Supersensitive and Specific Detection of Citrus Greening and Phytoplasmal Pathogens with RPA/Cas12a

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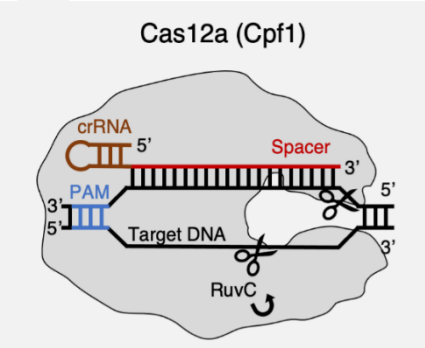
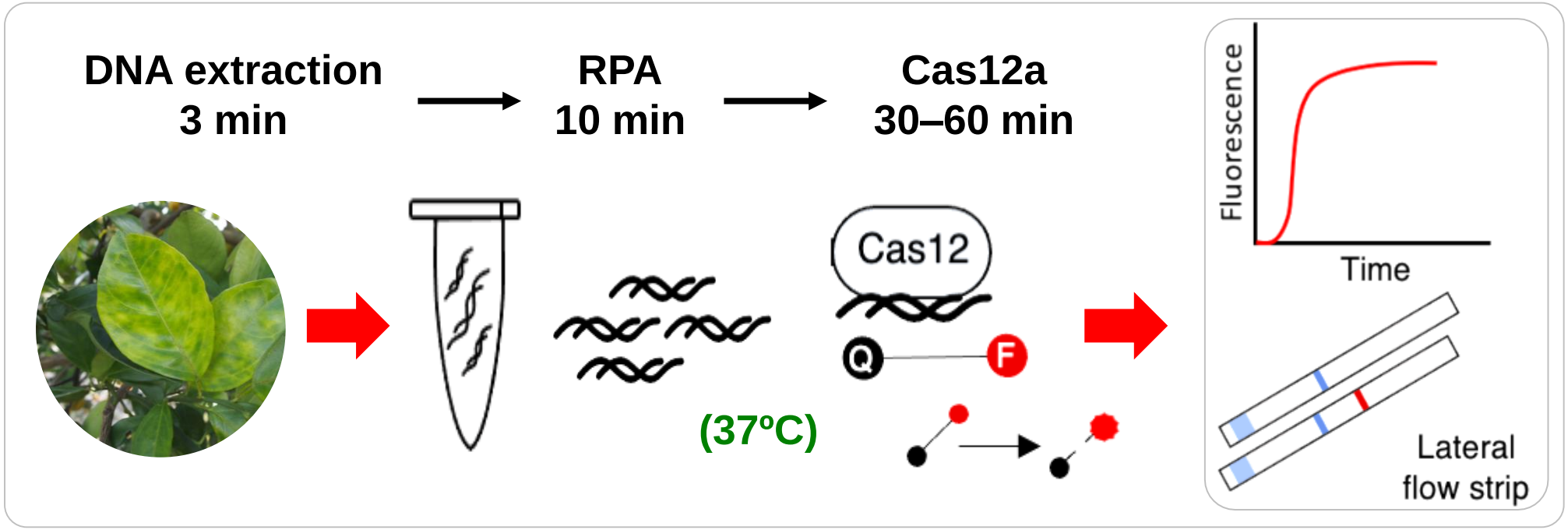
PennState



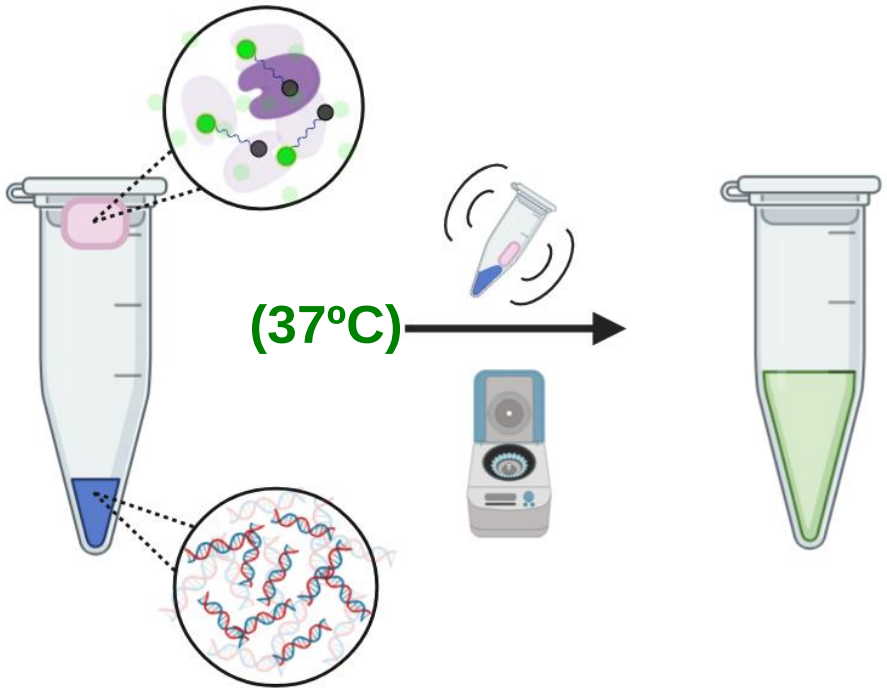
Isothermal RPA/Cas12a Assay for Plant Disease Diagnostics



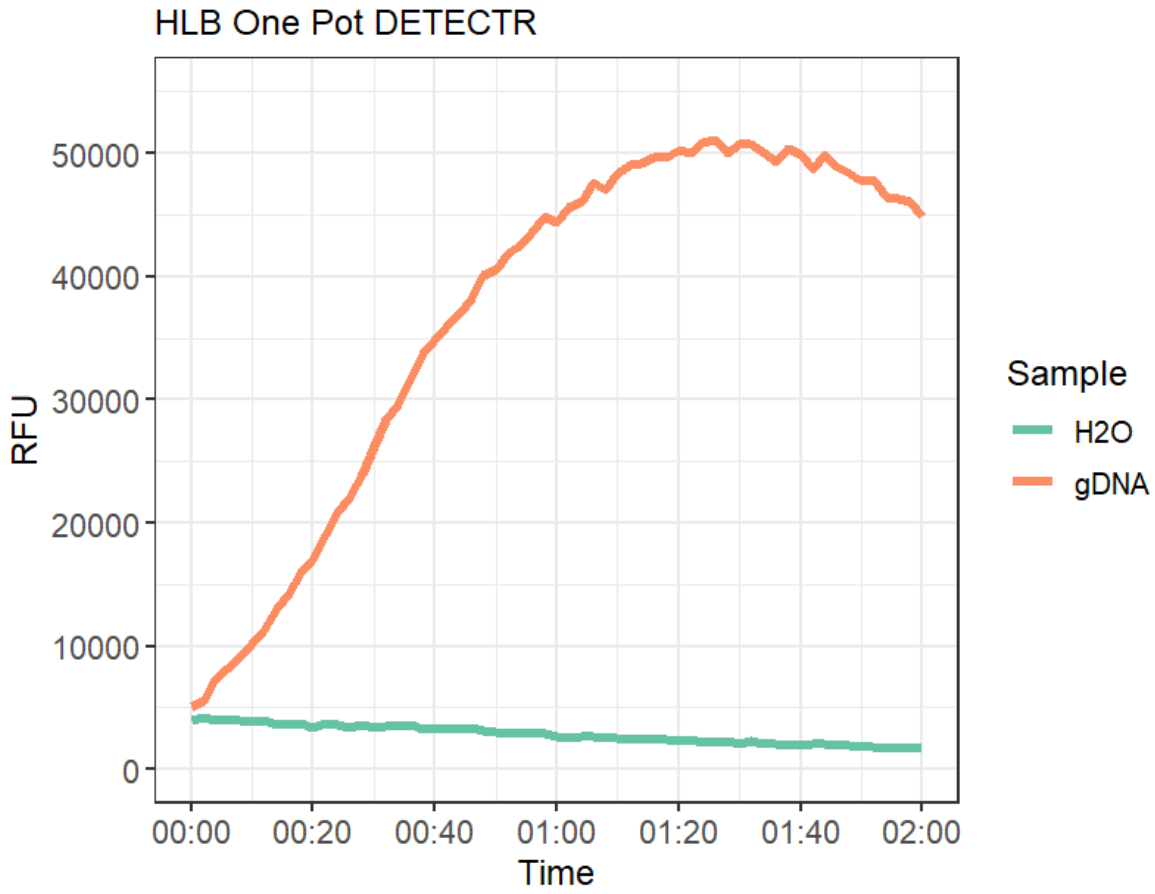
RPA/Cas12a: recombinase polymerase amplification/Cas12a nuclease
DETECTR: DNA endonuclease-targeted CRISPR trans reporter

