

Submission ID #: 68841

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=20996553>

**Title: Combined Recombinase Polymerase Amplification
CRISPR/Cas12a Assay for Detecting *Fusarium oxysporum* f. sp.
cubense Tropical Race 4**

Authors and Affiliations:

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **12 February 2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 23

Number of Shots: 38

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Jos Jansen van Vuuren**: We are interested in rapid and accurate detection of the *Fusarium* wilt of banana pathogen, known as Foc TR4.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

AUTHOR'S NOTE: Shot in 3 takes

~~What technologies are currently used to advance research in your field?~~

- 1.2. **Jos Jansen van Vuuren**: The combined use of recombinase polymerase amplification and CRISPR negates the use of expensive machines and improves the accuracy of current assays.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

AUTHOR'S NOTE: Shot in 4 takes

CONCLUSION:

~~What significant findings have you established in your field?~~

- 1.3. **Jos Jansen van Vuuren**: We have developed the first assay that employs CRISPR technology for detection of Foc TR4.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1*

AUTHOR'S NOTE: Shot in 2 takes

~~What advantage does your protocol offer compared to other techniques?~~

- 1.4. **Jos Jansen van Vuuren**: Once optimised, the assay offers faster, more accurate detection on inexpensive machines than existing commercial assays.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

AUTHOR'S NOTE: Shot in 2 takes

~~What questions will future research focus on?~~

1.5. **Jos Jansen van Vuuren**: Our further work will explore a smartphone-linked fluorescent detector to be combined with the assay.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

AUTHOR'S NOTE: Shot in 4 takes

Protocol

2. Simplified DNA Extraction from Plant Material

Demonstrator: Jos Jansen van Vuuren

2.1. To begin, transfer 250 milligrams of each piece of symptomatic pseudostem into a separate extraction bag [1].

2.1.1. WIDE: Talent placing pieces of symptomatic pseudostem into individual extraction bags.

AUTHOR'S NOTE: Shot in 2 takes in different angles

2.2. Add 2 milliliters of 0.5 molar sodium hydroxide-polyvinylpyrrolidone solution into each extraction bag containing plant material [1]. Using a manual grinder, such as a pestle, grind the infected vessels thoroughly inside the extraction bag [2].

2.2.1. Talent pipetting 2 milliliters of sodium hydroxide-polyvinylpyrrolidone solution into the extraction bag.

AUTHOR'S NOTE: Shot in 1 take

2.2.2. Talent grinding the plant material inside the bag using a pestle.

AUTHOR'S NOTE: Shot in 2 takes in different angles

2.3. Then, add 195 microliters of 100 millimolar Tris-HCl buffer at pH 8 into 1.5-milliliter tube [1]. Pipette 5 microliters of the plant extract from the extraction bag into the 1.5-milliliter tube containing Tris-HCl buffer to dilute the extract 40-fold [2].

2.3.1. Talent pipetting 195 microliters of Tris-HCl buffer into multiple 1.5 milliliter microcentrifuge tube.

AUTHOR'S NOTE: Shot in 4 takes in different angles

2.3.2. Talent pipetting 5 microliters of extract into a tube containing Tris-HCl buffer.

AUTHOR'S NOTE: Shot in 5 takes including cutoff

2.4. Vortex the tube for 15 to 30 seconds [1] and store it at minus 20 degrees Celsius until further use [2].

2.4.1. Talent vortexing each microcentrifuge tube for 15 to 30 seconds.

AUTHOR'S NOTE: Shot in 1 take

2.4.2. Talent placing the tube inside a minus 20 degree Celsius freezer.

AUTHOR'S NOTE: Shot in 1 take

3. crRNA Synthesis and Quality Control

3.1. Using the RNA transcription kit, add the given components to a 0.2-milliliter PCR tube [1].

3.1.1. TEXT ON A PLAIN BACKGROUND

10x T7 Reaction buffer: 1.5 µL

dNTPs: 1.5 µL each

T7 DNA polymerase mix: 1.5 µL

Nuclease-free H₂O: 1 µL

100 µM T7 template DNA: 10 µL

Total Volume: 20 µL

3.2. Incubate the prepared tube at 37 degrees Celsius in a water bath or PCR machine for at least 4 hours [1].

3.2.1. Talent placing the PCR tube into a water bath/PCR machine set to 37 degrees Celsius.

AUTHOR'S NOTE: 6 Takes (different stations and angles)

Takes 1-2 = Water bath

Takes 3-6 = PCR machine

3.3. Now, use a spectrophotometer to quantify the transcribed RNA [1]. Dilute the RNA to a concentration of 250 nanograms per microliter, in a final volume of 40 microliters using either nuclease-free water [2].

