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Development of a reverse transcriptase loop mediated isothermal amplification (RT-LAMP) method for detection of *Sugarcane yellow leaf virus* (SCYLV)

Emmanuel Fernandez

Mixed Research Unit in Plant-Pathogen Interactions
Cirad-Inra-Montpellier SupAgro, Montpellier-France

Amplification method: (Notomi et al. 2000 – Eiken Chemical Co., Ltd)

Isothermal amplification: Incubation at a single temperature (60 to 65°C)

PCR machine 96 well

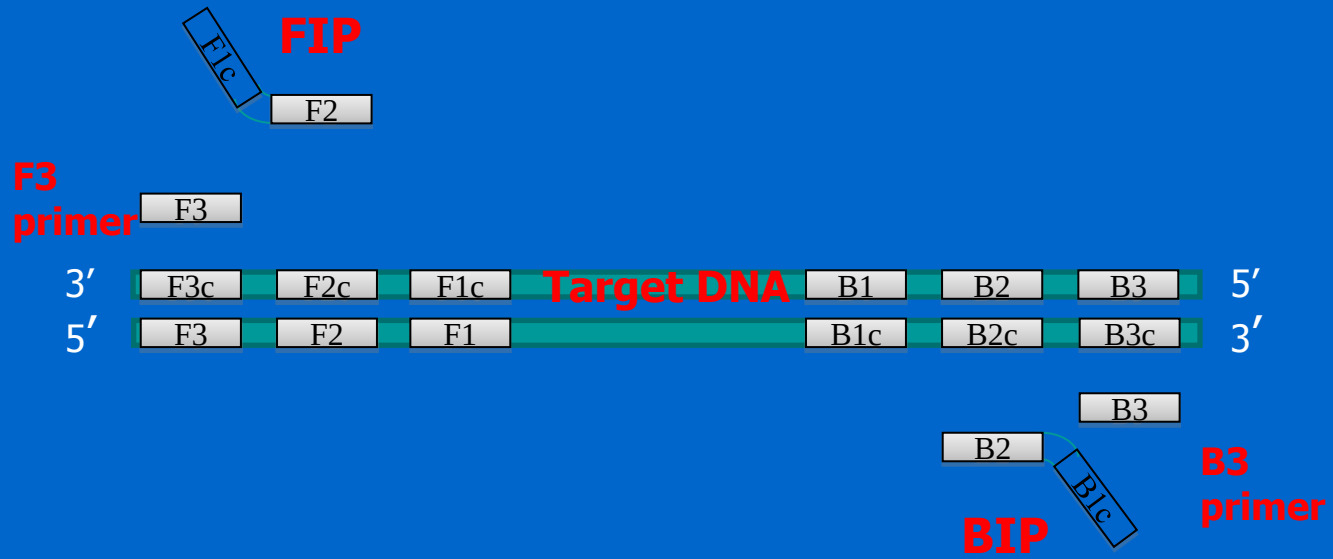
Incubator

Dry bath 96 well

Water bath

Amplification methods:

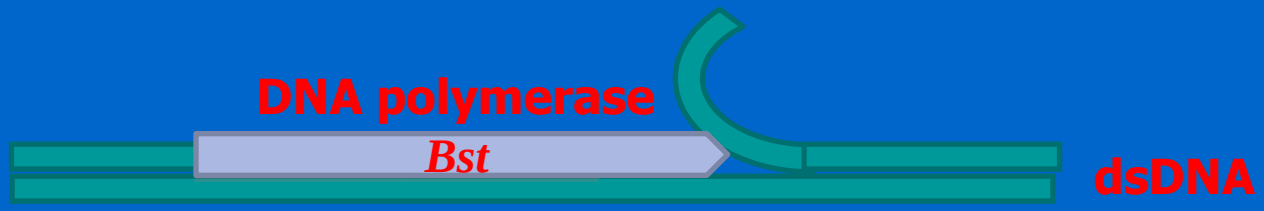
Use of 4 different primers: recognition of 6 distinct locations on the target gene



high specificity of the amplification

Amplification methods:

DNA polymerase with high strand displacement activity (Bst DNA pol)



DNA is amplified up to 10^9 to 10^{10} copies of target in 15 - 60 min

RNA can be amplified by adding reverse transcriptase enzyme

Amplification methods:

Tolerates low concentrations of PCR inhibitors

Works with rapid extraction methods

Closed system:

Reduced level of contamination post amplification

The principle of LAMP method:

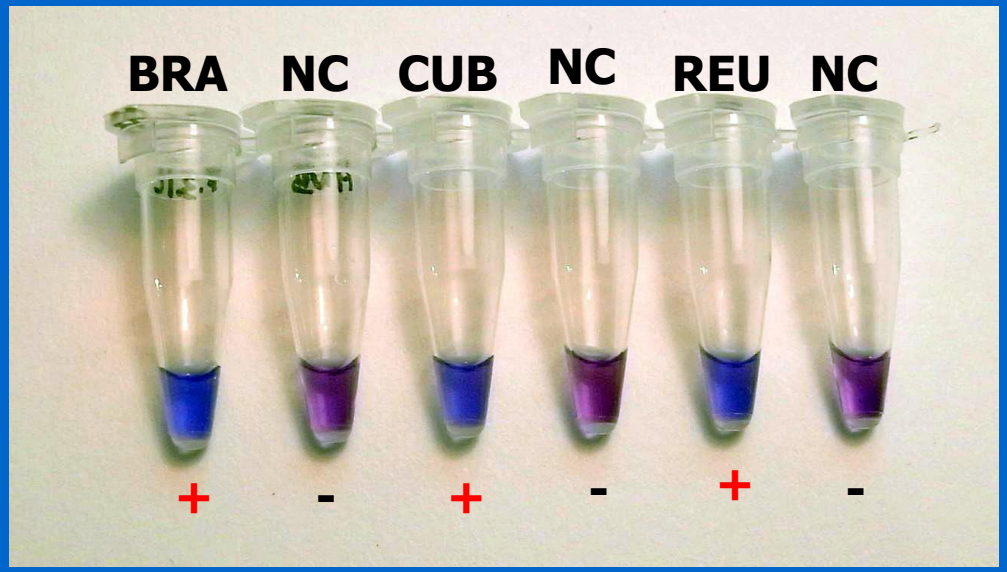
http://loopamp.eiken.co.jp/e/lamp/index.html

Detection methods:

Colorimetric detection of RT-LAMP by using Hydroxy Naphtol Blue (HNB)

HNB: titration of [Mg^{2+}] in a solution : modifies the color of the mix solution

RT-LAMP amplification:
pyrophosphate + Mg^{2+} = non soluble Mg-pyrophosphate



Detection of 3 strains of SCYLV by RT-LAMP /HNB

Perspectives

1- To determine the specificity and the sensitivity of the detection method

All the SCYLV genotypes and other polerovirus species

2 - To compare the reliability and the cost of this method to our classical methods of diagnosis (End-point RT-PCR / TBIA)

3 - To assess portability of this method at the laboratory and field levels

Thanks

Emmanuel Fernandez

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