One-pot RPA/Cas12a Detection Assay

10 µl Cas12a reagent mix



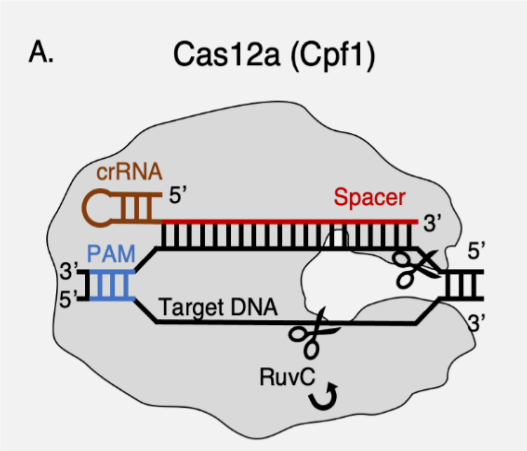
10 µl RPA reaction

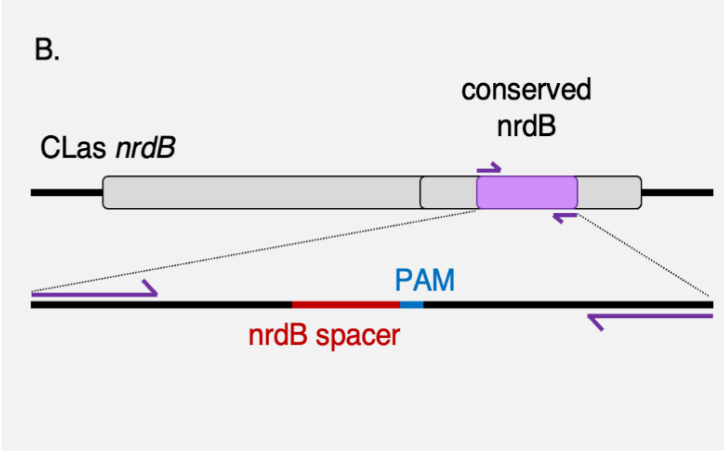


RPA/Cas12a Detection of Citrus Greening Pathogen

Candidatus Liberibacter asiaticus (CLas)

- ✓ ***CLas*** **contains five copies of** ***nrdB*** **that encodes the β-subunit of conserved ribonucleotide reductase (RNR)**
- ✓ ***nrdB*** **loci enable robust detection of** ***CLas*** **using qPCR with 3 times more sensitivity than 16S rDNA**

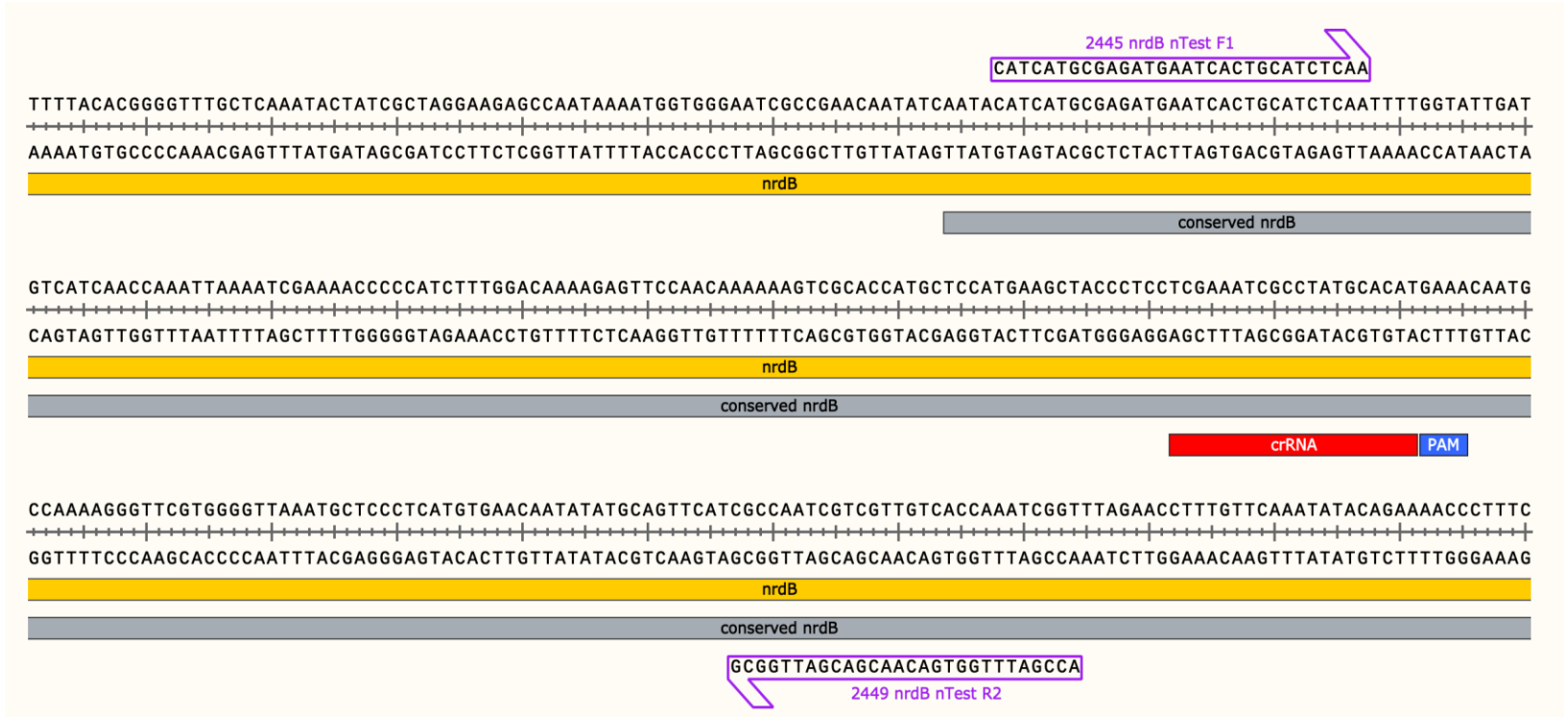




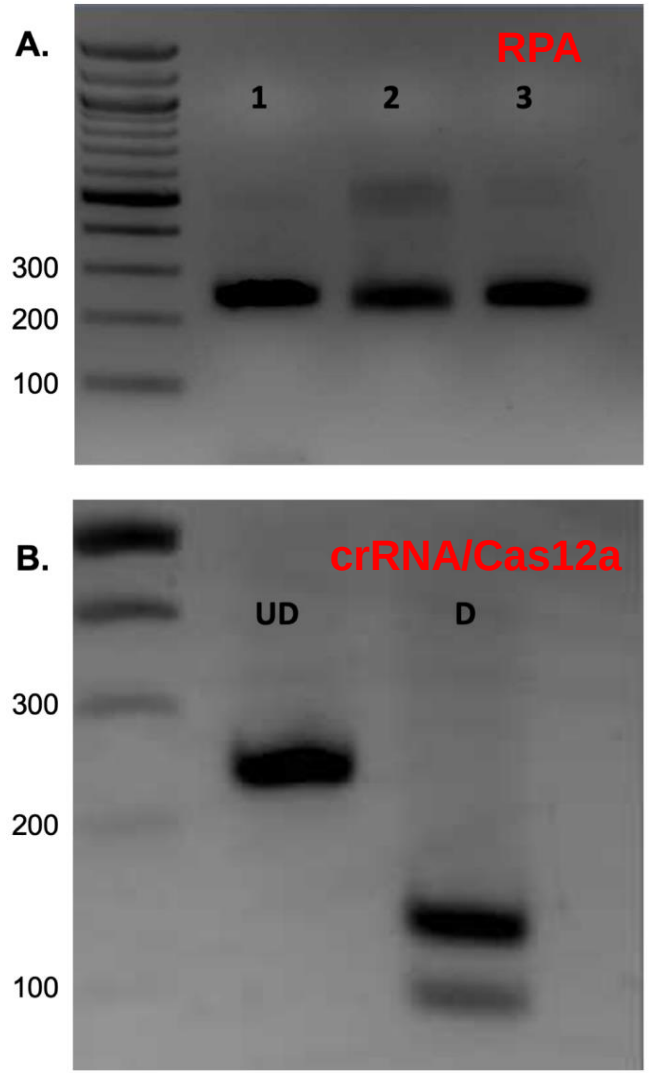
(Zheng et al 2016 Scientific Reports)

(Wheatley and Yang 2021 Phytopathology)

