Smart DART/LAS v1.0

Home-Test for HLB Detectioin
Citrus Huanglongbing Bacteria in psyllids

A collaborative Project

Smart DART/LAS v1.0

USDA ARS National Clonal Germplasm repository for Citrus & Dates, Riverside, CA	University of Hawaii Department of Molecular Biosciences and Bioengineering Honolulu, HI
Manjunath Keremane Chandrika Ramadugu Richard Lee	Daniel Jenkins
USDA ARS US Horticultural Research Laboratory Fort Pierce, FL	Diagenetix, Inc. Honolulu, HI
David Hall Yongping Duan	Scott Shibata Ryo Kubota

What is LAMP?
Loop mediated Isothermal amplification

- LAMP reaction involves an enzyme that displaces the old DNA strand instead of chewing up as in regular PCR
- LAMP reaction requires 4 primers
- The following links may give you a feel for the reaction

http://loopamp.eiken.co.jp/e/lamp/anim.html

http://www.youtube.com/watch?v=g0fykG8L3Wg

- Isothermal amplification is a very fast developing field
- May replace most of the PCR reactions in the next few years
- May turn out to be much cheaper and efficient than PCR

Introduction

- Smart-DART/LAS v1.0 technology provides an easy-to use tool for growers, nurserymen and others to test the psyllids in field or at home **without** need for:
  - a lab
  - an expert technician
  - a PCR machine
  - computer
  - not even a pipet

USDA UNIVERSITY of HAWAI'I MĀNOA

Smart DART/LAS v1.0

The Tools

Connected
via bluetooth

Smart-DART device

Your smart phone (any android device)

8-strip mini centrifuge

Smart-DART device

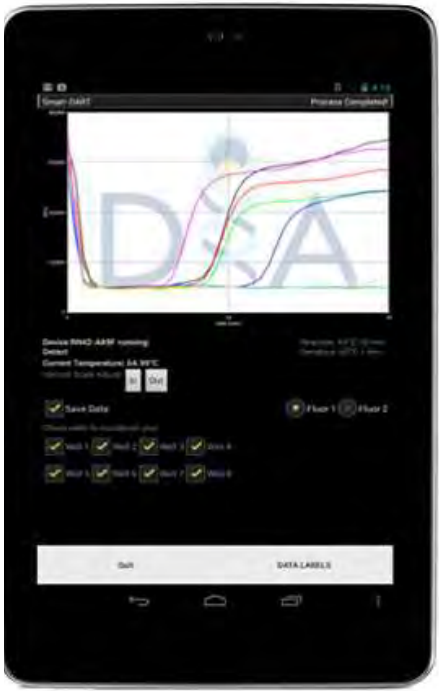
Smart DART/LAS v1.0

Current Prototype

- 3” x 4” footprint
- *Runs with 110 volts or a 12 v battery
- *Charge enough for an entire day
- Tests 8 samples at a time (e.g. 6 test samples and 2 controls)
- Completes the test in less than 30 minutes
- Connects wirelessly to phone via blue-tooth

Android device

Smart DART/LAS v1.0



- Your smart phone or any android device
- Smart-DART® software is uploaded to your phone
- Smart-DART is operated from your phone
- Test results stored on your phone
- Test results can be emailed instantly

Kit contents

Smart DART/LAS v1.0

- 8-strip white tubes with individual caps
- With extraction buffer
- Store at room temperature

- 8-strip clear tubes with individual caps
- With reaction solution
- Store frozen and bring to room temperature when needed

- Disposable plastic sticks with a loop at one end
- Used for one-time transfer of a small quantity of extraction from tube A to tube B

Kit contents cont..

Smart DART/LAS v1.0

- Filter paper squares
- Benchtop cover paper
- Rack for tubes
- Plastic disposable droppers for transferring psyllids from alcohol to filter paper
- Marker
- Instructions for using the kit

Huanglongbing

- HLB has become a major problem to the citrus industry in the USA and all countries in Central and Southern America
- Testing psyllids is an effective way for monitoring HLB
- The bacterium can be detected in psyllids about an year or two before the onset of symptoms in plants

HLB symptoms on mature trees (A), nursery trees (B), fruit (C). Asian citrus psyllid (D).

