MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2017) **17**, 138–141 doi: 10.1111/1755-0998.12548

NEWS AND VIEWS

PERSPECTIVE

DNA amplification in the field: move over PCR, here comes LAMP

PATRICIA L. M. LEE*†
Deakin University, Geelong, Vic., Australia, †School of Life and Environmental Sciences, Centre for Integrative Ecology, Warrnambool Campus, Princes Hwy, Sherwood Park, PO Box 423, Warrnambool Vic. 3280, Australia

It would not be an exaggeration to say that among molecular technologies, it is PCR (polymerase chain reaction) that underpins the discipline of molecular ecology as we know it today. With PCR, it has been possible to target the amplification of particular fragments of DNA, which can then be analysed in a multitude of ways. The capability of PCR to amplify DNA from a mere handful of copies further means that conservationists and ecologists are able to sample DNA unobtrusively and with minimal disturbance to the environment and the organisms of interest. However, a key disadvantage of PCR-based methods has been the necessity for a generally non-portable, laboratory setting to undertake the time-consuming thermocycling protocols. LAMP (loop-mediated isothermal amplification) offers a logistically simpler protocol: a relatively rapid DNA amplification reaction occurs at one temperature, and the products are visualized with a colour change within the reaction tubes. In the first field application of LAMP for an ecological study, Centeno-Cuadros *et al.* (2016) demonstrates how LAMP can be used to determine the sex of three raptor species. By enabling DNA amplification in situ and in 'real-time', LAMP promises to revolutionize how molecular ecology is practised in the field.

Keywords: conservation genetics, eDNA, museum DNA, non-invasive, real-time DNA amplification, sex identification

Received 9 May 2016; accepted 26 May 2016

LAMP was first developed over 15 years ago (Notomi *et al.* 2000), but while its clinical use has blossomed (Notomi *et al.* 2015), it has yet to be applied in ecological studies. Its key advantage lies in the simple and rapid protocol, which means that on-the-spot diagnostics is possible. The LAMP reaction uses a single enzyme, a DNA polymerase with strand displacement activity that is able to massively amplify from only a few copies of DNA. The amplification protocol requires only a single temperature for the reaction (which can be provided by a thermoblock), and amplification success is diagnosed without the need for electrophoretic techniques. Thus, LAMP can be carried out with relatively inexpensive equipment that is portable (Fig. 1). While implementing LAMP requires simpler logistics than PCR, the principle of the method is more complex, requiring four primers that target six regions on the DNA (Fig. 1).

The LAMP method, based on autocycling strand displacement, is a two-step procedure. First, four primers are utilized to produce a dumbbell-shaped structure (Fig. 1). Second, this structure is used as a template in further amplification cycles which proceeds with self-primed DNA synthesis with the two inner primers. The LAMP process, wholly undertaken under isothermal conditions, then leads to production of stem-loop DNA of different lengths and cauliflower-like structures consisting of a growing number of concatenated amplicons (see Notomi *et al.* 2000; Tomita *et al.* 2008 for full descriptions of the LAMP procedure).

While the second step and the end products are complex, the key to the LAMP reaction lies with the first step, which involves all four primers. Just as for PCR, preparation and testing of primers prior to field deployment are critical for success. The two inner primers (FIB/BIP; Fig 1) are designed such that a looped structure is produced every time a single-stranded DNA is synthesized. These primers consist of two sets of sequences corresponding to the sense and antisense sequences of the target DNA, with a TTTT spacer between the sets of sequence. The two outer primers (F3/B3; Fig 1) serve to initiate strand displacement DNA synthesis to release the FIB/BIP-linked single strand, which is then able to

Correspondence: Patricia L. M. Lee, Fax: +61 3 55633462; E-mail: p.lee@deakin.edu.au