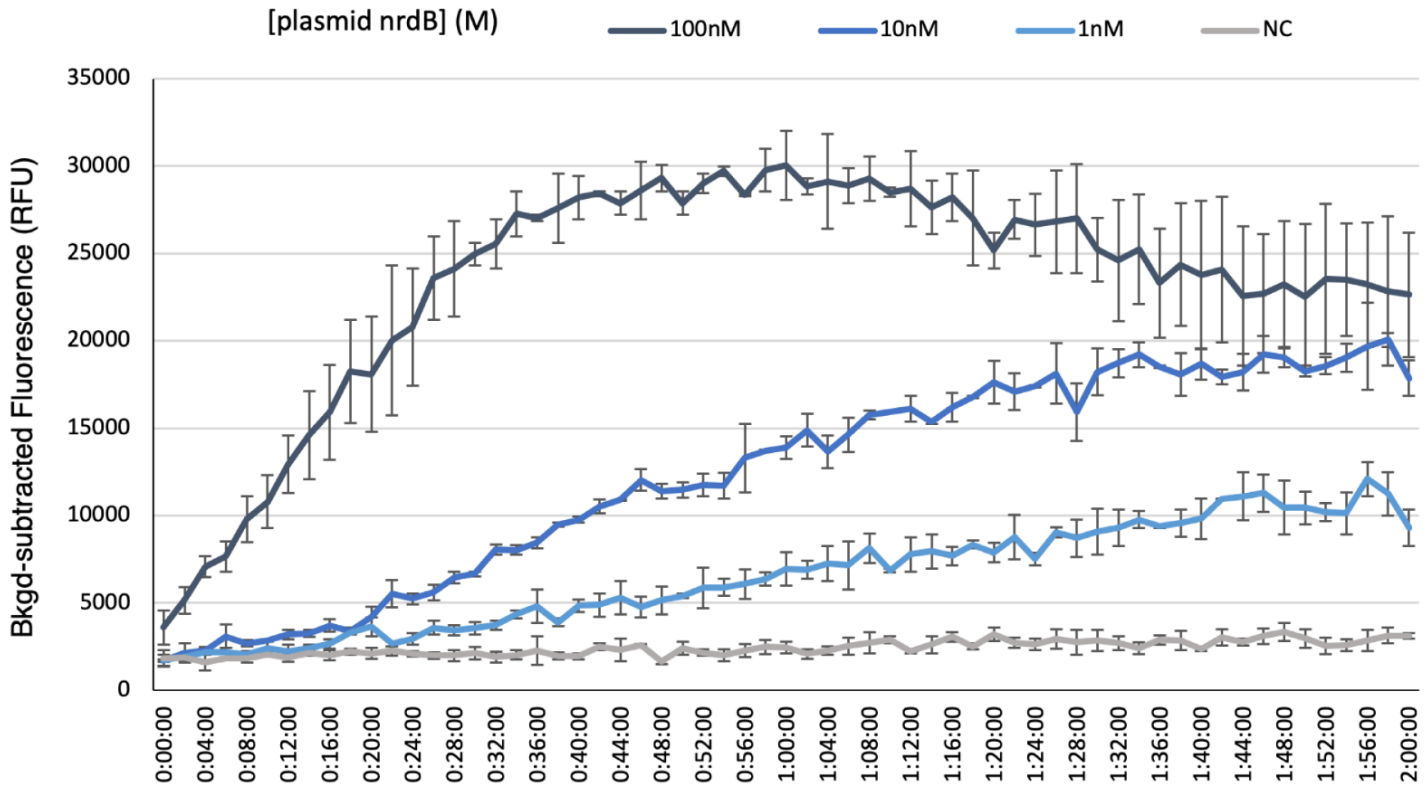
Evaluation of RPA Primers and crRNA/Cas12a DNA Cleavage Efficiency

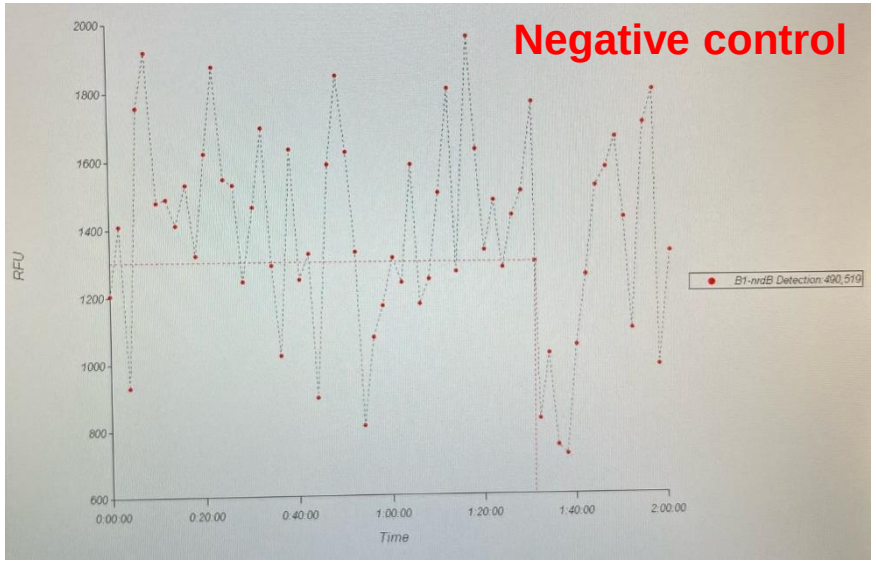


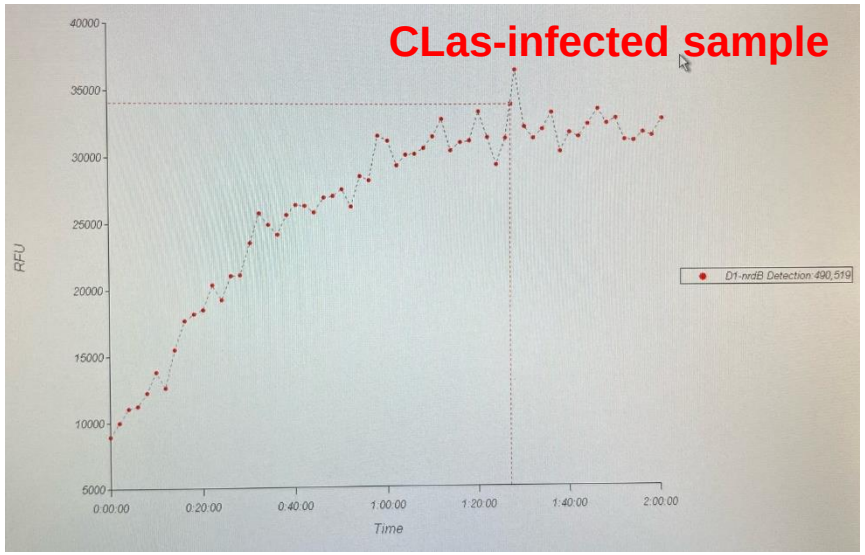
247 bp amplicon

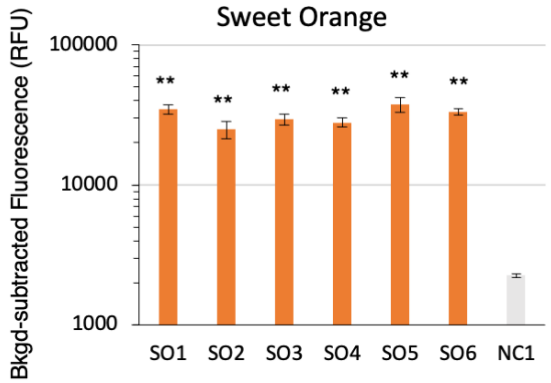


Cas12a Detection of *nrdB* DNA



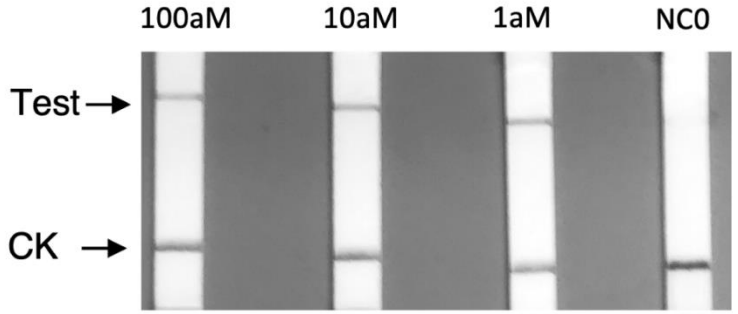


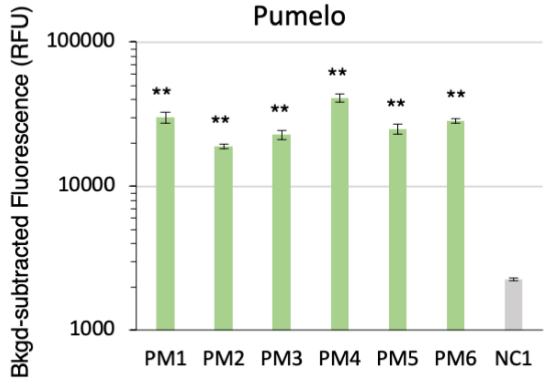


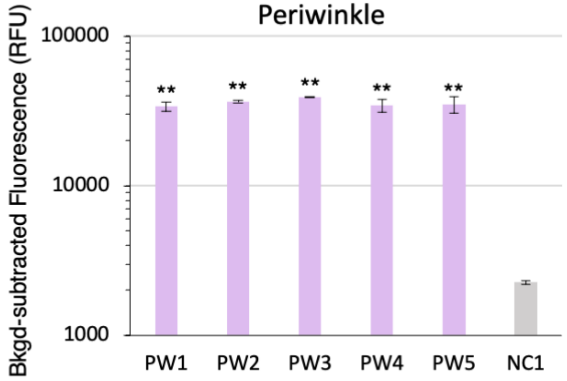


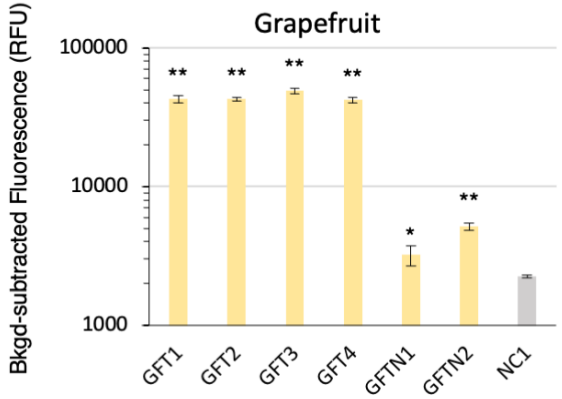
(Wheatley et al 2021 Phytopathology)

