An overview of isothermal amplification platforms in general and AmplifyRP® specifically

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Why Isothermal?

- Molecular detection is all the rage
  - "I've got a PCR for that!"
  - Lateral flow or ELISA are not always enough
  - Confirmation of immunological results
  - Sometimes only game in town
    - Hybridization, PCR and qPCR are lab based methods
      - Labor intensive, time consuming, and expensive
  - Easy, fast, cheap (relatively)
  - Potential for being a Point of Care technology

Various isothermal detection platforms

Table 1 Various isothermal detection platforms and components of the assay that are amplified

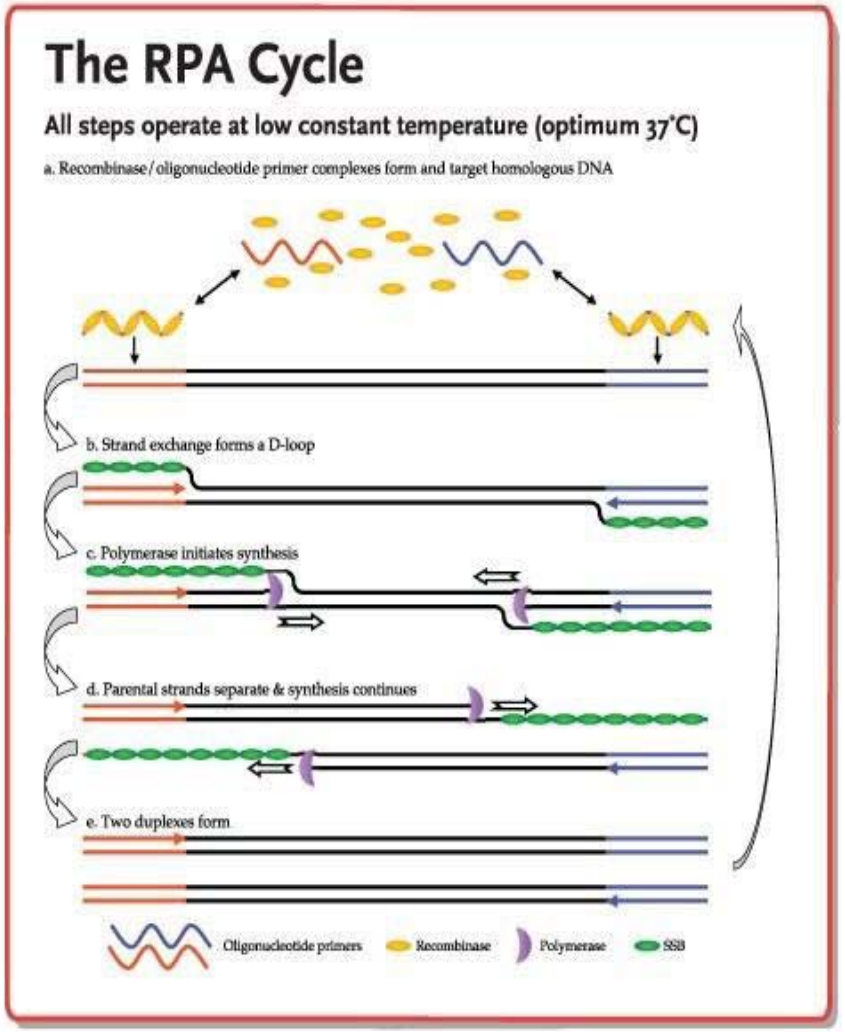
Section	Platform	Amplified component	Amplification catalyst
3.01	NASBA	Complementary sequence of target (RNA)	Enzymatic
3.02	SDA	Probe	Enzymatic
3.03	LAMP	Probe	Enzymatic
3.04	Invader assay	Signal	Enzymatic
3.05	RCA	Probe	Enzymatic
3.06	SMART	New RNA	Enzymatic
3.07	HDA	Target and the complementary sequence	Enzymatic
3.08	RPA	Target and the complementary sequence	Enzymatic
3.09	NESA, NEANA	Signal	Enzymatic
3.10	Exo III aided target recycling	Signal	Enzymatic
3.11	Junction or Y probes	Signal	Enzymatic
3.12	Reactivation of enzymatic activity	Signal	Enzymatic
3.13	Ultrasensitive miRNA detection	Probe and signal	Enzymatic
3.14	Split DNAzyme	Signal	Non-enzymatic
3.15	RNA cleaving deoxyribozymes (CMB, TASC)	Signal	Non-enzymatic
3.16	Template-directed chemical reaction	Signal	Non-enzymatic
3.17	Non-covalent DNA catalytic reaction	Signal	Non-enzymatic
3.18	HCR	Signal	Non-enzymatic
3.19	Self-assembled DNA	Signal	Non-enzymatic

