Agriculture and Agri-Food Canada
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Identification and classification of phytoplasmas using *cpn60*

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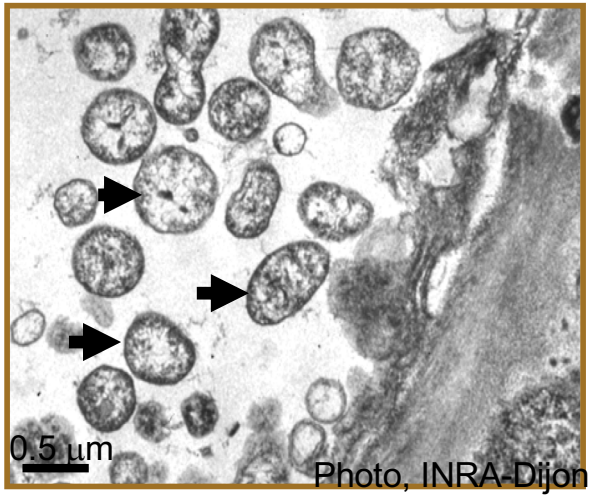
August 3, 2019

Canada

‘*Candidatus* Phytoplasma’ spp.



Characteristics

Unculturable

- Insect vectored plant pathogens

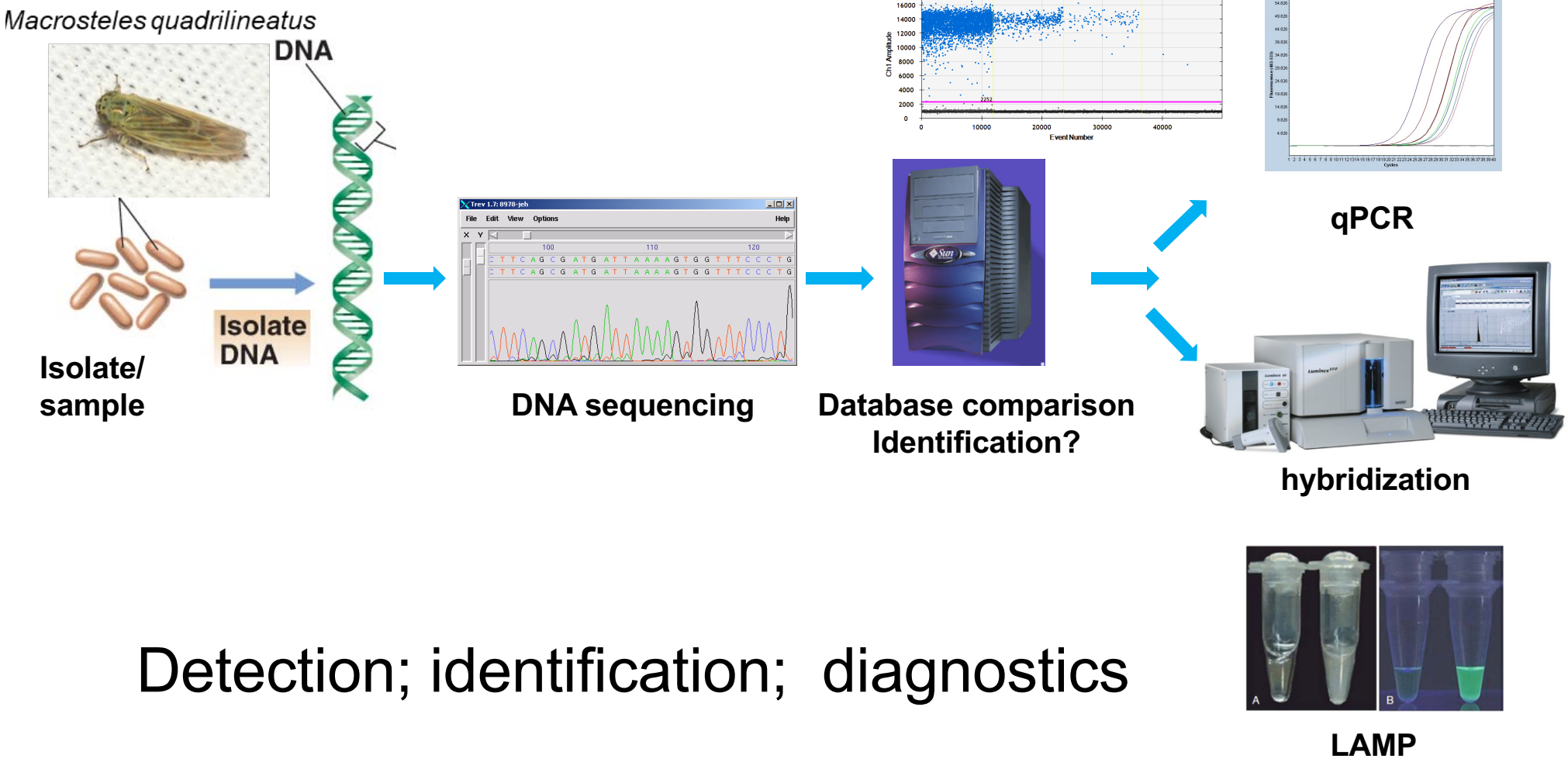
- Infect most crop species
- Cause developmental abnormalities that result in poor seed set, altered plant architecture, abnormal fruit development

Detection of phytoplasma DNA:

in insects: critical for disease management – speed is essential

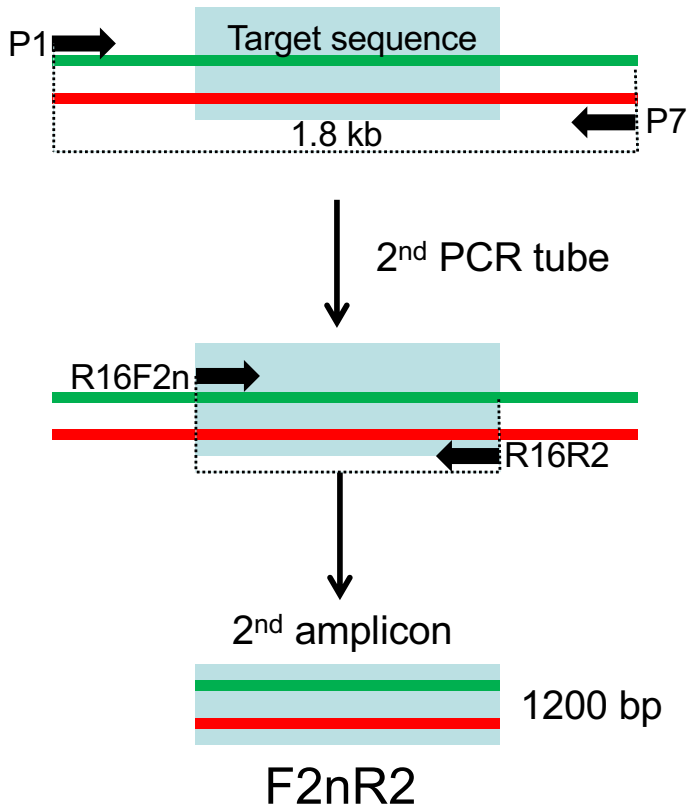
in plants: provides data on infection rates and disease epidemiology