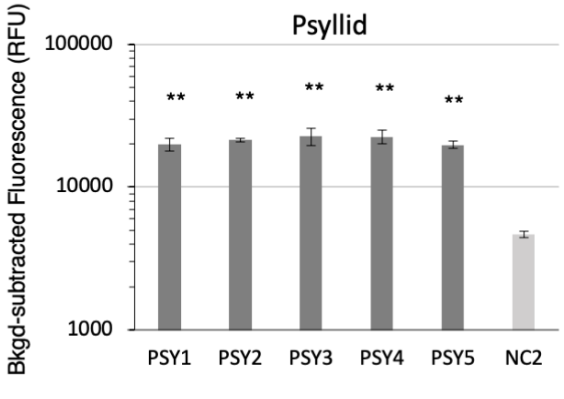
* = p < 0.05
** = p < 0.001

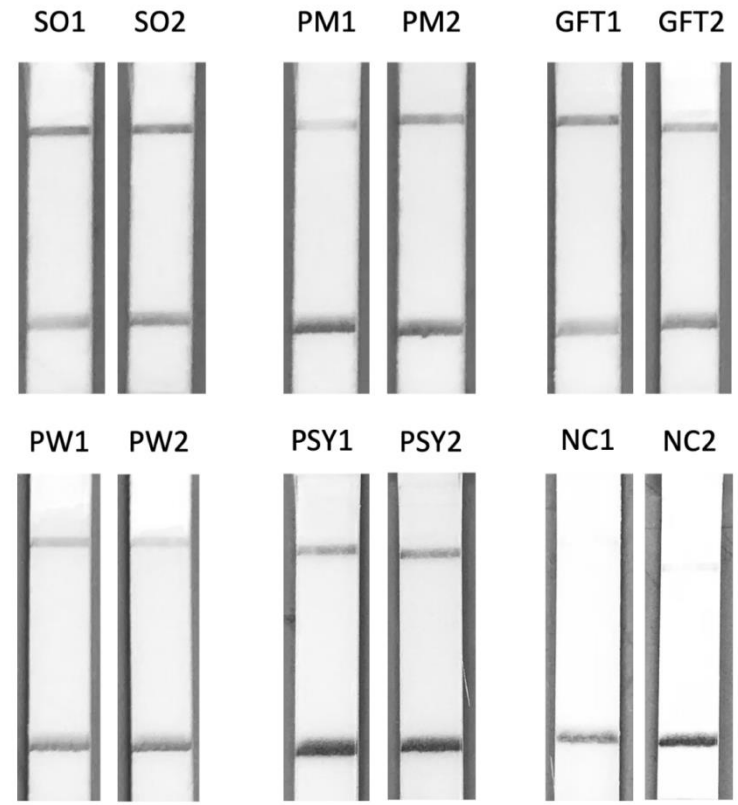








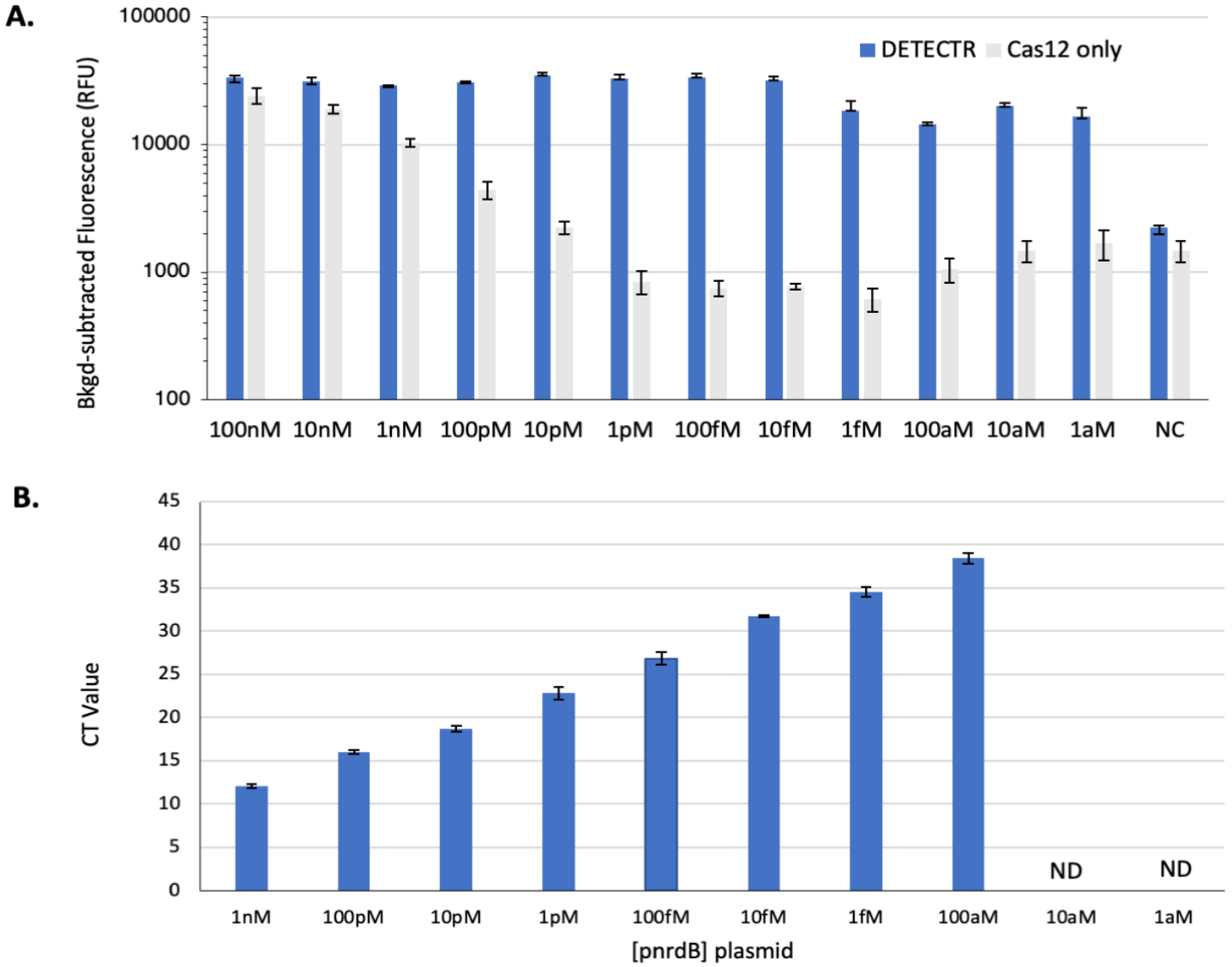




Fluorescence Microplate Reader

Lateral Flow Assay

Detection Sensitivity of RPA/Cas12a vs SYBR Green qPCR



DETECTR: DNA endonuclease-targeted CRISPR trans reporter (i.e., RPA/Cas12a)

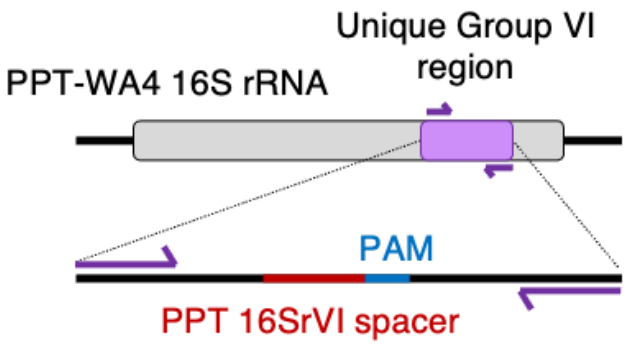
NC: negative control

ND: not detected

Error bars represent mean ± s.d. when *n* = 3 replicates

(Wheatley et al 2021 Phytopathology)

