Loop-Mediated Isothermal Amplification (LAMP) And Its Applications

Panan Kanchanaphum[1]

[1]Biochemistry Unit, Department of Medical Science,
Faculty of Science, Rangsit University, Patumthani 12000, Thailand

Corresponding author : Panan Kanchanaphum (panan.k@rsu.ac.th)

Abstract

Loop-mediated isothermal amplification (LAMP) is an efficient and rapid method of amplifying DNA under isothermal conditions. It consists of 4 specific primers based on 6 specific sequences on the target gene. The final products of the LAMP technique are stem-loop DNA, which resemble cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the target in the same strand. LAMP products can be detected by electrophoresis, reveals a smeared band of LAMP products. In addition, a white precipitate of magnesium pyrophosphate, a LAMP by-product, can be observed directly with the naked eye. Fluorescent dyes can be used to detect LAMP products instead of electrophoresis or magnesium-pyrophosphate precipitation. The LAMP technique has several applications in infectious-disease detection. The LAMP technique can also be used to determine the sex of animals and plants.

Key words: Application, LAMP, Loop-mediated isothermal amplification

Introduction

Loop-mediated isothermal amplification (LAMP) is a novel molecular technique for amplifying DNA developed a decade ago by Notomi *et al* (Notomi, Okayama *et al*. 2000). It consists of 4 specific primers based on 6 specific sequences on the target gene. Primer design is key to the LAMP reaction. Factors such as guanine-cytosine (GC) content, primer distance, and Tm must be strictly controlled (Notomi, Okayama *et al*. 2000; Song, Li *et al*.2012). Fortunately, primer-design software for LAMP is freely available. The PrimerExplorer V4 software, available on the Eiken Chemical Co. Ltd. website (http://primerexplorer.jp/e/), is often used to design 4 specific LAMP primers. The first primer pair, the inner primer, contains the sequences of the sense and antisense strands of target DNA for initiating the LAMP reaction. The following strand displacement DNA synthesis is primed by the second inner and outer primers that hybridize to the other end of the target, which produces the product, a stem-loop DNA structure. Then, the LAMP cycle one inner primer hybridizes to the loop on the product and initiates displacement DNA synthesis. The final products are stem-loop DNA with several inverted repeats of the target and cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the target in the same strand.

The LAMP method takes about one hour and is performed at a constant temperature of 60-65°C in the presence of Bst DNA polymerase, a DNA polymerase type with strand displacement activity (Notomi, Okayama *et al*. 2000). LAMP products can be detected by electrophoresis. The results as a smeared band of LAMP products (Notomi, Okayama *et al*. 2000). However, compared with PCR, LAMP has many advantages. One of the most important advantages is that the amplified product can be observed directly due to the presence of a white magnesium-pyrophosphate precipitate (see equation 1).

$$(DNA)_{n-1} + dNTPs \longrightarrow (DNA)_n + P_2O_7^{-4}$$

$$P_2O_7^{-4} + 2Mg^{2+} \longrightarrow Mg_2P_2O_7$$

Equation 1. Magnesium pyrophosphate formation in the LAMP product.

Besides the formation of magnesium pyrophosphate in the amplification product, a fluorescent dye can also be used in a LAMP reaction. The former method can be used in the

field without any special equipment, while the addition of CuSO4 to negative samples can be used to confirm negative samples (Zoheir and Allam 2010). When a positive reaction appears, high levels of pyrophosphate ions are released during amplification, which produce a visible precipitate. When CuSO4 is added to a negative sample, the dNTPs remain almost unchanged; these combine with CuSO4 to form white precipitates of Cu(OH)2 that formed a right-shaped deposit (Chan, Liu *et al*. 2012). The latter reaction can be partially attributed to the fact that the concentration of dNTPs used in LAMP is much higher than PCR (Notomi, Okayama *et al*. 2000; Chan, Liu *et al*. 2012).

An amino acid analog betaine is often added to PCR reaction mixtures and LAMP reactions to increase the yield of a specific PCR product. It stabilizes AT base pairs and reduces steric hindrances caused by secondary structures occurring in high GC DNA (Jensen, Fukushima *et al*. 2010). However, when the betaine concentration is > 0.8 M, the LAMP reaction is inhibited, which differs from PCR, where no such effect has been reported (Nagamine, Hase *et al*. 2002).