Molecular “barcoding” using DNA



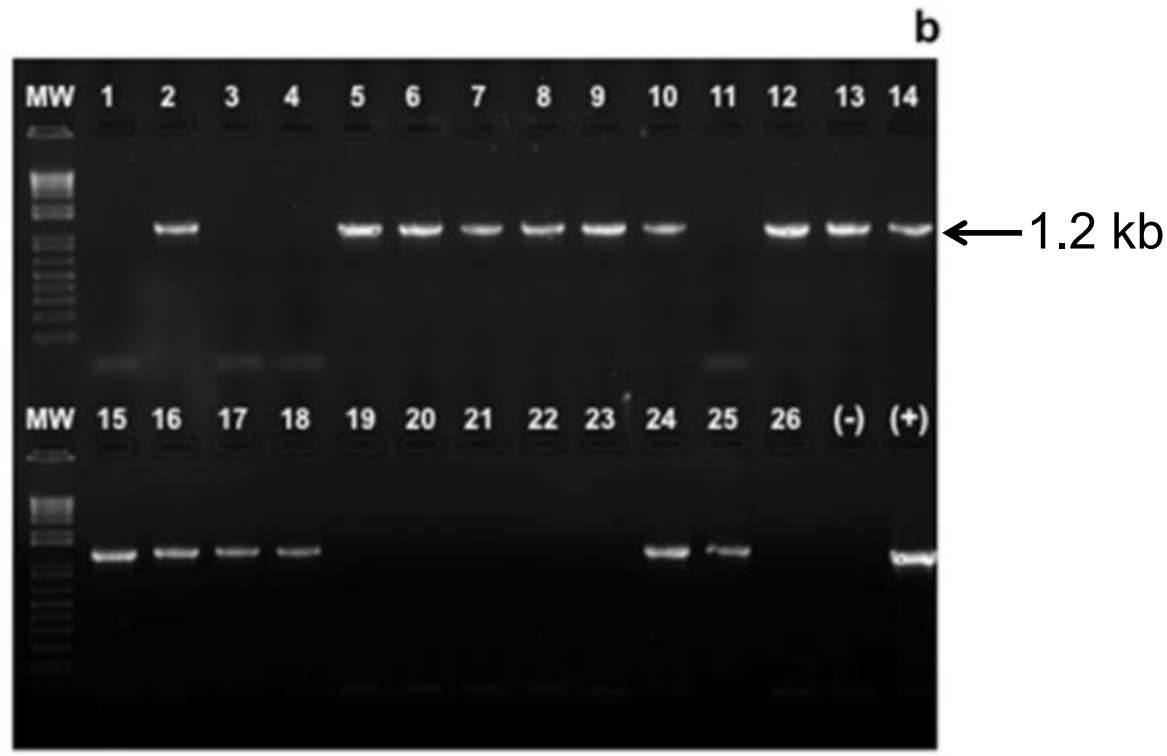
Phytoplasma detection, classification and phylogeny

"nested" PCR based on 16S rRNA-encoding locus (F2nR2)



F2nR2

~5hr



~45 min

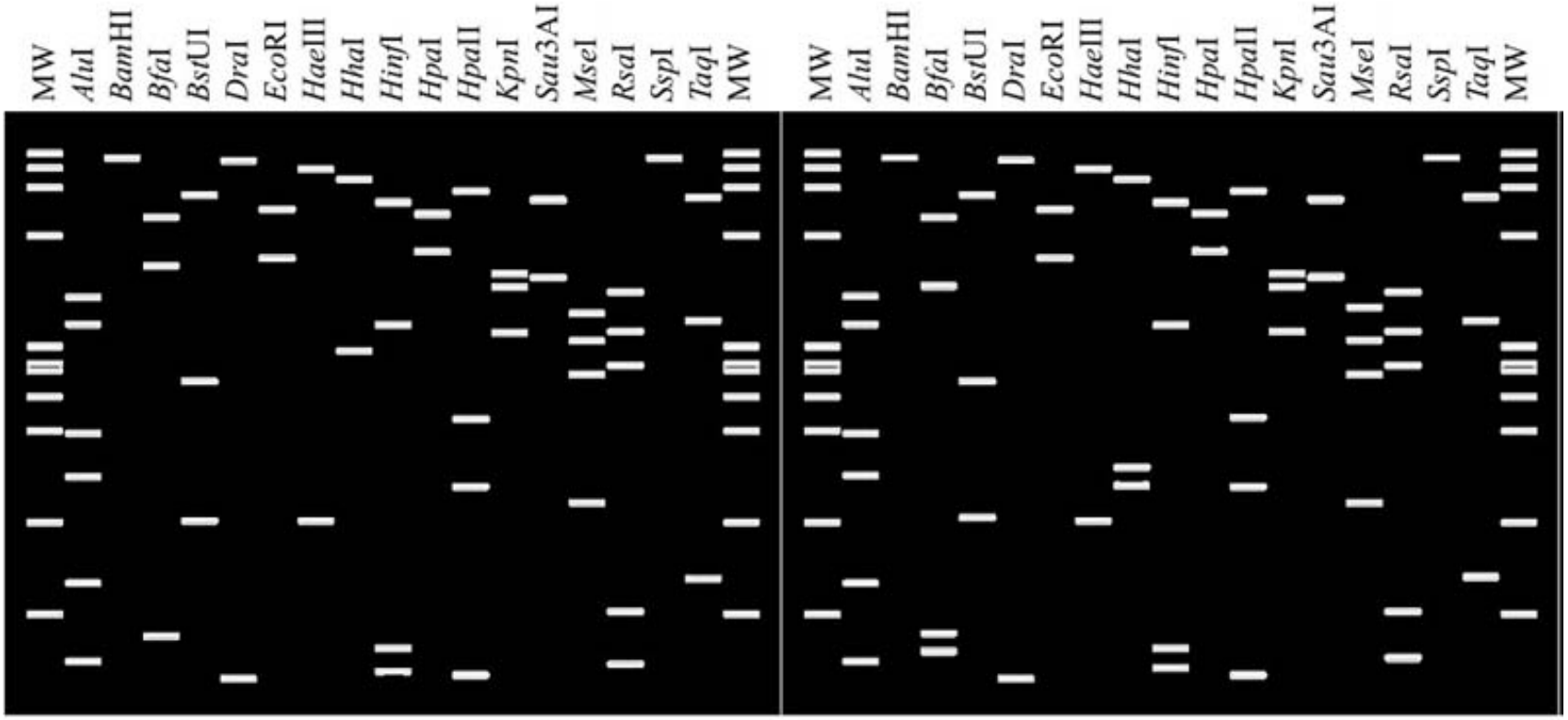
Gundersen, D. E., & Lee, I. M. (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathologia Mediterranea, 35(3), 144-151. doi:10.2307/42685262

