Development of the loop mediated isothermal amplification (LAMP) method for detection of *Cauliflower mosaic virus*

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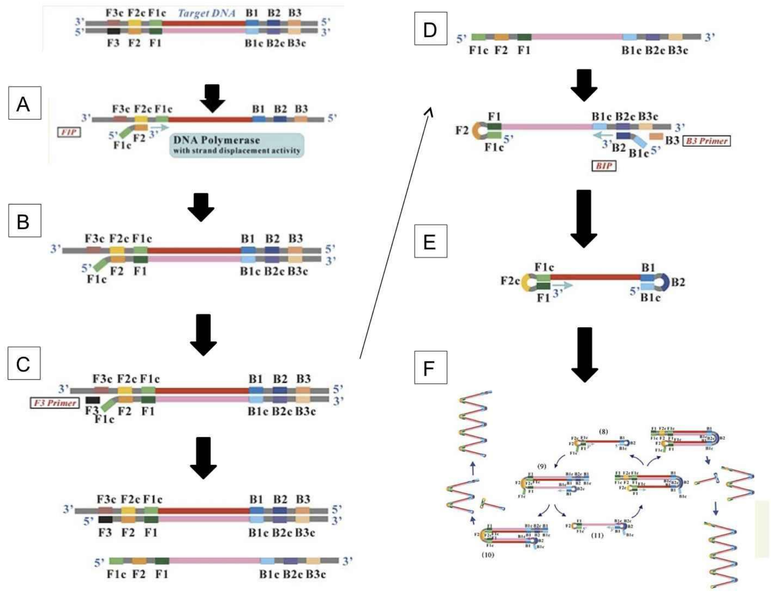
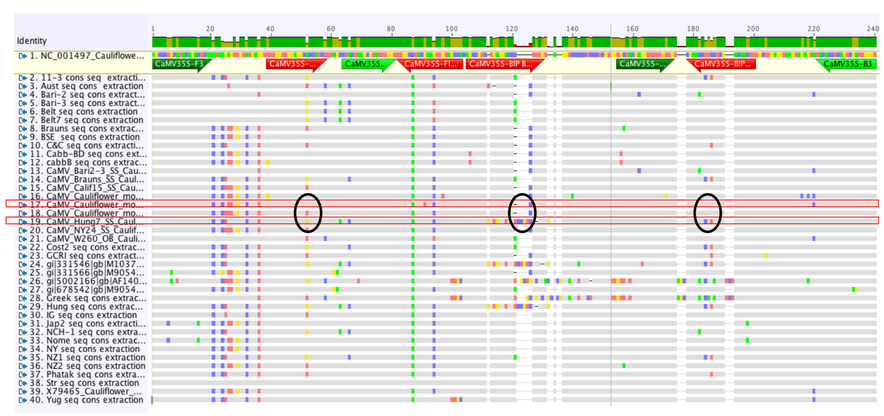
Are these plants infected by CaMV ?

Method

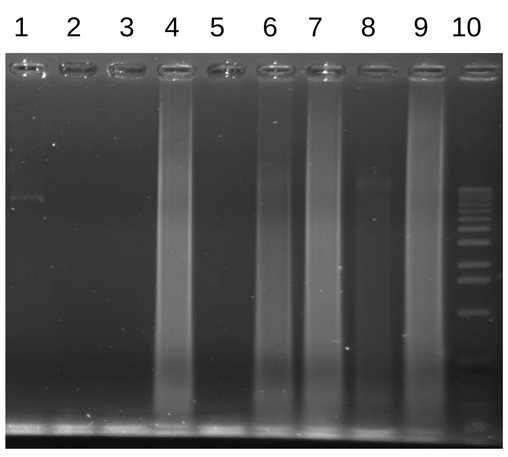
The principle of the Isohermal Loop-Mediated Amplification (LAMP; Notomi et al, 2000) is to amplify the target DNA under isothermal conditions (60 ° C to 65 ° C) for about an hour with four primers that will recognize six regions of the target DNA. When amplifying DNA, structures called dumbbell-like are made and the hybridization of the different primers happen as DNA polymerizes. Such process allows the amplification to take place continuously. The LAMP process thus produces concatenated DNA stem-loop structures with different sizes. Thus, one way of revealing if LAMP happend is to observe smear on an agarose gel. Other ways of revealing the amplification are to use colorimetric tests such as Hydroxy-naphtol Blue (HNB) which turns blue when LAMP happens (due to pyrophosphate production during the reaction).



Results



Alignment of CaMV 35S promoter sequences with representation of the LAMP primer (green & red arrows on top of sequences) designed by Fukuta et al. 2004
Sequences 17: Bji, Sequence 19: Hung7



1: negative control - plasmide pGreen (without 35S promoter)
2: negative control - H_2O
3: positive control - transgenic plant with the 35S promoter
4: negative control - Arabidopsis thaliana col-0
5: Brassica rapa infected by the CaMV isolate « Hung »
6: Brassica rapa infected by the CaMV isolate « B-ji »
7: Brassica rapa infected by the TuMV
8: positive control - plasmide pCambia (with 35S promoter but without CaMV)

LAMP amplification revealed after agarose gel electrophoresis

1: Brassica rapa infected by the CaMV isolate « Hung »
2: pUC19 + CaMV genome (CaMV isolate « Hung »)
3: negative control - H_2O

LAMP amplification revealed by colorimetric test (HNB)

Conclusion

The Isohermal Loop-Mediated Amplification (LAMP) works on *Cauliflower mosaic virus* 35S promoter and is robust face to viral polymorphism.

Reference

Notomi *et al.* 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. **28**: 63
Fukuta *et al.*, 2004 Real-time loop-mediated isothermal amplification for the CaMV-35S promoter as a screening method for genetically modified organisms. Eur. Food Res. Technol. **218:** 496-500