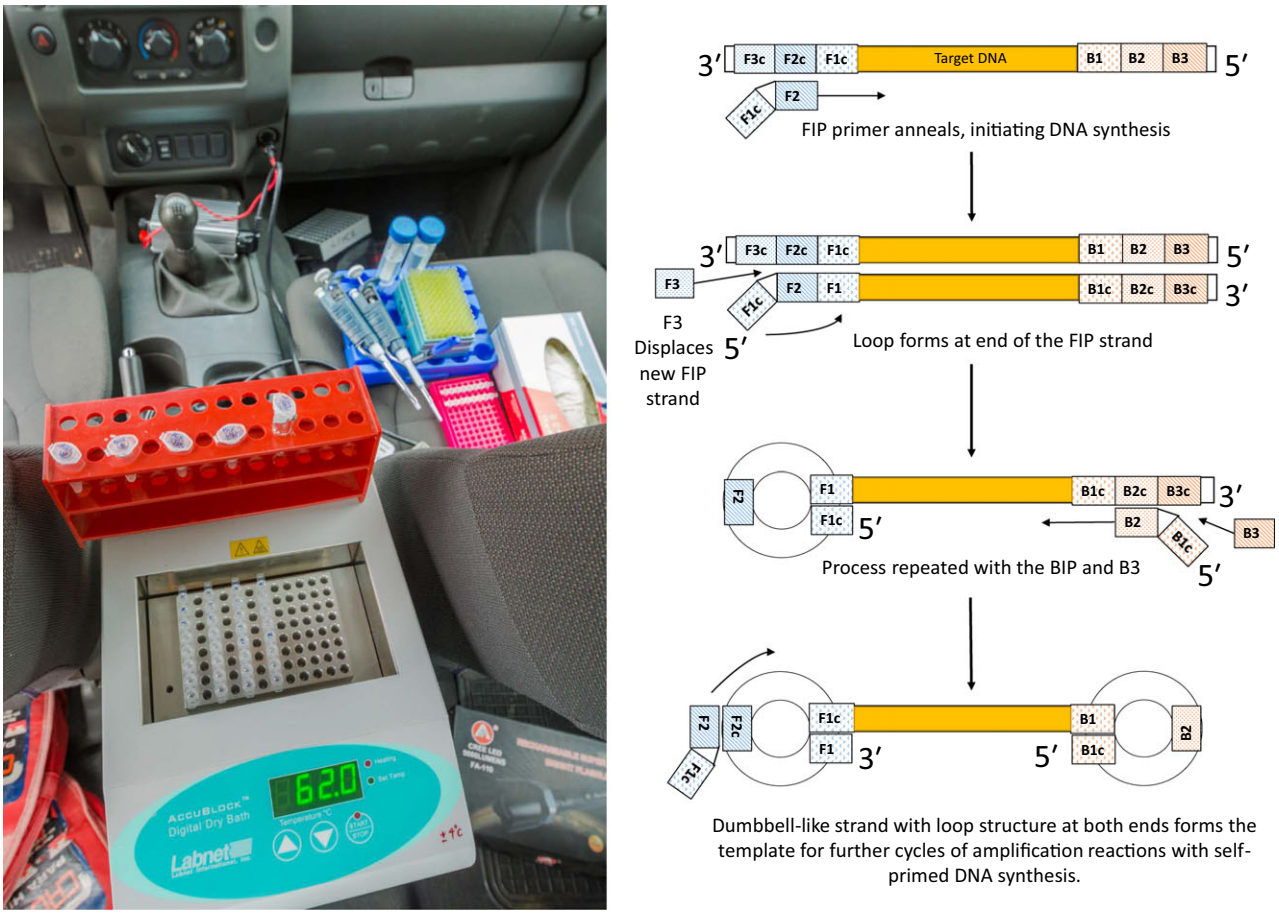

Fig. 1 The LAMP method: (left) equipment can be modified for portable field use such as in this example, where the cigarette-lighter port of an automobile provides the power for the thermoblock (Photo credit: Alejandro Centeno-Cuadros); (right) schematic representation of the first step of the LAMP mechanism, which requires four primers: FIP, BIP, F3 and B3 primers that target 6 sequences (F1, F2, F3, B1, B2 and B3, with 'c' representing the complementary sequence).

form the loop structure at one end. Tomita *et al.* (2008) advices on the appropriate primer design and methodology for LAMP.

In the study presented in this issue of Molecular Ecology Resources, Centeno-Cuadros *et al.* (2016) developed a LAMP protocol for sexing raptor species of the *Accipitridae* family (Fig. 2), by designing the primers to target a fragment on the CHD (Chromo-Helicase-DNA binding protein) gene located on the sex-linked W (unique to females) and Z chromosomes. They designed two sets of primers – one targeting the CHD on the W chromosome, and the other, the CHD on the Z chromosome. Female samples will be positive for both. A negative result for the former, but positive for the latter provides a controlled test for males.