Phytoplasma classification and phylogeny

Ribosomal groups: defined by RFLP analysis (*in silico*) of F2nR2 sequences

iPhyClassifier

Zhao et al. Int J Syst Evol Microbiol 2009;59(Pt 10):2582-2593.



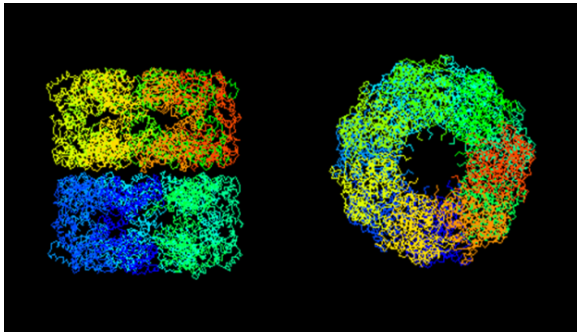
16SrI-A Aster yellows witches'-broom phytoplasma rrnA/rrnB (NC_007716)

16SrI-B Onion yellows phytoplasma mild strain rrnA/rrnB (NC_005303) and '*Ca.* Phytoplasma asteris' (M30790)

- relatedness index F=2Nxy/(Nx+Ny) → 36 ribosomal groups, >100 subgroups
- 2 copies of 16S rRNA-encoding locus in phytoplasma genome
- Not always the same (**heterogeneity**)

Chaperonins: an alternative barcode

•molecular chaperones present in all bacteria, archaea and in the cytoplasm, plastids and mitochondria of eukaryotes



• required for protein folding

• stress inducible

• type I: *cpn60*
• type II: CCT/TriC

groEL=cpn60=hsp60

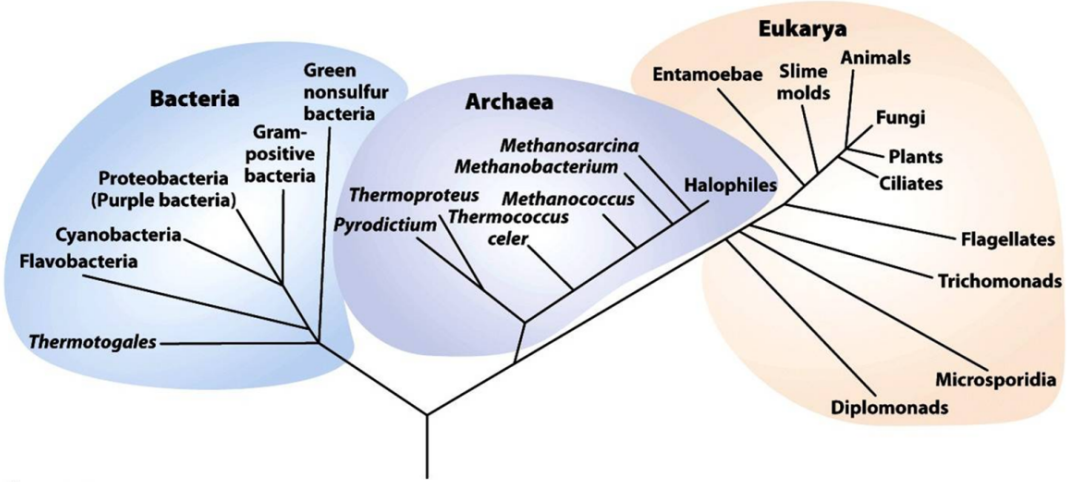
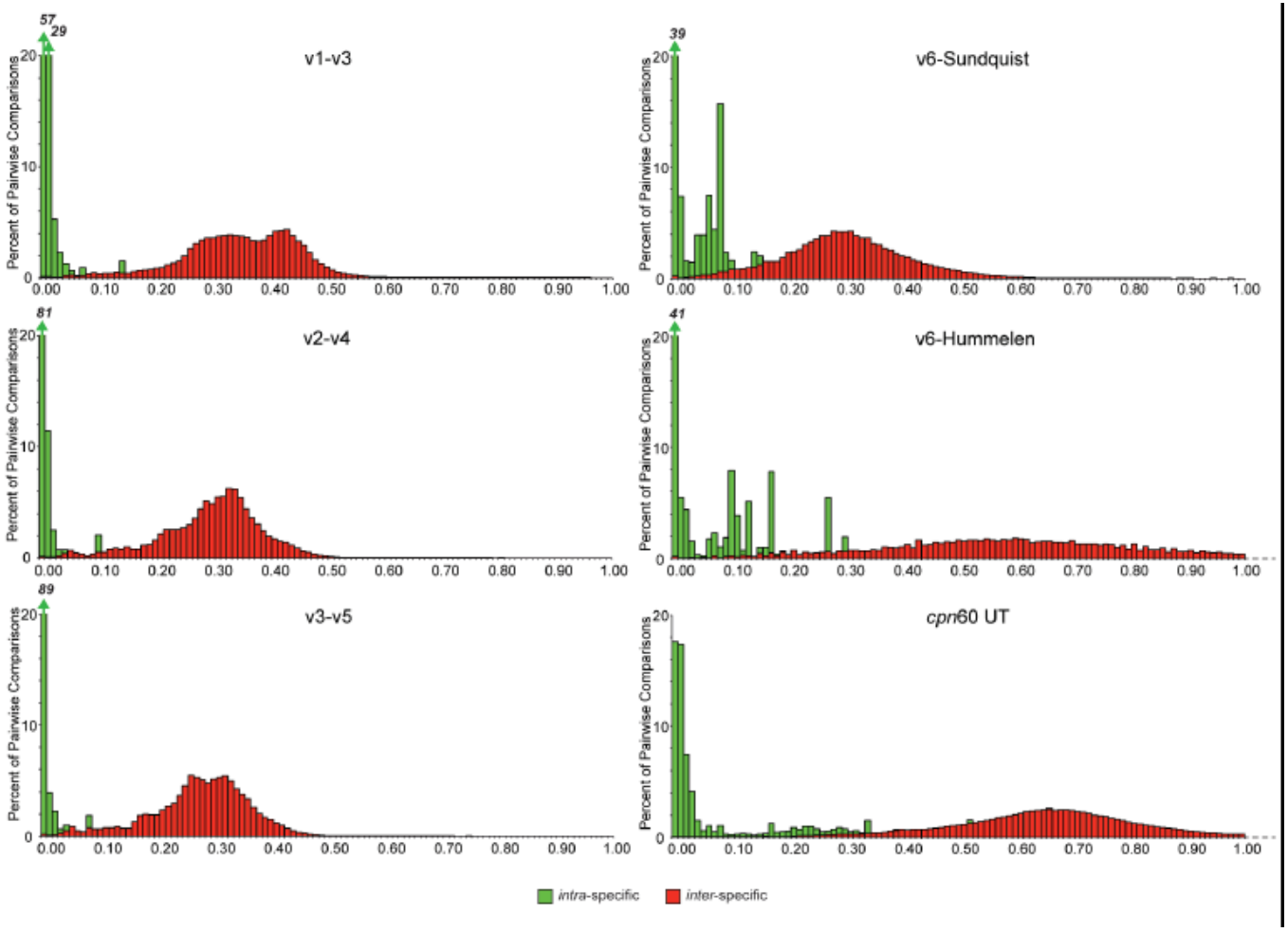