3.3.1. Talent placing the RNA sample into the spectrophotometer and initiating the measurement.

AUTHOR'S NOTE: 4 Takes (different angles and result of ~ 4000 ng/uL before cleaning)

3.3.2. Talent pipetting nuclease-free water into the tube to dilute the RNA.

AUTHOR'S NOTE: 3 Takes including a cutaway

3.4. Then, treat the RNA sample with DNase I (*D-N-aze-1*) provided in the RNA cleaning kit, following the manufacturer's instructions [1].

3.4.1. Talent adding DNase I to the RNA sample.

AUTHOR'S NOTE: 3 Takes

3.5. After that, clean the RNA sample using the RNA cleaning kit [1].

3.5.1. Talent pipetting the clean-up reagent in the tube with the RNA cleaning kit placed in the frame.

AUTHOR'S NOTE: 2 Takes for reagent 1 (RNA Binding Buffer)

1 Take for reagent 2 (100% EtOH)

3 Takes for reagent 3 (RNA Prep Buffer)

1 Take for reagent 4 (RNA Wash Buffer)

1 Take for reagent 5 (Water)

3.6. Use a Nanodrop device to determine the quantity and quality of the cleaned crRNA [1]. Then, dilute the RNA to 100 nanograms per microliter with nuclease-free water [2-TXT].

3.6.1. Talent placing the cleaned RNA on the Nanodrop device and reviewing the results.

AUTHOR'S NOTE: 4 Takes (different angles)

Note: Screen shows ~ 100 ng/uL instead of ~ 250 ng/uL according to the protocol

3.6.2. Talent diluting the RNA with nuclease-free water. **TXT: Store the RNA sample at -80 °C until use**

AUTHOR'S NOTE: 2 Takes(Different angles)

3.7. Now heat the crRNA (*C-R-R-N-A*) mixed with RNA loading dye to 70 degrees Celsius for 10 minutes in a water bath [1] and run the sample on a 1.5 percent agarose gel to visualize the crRNA through electrophoresis [2].

3.7.1. Talent placing the RNA sample with loading dye into a water bath set to 70 degrees Celsius for 10 minutes.

AUTHOR'S NOTE: 2 Takes(Different angles)

3.7.2. Talent loading the sample onto a 1.5 percent agarose gel and initiating electrophoresis.

AUTHOR'S NOTE: 4 Takes

Take 1-3 = Bottom angle

Take 4 = Top angle

4. Recombinase Polymerase Amplification (RPA)-Cas12a One-Tube Reaction for In Vitro Foc TR4 Detection

- 4.1. Prepare 20 microliters of Cas12a (*Cas-Twelve-A*) reaction mixture in a separate 0.5-milliliter tube using the given components [1].

4.1.1. TEXT ON A PLAIN BACKGROUND

10x NEBuffer r2.1: 2 μ L
10 μ M LbCas12a protein: 0.4 μ L
100 ng/ μ L crRNA: 0.6 μ L
10 μ M reporter: 0.4 μ L
Deionized water: 16.6 μ L

AUTHOR'S NOTE: Additional footage:

4.1.1.. Take 1-3 (pipetting CRISPR reagent into a clean 0.5 mL tube from different angles)

4.1.1. Take 4 (CRISPR reagents in ice box)

- 4.2. Next, prepare 9.5 microliters of RPA reaction mixture in a separate 0.5-milliliter tube [1] with the components shown here [2].

4.2.1. Talent pipetting a reagent into a clean 0.5 milliliter tube.

AUTHOR'S NOTE: 2 takes (different angles)

Additional footage:

4.2.1. Take 3 (RPA reagents in ice box)

4.2.1. Take 4 (RPA and CRISPR reagents in ice box)

4.2.1. Take 5 (CRISPR reaction mix tube)

4.2.1. Take 6 (RPA reaction mix tube)

4.2.2. TEXT ON A PLAIN BACKGROUND

2x Reaction Buffer: 5 μ L
dNTP Mix: 1.6 μ L each
10x Basic E-Mix : 1 μ L
RPA primer at 10 μ M: 0.5 μ L each
20x Core Reaction Mix: 0.5 μ L
Deionized water: 0.4 μ L

- 4.3. Place an 8-tube strip into a tube rack set on ice [1] and transfer the 20 microliters of

Cas12a reaction mixture into the base of each tube in the strip [2].

4.3.1. Talent positioning the 8-tube strip into the rack on an ice-filled container.

AUTHOR'S NOTE: 2 Takes(Different angles)

4.3.2. Talent pipetting the Cas12a reaction mix into the bottom of each tube in the 8-tube strip.

AUTHOR'S NOTE: 2 Takes(Different angles)

4.4. Then, transfer the 9.5 microliters of RPA reaction mixture into the lid of each tube in the strip [1]. Add 0.5 microliters of 280 nanomolar magnesium acetate to the RPA mix in the lid and mix by pipetting up and down [2].

