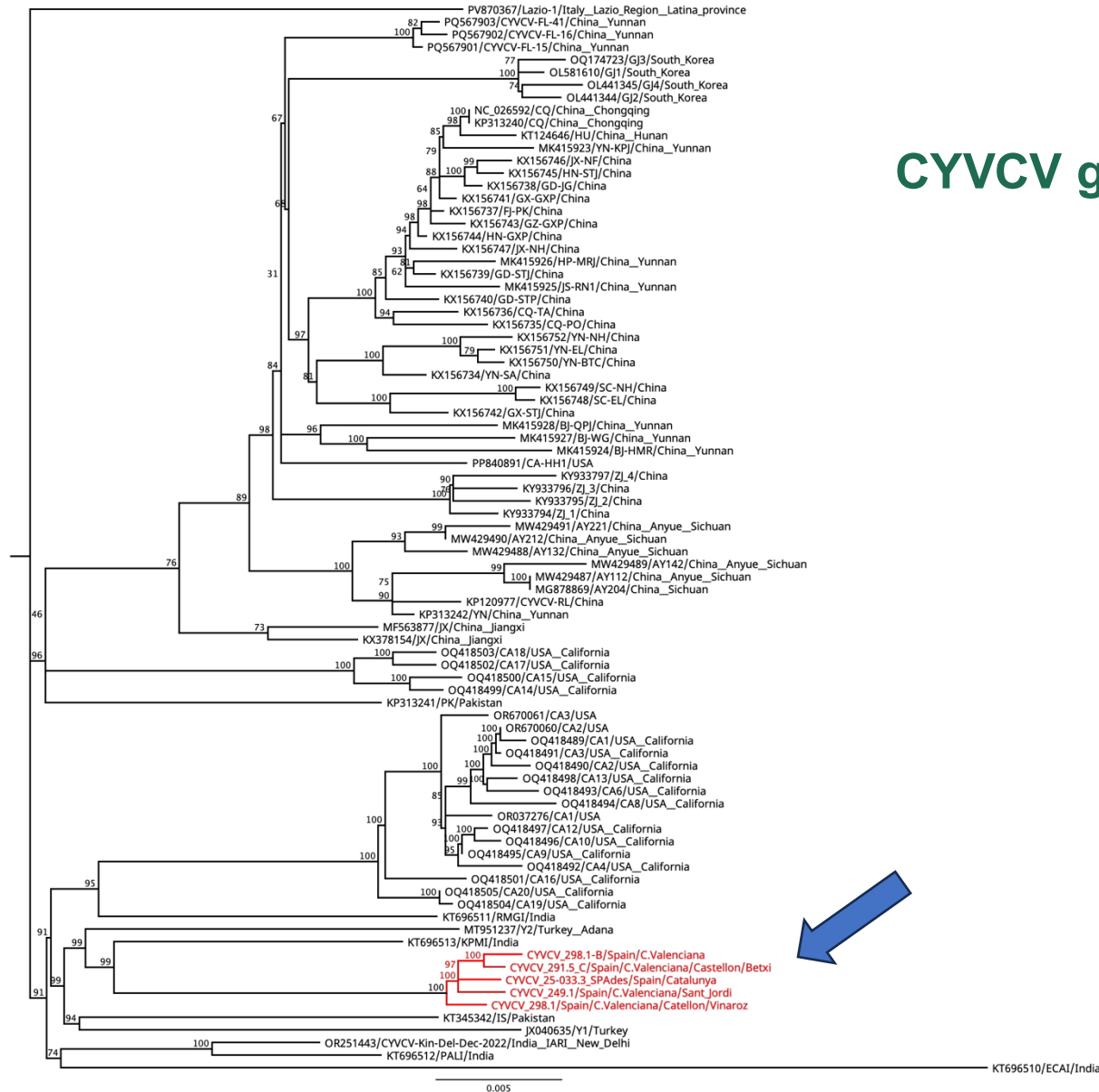


NovaSeq X (Illumina)