USDA UNIVERSITY of HAWAI'I MANOA DNA DIAGNOSTICS INC.

Smart DART/LAS v1.0

Testing psyllids

Smart DART/LAS v1.0

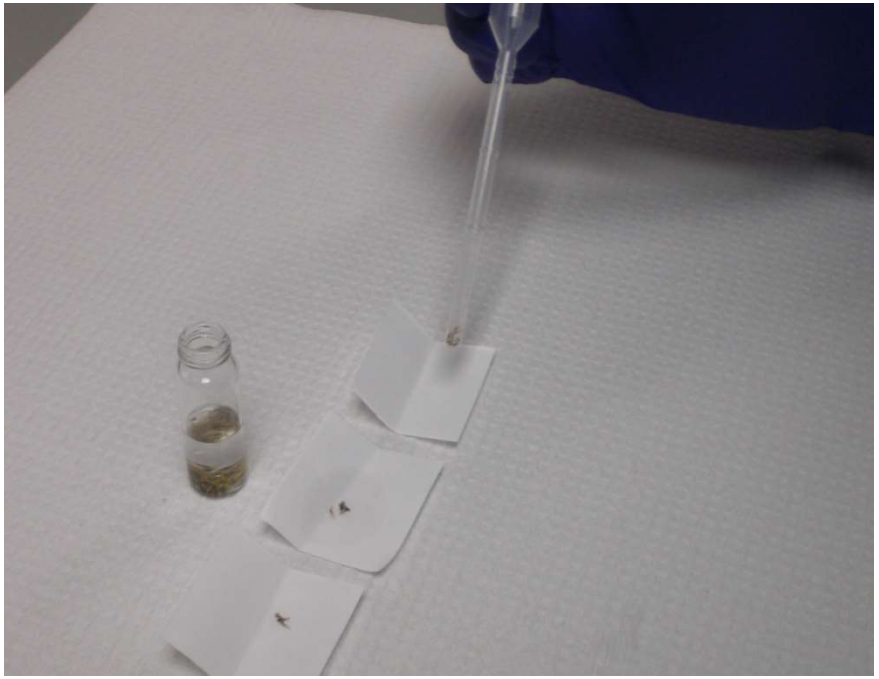
- Freshly captured psyllids can be directly assayed.

- Psyllids in ethanol stored frozen can also be used.

Procedure

Smart DART/LAS v1.0

1. Use a clean bench and place benchtop paper.
2. Place 8 filter paper squares.
3. Take out psyllids and place on filter paper.
4. Air-dry for 5 min.



Procedure cont..