Lei Yan *et al.* Mol. BioSyst., 2014, 10, 970

RPA Method of Detection - AmplifyRP™

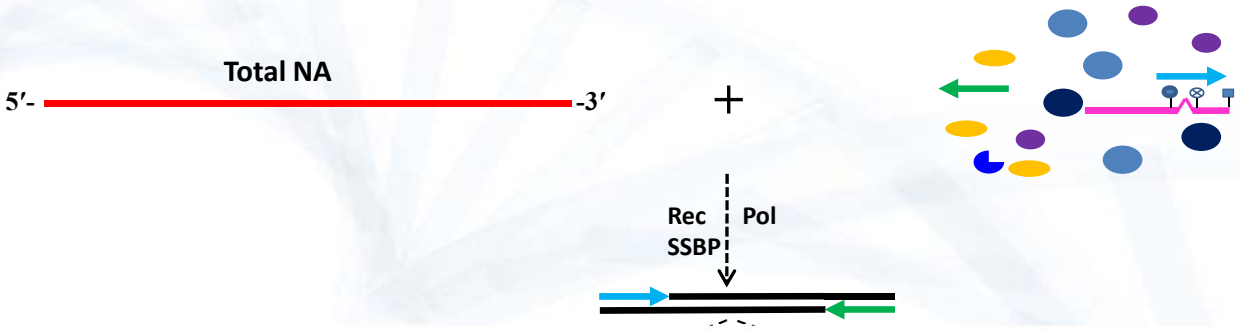
- Based upon the recombinase-polymerase methodology
  - Amplify DNA at a constant temperature (e.g. 37-39^{0}C)
- Uses a mix of enzymes/reagents in a lyophilized pellet
  - Recombinases, DNA polymerase, SSBP and other proteins
- Rapid detection of DNA or RNA
  - Positive results can be seen between 5-20 minutes, depending on detection method

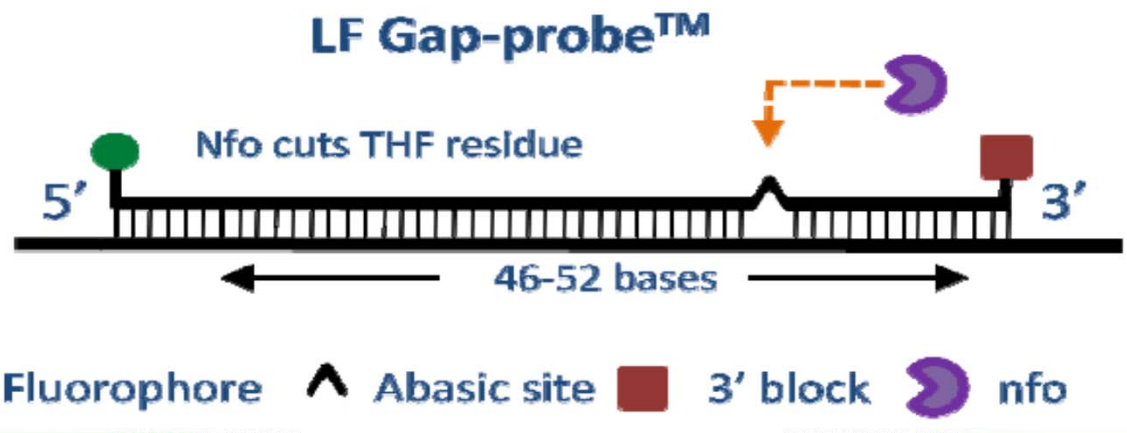
AmplifyRP Basics



Citation: Piepenburg O, Williams CH, Stemple DL, Armes NA (2006) DNA detection using recombination proteins. PLoS Biol 4(7): e204. DOI: 10.1371/journal.pbio.004020