Potato Purple Top Disease



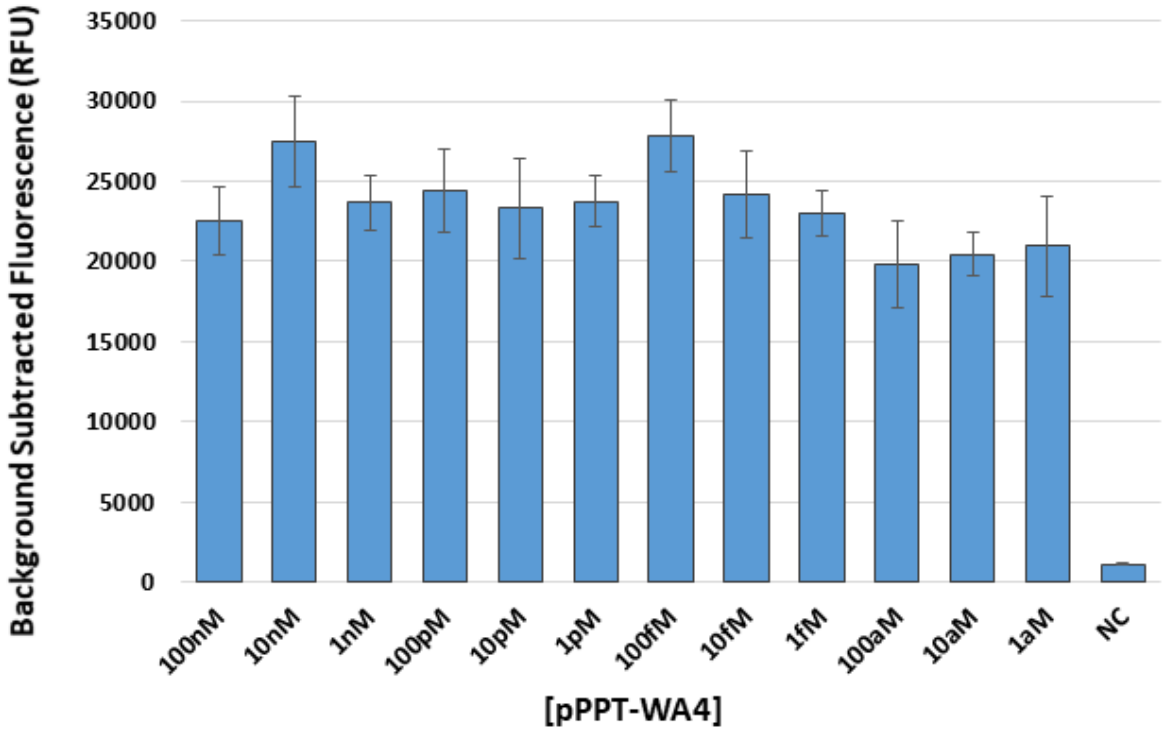
PPT 16S-23S ITS Amplicon – 156 bp

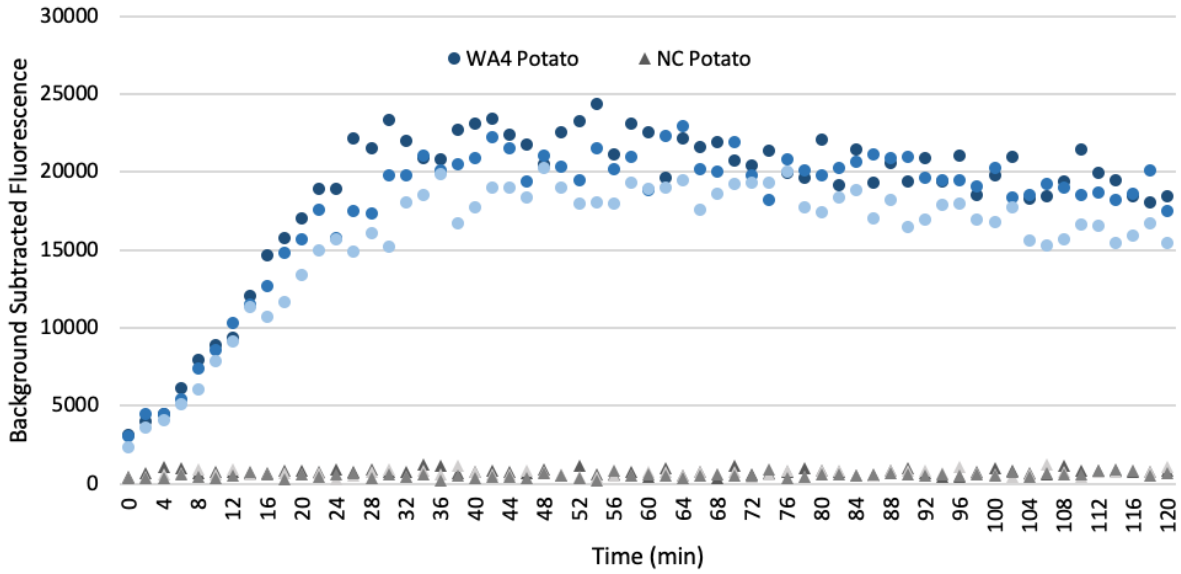
TGATAAGCGTGAGGTCGGTGGTTCAAGT
CCATTTAGGCCCACCAAAAAAAGGTCCT
GCTTAAGAAGTTCTTTGAAAAGTAGGTA
AACATGCTTTAAAATTTTCATAAAGTTG
AAGGAAGTAAGGGCATATAGTGGATGCC
TTGGCACTAAGA

***Candidatus* Phytoplasma trifolii related strains (16SrVI)**

Supersensitive and Specific Detection of 16S-23S ITS Target from PPT-WA4

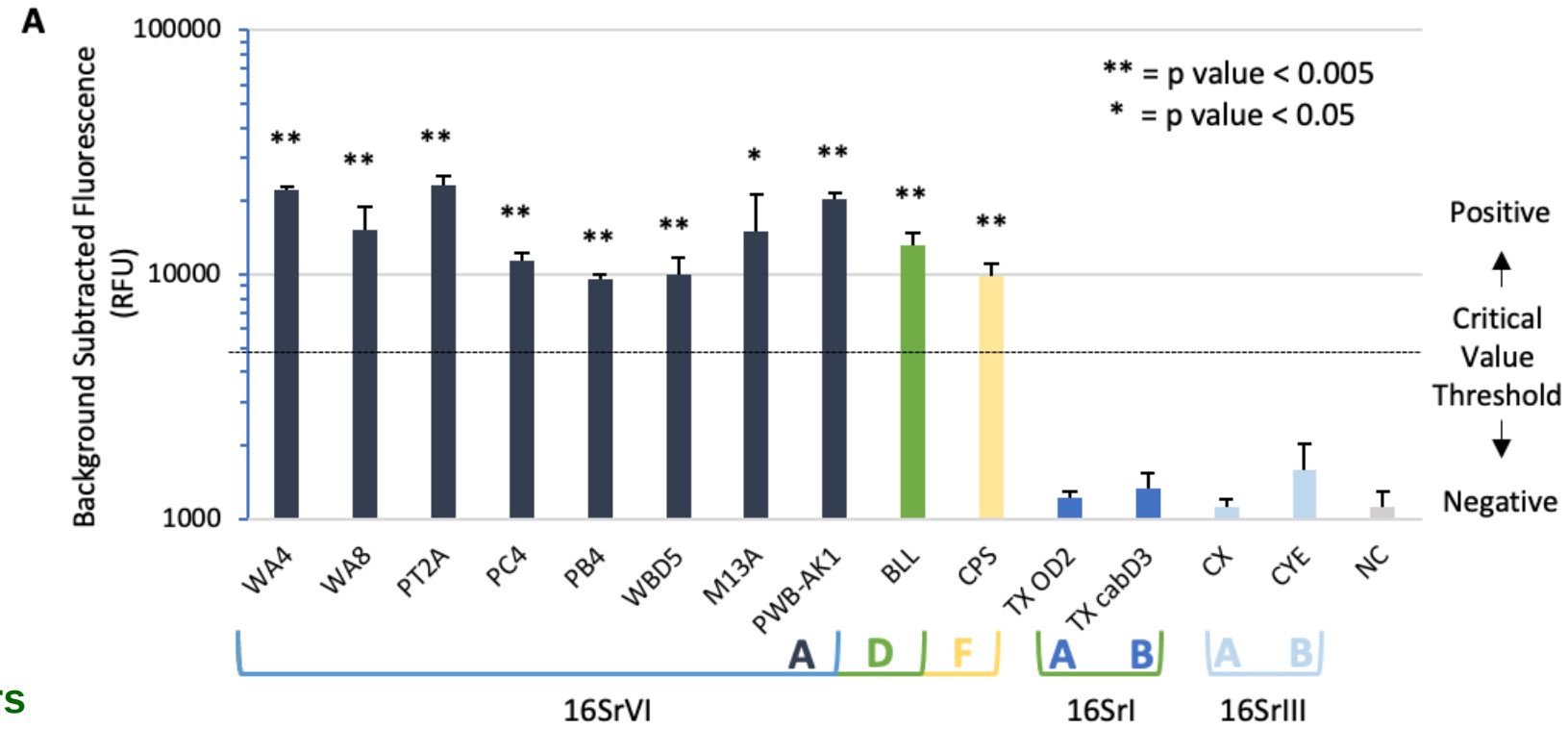
(Wheatley et al 2022 Plant Disease)