4.4.1. Talent pipetting the RPA mixture into the lids of each tube.

AUTHOR'S NOTE: 2 Takes(Different angles)

4.4.2. Talent adding magnesium acetate and mixing the solution by pipetting up and down in the lid.

AUTHOR'S NOTE: 3 Takes(Different angles)

4.5. Next, add 0.5 microliters of deionized water to the lid of the non-template control tube and close it carefully to prevent the RPA and Cas12a reaction mixtures from mixing [1].

4.5.1. Talent pipetting deionized water into the non-template control tube lid and sealing it securely.

AUTHOR'S NOTE: 3 Takes(Different angles): Take 2 – the cap wasn't sealed

4.6. Now, add 0.5 microliters of template DNA to the 10 microliters of RPA reaction mixture containing magnesium acetate in the tube strip lid [1-TXT]. Mix the contents by pipetting up and down [2].

4.6.1. Talent pipetting 0.5 microliters of template DNA into the lids of tubes containing RPA reaction mix, omitting the non-template control. **TXT: For NTC, add deionized H₂O; Add Foc TR4 DNA in one well as a +ve control**

AUTHOR'S NOTE: 2 Takes

Take 1 – each cap closed individually after DNA is added

Take 2 – the 4 tubes with negative DNA are added and caps closed, then the 3 tubes with positive DNA are added and caps closed.

Tube 1 = NTC

Tubes 2-5 = Negative DNA

Tubes 6-8 = Positive DNA

This is according to the tube strip used in the protocol. We made it the same for demonstration purposes. Therefore there are 3 wells for positive DNA and not just 1.

Talent pipetting up and down to mix the contents in each tube lid.

AUTHOR'S NOTE: 3 Takes(Different angles)

- 4.7. Carefully close all tube strip lids to ensure that the RPA and Cas12a reaction mixtures do not mix [1].

- 4.7.1. Talent gently closing the lids of the 8-tube strip to keep the reaction mixtures separated.

AUTHOR'S NOTE: 1 Take (the other takes are combined with Step 4.6.1.); Additional footage:

4.7.1. Take 2 (Showing no run down of the top reaction mix into the bottom reaction mix)

- 4.8. Afterward, switch on the portable fluorometer and create a **Run profile** for the RPA-Cas12a assay [1]. Open the saved **Run profile** [2] and place the tube strip inside the fluorometer to incubate for 30 minutes at 37 degrees Celsius [3].

- 4.8.1. Show the fluorometer display as the talent navigates the menu to create the Run Profile for the RPA-Cas12a assay.

AUTHOR'S NOTE: 1 Take

- 4.8.2. Talent opens the saved run profile

AUTHOR'S NOTE: 1 Take

- 4.8.3. Talent placing the tube strip into the fluorometer and starting the 30-minute incubation at 37 degrees Celsius.

AUTHOR'S NOTE: 2 Takes

- 4.9. After 30 minutes, remove the tube strip from the fluorometer [1]. Invert the tubes six times to mix the RPA and Cas12a reactions [2] and briefly spin the strip down in a mini centrifuge for 5 seconds [3].

- 4.9.1. Talent taking the tube strip out of the fluorometer.

AUTHOR'S NOTE: 1 Take

- 4.9.2. Talent inverting the tubes to allow mixing of the contents.

AUTHOR'S NOTE: : 1 Take

- 4.9.3. Talent placing the tube strip in a mini centrifuge and spinning it for 5 seconds.

AUTHOR'S NOTE: 2 Takes (Take 1: Not spun long enough)

4.10. Then, return the tube strip to the fluorometer and incubate for another 30 minutes at 37 degrees Celsius using the saved Run profile [1].

4.10.1. Talent placing the mixed and spun-down tube strip back into the fluorometer and starting incubation.

AUTHOR'S NOTE: 2 Takes

Take 1 – spun down tube, put back into fluorometer, but did not start incubation

Take 2 – spun down tube, put back into fluorometer, did start incubation

4.11. Interpret the average relative fluorescence units measured on the portable fluorometer [1].

4.11.1. Show the fluorometer display with average relative fluorescence units.

AUTHOR'S NOTE: 4 Take; Take 1 – no highlighting

Take 2 – highlighting all samples

Take 3 – highlighting NTC and negative samples

Take 4 – highlighting positive samples

4.12. Finally, visualize the reaction tubes under LED blue light at an excitation wavelength of 465 nanometers using a blue light transilluminator [1] and capture images of the fluorescent signal using a phone camera [2].

