Instructions for Use of LAMP Primers and Positive Control for Detection of Banana Wilt Pathogen (Foc)

1. Preparation of LAMP Primer*1 and Positive Control for Banana Wilt Pathogen Detection

1.1 Preparation of Primer Solutions (100 µM each)

Add the required volume of 10 mM Tris/HCl (pH 8.0) Buffer *2 to each primer tube to prepare the primers at 100 µM, according to the Data Sheet. After vortex, centrifuge briefly (spin down).

*1: This product was jointly developed with Tokyo University of Agriculture and Technology.

*2: Use molecular biology grade reagents.

1.2 Preparation of Primer Mix

Add the following volumes of each 100 µM primer solution prepared in Section 1.1 into a sterile microtube. Vortex and briefly centrifuge. The resulting solution is referred to as the 「**10× Primer Mix**」. Store at −20℃ after preparation.

	1 Test	**100 Tests**
100 µM FIP	0.40 µL	40 µL
100 µM BIP	0.40 µL	40 µL
100 µM F3	0.05 µL	5 µL
100 µM B3	0.05 µL	5 µL
100 µM LF	0.20 µL	20 µL
100 µM LB	0.20 µL	20 µL
ddWater	1.20 µL	120 µL
Total Volume	**2.50 µL**	**250 µL**

1.3 Preparation of Positive Control

Add 10 µL Positive Control Dissolve Solution, centrifuge briefly, and leave at room temperature for 5 minutes. Vortex again and briefly centrifuge. The solution is then ready for use as the 「**Positive Control**」.

The positive control tube contains 5×10^8 copies of each target DNA fragment for the *Foc* primers. Store at −20℃ after preparation.

1.4 Processing Banana Samples

Extract DNA using a commercially available kit. For a simplified DNA extraction protocol from plant tissues, please contact Nippon Gene Co., Ltd.

The following describes the procedure for use with DryADD™ LAMP Master Mix (Turbidity/Visible Dye). For further details, please refer to the Instructions for Use.

2. Preparation of Reaction Mixture

2.1 Preparation of Master Mix

Prepare the required amount of reagents according to the number of tests in a reagent preparation tube, and dispense 23 µL into each DryADD™ LAMP Master Mix tube. **Perform all procedures on ice.**

	Per Test	8+1 Tests
10× Primer Mix	2.5 µL	22.5 µL
LTV Dissolve Solution*3	5.0 µL	45.0 µL
ddWater	15.5 µL	139.5 µL
Total Dry Reagent Dissolve Solution	**23.0 µL**	**207.0 µL**

*3: This solution is included in DryADD™ LAMP Master Mix (Turbidity/Visible Dye).

2.2 Dissolving the Dry Reagents

After dispensing, close the caps and allow the tubes to stand for 2 minutes to dissolve the dried reagents.

2.3 Addition of Template DNA

Add 2.0 µL template DNA and close the cap. Mix by gentle inversion and centrifuge briefly. Avoid generating bubbles during mixing.

To prevent concentration of the reaction mixture due to evaporation, add 20.0 µL Mineral Oil. If using a thermal cycler equipped with a heated bonnet, mineral oil is not required.

Caution:

When using Mineral Oil, ensure that the dried reagents have completely dissolved before adding it. Contact between Mineral Oil and undissolved dried reagents may render the reagents insoluble.

3. Assay Procedure

3.1 LAMP Reaction

Incubate the prepared tubes for 60 minutes at the specified temperature using an incubator or turbidity measurement instrument.

Primer	Reaction Temperature (℃)
Foc Ce15	64.5
Foc SIX8	64.5
Foc SIX7	64.5
Foc SIX6	64.0

3.2 Termination of the LAMP Reaction

Stop the reaction by heating at 80℃ for 2 minutes.

4. Interpretation of Results

Observe the color change of the reaction mixture.

- **Positive:** The solution changes to a **bright yellow-green** color.
- **Negative:** The solution remains **pale red** with no color change.

5. Race Identification

The pathogen race is determined based on the gene retention pattern indicated by each primer result.

Fusarium oxysporum f. sp. *cubense*	*Foc Ce15*	*Foc SIX6*	*Foc SIX7*	*Foc SIX8*
Race 1	+	−	−	−
Race SR4	+	−	+	+
Race TR4	+	+	−	+
Other variants	−	±	±	±