Loop-mediated isothermal amplification of DNA

Paper Notomi *et al*. 2000, *Nucleic Acids Res*. 28, e63

LAMP
Specific, fast, efficient (10^9 copies in 1 h), isothermal!
High selectivity because 4 primers recognize 6 target sequences.

DNA polymerase with strand displacement activity (***B****acillus* ***st****earothermophilus*)

Efficiency increased by:
The addition of 0.5-1.5 M betaine (N,N,N-trimethylglycine) – reduces base stacking

Not for long targets – 300 bp is the limit

Not for multiple targets, no downstream applications and reconfirmation (e. g. product sequencing).

RNA templates can be used along with reverse transcriptase.

Simple to perform, no machinery needed.

Accelerated LAMP

Paper Nagamine *et al*. 2002, *Molecular and Cellular Probes* 16, 223-229.

The use of additional primer pair (loop primers) is the basis for „acceleration”.

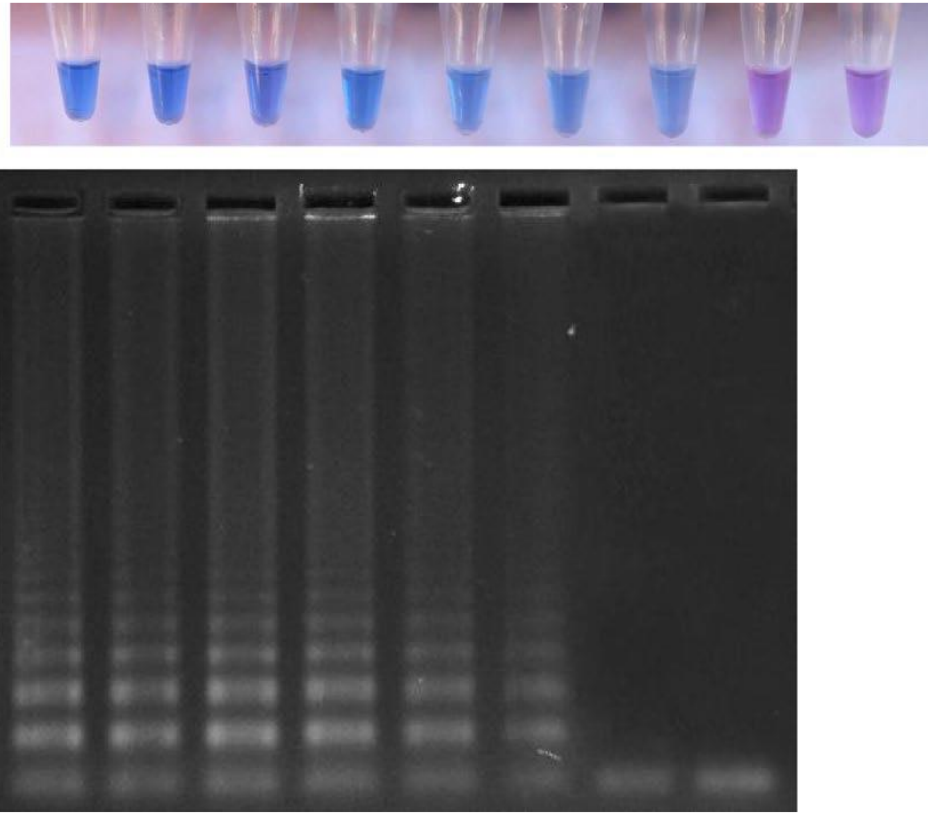
Reaction can be performed in half of the time needed for LAMP.

The concept and the primer design is a bit complicated but the procedure is simple!

The result is not one band but amplicons of different sizes and structures.

Detection is easy – with or without electrophoresis

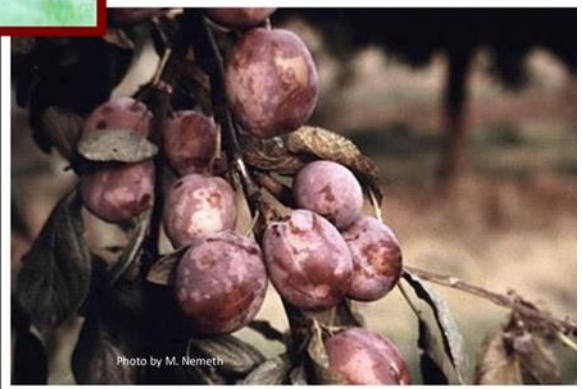
Intercalating dyes (ethidium bromide, sybr green, etc…), magnesium pyrophosphate turbidity, or hydroxy naphthol blue; (HNB) in tubes (Figure),
fluorescent dyes in real-time PCR machine.



Marimuthu *et al*. 2020, Loop-mediated isothermal amplification assay for the detection of *Plasmopara viticola* infecting grapes. *Journal of Phytopathology*, 168: 144–155.