4.12.1. Talent placing the tube strip on the blue light transilluminator.

AUTHOR'S NOTE: 1 Take; Additional Footage: 4.12.1. Take 2 (showing tubes with the lights off)

4.12.2. Talent using a phone camera to take images of the illuminated reaction tubes.

AUTHOR'S NOTE: 3 Takes(Different angles)

Results

5. Results

- 5.1. Positive samples produced visible fluorescence in the Genie III (*Three*) portable fluorometer following RPA-Cas12a detection Foc TR4 (*F-O-C-T-R-4*) DNA [1], while negative samples yielded low endpoint fluorescence [2].
 - 5.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the upward fluorescence amplification curves and bars labeled 6, 7, and 8 on the left and right graphs.*
 - 5.1.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the flat or low-level curves labeled 1 to 5 on the left and right graphs.*
- 5.2. When viewed under LED blue light, the sample tubes containing DNA from Foc TR4-infected plant material displayed visible fluorescence [1], while tubes with DNA from uninfected tissue did not show any fluorescence [2].
 - 5.2.1. LAB MEDIA: Figure 4B. *Video editor: Highlight tubes 6, 7, and 8 with visibly glowing contents.*
 - 5.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight tubes 1 to 5, which show no visible glow.*

Pronunciation Guide:

Recombinase

Pronunciation link: <https://www.merriam-webster.com/dictionary/recombinase>

IPA: /ri:'kɑ:mbəˌneɪs/

Phonetic Spelling: ree·kom·buh·nays

Polymerase

Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>

IPA: /pə'liːməˌreɪs/

Phonetic Spelling: puh·lim·uh·rays

Amplification

Pronunciation link: <https://www.merriam-webster.com/dictionary/amplification>

IPA: /ˌæmpləfə'keɪʃən/

Phonetic Spelling: am·pluh·fuh·kay·shuhn

CRISPR

Pronunciation link: <https://www.merriam-webster.com/dictionary/CRISPR>

IPA: /'krɪspər/

Phonetic Spelling: kris·per

Cas12a

Pronunciation link: No confirmed link found

IPA: /kæz twelv eɪ/

Phonetic Spelling: kaz·twelv·ay

Fusarium

Pronunciation link: <https://www.merriam-webster.com/dictionary/Fusarium>

IPA: /fjuː'zæəriəm/

Phonetic Spelling: fyoo·zair·ee·uhm

Oxysporum

Pronunciation link: <https://www.merriam-webster.com/dictionary/oxysporum>

IPA: /ˌɑːksi'spɔːrəm/

Phonetic Spelling: ok·see·spor·uhm

Cubense

Pronunciation link: No confirmed link found

IPA: /kjuː'benseɪ/

Phonetic Spelling: kyoo·ben·say

Pseudostem

Pronunciation link: <https://www.merriam-webster.com/dictionary/pseudostem>

IPA: /'suːdɒʊˌstem/

Phonetic Spelling: soo·doh·stem

Polyvinylpyrrolidone

Pronunciation link: <https://www.merriam-webster.com/dictionary/polyvinylpyrrolidone>

IPA: /ˌpɑːliˌvaɪnəlˌpɪrə'liːdoʊn/

Phonetic Spelling: pol·ee·vy·nuhl·pir·uh·lih·dohn

Spectrophotometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>

IPA: /ˌspektrəfoʊ'tɑːmətər/

Phonetic Spelling: spek·truh·foh·tom·uh·ter

DNase

Pronunciation link: <https://www.merriam-webster.com/dictionary/DNase>

IPA: /'diː,ɛn,ɛz/

Phonetic Spelling: dee-en-ayz

Electrophoresis

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>

IPA: /ɪ,lektroʊfə'riːsɪs/

Phonetic Spelling: ih-lek-troh-fuh-ree-suhs

Nanodrop

Pronunciation link: <https://www.merriam-webster.com/dictionary/Nanodrop>

IPA: /'nænəʊ,drɑːp/

Phonetic Spelling: nan-oh-drop

Fluorometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorometer>

IPA: /flʊ'rɑːmɛtər/

Phonetic Spelling: floor-om-uh-ter

Fluorescence

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>

IPA: /flʊ'rɛsəns/

Phonetic Spelling: floor-es-uhns

Transilluminator

Pronunciation link: <https://www.merriam-webster.com/dictionary/transilluminator>

IPA: /ˌtrænzɪ'luːmə,neɪtər/

Phonetic Spelling: tranz-ih-loo-muh-nay-ter

Fluorometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorometer>

IPA: /flʊ'rɑːmɛtər/

Phonetic Spelling: floor-om-uh-ter