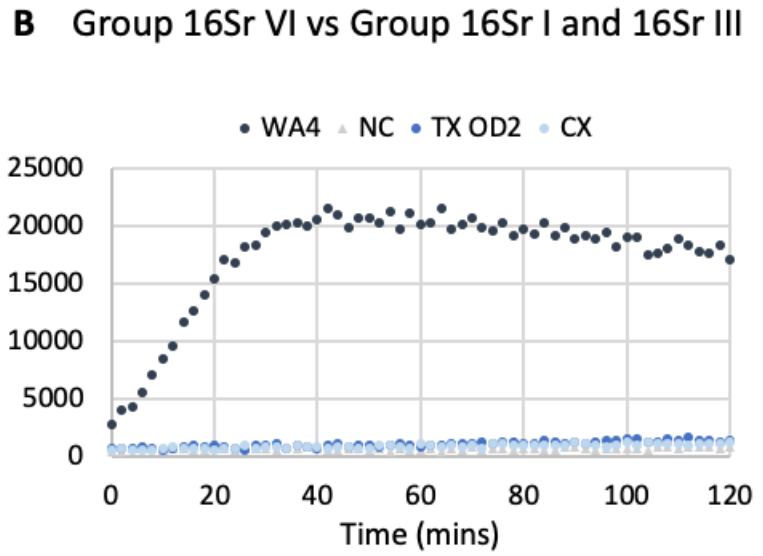


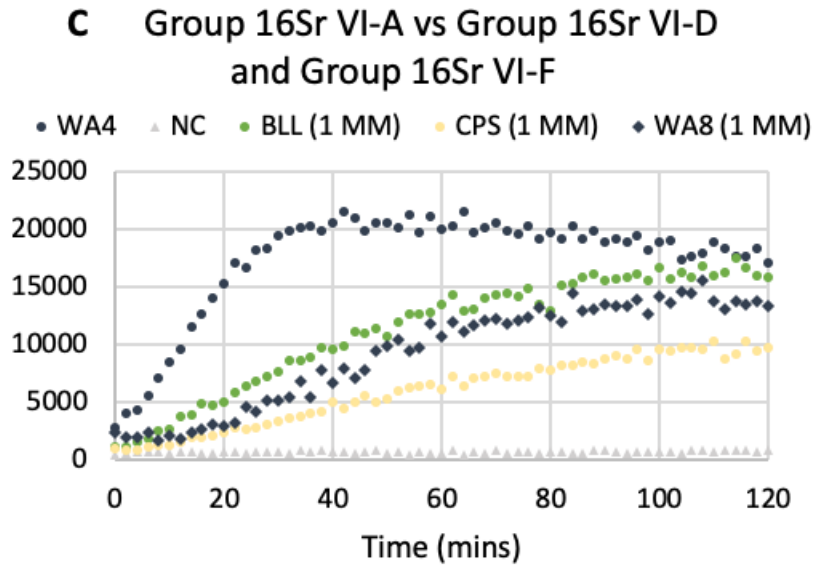


Specific Detection of Group 16SrVI Phytoplasma by RPA/Cas12a

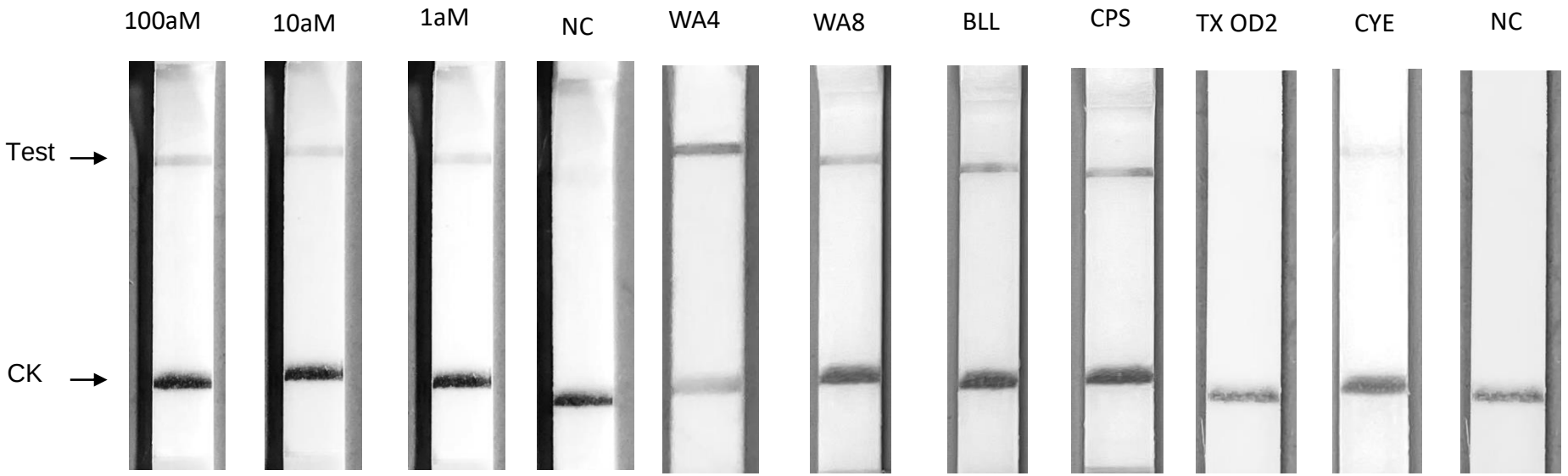
- Cas12a can still cleave protospacers with a single mismatch (MM)
- Turnover is lower, resulting in delayed signal intensity
- More than a single MM abolishes cleavage activity







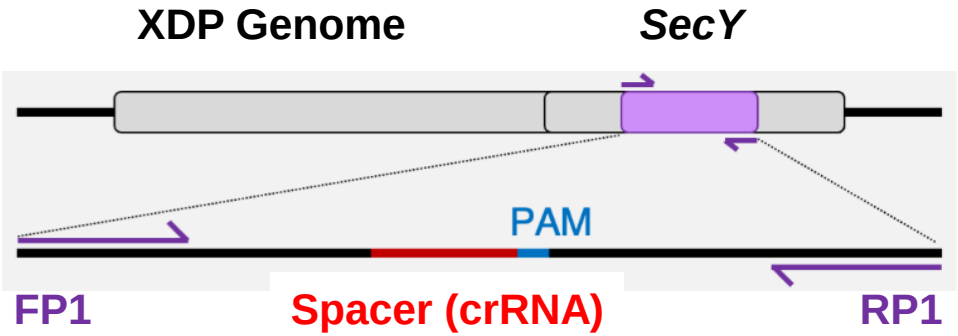
RPA/Cas12a Detection with Lateral Flow Assay



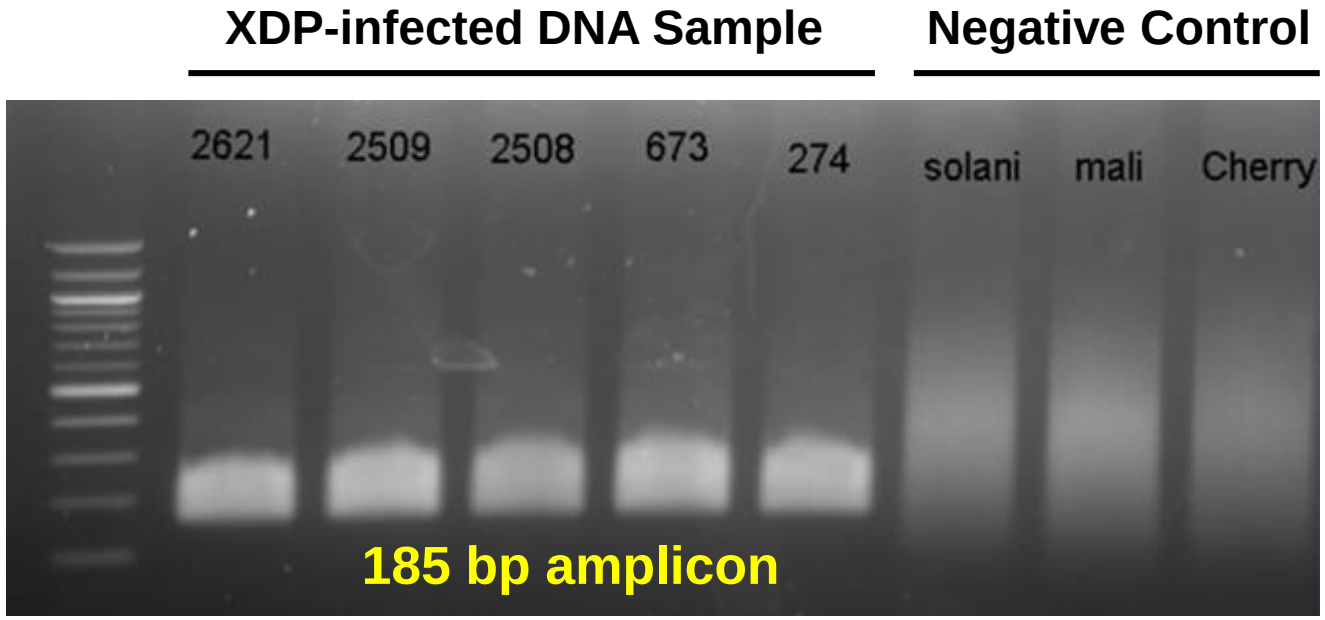
(Wheatley et al 2022 Plant Disease)

