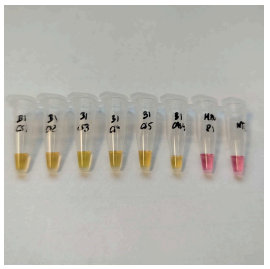
May 07, 2025

RT-LAMP for BYV detection



DOI
https://dx.doi.org/10.17504/protocols.io.261geeo1og47/v1

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Protocol Citation: Marta Niedzicka, Stephen Byrne 2025. RT-LAMP for BYV detection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261geeo1og47/v1

Protocol status: Working
We use this protocol and it's working

Created: April 02, 2025

Last Modified: May 07, 2025

Protocol Integer ID: 125978

Keywords: beet yellows virus, BYV, RT-LAMP, plant virus, detecting beet yellows virus, symptomatic beet samples from ireland, byv genome, lamp for byv detection, symptomatic beet sample, byv detection, virus, byv, rna, easy colorimetric assay, yellow color, total rna, assembled genome, genome

Funders Acknowledgements:
Horizon Europe - Marie Sklodowska-Curie Actions
Grant ID: 101106728

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Abstract

This protocol describes the process of detecting beet yellows virus (BYV) in plant material utilising a quick and easy colorimetric assay. The starting point is total RNA extracted from the material. The positive results are shown in yellow color, while negative samples stay bright pink - an example of these results is provided at the end of the protocol. The basis for development of the assay was assembly of a BYV genome from symptomatic beet samples from Ireland - the sequence data and assembled genome have been deposited in a public repository.

Materials

WarmStart® Colorimetric LAMP 2X Master Mix **New England Biolabs Catalog #**M1800

Nuclease-free water **Merck MilliporeSigma (Sigma-Aldrich)**

Protocol materials

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Safety warnings

Please read SDS associated with various consumables and kits used in this protocol and wear appropriate PPE. A site specific procedural risk assessment should be carried out prior to introducing this protocol to the lab.

Before start

This protocol assumes that you have already isolated Total RNA from plant material.

Primers

1 Primers were designed using NEB LAMP Primer design tool: **https://lamp.neb.com/#!/**
The BYV genome sequence used for the design is deposited in GenBank (accession no. PV593736)

	A	B
	BYV_F3_P243L1	AGTCGATGAAAGAGTGATCG
	BYV_B3_P243L1	AGCAGTGTCAAACTTCTCG
	BYV_FIP_P243L1	ATGAATAGCACACAGGGCGGTGACGCATTTGGTTCACAG
	BYV_BIP_P243L1	TTGTACCCTAAAGTTAAGGGTTGGAACAGTTCGCGAATTTGC
	BYV_LF_P243L1	TCACCACTTTCGTACCCGTACTTA
	BYV_LB_P243L1	TACGGTAAACTGAAATTTGTGCTGC

Designed primers within ORF1a/b gene of BYV

gBlock sequence ordered and used for testing the primers:
CAAAGTTTTTCGTTATGACCGGTCACGACGTTTTCTTCGTGCCTGACCCTTATAAACTTTTA
GTGAAATTGGGAGCTTCTAAGGATGAAGTGGACGATGAGTTTCTGTTTGAAGTGTTCACCTC
TTTTCGCGATTTAACGAAAGATTTAGTCGATGAAAGAGTGATCGAACTCTTGACGCATTTGG
TTCACAGTAAGTACGGGTACGAAAGTGGTGACACGTACGCCGCCCTGTGTGCTATTCATTGT
ATTCGTTCAAACTTTTCATCGTTCAAGAAATTGTACCCTAAAGTTAAGGGTTGGGTCGTTCA
CTACGGTAAACTGAAATTTGTGCTGCGCAAATTCGCGAACTGTTTTCGCGAGAAGTTTGAC
ACTGCTTTCGGCGAAGCGTACTTTCTTACTTACGACGAAGCTTGAGACTGTGTGGTAACTC
GGTTGTTGTTTTTGTTTGTTTGTGTGTCTGTAGTCCTACTTCGCGGTGATGGACTGTGTACT
CCGCTC

Primer mix

2 We recommend making stockings of the LAMP primers at a useable concentrations. Here, we suggest a 10X Primer Mix containing all LAMP primers.

	A	B	C
	Primer	10X Concentration (Stock)	1X Concentration (Final)
	FIP	16 µl	1.6 µl
	BIP	16 µl	1.6 µl
	F3	2 µl	0.2 µl
	B3	2 µl	0.2 µl
	Loop F (LF)	4 µl	0.4 µl
	Loop B (LB)	4 µl	0.4 µl

LAMP Primer Mix

3 Prepare primer stocks in nuclease free water and store at -20 °C for up to 2 years.

Note: Make primer stock in molecular biology graded water rather than other buffer to avoid carryover of additional buffer to the LAMP reaction

Reaction mix

40m

4 Thaw all components to be used at Room temperature and place on ice. Vortex briefly to mix thoroughly. Centrifuge to collect material and place On ice.