Not Just the Basics – Two Formats – They Differ How?





Th

Acceler8™

Think Nested PCR

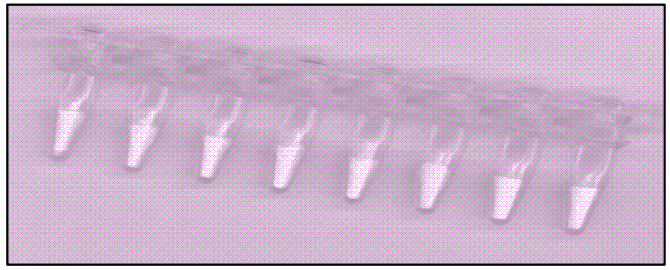
Tube Scanner

Immunostrip

What does it look like?

Procedure

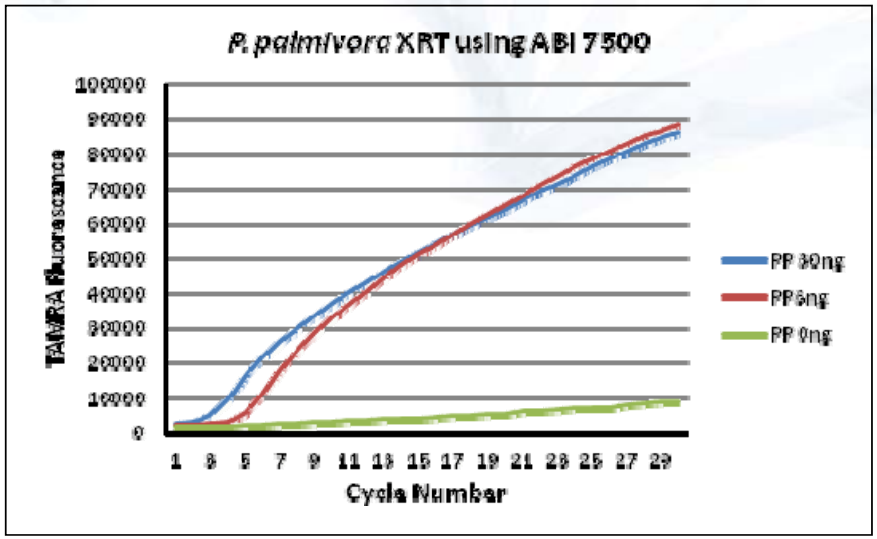
- Extract sample (1:10 – 1:20 w/v) using sample extraction buffer
- Mesh bags, tube and pestle, bead beater, or any method will work

