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Rapid On-Site Detection of Crop RNA Viruses Using CRISPR/Cas13a V.1

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

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Abstract

CRISPR/Cas technology is an emerging tool for the identification of nucleic acids sequences. Here, we present a user-friendly, extraction-free, rapid protocol for specific on-site detection of plant viruses using CRISPR/Cas13a.

Materials

Before starting:

- crRNAs – synthetic RNA oligos
- LbuCas13a
- RNaseAlert® substrate (IDT, Coralville, USA)
- Lysis buffer (15% polyethylene glycol 4000, 20 mM NaOH)
- **Reaction buffer:**

	A	B
	407 Hepes pH 6.8	20 mM
	KCl	50 mM
	MgCl2	5 mM
	Glycerol	5%

- A healthy plant to serve as control.

Equipment:

- P51™ Molecular Fluorescence Viewer from MiniPCR Bio (Cambridge, MA, USA) Mobile phone with camera

Nuclease assay:

Mix the following

	A	B
	Reaction buffer	5 µL
	RNPs	1 µL (100 nM final volume)
	RNaseAlert ® substrate (IDT, Coralville, USA)	1 µL (200 µM final volume)
	Crude RNA	3 µL

crRNAs

1 Design 23-long crRNAs that complement the RNA sequence of the virus of interest.

2 Order the crRNAs fused to the 3' end of the LbuCas13a stem (5'GACCACCCCAAAAAUGAAGGGGACUAAAAC 3').

3 Resuspend crRNA oligos with molecular grade water to [M] 1 micromolar (µM) .

RNP Assembly

30m

4 Mix crRNA with LbuCas13a at a 1:1 ratio. Incubate at Room temperature for 00:30:00 .

30m

5 RNPs can be stored at -20 °C for up to at least 6 months.

On-site: Crude RNA Extraction

5m 5s

6 Punch a 1 cm leaf disc and place in 300 µL of lysis buffer.

7 Incubate at Room temperature for 00:05:00 .

5m

8 Shake vigorously for 00:00:05 .

5s

Note

Use immediately or place On ice .

On site: Nuclease Assay

10m

9 Mix the following in a PCR tube:

	A	B
	Reaction buffer	5 µL
	RNPs	1 µL (100 nM final volume)
	RNaseAlert ‰ substrate (IDT, Coralville, USA)	1 µL (200 µM final volume)
	Crude RNA	3 µL

10 Incubate at Room temperature for 00:10:00 . 10m

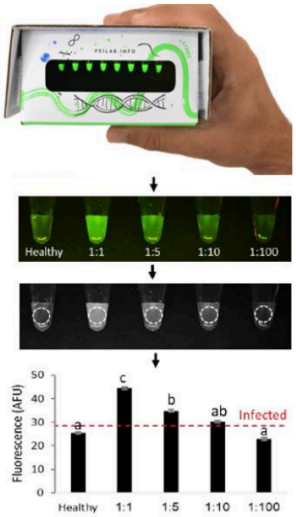
11 View fluorescence using P51™ Molecular Fluorescence Viewer. Since exposure to light interferes with fluorescence, it's best to place the Fluorescence Viewer in a dark box (we use a small shoebox).

12 Take a photo with any mobile phone camera. It is better to set the brightness to a medium level.

13 Compare fluorescence levels of tested plants with that of the control.

14 **Optional:** Fluorescence quantification using ImageJ (https://imagej.net/ij/).

Select an area of the liquid in the tube. Be careful not to include reflections of the LED lights. Measure grayscale by clicking Analyze → Measure. Compare Mean value with that of a control sample.


Healthy
1:1
1:5
1:10
1:100
Fluorescence (AFU)
50
40
30
20
10
0
a
c
b
ab
a
Infected
Healthy
1:1
1:5
1:10
1:100