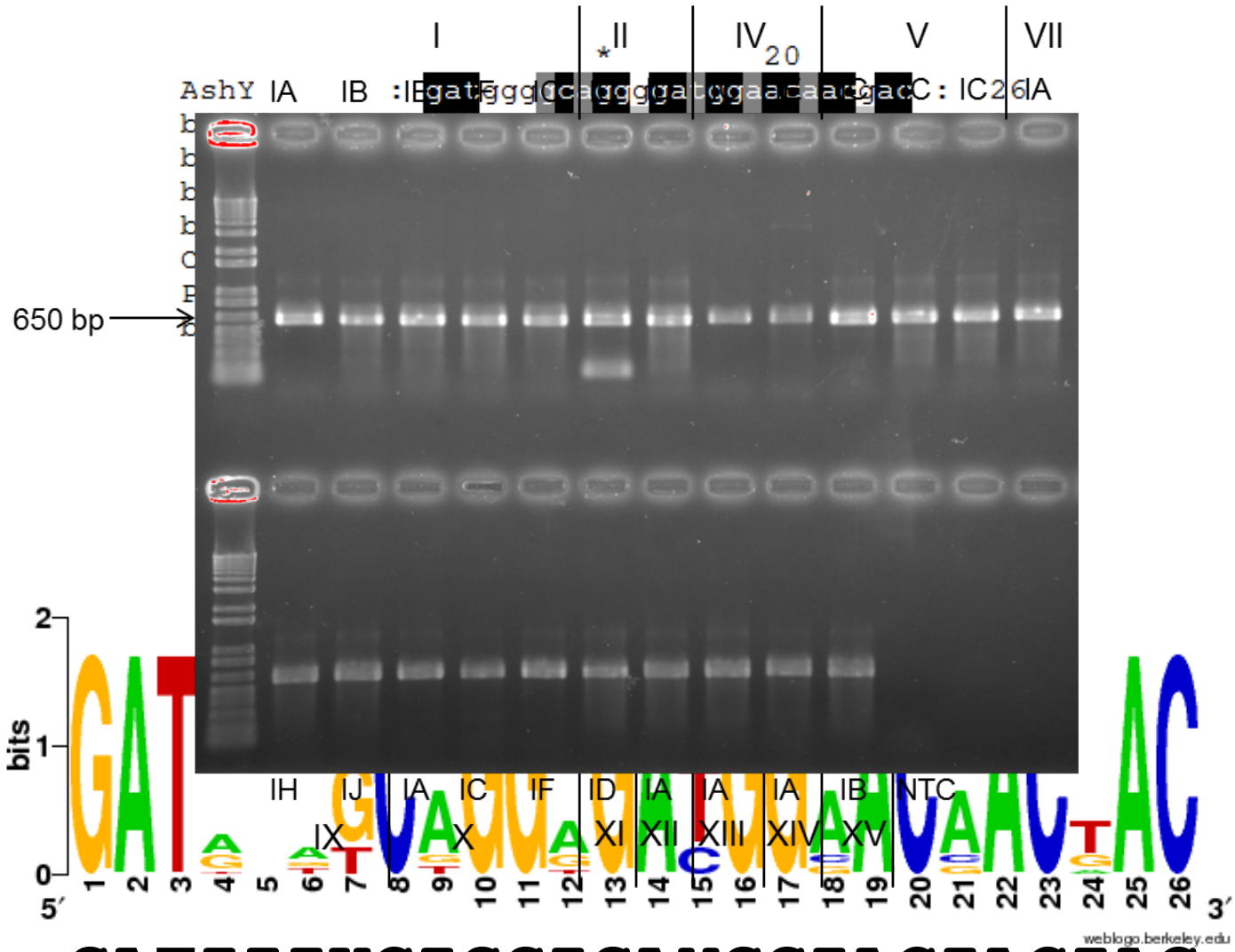
Figure 1-4
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

Assessing the “barcode gap”



Links et al. The chaperonin-60 universal target is a barcode for Bacteria that enables de novo assembly of metagenomic sequence data PLOS One: **7:**e49755, 2012