However, for field deployment, two further processes also need to be considered in addition to a rapid DNA amplification method: first is DNA purification and second is visualization of the DNA amplification products. The latter is solved by adding Sybr Green nucleic acid stain to the LAMP reactions, which when interacting with magnesium pyrophosphate (a by-product of the DNA amplification process) changes the colour of the stain from orange to yellow-green. The colour change is visible to the naked eye, and can be further confirmed with irradiation with a portable UV lamp. For a rapid and portable DNA extraction method, Centeno-Cuadros *et al.* (2016) opted for an NaOH-based protocol that can be completed in 10 minutes (Truett *et al.* 2000), although other methods may also be suitable (e.g., FTA papers). Furthermore, they also considered how the various reagents and primers can be transported and stored without the need for refrigeration or freezing, as well as a solution for powering the thermoblock in the field (Fig. 1). Altogether, Centeno-Cuadros *et al.* (2016) designed a complete protocol for a simple and efficient

Fig. 2 Raptor species of the *Accipitridae* family: (left) *Gyps fulvus* Griffon Vulture (photo credit: Manuel de la Riva), (top right) *Milvus migrans* Black Kite (photo credit: Fabrizio Sergio) and (bottom right) *Neopron percnopterus* Egyptian Vulture (photo credit: Manuel de la Riva).

field deployment of a DNA amplification-based diagnostic tool.

This protocol will revolutionize molecular ecology in two ways. First, raptor birds can now be sexed in the field, in 'real-time'. Molecular ecologists wanting to sex other non-ratite bird species with LAMP will of course have to tweak the design of the primers for their species of interest, but Centeno-Cuadros *et al.* (2016) have proven the principle. Birds are one of the most important models for understanding animal behaviour, ecology and evolution. Being charismatic, many are 'keynote' species and the foci of conservation study. Knowing the sex of individuals is important where there is different treatment for the sexes. The example presented by Centeno-Cuadros *et al.* (2016) was a case where only females needed to be tagged with costly GPS and accelerometer tags. Sex is not only a vital variable for conservation management, but also for experimental manipulation. Often, particularly for natural populations, the sex of individuals was not known at the outset of an experiment, and could only be statistically controlled for after the experiment has been completed, once sex has been determined in a laboratory using the traditional PCR-based method. However, with the LAMP-based sexing test, researchers will now be able to directly factor in sex or manipulate sex as part of the experimental design. Sex itself poses classic questions in ecology and evolution – for example, whether parents allocate resources depending on the sex of their offspring, or indeed manipulate offspring sex ratios.

The impact of the study by Centeno-Cuadros *et al.* (2016), however, goes beyond sexing birds. The second, and perhaps more important impact, is the demonstration that the entire process of a DNA amplification-based diagnostic tool, from DNA extraction to visualizing the results, can be slim-lined for field implementation. This is an exciting prospect for applications that have traditionally been PCR based. Many applications could benefit from a field protocol, including the identification of cryptic species, and the monitoring of wildlife diseases and parasites. Furthermore, LAMP is reported to be ten times more sensitive than PCR (Hamburger *et al.* 2013). This holds particular promise for applications where sensitivity is important, such as non-invasive sampling (Kohn & Wayne 1997) or sampling from museum specimens (Bantock *et al.* 2008). One application rising in popularity that further comes to mind is the so-called 'eDNA' (environmental DNA) touted as the next new survey tool in ecology (Rees *et al.* 2014). The analysis of eDNA extracted from an environmental sample for short, species-specific DNA fragments to reveal the presence of specific species or assemblages of closely related species is being used for a variety of applications from tracking rare, invasive or pathogenic organisms, to assessing species composition and diversity. Combining LAMP and eDNA presents interesting prospects for in situ detection of specific species in the field. This could speed up the implementation of management actions, either to protect or eradicate the organism of interest.

Finally, resource-poor locations are frequently also areas of conservation concern and rich biodiversity. Another advantage of LAMP-based diagnostic tools is that, once developed, the protocols are easily learnt and

carried out by field operatives without the need for prior molecular experience or laboratory infrastructure (Cuadros *et al.* 2015). Cheap, easily implemented but accurate DNA amplification diagnostic tools could help empower local researchers to conduct their own studies, and to take credit and profit for the biological/ecological discoveries in their own backyard.

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This paper was conceived and written by P.L.M.L.