RPA/Cas12a Detection of Cherry X Disease Phytoplasma

Candidatus Phytoplasma pruni (16SrIII)

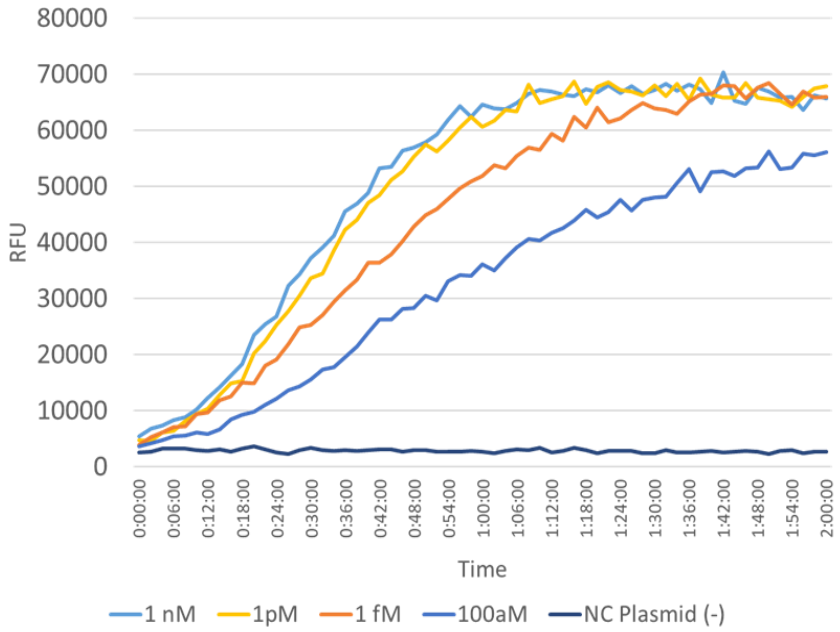


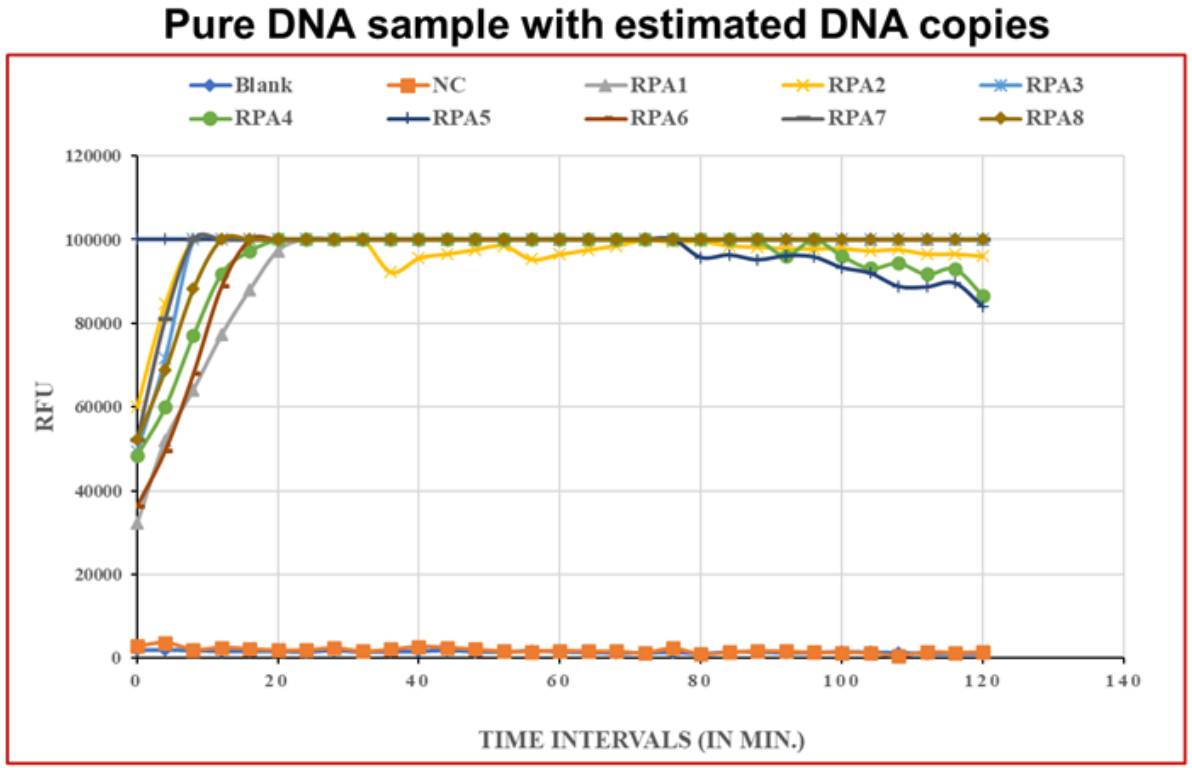
- ✓ Alignment of *SecY* sequences across the 16SrIII subgroups of phytoplasmas
- ✓ Identification and evaluation of highly specific RPA primer pairs and crRNAs



Recombinase Polymerase Amplification of XDP DNA

Development of Specific RPA/Cas12a Assay for Sensitive Detection of XDP DNA





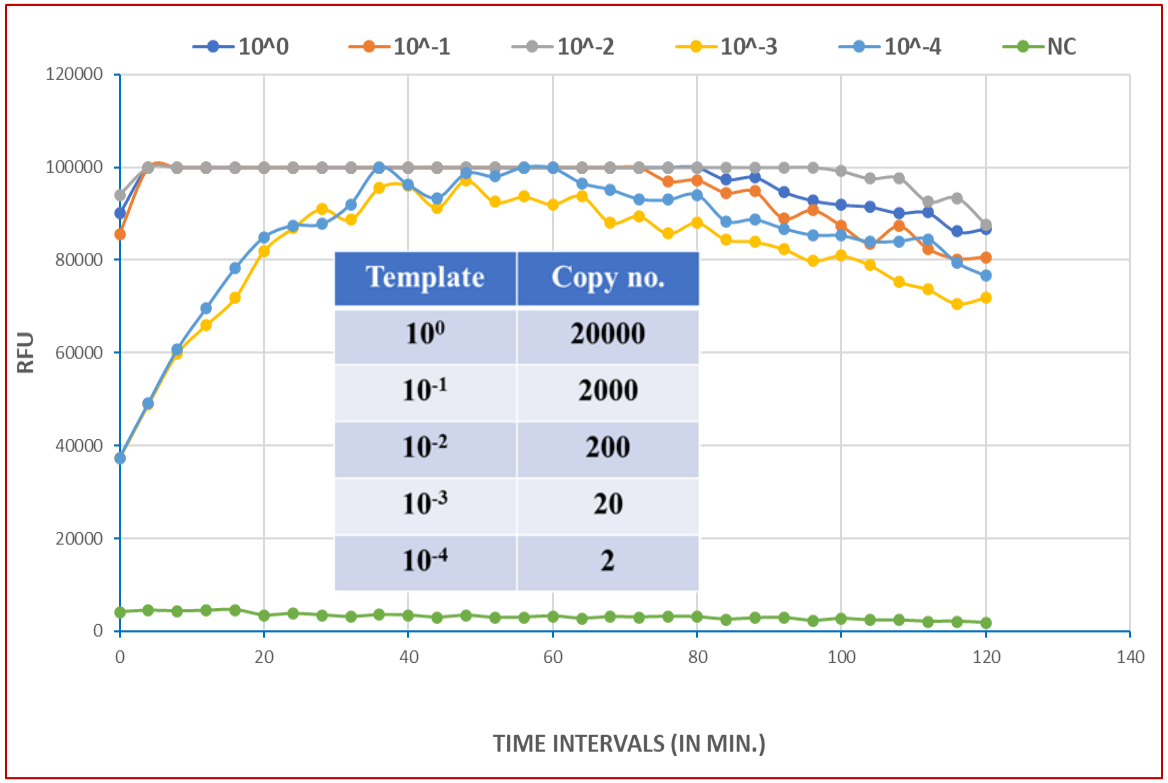
NC: Negative control (non-infected sample); **Blank:** Water.