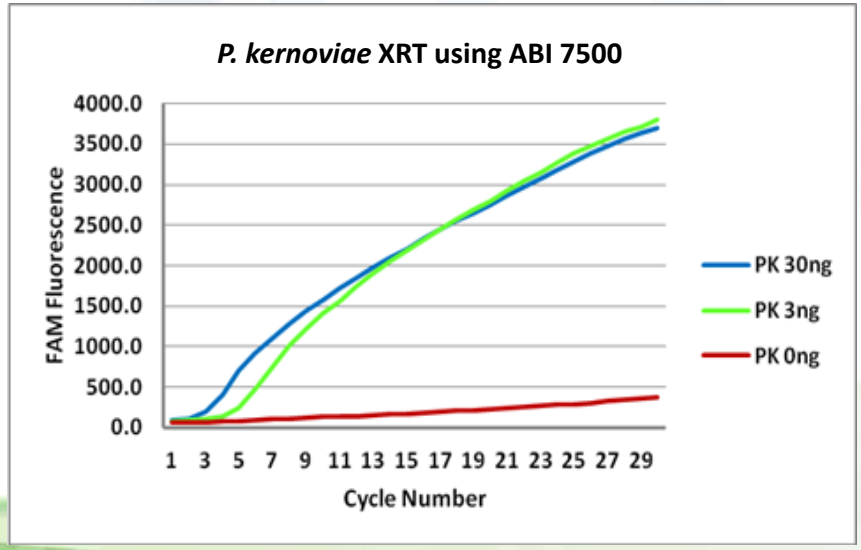


- Rehydrate reaction pellet

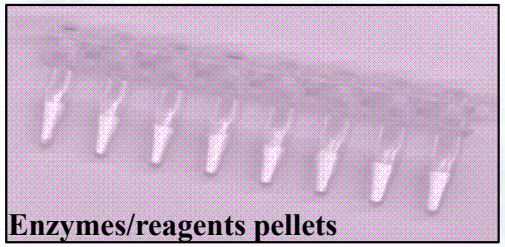
- Transfer 1ul of crude sample extract to the rehydrated reaction pellet
- Mix

Detection of AmplifyRP XRT - Instrument Agnostic

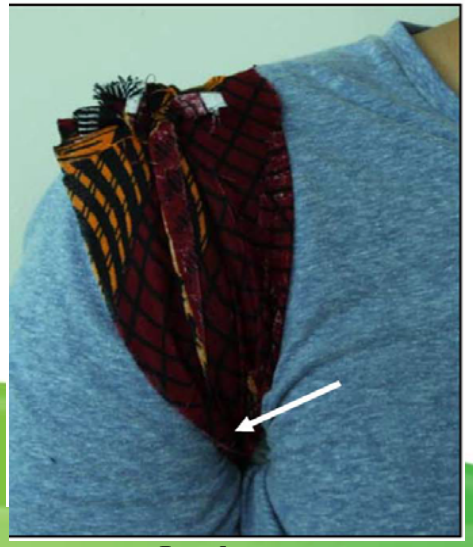




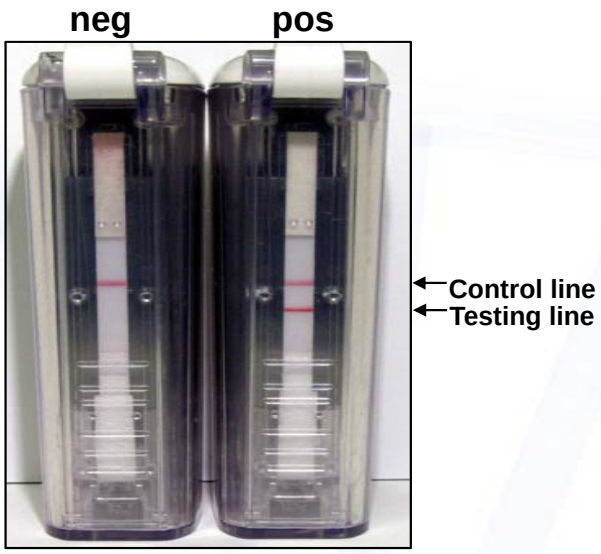
Acceler8™ is endpoint detection by ImmunoStrip®