Components:

- Target nucleic acid
- LAMP Primer 10X
- WarmStart® Colorimetric LAMP 2X Master Mix **New England Biolabs Catalog #**M1800
- Nuclease-free water **Merck MilliporeSigma (Sigma-Aldrich)**

5 Prepare reaction mix as described below using **Colorimetric LAMP MasterMix, LAMP primers** and **nuclease-free water** (1-3 positions from the table below) for your number of samples. The final volume with target included per sample is 25 µL

	A	B
	WarmStart Colorometric LAMP 2X Master Mix	12.5 µl
	LAMP Primer Mix 10X	2.5 µl
	dH2O	9 µl
	Target (DNA, RNA, or water for NTC)	1 µl
	Total volume	25 µl

Reaction mix per 1 sample - final concentration

6 Vortex reaction mix and centrifuge to collect material.

7 Pipet 24 µL per reaction into desired reaction vessels and add 1 µL of sample.
Mix by vortexing or by pipetting if using a plate or similar vessel, centrifuge to collect if necessary.
Check that reaction solutions have a bright pink color, which indicates initial high pH required for successful pH-LAMP reaction.

8 Seal reaction vessels.

9 Incubate at 65 °C for 00:30:00 . 30m

10 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 00:10:00 . Reactions can be examined earlier if desired, and high copy of input reactions can exhibit full color change in as little as 10–15 minutes. 10m

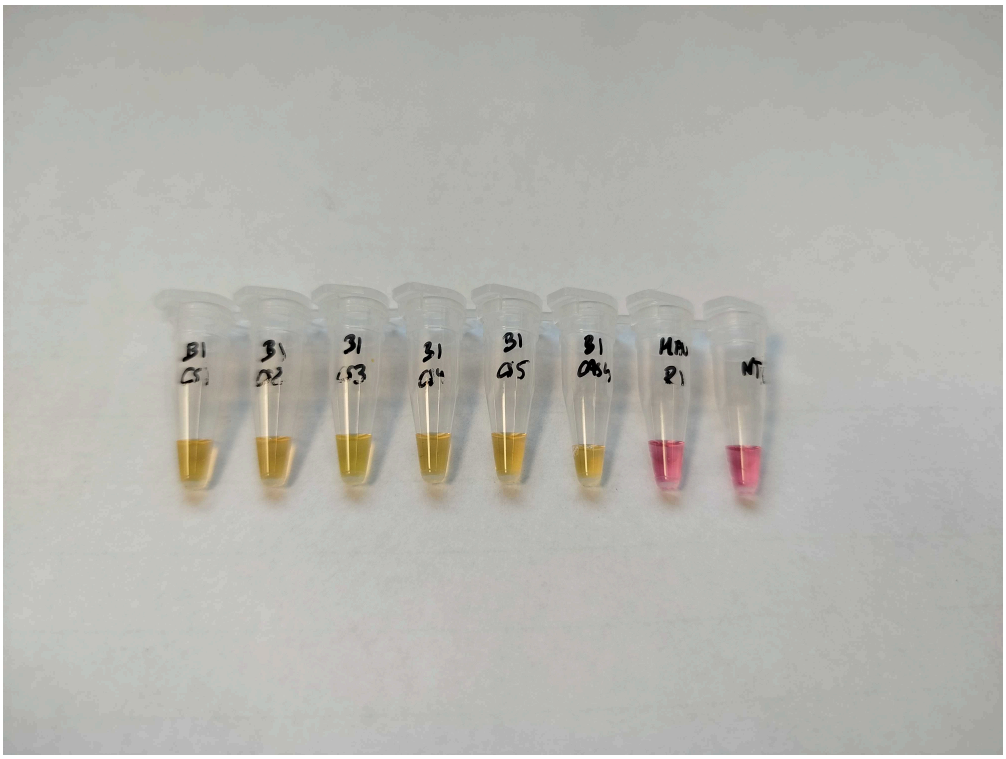
Color will be visible directly on removal from incubation temperature, but can be intensified by allowing reaction to cool to room temperature.

Results

40m

11 The result can be photographed or scanned to record the colorimetric results, or simply kept at room temperature in the reaction vessel (the colour change remains stable).

Example result after 00:30:00 incubation



Example RT-LAMP results: 6 positive samples, negative sample (RNA from barley infected with BYDV) and NTC