The max fluorescence reading is 100000 RFU.

Sample	Copy no.
RPA1	1
RPA2	10
RPA3	100
RPA4	1000
RPA5	10000
RPA6	100000
RPA7	1000000
RPA8	10000000

Supersensitive and Rapid Detection of XDP from Infected Cherry Tree Samples Using RPA/Cas12a Method

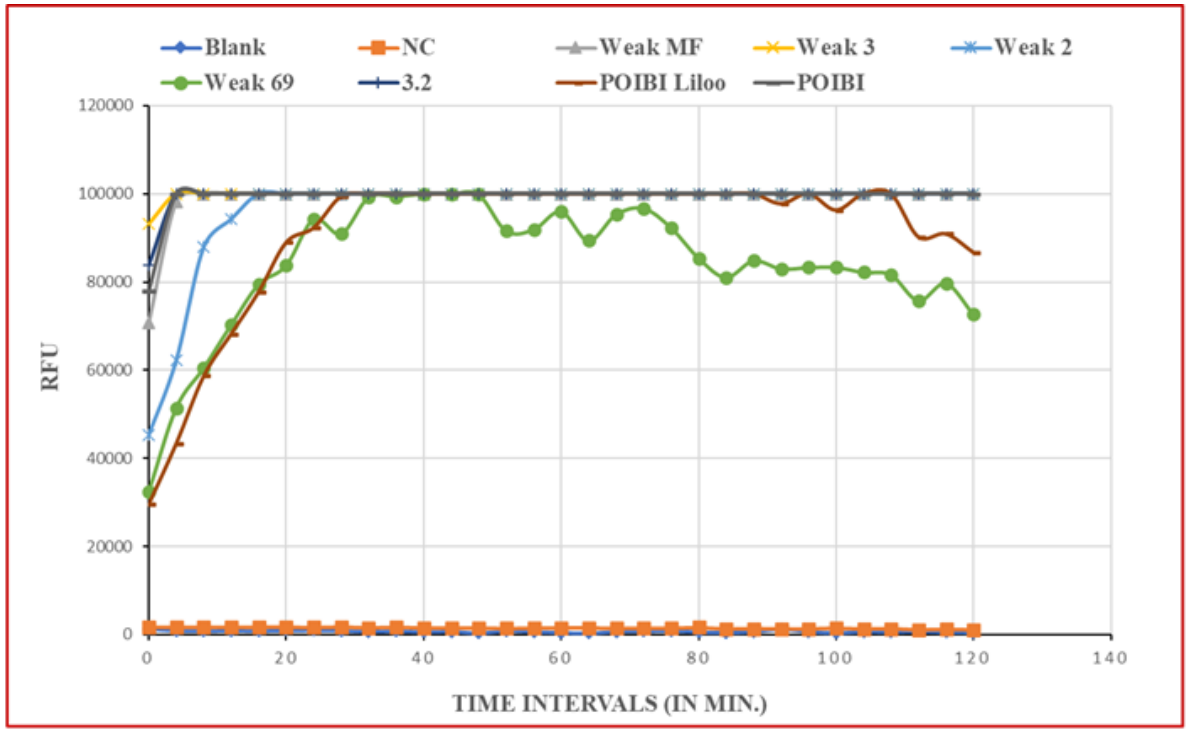
XDP infected Cherry DNA sample



Template	Copy no.
10^{0}	20000
10^{-1}	2000
10^{-2}	200
10^{-3}	20
10^{-4}	2

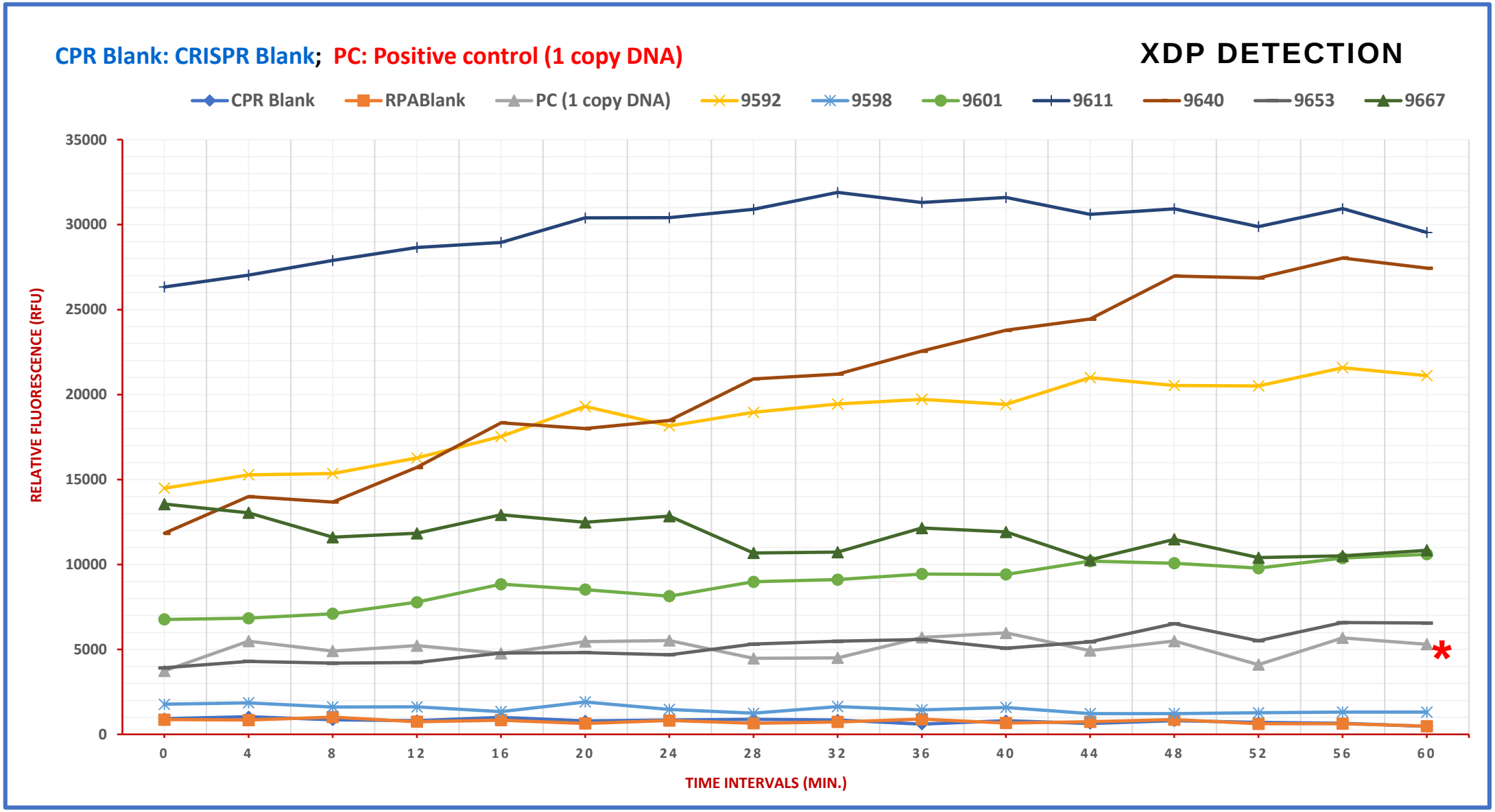
NC: Negative control (non-infected sample)

The max fluorescence reading is 100000 RFU.



NC: Negative control (non-infected sample); **Blank:** Water.

The max fluorescence reading is 100000 RFU.



RPA/Cas12a detection of XDP in cherry DNA samples that are negative in TaqMan qPCR

Conclusions and Perspectives

RPA/Cas12a assay is particularly appealing for early detection of low titer plant pathogens such as *Candidatus* Liberibacter asiaticus and phytoplasmas in asymptomatic tissues with the following advantages.

- ✓ **Supersensitivity at the attomolar level (10^{-18} or 0.6 copy DNA per µl)**
- ✓ **High specificity at the single nucleotide level**
- ✓ **Isothermal RPA and Cas12a reactions at 37- 42 ºC**
- ✓ **Rapid assays within 1 or 2 hr**
- ✓ **High throughput with a 384 fluorescence microplate reader or real-time PCR system**
- ✓ **Field deployable with a portable Genie III, lateral flow immunostrip or colorimetric assay**
- ➢ **One-pot assay to simplify the detection protocol and reduce potential contamination**
- ➢ **Amplification-free methods**
- ➢ **Microfluidics, electrochemical sensors and mobile phone platforms**

Acknowledgments

Penn State University: Matthew Wheatley, Sydney Ostlund, Qin Wang
USDA/APHIS: Jarred Yasuhara-Bell, Eric Newberry, Yazmín Rivera
USDA/ARS: Yong-ping Duan, Yan Zhao, Wei Wei, Stefano Costanzo
Washington State University: Youfu Zhao, Scott Harper

USDA APHIS

USDA ARS