Applications in infectious-disease detection

LAMP is a powerful technique for the detection of infectious diseases. Bacterial infections are one good example of this application. It is used for detecting Brucella sp., facultative intracellular bacteria that infect humans and animals, in milk and blood samples (Song, Li *et al*. 2012). Bacillus anthracis is another bacterium detected by LAMP (Kurosaki, Sakuma *et al*. 2009). Song Q. *et al* developed a LAMP technique to detect Mycoplasma wenyonii, a wall-less hemotrophic prokaryote with worldwide distribution, which causes disease in cattle (Song, Wang *et al*. 2012), Vibrio parahaemolyticus, a bacterium commonly found in seafood, and Streptococcus iniae, a bacterium that infects fish (Han, Jung *et al*. 2011). LAMP can be used to detect methicillin-resistant Staphylococcus aureus (MRSA) in blood culture (Misawa, Yoshida *et al*. 2007). The LAMP technique is also used to detect fungi and eukaryotic cells. In the detection of fungi, Fusarium graminearum and the closely related F. culmorum have been found associated with excessive foaming in bottled beer, when brewing malt is contaminated; these fungi can be screened by LAMP (Denschlag, Vogel *et al*. 2012). Regarding applications for parasitic infections, Paragonimus westermani, the most prevalent lung fluke in Asia and South America, can be detected by LAMP (Chen, Ai *et al*. 2010). Babesia gibsoni, a parasite of canines, has also been detected by LAMP (Ikadai, Tanaka *et al*. 2004), while the African trypanosome, Trypanosoma brucei, has been detected in human cerebrospinal fluid (CSF) and blood using this technique (Grab, Nikolskaia *et al*. 2011). LAMP assays have been developed and validated for the detection in adult, metacercariae, and eggs of the lung fluke, Paragonimus westermani, in human and animal samples (Chen, Ai *et al*. 2010). Many research studies have used the LAMP technique to detect Plasmodium spp. Lau Y.L. *et al* developed a LAMP method to detect P. knowlesi in blood samples (Lau, Fong *et al*. 2011). P. vivax infection in the Republic of Korea has been detected by LAMP (Chen, Lu *et al*.). In September-November 2009, in Sao Tome, Lee P.W. *et al* used LAMP to amplify and follow up a study of malaria patients (Lee, Ji *et al*. 2012). LAMP can be used to detect viral infections; e.g., mink enteritis virus (MEV) infection has been detected (Wang, Cheng *et al*. 2012). Moreover, LAMP can be combined with reverse transcription (reverse transcription LAMP or RT-LAMP) for use with viral RNA. Tohru M. *et al* detected yellow-head virus in Penaeus shrimp by RT-LAMP (Mekata, Kono *et al*. 2006). Taura syndrome virus, a virus found in Penaeus shrimp, was detected by RT-LAMP (Kiatpathomchai, Jareonram *et al*. 2007). Arita M. *et al* developed RT-LAMP for the highly sensitive detection of Enterovirus in stool samples of acute flaccid paralysis cases (Arita, Ling *et al*. 2009). Zheng F. *et al* used RT-LAMP to detect bovine ephemeral fever virus (BEFV) in blood samples (Zheng, Lin *et al*. 2010).

Application in sex determination

The LAMP technique can be used to determine sex. Chan K.W. *et al* used LAMP to identify the sex of Columbidae birds (pigeons & doves), by amplifying female-specific sequence and 18S ribosomal RNA sequence of the rock pigeon, Columba livia (Chan, Liu *et al*. 2012). A LAMP embryo kit to analyze chromosomal chimerism in heterosexual twin female calves, has been developed by Hirayama, H. *et al* (Hirayama, Katagiri *et al*. 2007). A rapid method for sexing bovine embryos using LAMP was announced by Zoheir K.M. *et al* (Zoheir and Allam 2010).

Species and sex identification of Formosan landlocked salmon (Oncorhynchus masou formosanus) is a very important parameter for conservation management, albeit difficult to perform; however, the LAMP technique can help identify the species and sex of Formosan landlocked salmon (Hsu, Adiputra *et al*. 2011). To determine sex, dental-pulp DNA was examined by LAMP (Nogami, Tsutsumi *et al*. 2008), using 32 dental-pulp DNA samples removed from permanent teeth stored at room temperature for 1-25 years after extraction. Since the LAMP technique can be used to determine sex, it is also very useful for identifying fatalities in mass disasters (Nogami, Tsutsumi *et al*. 2008). The LAMP method can also be used to detect the sex of the transgenic fruit, the papaya (Carica papaya) (Hsu T.H. *et al*) (Hsu, Gwo *et al*.).

Conclusion

LAMP can amplify DNA very efficiently under isothermal conditions; it is simple and suitable for use under field conditions for rapid detection without a thermocycler or PCR machine. The existence of an amplified LAMP product, demonstrated by magnesium pyrophosphate precipitation, is easily observed with the naked eye. The LAMP assay is highly specific for the target sequence because it uses 4 primers to recognize 6 specific DNA sequences and Bst DNA polymerase for loop synthesis. The technique can be used in many fields, such as infectious diseases, the determination of sex in animals and humans, and in forensic science.

Refernces

Arita, M., H. Ling, *et al*. (2009). "Development of a reverse transcription-loop-mediated isothermal amplification (RT-LAMP) system for a highly sensitive detection of enterovirus in the stool samples of acute flaccid paralysis cases." BMC Infect Dis 9: 208.

Chan, K. W., P. C. Liu, *et al*. (2012). "A novel loop-mediated isothermal amplification approach for sex identification of Columbidae birds." Theriogenology 78(6): 1329-38.

Chen, J. H., F. Lu, *et al*. "Detection of Plasmodium vivax infection in the Republic of Korea by loop-mediated isothermal amplification (LAMP)." Acta Trop 113(1): 61-5.