Plum Pox Virus aka Sharka

Most feared viral disease of Prunus

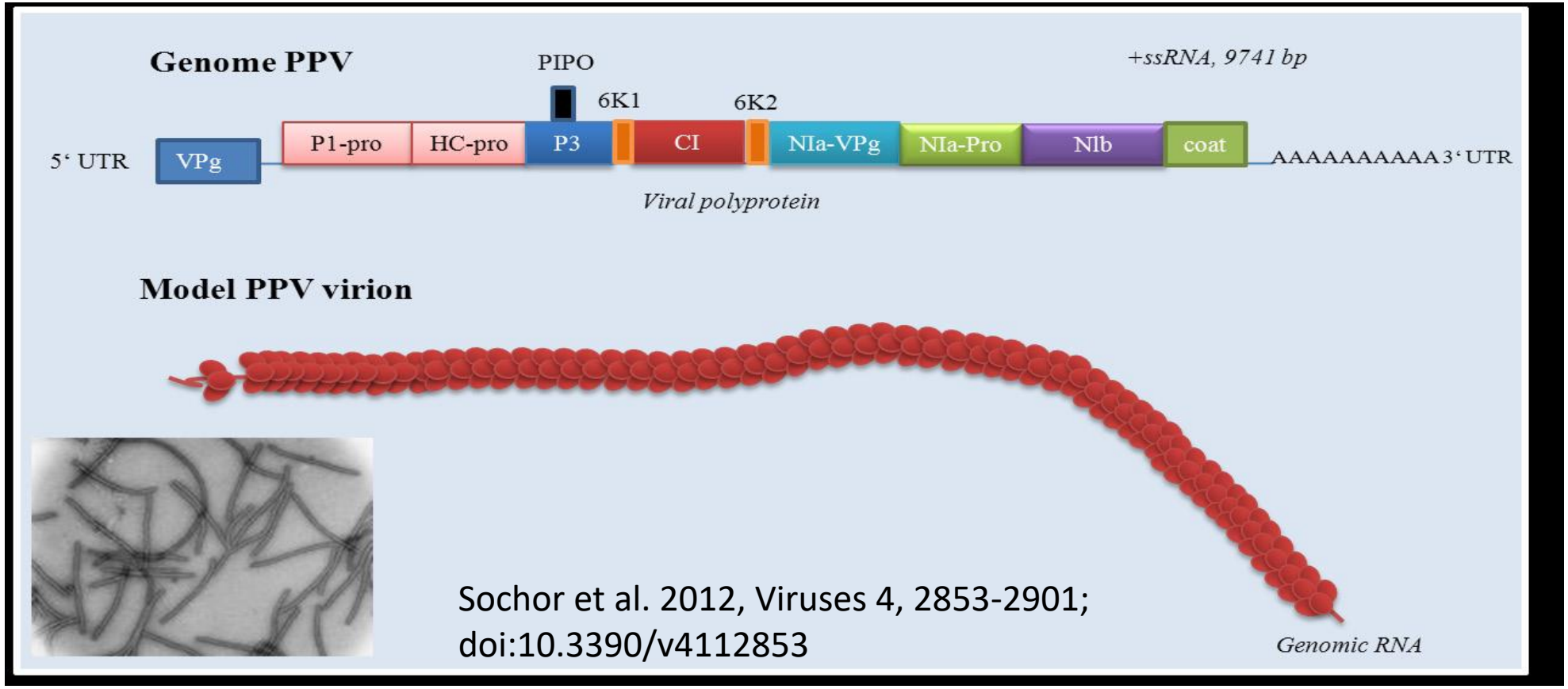


Sochor et al. 2012, Viruses 4, 2853-2901; doi:10.3390/v4112853

Use of reverse transcription loop-mediated isothermal amplification for the detection of *Plum pox virus*

Varga & James, 2006, *J. Virol. Meth.* 138: 184–190.

Plum pox virus (PPV), *Potyviridae*



Sochor et al. 2012, Viruses 4, 2853-2901; doi:10.3390/v4112853

Targets C-terminus of the PPV coat protein gene.

RT-LAMP detects isolates five (out of currently ten known Dideron (D), Marcus (M), Recombinant (Rec), Cherry (C), Cherry Russian (CR), EL Amar (EA), Winona (W), Turkish (T), Ancestral (An) and Cherry Volga (CV)) PPV strains including D, M, EA, C, and W.

Procedure:

1. Do the list of samples.
2. In 2ml Eppendorf tubes prepare 100 mg fresh tissue (flowers, leaves).
3. Homogenize in liquid nitrogen (with a teflon pistile) and add 1 ml of extraction buffer.
4. Centrifuge for 1 min in a refrigerated centrifuge at 5000 G or more.
5. Put 1 microliter of extract in a 0.5 ml tube and add 9 µl of PCR-water.
6. Prepare master mix for all samples according to the table based on the paper.
7. Prepare the PCR tubes, number them, include controls, distribute mix in PCR-tubes (20 µl aliquots in 0.2 ml tubes).
8. Add 5 µl of 10x diluted extract to a respective tube.
9. Run the RT-LAMP in a PCR cycler (1 step if a robust RT is used: 63°C, 1h; 80°C, 2 min, hot top), or 2-step if not (50°C, 10 min followed by the described conditions for 1-step).
10. Do the electrophoresis in 1% agarose gel (9 V/cm, 45 min, in TBE) and visualise products or visualize in a tube.
11. Record and analyze the results.

Table for PPV RT-LAMP master mix

Mix	1x	
Water	1.98	
10 x BST Buffer	2.5	
50 mM $MgSO_4$	3	
5 M Betain	4	
25 mM dNTP	1.4	
F3 5 uM	1	
B3 5 uM	1	
FIP 40 uM	1	
BIP 40 uM	1	
F-loop 20 uM	1	
B-loop 20 uM	1	
Rnase Out 40 U/ul	0.02	
Superscript III RT 200U/ul	0.1	
BST polymerase 8U/ul	1	
Aliquot	20	
Template	5	

Cycling :
1 Step
63°C for 60 min
80°C for 2 min

hot top at 85°C