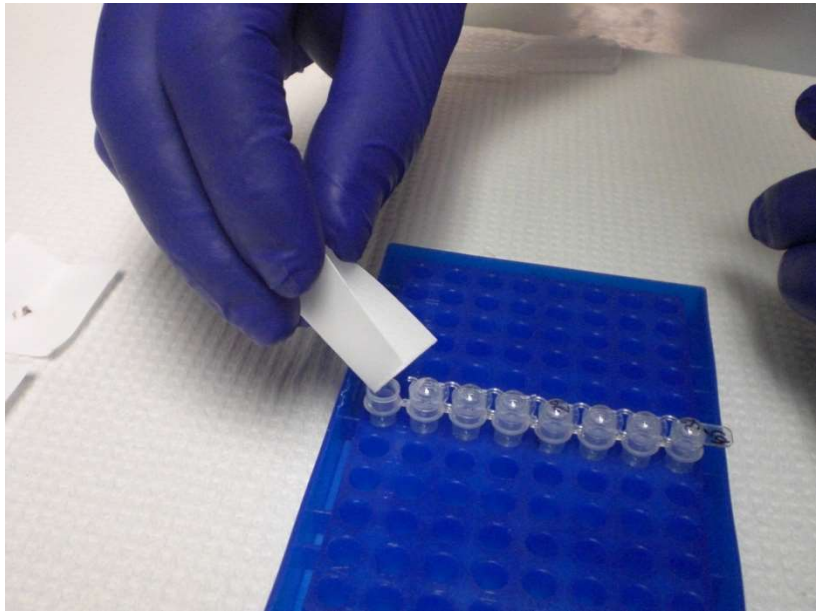
Smart DART/LAS v1.0

5. Take one blue 8-tube strip, place on a rack.

6. Open one tube at a time and drop dried psyllids into the blue tube and close the cap.

7. Record sample information and tube numbers.

8. Using Smart-DART, heat the tubes at 95 **o**C for 5 min.



Procedure cont..

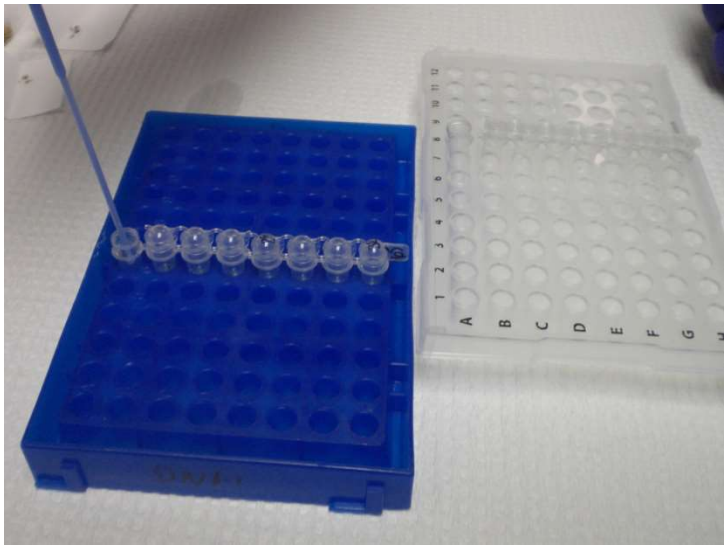
Smart DART/LAS v1.0

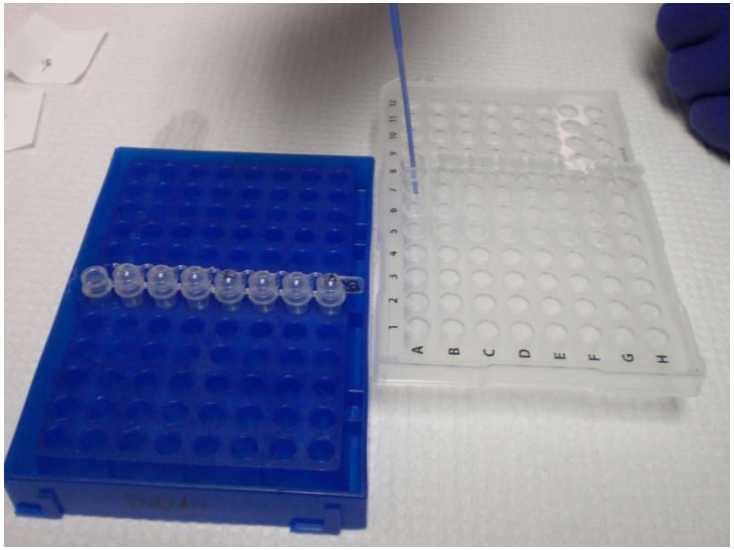
9. Place blue tube strip back on the rack.