CYVCV genomic diversity including Spanish isolates

Design of an inclusive real time RT-qPCR
EPPO Validation
Reference method

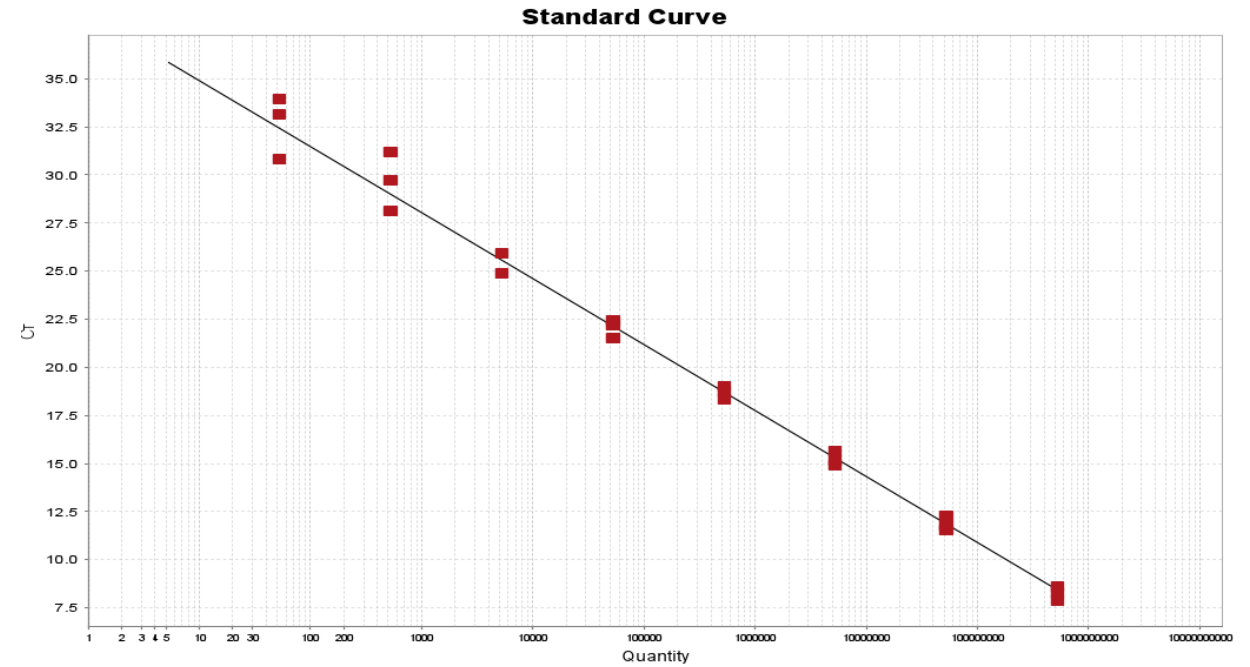
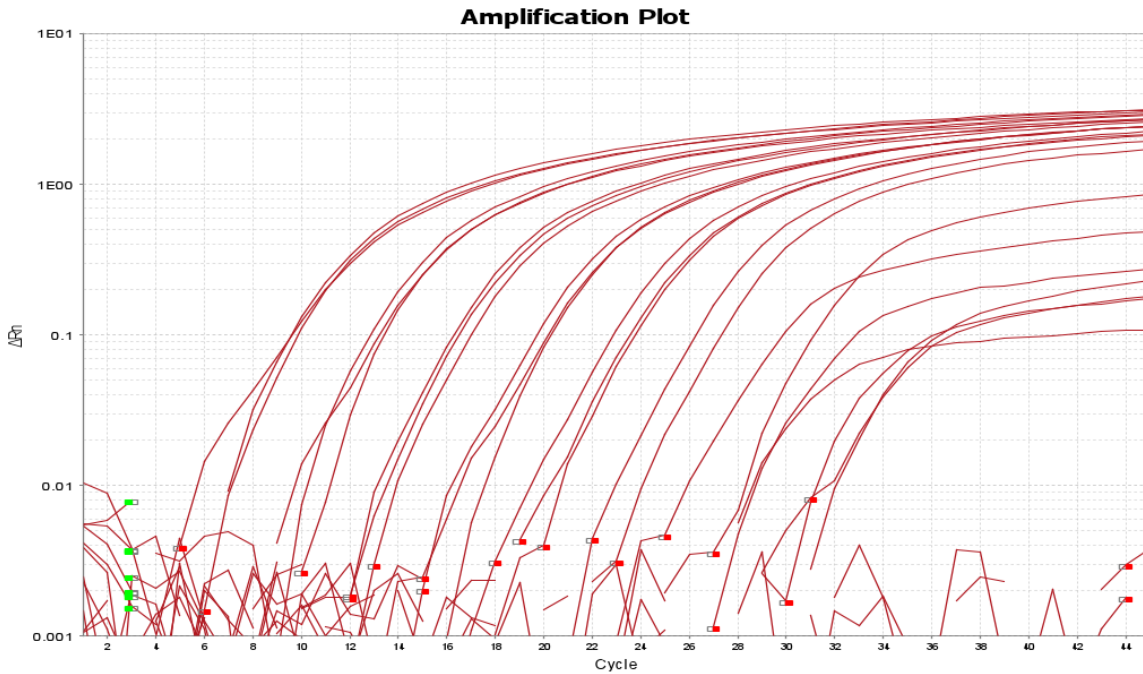