Phytoplasma classification and phylogeny tools using *cpn60*

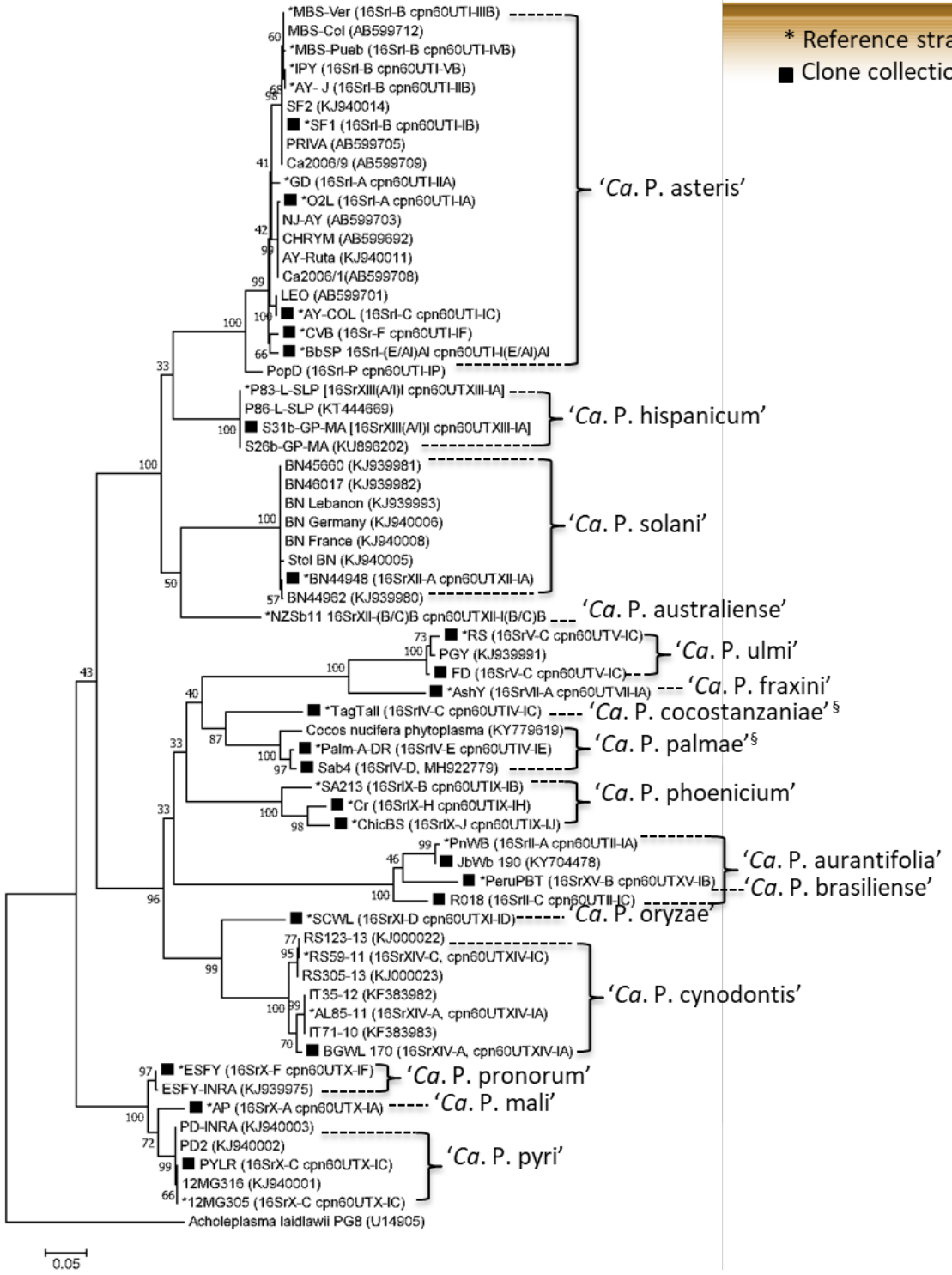


GATIIIKCIGGIGAYGGIACIACIAC

H279p→ ***cpn60***

Dumonceaux TJ et al. *PLOS ONE 2014;9(12):e116039.* ← **H280p** 552-555 bp of template-derived sequence

