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RT-LAMP for BYDV-PAS and BYDV-MAV detection

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We are still developing and optimizing this protocol

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Abstract

A rapid test was developed primarily to support QC of lab colonies, and testing colonies prior to starting lab experiments. It could also be utilised for general BYDV testing of aphids from trap samples for rapid surveillance. An RT-LAMP assay was developed to detect the presence of *Luteovirus pashordei* (BYDV-PAS) and *Luteovirus mavhordei* (BYDV-MAV) in samples - these are the two BYDV species routinely utilised in our lab experiments.

Safety warnings

- While the assay was designed to detect BYDV-MAV - it will also likely detect BYDV-PAV. The assay was designed in target regions to distinguish between BYDV-MAV and BYDV-PAS.

Primers

1 The primers for RT-LAMP were designed for *Luteovirus mavhordei* from the sequence OQ686659, and for *Luteovirus pashordei* from the sequence OQ686684.

	Primer Name	Sequence (5' - 3')	Scale	Desalting
	F3-MAV001	ACAGCTTCACAGAGGTCAAG	25nm	STD
	B3-MAV001	ATGGTGGCAAGAGACAGTTC	25nm	STD
	FIP-MAV001	TTCCATGATGGGCTCGAGGTGTGCCTCATGAAGGAGGTCGT	25nm	STD
	BIP-MAV001	GCCGCCTCCACCTGGATAACATCTGCGTTAAGCGTTGAGT	25nm	STD
	LF-MAV001	TCTTCTTCGCCGTTCTTCTCCT	25nm	STD

Primers as ordered from Integrated DNA Technologies (IDT) for *Luteovirus mavhordei* (BYDV-MAV) assay.

	Primer Name	Sequence (5' - 3')	Scale	Desalting
	F3-PAS001	GGGTTTTTAGAGGGGCTCTG	25nm	STD
	B3-PAS001	TGTCCCCCTTACCAACTGTA	25nm	STD
	FIP-PAS001	CGGGCTCCGTCTGTGACCTTAAGCCTCCTCCGGTTTTGAAAG	25nm	STD
	BIP-PAS001	CCTAACCCAAACCCACCTCGGCTGCAACCAAGGCATTGTG	25nm	STD
	LF-PAS001	CCGGCAGTCCTAATAGAGAAAAGG	25nm	STD

	Primer Name	Sequence (5' - 3')	Scale	Desalting
	LB-PAS001	GGTATACAAAGCC CCAAACGCC	25nm	STD

Primers as ordered from Integrated DNA Technologies (IDT) for *Luteovirus pashordei* (BYDV-PAS) assay.

2 A 10X LAMP primer mix was prepared for each of the two assays according to the following tables:

	Primer Name	10X stock (µM)	1X reaction (µM)
	F3-MAV001	2	0.2
	B3-MAV001	2	0.2
	FIP-MAV001	16	1.6
	BIP-MAV001	16	1.6
	LF-MAV001	4	0.4

Preparation of primer mix for *Luteovirus mavhordei* (BYDV-MAV) assay. This is labelled as **MAV001 10X** mix.

	Primer Name	10X stock (µM)	1X stock (µM)
	F3-PAS001	2	0.2
	B3-PAS001	2	0.2
	FIP-PAS001	16	1.6
	BIP-PAS001	16	1.6
	LF-PAS001	4	0.4
	LB-PAS001	4	0.4

Preparation of primer mix for *Luteovirus pashordei* (BYDV-PAS) assay. This is labelled as **PAS001 10X** mix.

Gene Fragments

3 A gene fragment was synthesised for each target region to act as a positive control.

gBlocks.txt 908B

These were ordered from Integrated DNA Technologies and diluted to 10 ng/µl with IDTE ([M] 10 millimolar (mM) Tris, [M] 0.1 µg/µL EDTA). Working solutions for testing were prepared by carrying out serial dilutions of the stock.

RT-LAMP Reaction

4 Thaw all components at RT and place on ice (WarmStart Colorimetric LAMP 2X Master Mix; 10X LAMP primer mix; Target RNA or gBlock (0.1 ng/µl) controls.

5 Prepare reaction mix according to table below for the number of target samples (plus positive control(s) and no template and/or negative control(s)):

	Component	Amount (µl)
	WarmStart Colorimetric LAMP 2X Master Mix	12.5
	LAMP Primer Mix (10X) [MAV0001 or PAS0001]	2.5
	dH2O	9

Vortex reaction mix and briefly spin contents down in centrifuge.

6 Pipette 24 µL into PCR 8-strips (e.g. 72.991.002 from Sarstedt) and add 1 µl of target RNA, gBlock, control, or water for no-template-control. Mix by pipetting up and down, and briefly centrifuge to collect contents. Confirm that reaction solutions have a bright pink color, which indicates an initial high pH, which is required for a successful pH-LAMP reaction.

7 Seal reaction vessels.

8 Incubate at 65 °C in a thermocycler or heating block for 30 minutes.

9 Remove tubes or vessels from incubation and examine by eye. Positive reactions will have turned yellow while negative controls should remain pink. If color change is not robust, e.g. an orange color is visible, return reactions to 65°C for an additional 10 minutes. When extending the reaction time it is important to monitor the no-template and negative controls closely to ensure false positive reactions are not present. Color will be visible directly on removal from incubation temperature, but can be intensified by allowing the reaction to cool to room temperature.

10 The result can be photographed to record the colorimetric results, or simply kept at room temperature in the reaction vessel.