Enzymes/reagents pellets



Incubator



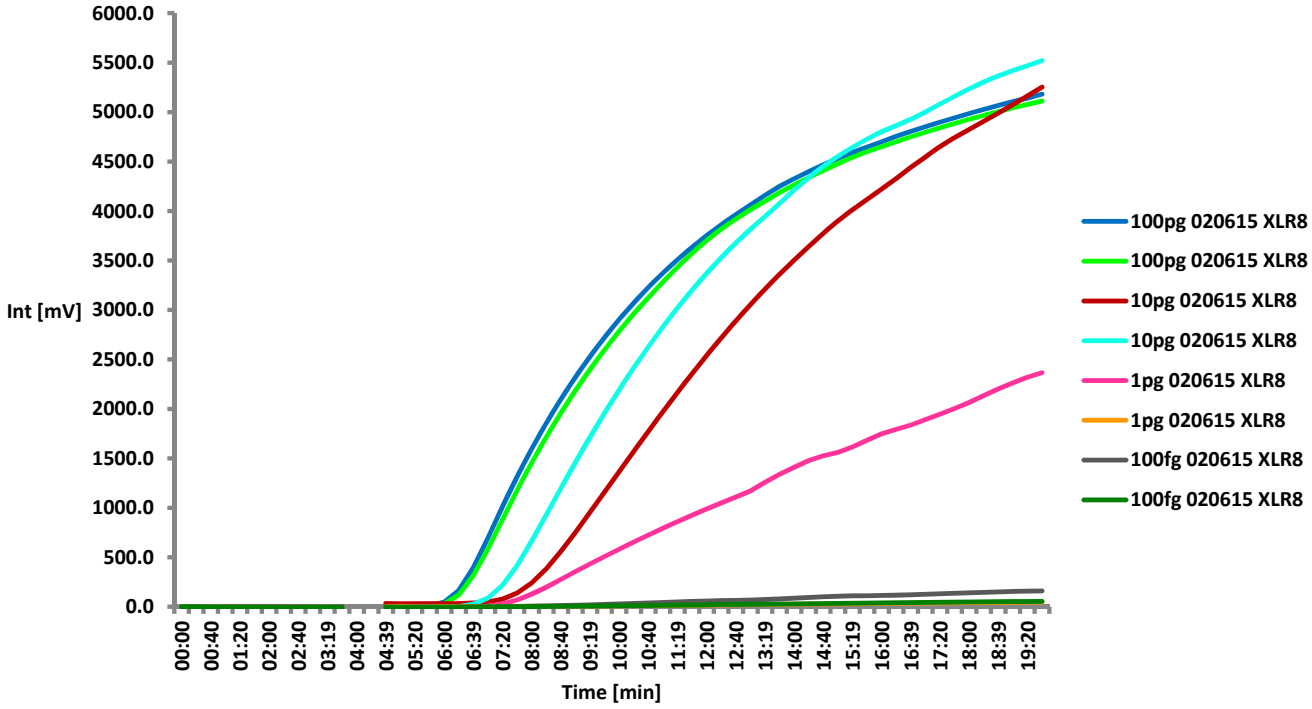
Detection Result

- How it works:
  - Uses a labelled streptavidin to capture the biotinylated 3’ primer and monoclonal anti-dye to capture FITC 5’ primer of the amplimer.

