Use of isothermal amplification assays in seed testing

Rolland M., Andro C., Potier A., Delaunay A., Mathis R.

Corresponding author : *mathieu.rolland@geves.fr*

Introduction

In the field of molecular biology, **Loop mediated isothermal AMPlification (LAMP)** is one of the most promising techniques for diagnostic purpose. This technology, first published in 2001, is used in an increasing number of published methods and presents many characteristics corresponding to the expectations of the seed testing laboratories. This technique is **extremely sensitive** (more than the usual polymerase chain reaction). It **does not rely on thermocycling** and **requires less equipment** than regular molecular biology diagnostic tools. It is **easy to use** and can be performed in basic conditions. For seed testing, the essential characteristic of LAMP remains its **important ruggedness**. In some cases it allows the amplification of nucleic acids from raw samples. The main drawback of the technique however, is the complexity of the design of a new assay. The design of LAMP primers indeed requires 6 or 8 specific regions. Due to the extreme sensitivity, some contamination issues have also been reported.

Principle

Amplification

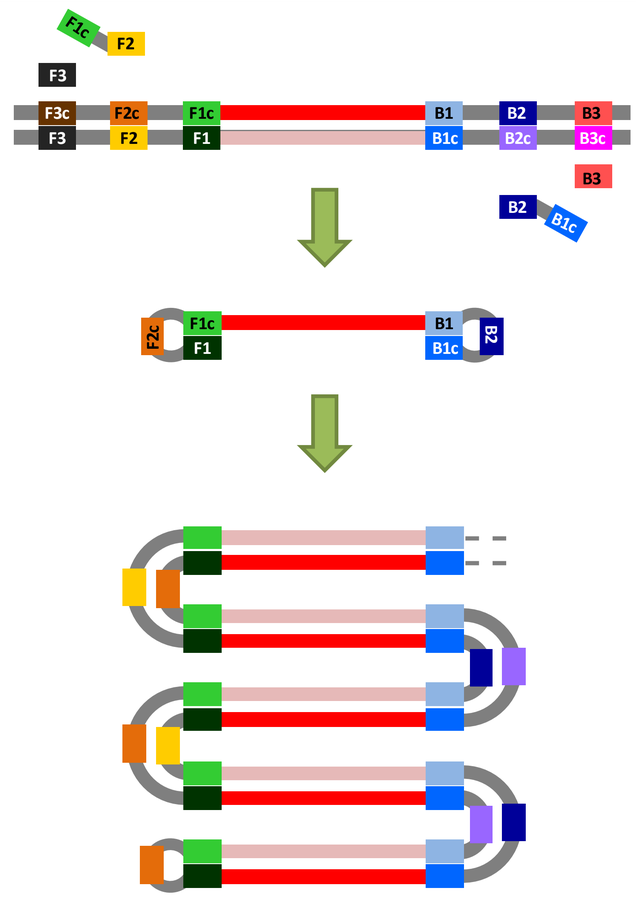
- Constant temperature : 30 – 60 min at 65°C.
- Loop structures allow a continuous amplification, no denaturation step is required
- Enzyme is a Bst polymerase (5´ → 3´ polymerase activity but no 5´ →3´ exonuclease activity)

Primers

- A minimum of 6 specific regions is required to design four complex primers
- Optional « loop » and « stem » primers can be used (loop: between regions 1 and 2, stem: in the central region). These primers improve the specificity and speed up the amplification.

Reading

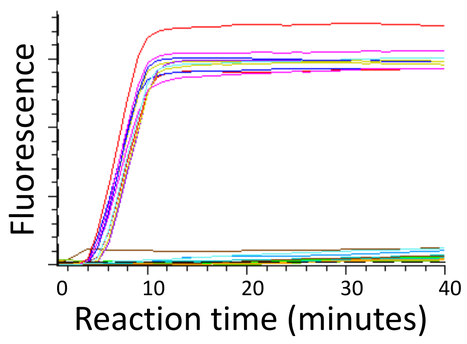
- Electrophoresis : Produced DNA can be visualized on gels.
- Turbidity : A positive LAMP reaction causes the solution to become cloudy due to the formation of a magnesium pyrophosphate byproduct. Turbidity can be measured using specific equipment or directly visualised by eye.
- Colorimetric reaction : Some colorimetric reagents (such as hydroxy naphthol blue) can be added to the reaction mix to detect positive reactions.
- Fluorescence : The use of an intercalating dye allows the detection of positive results and the real-time measure of accumulation of DNA. In this case a specific equipment (such as a real time thermocycler) is required.



