Article Id
AL04278

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Divya Shivani Thangi

Email
divyashivani4299@gmail.com

Department of Genetics and Plant Breeding, Acharya N G Ranga Agricultural University, Bapatla, India

The invention of the polymerase chain reaction (PCR) method by Kary B. Mullis in 1983, enabling genetic study to be carried out even with small amounts of the targeted biological material, is one of the most notable achievements of molecular biology. The demand for a high precision thermal cycler in PCR prohibits this potent technology from being utilised extensively, such as in private clinics as a routine diagnostic tool, despite the simplicity and the attainable volume of amplification. Lately a unique technique was developed that can amplify a few copies of DNA to 10^9 in less than an hour while maintaining improved specificity, calledloop-mediated isothermal amplification (LAMP).

Loop Mediated Isothermal Amplification (LAMP)

LAMP is an isothermal nucleic acid amplification technique designed and developed by Notomi*et al* which helps in the diagnostic field, healthcare, agriculture, and food industries, with methods like the screening of viral and bacterial strain mutations, analysis of fungicide resistant mutations, analysis of micro RNAs, identification of herbal medicines, identification of plant pathogen vectors, single nucleotide polymorphisms analysis, and detection of genetic variants.

Principle: The LAMP technique is based on auto cycling and high DNA strand displacement activity mediated by *Bst* polymerase from *Geobacillus stearothermophilus*, under isothermal conditions. There are two steps in the reaction:

i) Initial step : At 65 °C temperature, the forward inner primer combines with the original reverse target sequence and synthesize of the new forward strand starts at the 3′ end. The synthesis of this new forward strand proceeds until the enzyme locates the 5′ end of the initial strand made with the usage of the inner primer, at which point the forward outer primer

hybridises once more with the identical original reverse target sequence. Using the forward inner primer results in the displacement of the first forward strand. Due to the complementarity of the reverse sequence from the inner primer to the target sequence, this split strand develops a self-hybridizing loop at one end. It also serves as a template for the reverse inner primer and the reverse outer primer, which, in a similar way, will produce the strand displacement of the forward strand, thus creating a dumbbell-like DNA structure.

ii) Combination of a cycling amplification step with an elongation/recycling step: In order to create a new strand with an inverted copy of the target sequence in the stem region and a loop on the opposite side, the forward inner primer hybridises to the loop of the strand generated during an initial step. The two products of self-primmed strand displacement DNA synthesis are a complementary strand and a second strand with a double-elongated stem that is the same length as the first and the loop on the opposite site. The ensuing elongation and recycling stages utilise both strands as templates for the reverse-primed strand displacement synthesis. As a result, the target sequence can be amplified three times during each half of the cycle. Due to the *Bst* DNA polymerase's strong displacement activity, a significant quantity of DNA with a high molecular weight is quickly produced. As a result, target DNA may be amplified up to 10^9 copies in less time. Finally, several looped stem-loop DNAs with varying lengths are created to resemble cauliflower-like formations.

LAMP Primers

In LAMP, a set of four specific primers, which can recognize six distinct regions on the target region. Basically, the LAMP primers consist of two outer primers (F3 and B3) and two inner primers, termed forward inner primer (FIP) and backward inner primer (BIP). Each inner primer of LAMP includes two regions; F2 and F1c on FIP, and B2 and B1c on BIP; in which F2 and B2 are complementary to F2c or B2c on the target, while F1c and B1c have similar sequences to the target.

Limitations of LAMP

Despite its brilliant advantages, there are some limitations like using of 4-6 primers for detecting a particular target can be a challenging especially for the short target sequence containing high mutation points such as RNA viruses or micro RNA. Primer dimers may occur when four to six long-sequence primers are used in high quantities, which might lead to a false-positive LAMP result. Due to carry-over contamination, the large yield of dsDNA

produced following amplification in conjunction with the test's high sensitivity may provide false positive findings in the LAMP assay.

Conclusions

In the future it has every chance of becoming a full-fledged alternative to PCR in the field of molecular diagnostics of pathogens, and new modifications of LAMP will certainly help this interesting amplification method find its unique scope of application.

References

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids Research*. 28(12), 63-63.

Panno, S., Matić, S., Tiberini, A., Caruso, A.G., Bella, P., Torta, L., Stassi, R., and Davino, S.(2020). Loop mediated isothermal amplification: principles and applications in plant virology. *Plants.* 9(4),461.