AmplifyRP Hybrid Assays

Same result on DNA dilution series: 100fg – 1pg sensitivity

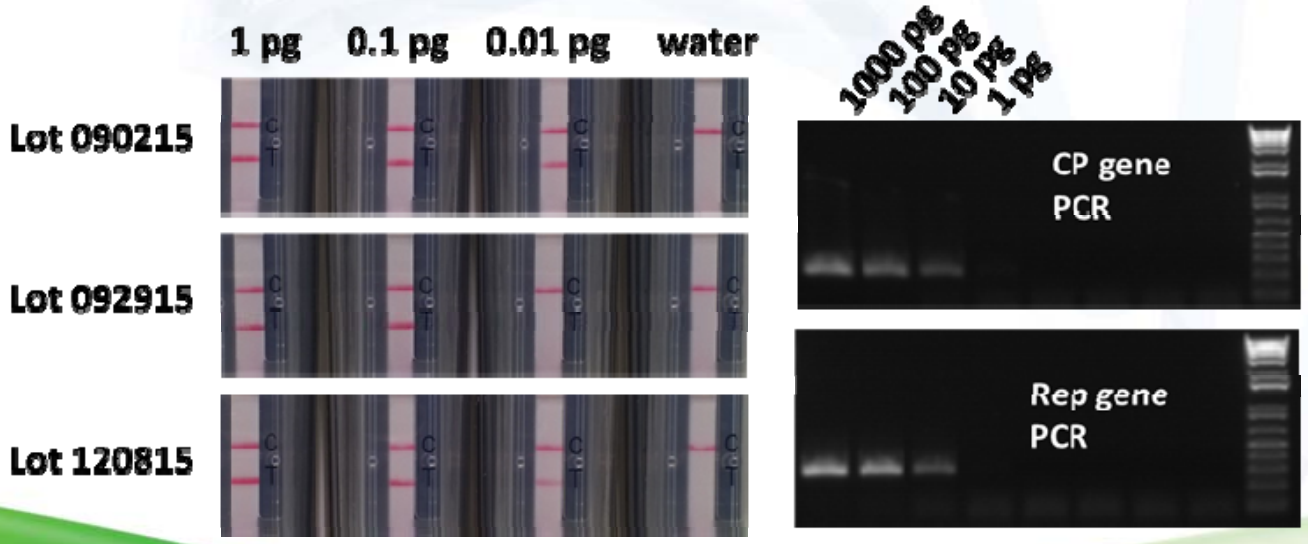
Real-time fluorescent detection



Endpoint Immunostrip detection

Sensitivity: Grapevine Red Blotch AmplifyRP vs. PCR

AmplifyRP Acceler8 is at least 100X more sensitive than PCR when testing purified GRBaV-infected grapevine leaf DNA

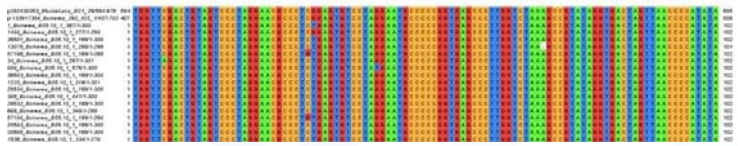


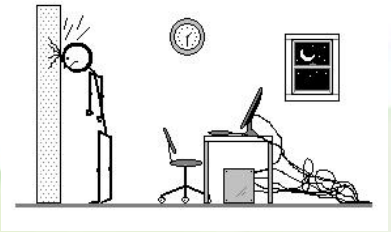
Candidatus liberibacter asiaticus (Las)

- Citrus Greening (HLB)
  - Kills citrus trees.
  - Asia, Africa, S. America (Brazil), Mexico, USA (Florida), Europe (?).
  - 3 strains Las, Lam, Laf.
  - Insect vector (psyllid).

Assay Development

- Product definition
  - Specificity, sensitivity, cross-reactivity, test format.
  - Other tests on the market.
  - Collaborators and sources of materials (development and validation).
- Primer/probe design
  - Sequence data, lots of it.
  - Lineups, blasts, choice of target region.
  - Choose (no algorithm) and test combinations, (repeat).
  - Optimize.
- Produce pilot lots and validate.