Real Time RT-qPCR for CYVCV detection

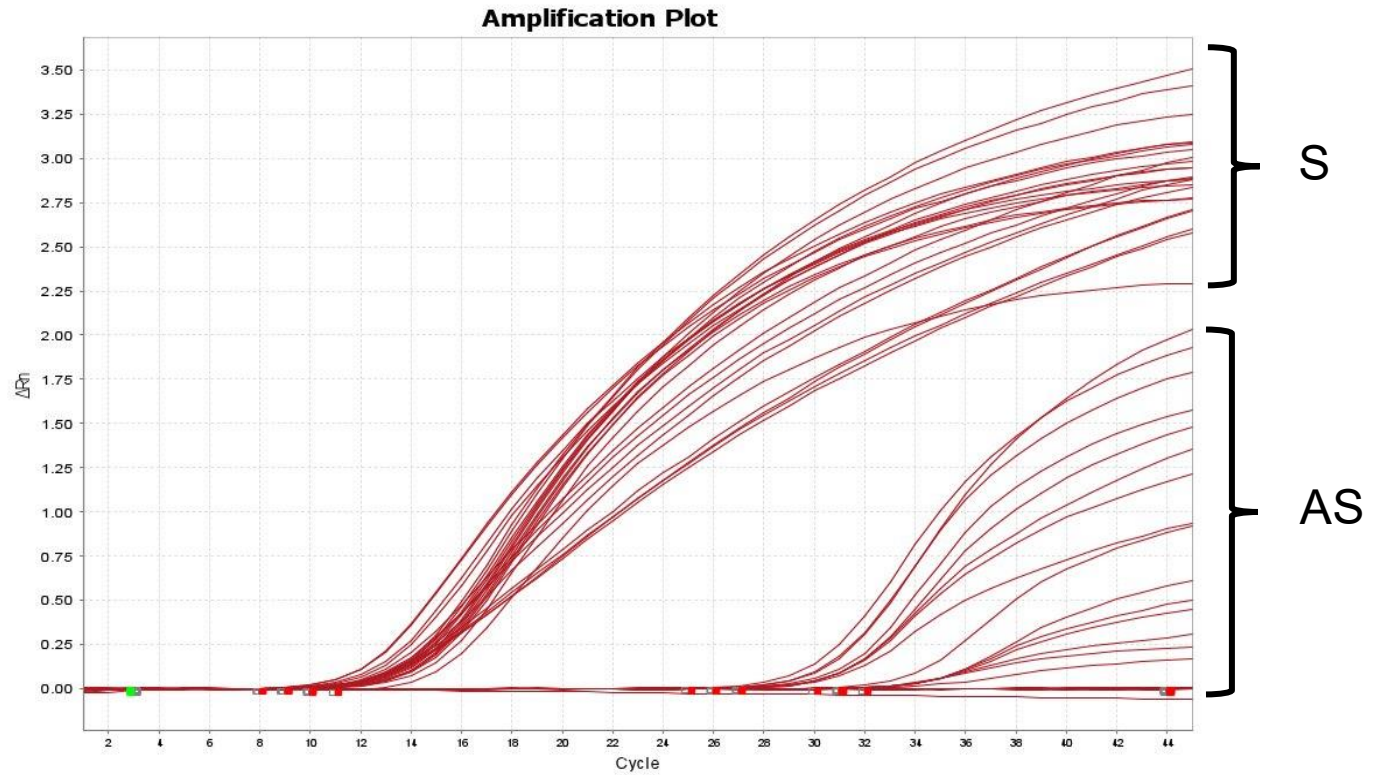


Analytical sensitivity of up to 3 copies with transcripts
and 30 viral copies in plant material

Efficiency: 99%



Sampling is a critical step



Asymptomatic
Branch (AS)