Chen, M. X., L. Ai, *et al*. (2010). "Sensitive and rapid detection of Paragonimus westermani infection in humans and animals by loop-mediated isothermal amplification (LAMP)." Parasitol Res 108(5): 1193-8.

Denschlag, C., R. F. Vogel, *et al*. (2012). "Hyd5 gene-based detection of the major gushing-inducing Fusarium spp. in a loop-mediated isothermal amplification (LAMP) assay." Int J Food Microbiol 156(3): 189-96.

Grab, D. J., O. V. Nikolskaia, *et al*. (2011). "Using detergent to enhance detection sensitivity of African trypanosomes in human CSF and blood by loop-mediated isothermal amplification (LAMP)." PLoS Negl Trop Dis 5(8): e1249.

Han, H. J., S. J. Jung, *et al*. (2011). "Rapid and sensitive detection of Streptococcus iniae by loop-mediated isothermal amplification (LAMP)." J Fish Dis 34(5): 395-8.

Hirayama, H., S. Katagiri, *et al*. (2007). "Rapid sex chromosomal chimerism analysis in heterosexual twin female calves by Loop-mediated Isothermal Amplification." Anim Reprod Sci 101(1-2): 38-44.

Hsu, T. H., Y. T. Adiputra, *et al*. (2011). "Species and sex identification of Formosa landlocked salmon using loop-mediated isothermal amplification." Mol Ecol Resour 11(5): 802-7.

Hsu, T. H., J. C. Gwo, *et al*. (2012)"Rapid sex identification of papaya (Carica papaya) using multiplex loop-mediated isothermal amplification (mLAMP)." Planta 236(4): 1239-46.

Ikadai, H., H. Tanaka, *et al*. (2004). "Molecular evidence of infections with Babesia gibsoni parasites in Japan and evaluation of the diagnostic potential of a loop-

mediated isothermal amplification method." J Clin Microbiol 42(6): 2465-9.

Jensen, M. A., M. Fukushima, *et al*. (2010). "DMSO and betaine greatly improve amplification of GC-rich constructs in de novo synthesis." PLoS One 5(6): e11024.

Kiatpathomchai, W., W. Jareonram, *et al*. (2007). "Rapid and sensitive detection of Taura syndrome virus by reverse transcription loop-mediated isothermal amplification." J Virol Methods 146(1-2): 125-8.

Kurosaki, Y., T. Sakuma, *et al*. (2009). "A simple and sensitive method for detection of Bacillus anthracis by loop-mediated isothermal amplification." J Appl Microbiol 107(6): 1947-56.

Lau, Y. L., M. Y. Fong, *et al*. (2011). "Specific, sensitive and rapid detection of human plasmodium knowlesi infection by loop-mediated isothermal amplification (LAMP) in blood samples." Malar J 10: 197.

Lee, P. W., D. D. Ji, *et al*. (2012). "Application of loop-mediated isothermal amplification for malaria diagnosis during a follow-up study in Sao Tome." Malar J 11(1): 408.

Mekata, T., T. Kono, *et al*. (2006). "Detection of yellow head virus in shrimp by loop-mediated isothermal amplification (LAMP)." J Virol Methods 135(2): 151-6.

Misawa, Y., A. Yoshida, *et al*. (2007). "Application of loop-mediated isothermal amplification technique to rapid and direct detection of methicillin-resistant Staphylococcus aureus (MRSA) in blood cultures." J Infect Chemother 13(3): 134-40.

Nagamine, K., T. Hase, *et al*. (2002). "Accelerated reaction by loop-mediated isothermal amplification using loop primers." Mol Cell Probes 16(3): 223-9.

Nogami, H., H. Tsutsumi, *et al*. (2008). "Rapid and simple sex determination method from dental pulp by loop-mediated isothermal amplification." Forensic Sci Int Genet 2(4): 349-53.

Notomi, T., H. Okayama, *et al*. (2000). "Loop-mediated isothermal amplification of DNA." Nucleic Acids Res 28(12): E63.

Song, L., J. Li, *et al*. (2012). "Establishment of loop-mediated isothermal amplification (LAMP) for rapid detection of Brucella spp. and application to milk and blood samples." J Microbiol Methods 90(3): 292-7.

Song, Q., L. Wang, *et al*. (2012). "Detection of Mycoplasma wenyonii in cattle and transmission vectors by the loop-mediated isothermal amplification (LAMP) assay." Trop Anim Health Prod.

Wang, J., S. Cheng, *et al*. (2012). "Detection of mink enteritis virus by loop-mediated isothermal amplification (LAMP)." J Virol Methods.

Zheng, F., G. Lin, *et al*. (2010). "A reverse-transcription, loop-mediated isothermal amplification assay for detection of bovine ephemeral fever virus in the blood of infected cattle." J Virol Methods 171(1): 306-9.

Zoheir, K. M. and A. A. Allam (2010). "A rapid method for sexing the bovine embryo." Anim Reprod Sci 119(1-2): 92-6.