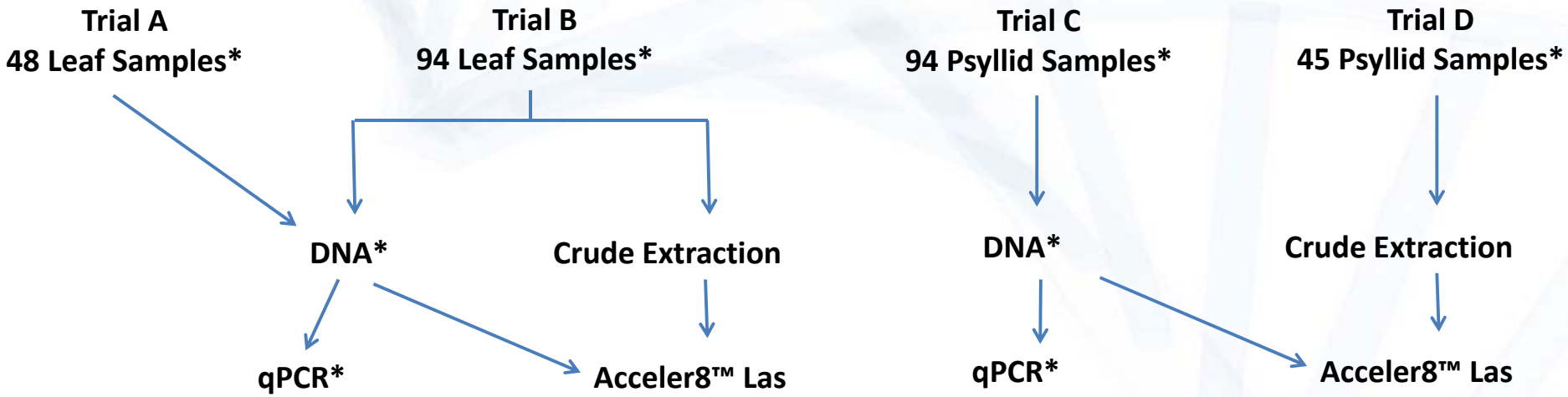
Las Acceler8™ Cross-reactivity

Control line →
Testing line →

Las cross-reactivity with HLB species and *Xanthomonas citri* strains.

Left to right: Las DNA positive control, host DNA negative control, Lam sample, Laf sample, Lsol sample, *X. citri* strain 1, *X. citri* strain 2, *X. citri* strain 3.

Comparison of Acceler8™ and qPCR



* Leaf and psyllid samples, DNA, and qPCR data courtesy of Dr. Mike Irey, US Sugar

Las Acceler8™ Real World Samples

Template		HLB Status			
		Positive (Cq range)		Negative (Cq range)	
		qPCR	Acceler8™	qPCR	Acceler8™
Trial A	**DNA**	18 (22.49 – 27.16)	18 (NA)	30 (40)	30 (NA)
Trial B	**DNA**	76 (21.52 – 30.1)	76 (NA)	18 (40)	18 (NA)
	Crude Extract	(NA)	76	(NA)	18
Trial C	**DNA**	16 (25.22 – 33.0)	16 (NA)	78 (40)	78 (NA)
Trial D	**Crude Extract**	ND*	31%	ND*	69%

* qPCR not done. Previous year's data gave an expectation of 20% positives, but is not confirmed this year.

Summary

- The test is robust: Works very well with extremely variable samples and targets using a crude extract
- Fast: 30 - 45 min start to finish, including sample prep
- Can be done on-site with ImmunoStrip® detection for a visual read or a portable reader for real-time results
- Specific for *L. asiaticus*; no cross-reactivity with other citrus pathogens
- Very sensitive assay: equivalent to qPCR with real world plant tissue and psyllid samples
  - Sensitivity detecting Purified PCR fragment = Approximately 48 copies

Research and Products

Pathogen	Format	
Phytophthora ramorum	[XRT]	[Acceler8™]
Phytophthora kernoviae	[XRT]	[Acceler8™]
Phytophthora palmivora	[XRT]	[Acceler8™]
Fusarium, Race 4		[Acceler8™]
Candidatus liberibacter asiaicus	[XRT]	[Acceler8™]
Candidatus liberibacter solanacearum		[Acceler8™]
Phytoplasma [aster yellows]	[XRT]	[Acceler8™]
Clavibacter michiganensis subsp. *michiganensis*	[XRT]	
Clavibacter michiganensis subsp. *sepidonicus*	[XRT]	[Acceler8™]
Pseudomonas syringae pv. tomato	[XRT]	[Acceler8™]
Xanthomonas campestris pv. *vesicatoria* (Xcv)	[XRT]	[Acceler8™]
Banana bunchy top virus	[XRT]	[Acceler8™]
Plumpox virus*	[XRT]	[Acceler8™]
Little cherry virus 2*		[Acceler8™]
Tomato chlorotic dwarf viroid*	[XRT]	[Acceler8™]
Potato spindle tuber viroid*	[XRT]	[Acceler8™]
Discovery Format	[XRT]	[Acceler8™]

* RNA test