10. Place clear 8-strip tubes next to it.

11. Opening only one tube at time, dip the loop into blue tube first and then into clear tube.

12. Discard the loop, and use fresh loop for the next sample.





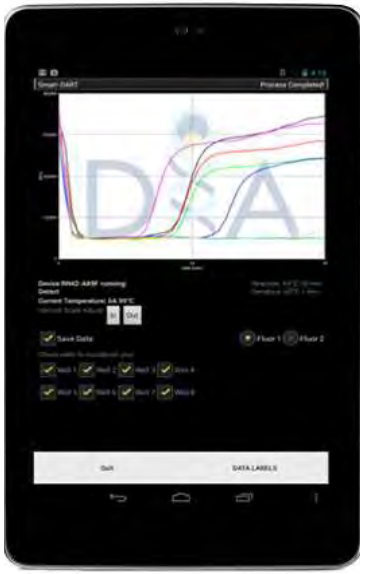
Procedure cont..

Smart DART/LAS v1.0

13. Place the clear 8-strip tube in Smart-DART and connect your smart phone software.

14. Start real-time PCR and get results real time and complete the reaction in 20 min.

15. Email results as needed.

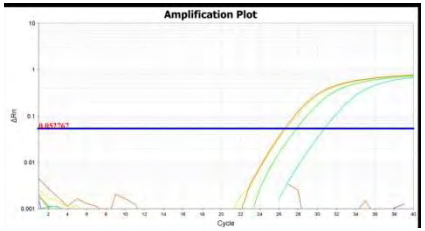


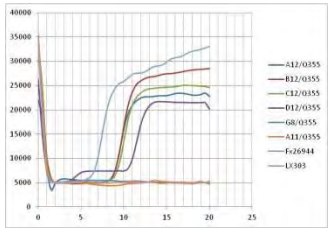
Comparison of standard qPCR with LAMP-PCR using two units of SmartDART

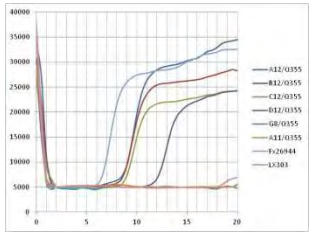
Smart DART/LAS v1.0

- DNA of single psyllids from Pakistan (4 Las positive and 2 Las negative) along with pos and negative controls were used.

Sample	Extrn	CT16s	SmartDART 1(min)	SmartDART 2(min)
PK psyllid1	Qia	26.554	9	7
PK psyllid2	Qia	26.760	9	7
PK psyllid3	Qia	27.810	9	7
PK psyllid4	Qia	30.708	12	13
PK psyllid5	Qia	40	0	0
PK psyllid6	Qia	40	0	0
W navel FP POS	Qia	25	7	7
W navel Riv NEG	Qia	40	0	0







Qiagen DNA from QMAG355
Pakistan single psyllids
A12, B12, C12, D12, G8, A11,
FX26944, LX303
QPCR from ABI Pakistan #7/Apr5, 13
SmartDART1 **LAS__2013-05-06 16_45_38.csv**
SmartDART2 **Detect__2013-05-06 16_47_50.csv**

Effect of pooling multiple psyllids

Smart DART/LAS v1.0

Category	infected D Hall	heallthy UCR	LAMP Extractions	POS	PERCENT	Min_threshold
1	1	0	24	9	37.5	8-14
2	1	4	16	2	12.5	9-11
3	1	9	16	4	25	9-12
4	1	19	16	3	18.75	12-20
5	0	1	16	0	0	0

In this study single psyllids from Las positive colony are mixed with varying numbers of healthy psyllids (from UCR quarantine).

Pooling experiments were not done until recently because of shortage of psyllid samples with high numbers of Las positives.

We have supply of Las positive psyllids from D Hall, and will pursue this study.

Conclusions

Smart DART/LAS v1.0

1. Smart-DART/LAS v1.0 provides a long awaited easy-to-use method that can be used to test psyllids for LAS at very low cost.

2. Please note that this is still in trial version and the methodology is in the field validation process.

3. Future versions would include detection of host gene for quality control, development of methods to test plants and providing a simple database for data management at the grower's level.