BSP Spring Meeting 2024

BSP Student Travel Award

Development of a LAMP Detection Assay for *Dictyocaulus viviparus* Lungworm

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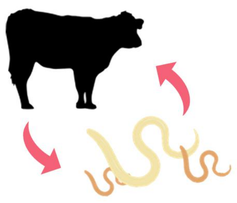
INTRODUCTION

Dictyocaulus viviparus - The bovine lungworm



- highly pathogenic, sporadic disease outbreaks
- parasitic bronchitis (dictyocaulosis) in cattle
- difficult to predict and manage; can be detected in faeces

Loop-mediated isothermal amplification (LAMP)

- a rapid and isothermal DNA amplification assay, which could be developed for field-based detection
- application in point of care diagnosis

METHOD

1. DNA preparation and sequencing

- Extracted DNA from single *D. viviparus* L3 larvae
- ITS2 amplification using PCR - primer Bisset ITS2 GF & GR (Bisset et al., 2014)

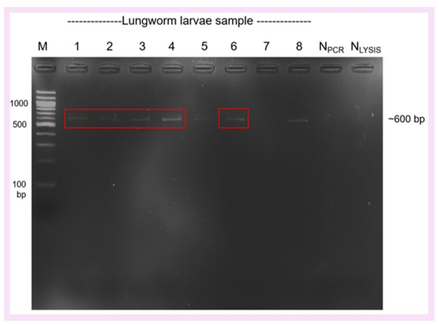
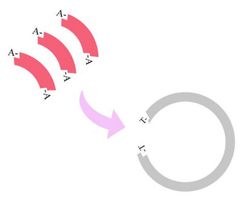


Fig. 1 PCR result for 8 lungworm larvae samples. The PCR product length approximately 600 bp.

Purified DviITS2-PCR product



DNA cloning and selection
- TOPO™ TA Cloning (pCR 2.1-TOPO vector) with One Shot™ TOP10 Chemical Competent *E. coli* cell
- Blue-White screening and colonies-PCR

DNA plasmid (DviITS2 Plasmid)
- extraction using Monarch Plasmid Miniprep Kit
- DNA sequencing
- used as DNA template for LAMP assay

DNA sequences

2. LAMP primer design

1 LAMP PRIMER SET (6 primers) = F3 + B3 + FIP + BIP + LF + LB

- the DNA sequence contains 5.8S ribosomal RNA gene partial sequence; internal transcribed spacer 2 complete sequence; and 28S ribosomal RNA gene partial sequence
- species confirmed by BLASTN-NCBI
- used as DNA template for LAMP primer design on Geneious Prime software

Fig. 2 DviITS2-DNA sequence alignment and the DviLAMP primer targets.

3. LAMP condition optimization and tests – colorimetry, gel electrophoresis, real-time, and lateral flow (LF)

- incubation at 64 °C for 45 / 60 / 90 min, and then at 80 °C for 10 min with various DNA template concentrations (20 ng to 1 pg)

RESULTS

- Various DNA concentrations of the DviITS2 plasmid including:
1 = 20 ng; 2 = 10 ng; 3 = 5 ng; 4 = 1 ng; 5 = 0.5 ng; 6 = 0.1 ng; 7 = 50 pg; 8 = 10 pg; 9 = 5 pg; and 10 = 1 pg.

Colorimetry and gel electrophoresis

Fig. 3 Observation for the DviLAMP results by naked eye (A) and gel electrophoresis (B) with different DNA template (the DviITS2 plasmid) concentration and different incubation times.

Real time LAMP (qLAMP)

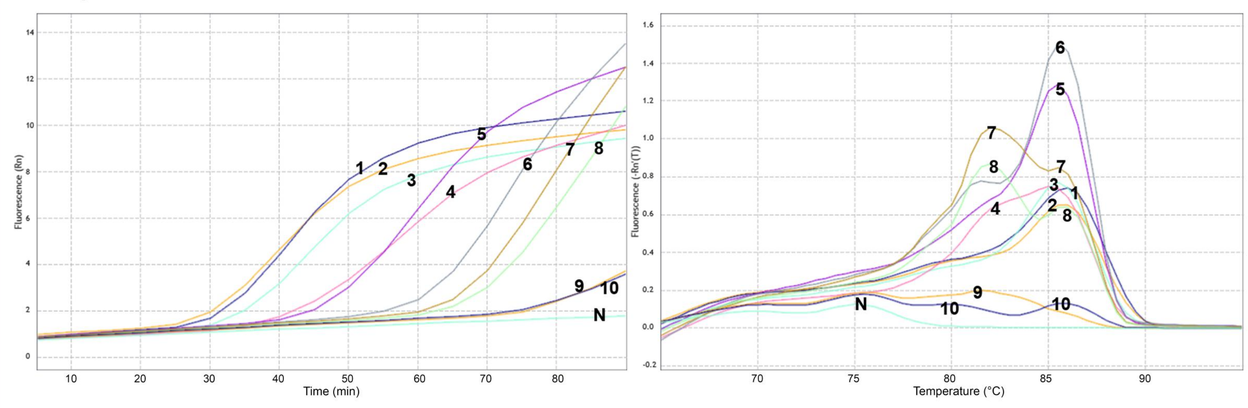


Fig. 4 Amplification plot and melting curve for the DviLAMP with the various DNA concentration.

LAMP-LF strip

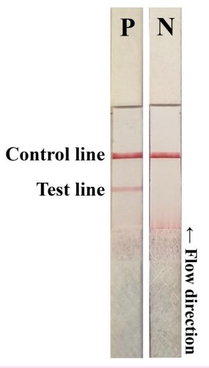


Fig. 5 The DviLAMP-LF strip test results for positive (P; the plasmid at 20 ng) and negative (N) after the incubation for 90 min.

CONCLUSION

- We have presented findings showing the rapid detection of *D. viviparus* ITS2 DNA within 1 hour using the DviLAMP.

- Therefore, the development of DviLAMP could significantly improve the sensitivity of lungworm diagnosis in the field.

"The DviLAMP project"

DISCLOSURE

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