cpn60-base

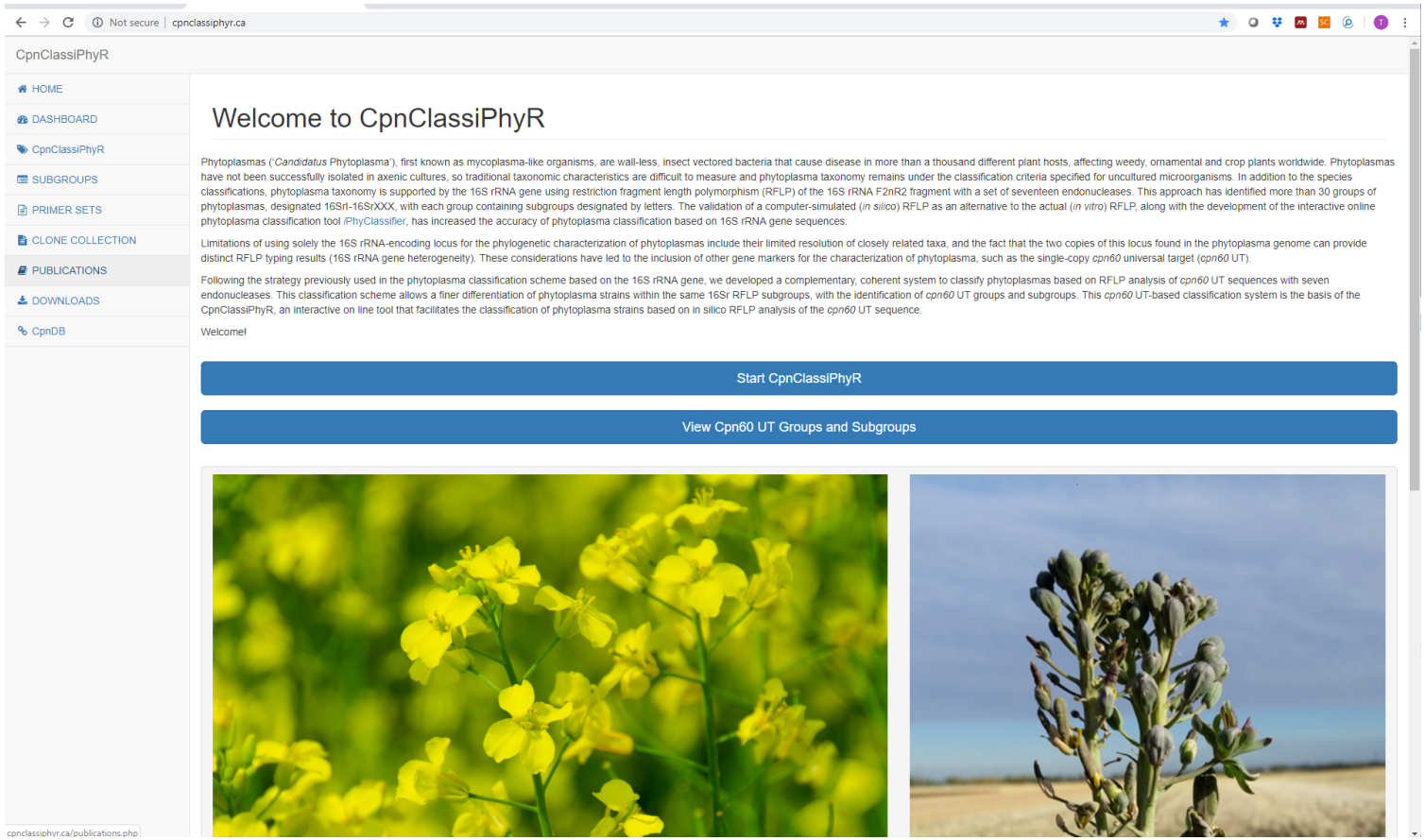

* Reference strain
■ Clone collection
*MBS-Ver (16SrI-B cpn60UTI-IIIB)
MBS-Col (AB599712)
*MBS-Pueb (16SrI-B cpn60UTI-IVB)
*IPY (16SrI-B cpn60UTI-VB)
*AY- J (16SrI-B cpn60UTI-IIB)
SF2 (KJ940014)
*SF1 (16SrI-B cpn60UTI-IB)
PRIVA (AB599705)
Ca2006/9 (AB599709)
*GD (16SrI-A cpn60UTI-IIA)
*O2L (16SrI-A cpn60UTI-IA)
NJ-AY (AB599703)
CHRYM (AB599692)
AY-Ruta (KJ940011)
Ca2006/1(AB599708)
LEO (AB599701)
*AY-COL (16SrI-C cpn60UTI-IC)
*CVB (16Sr-F cpn60UTI-IF)
*BbSP 16SrI-(E/AI)AI cpn60UTI-I(E/AI)AI
PopD (16SrI-P cpn60UTI-IP)
'Ca. P. asteris'
*P83-L-SLP [16SrXIII(A/I)I cpn60UTXIII-IA]
P86-L-SLP (KT444669)
S31b-GP-MA [16SrXIII(A/I)I cpn60UTXIII-IA]
S26b-GP-MA (KU896202)
'Ca. P. hispanicum'
BN45660 (KJ939981)
BN46017 (KJ939982)
BN Lebanon (KJ939993)
BN Germany (KJ940006)
BN France (KJ940008)
Stol BN (KJ940005)
*BN44948 (16SrXII-A cpn60UTXII-IA)
BN44962 (KJ939980)
'Ca. P. solani'
*NZSb11 16SrXII-(B/C)B cpn60UTXII-I(B/C)B
'Ca. P. australiense'
*RS (16SrV-C cpn60UTV-IC)
PGY (KJ939991)
FD (16SrV-C cpn60UTV-IC)
'Ca. P. ulmi'
*AshY (16SrVII-A cpn60UTVII-IA)
'Ca. P. fraxini'
*TagTall (16SrIV-C cpn60UTIV-IC)
'Ca. P. cocostanzaniae'§
Cocos nucifera phytoplasma (KY779619)
*Palm-A-DR (16SrIV-E cpn60UTIV-IE)
Sab4 (16SrIV-D, MH922779)
'Ca. P. palmae'§
*SA213 (16SrIX-B cpn60UTIX-IB)
*Cr (16SrIX-H cpn60UTIX-IH)
*ChicBS (16SrIX-J cpn60UTIX-IJ)
'Ca. P. phoenicium'
*PnWB (16SrII-A cpn60UTII-IA)
JbWb 190 (KY704478)
*PeruPBT (16SrXV-B cpn60UTXV-IB)
R018 (16SrII-C cpn60UTII-IC)
'Ca. P. aurantifolia'
'Ca. P. brasiliense'
*SCWL (16SrXI-D cpn60UTXI-ID)
'Ca. P. oryzae'
RS123-13 (KJ000022)
*RS59-11 (16SrXIV-C, cpn60UTXIV-IC)
RS305-13 (KJ000023)
IT35-12 (KF383982)
*AL85-11 (16SrXIV-A, cpn60UTXIV-IA)
IT71-10 (KF383983)
BGWL 170 (16SrXIV-A, cpn60UTXIV-IA)
'Ca. P. cynodontis'
*ESFY (16SrX-F cpn60UTX-IF)
ESFY-INRA (KJ939975)
'Ca. P. pronorum'
*AP (16SrX-A cpn60UTX-IA)
'Ca. P. mali'
PD-INRA (KJ940003)
PD2 (KJ940002)
PYLR (16SrX-C cpn60UTX-IC)
12MG316 (KJ940001)
*12MG305 (16SrX-C cpn60UTX-IC)
'Ca. P. pyri'
Acholeplasma laidlawii PG8 (U14905)
0.05

RFLP scheme for phytoplasmas based on *cpn60*:

- Perez-Lopez, E. et al. Int. J. Sys. Evol. Microbiol. 66, 5600 (2016)

The CpnClassiPhyR: a cpn(60-based) Classi(fier for) Phy(toplasmas using) R(FLP) analysis

Muirhead, K. et al. Plant Disease, in press doi 10.1094/PDIS-03-19-0454-A



Phytoplasma genome sequencing

AY-canola (Saskatoon, SK)