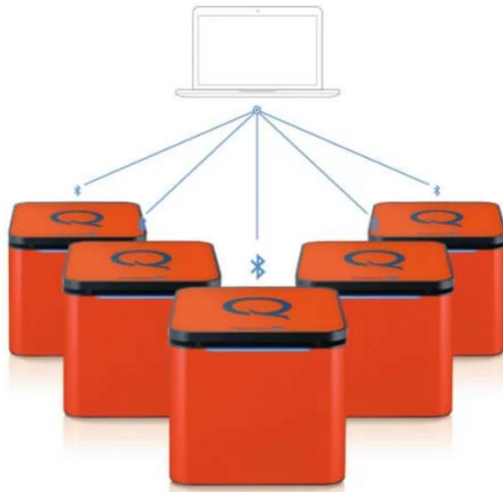


Symptomatic
Branch (S)

Detection can be challenging due to uneven distribution,
seasonal effects, and asymptomatic infections

Magnetic induction real-time PCR: a portable tool

Quantabio Q real-time thermal cycler



- Magnetic induction technology
- Compact portable equipment
- 48 samples / device
- Up to 10 devices connected



Dr. Campbell Costello, Queensland

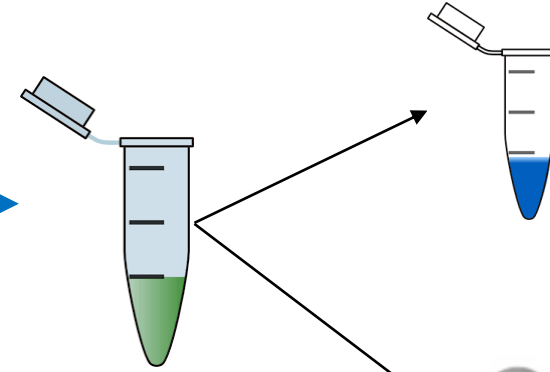
Magnetic induction real-time PCR for CYVCV detection



Field
sampling



Sample
processing



Diluted crude extract

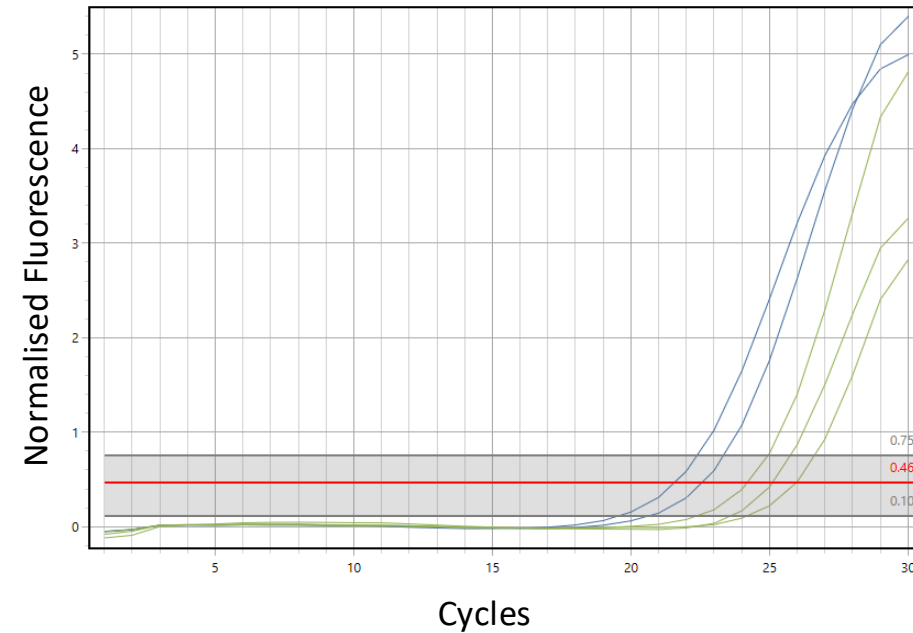


RNA purification





Purified RNA Crude extract



In less
than an
hour



Towards more accessible plant pathogen detection

-  Portable diagnostics are moving plant pathogen detection closer to where decisions need to be made
-  The examples presented here show that portable approaches can be applied across different pathogen groups. This includes bacteria, fungi, and viruses of clear phytosanitary relevance
-  Their main advantage is to enable rapid testing for surveillance, early detection, and phytosanitary response
-  Under those conditions, portable devices can make a meaningful contribution to modern plant health systems

Emergent Plant Disease Prevention and Management Team



Thank you for your attention