During the first steps of the process, annealing of the primers and amplification by Bst polymerase will produce a structure with stem-loops at each end. This "dumbbell shaped DNA" structure serves as a template for LAMP cycling that will produce various sized structures consisting of alternately inverted repeats of the target sequence

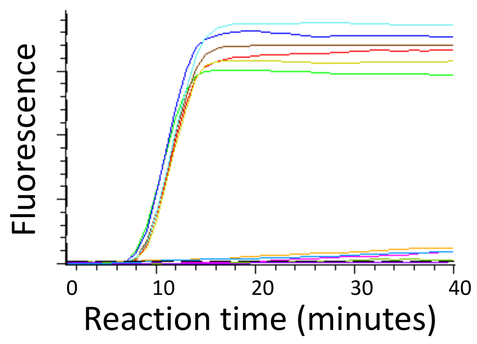
Detection of a seed-borne pathogenic bacteria using a LAMP assay (fluorescence reading)

On isolated colonies



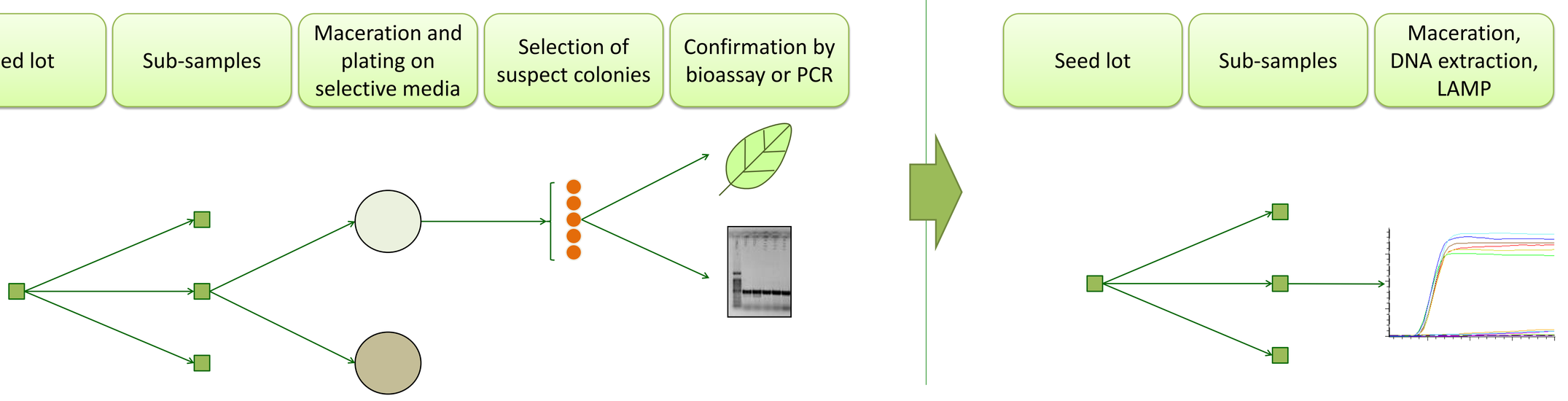
For all the colonies belonging to the target species, a strong fluorescence can be observed. Fluorescence increase is detectable from 5 min after the beginning of the reaction. No signal increase is observed for the non target colonies

On seed macerate



Conducted on seed macerates the same assay gives similar results: contaminated seed lots are found positive with a fluorescence increase detectable from 8 min. In healthy seed lots, no signal increase is detected.

Suggested impact on the analysis process



Conclusion

This new technique presents many interesting characteristics. Its important ruggedness allows direct testing on seed macerate. Its use should accelerate the analysis process. Only few methods are currently available, but more developments are expected in the years to come.

Pros

- Flexibility
- Specificity
- Robustness
- Ruggedness

Cons

- Difficulty to design new assays
- Contamination risks

GEVES - Variety and Seed Study and Control Group
BioGEVES - Molecular biology and biochemistry laboratory
25 rue Georges Morel, CS 90024, 49071 Beaucouzé cedex