F2nR2 (16S) amplification/sequencing:
16SrI-A
16SrI-B

cpn60 amplification/sequencing:
cpn60 I-IB

rp amplification/sequencing:
rpI-B

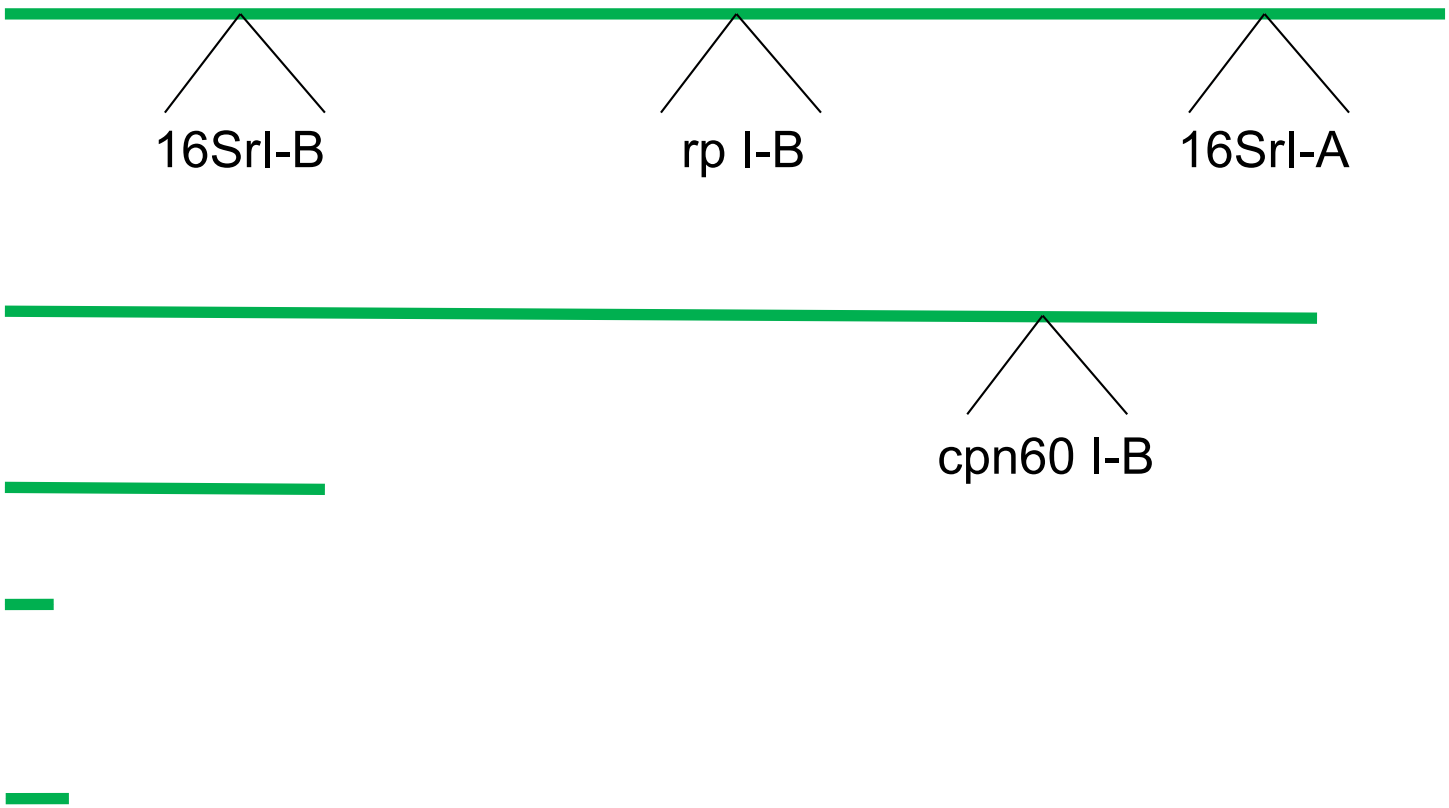
Heterogeneity??

Phytoplasma genome sequencing

AY-canola (Saskatoon, SK)

Sequencing strategy:

1. Illumina (MiSeq)
2. ONT MinIon (nanopore)



Length (bp)

373,899

336,406

24,285

3,909

5,099

Molecular diagnostic assays

cpn60-targeted LAMP vs. nested PCR (F2nR2):
-examined 256 samples by LAMP and by 16S nested PCR

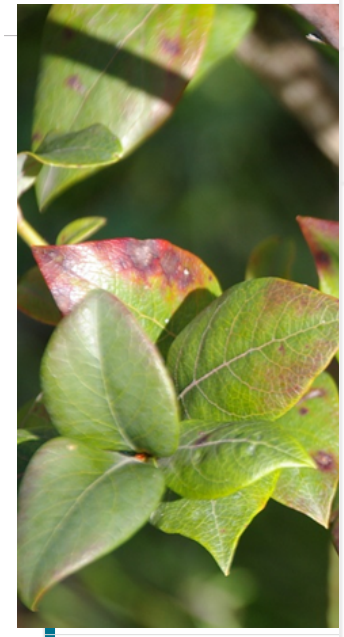
Sensitivity/specificity with nested PCR set as gold standard:

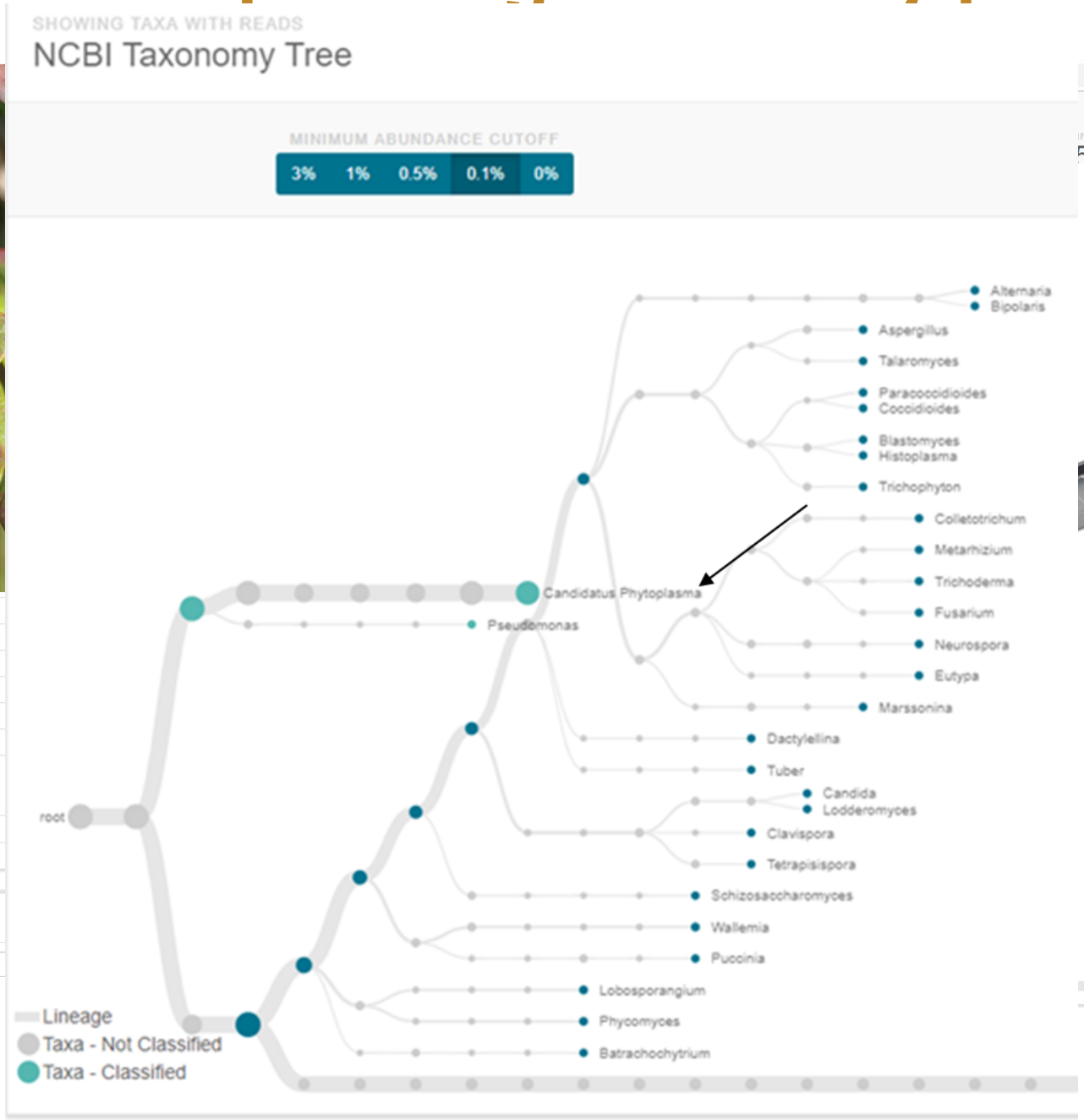
1. evaluating test performance using a 2x2 table

	reference standard test: 16S nested PCR			
test under evaluation: cpn60 LAMP	**positive**	**negative**	**total**	
positive	44	30	74	
negative	4	178	182	
total	48	208	256	
		95%CI	low	high
test sensitivity	0.917	0.078	0.838	0.995
test specificity	0.856	0.048	0.808	0.904

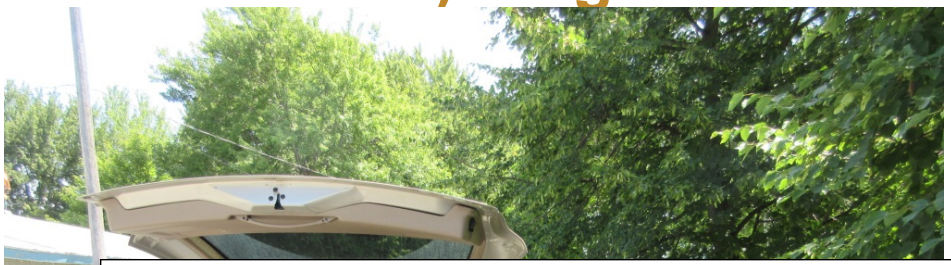
All rp PCR POSITIVE

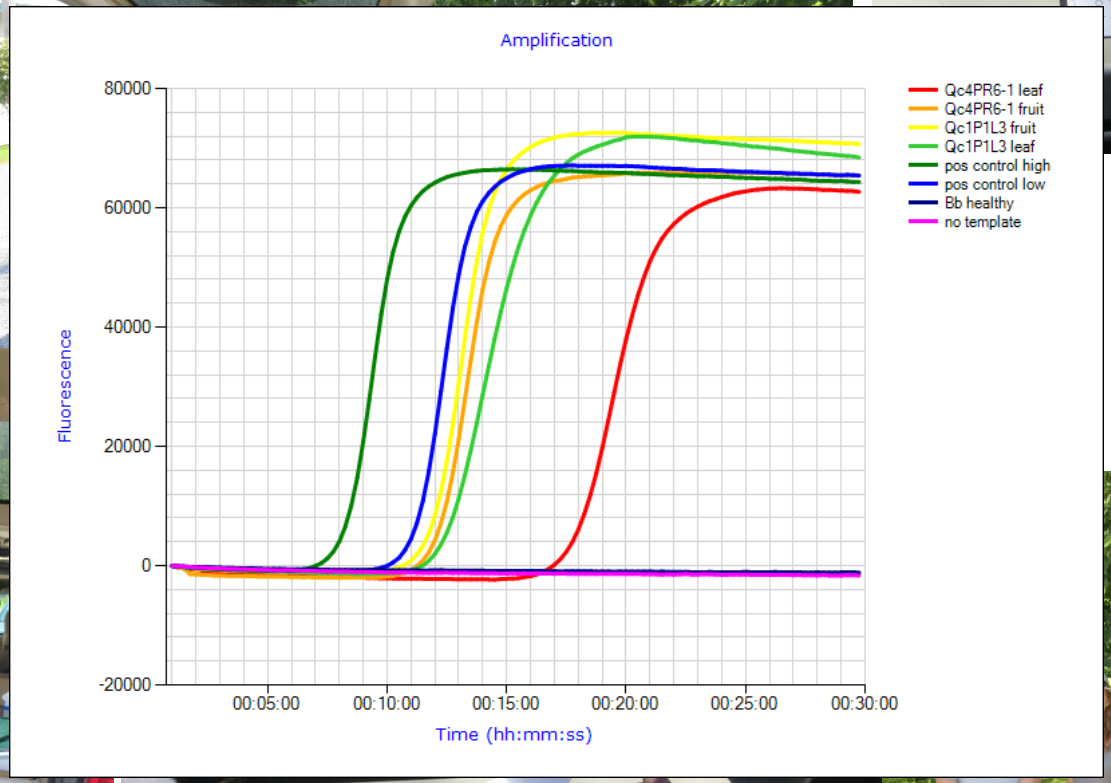
Rapid DNA sequencing to identify pathogens





In-field ("point-of-care") diagnostics





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