The Gnatwork
Intended use of resource / data

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Title of resource

GN_27: **Detection of *Onchocerca spp* infection in *Simulium damnosum s.l.* by loop-mediated isothermal amplification (LAMP).**

Authored by

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DOI

Description

A LAMP assay protocol developed for detecting (a) *Onchocerca spp* infection in *Simulium damnosum s.l.* populations using a genus-specific O-150 primer set and (b) *Onchocerca volvulus* infection in *Simulium damnosum s.l.* populations using a species-specific OvGST-1α primer set. Both methods are intended for use on pooled samples (n=100) of *S. damnosum s.l.* flies to determine infection status within the population of interest.

Intended use

Scientific research use and training purposes.

Restrictions on use

Content is not to be redistributed in the public domain (e.g. presentation, lecture, online or in publications).

Resource history

N/A

GN_27: Detection of *Onchocerca spp* infection in *Simulium damnosum s.l.* by loop-mediated isothermal amplification (LAMP).

When using this protocol, the following should be referenced:

Ya'cob, Z. Poole, C. Bah, GS. Carlow, T. Makepeace, BL. Graham Brown, J

Equipment & Consumables:

DNA extraction:
- 5mL eppendorfs
- Micropestle
- Dneasy Blood & Tissue kit (QIAGEN)

LAMP assay:
- Nuclease free water
- M1800 Warmstart Colorimetric LAMP 2x Master Mix (New England Biolabs)
- O-150 LAMP Primer set (New England Biolabs)
- OvGST1α LAMP Primer set (New England Biolabs)
- 0.2ml PCR tubes
- 10-200µL pipettes & filter tips
- Heat block

Sample preparation:

- Prior to performing LAMP assays, perform DNA extraction on pooled *S. damnosum s.l.* specimens (100 flies per pool) using Dneasy blood and tissue kit following manufacturer's recommendations (QIAGEN).
- Black flies (*Simulium damnosun s.l.*) collected in the field may be processed fresh, or following freezing at -20^{o}C for short-term storage.
- For samples collected in remote locations where fresh processing or freezing are not possible, store samples in molecular grade ethanol to ensure DNA preservation.
- Pooled samples of 100x flies can be analysed following DNA extraction from whole bodies or heads only.

Protocol A: Detection of *Onchocerca spp.* using O-150 primer set

1. Prepare 100µl of 10x O-150 Primer Mix

Primer	Volume (µL) of stock	10x concentration (µM)	Final working (1x) concentration (µM)
100 µM FIP	16	16	1.6
100 µM F3	2	2	0.2
100 µM BIP	16	16	1.6

100 µM B3	2	2	0.2
Nuclease free H_2O	64	---	---
Total volume	100	---	---

2. Prepare 1x concentration Colorimetric Master Mix for *n* reactions;

	Volume per reaction[1] (µL)
Nuclease free H_2O	8.0
M1800 Warmstart Colorimetric LAMP 2x Master Mix	12.5
O-150 Primer Mix (10x)	2.5
Subtotal[2]	**23**
Sample DNA /control	2
Total	**25**

[1]Samples run in triplicate require 3x reaction volume, 2x reaction volume for duplicate etc.
[2]Mix H_2O, 2x Colorimetric LAMP master mix and 10x Primer mix together at volumes required for *n* reactions then aliquot out 23µL per tube

3. Add 2µL of sample DNA to each tube. **N.B. It is recommended samples are run in triplicate.**
4. Include a positive and negative (blank elution buffer or nuclease free-water) control samples for each LAMP assay run, with negative control samples added after all other samples have been processed. **N.B. It is recommended controls are run in triplicate.**
5. Incubate reactions at 64°C for 80 min. Reactions may be removed from incubation periodically (every 15 to 20 min) and examined by eye as high DNA input may cause reactions to develop more quickly than the recommended 80 min.
6. Once complete, compare to positive and negative controls for interpretation, photograph the reactions and record the results. **N.B. <u>DO NOT</u> open LAMP assay tubes once complete. Opening completed LAMP assay tubes will contaminate the laboratory with DNA product.**

Protocol B: Detection of *Onchocerca volvulus* using OvGST-1α primer set

1. Prepare 100µL of 10x OvGST1a Primer Mix:

Primer	**Volume (µL) of stock**	**10x Concentration (µM)**	**Final working (1x) concentration (µM)**
100 µM FIP	16	16	1.6
100 µM F3	2	2	0.2
100 µM BIP	16	16	1.6
100 µM B3	2	2	0.2
100 µM LF	4	4	0.4
100 µM LB	4	4	0.4
Nuclease free H_2O	56	---	----
Total volume (µl)	100	---	----

2. Prepare 1x concentration Colorimetric Master Mix for *n* reactions;

	Volume per reaction[1] (µL)
Nuclease free H_2O	8.0
M1800 Warmstart Colorimetric LAMP 2x Master Mix	12.5
Primer OvGST-1α mix (10x)	2.5
Subtotal[2]	**23**
Sample DNA /control	2

Total	**25**

[1]Samples run in triplicate require 3x reaction volume, 2x reaction volume for duplicate etc.
[2]Mix H_2O, 2x Colorimetric LAMP master mix and 10x Primer mix together at volumes required for *n* reactions then aliquot out 23µL per tube

3. Add 2µL of sample DNA to each tube. **N.B. It is recommended samples are run in triplicate.**
4. Include a positive and negative (blank elution buffer or nuclease free-water) control samples for each LAMP assay run, with negative control samples added after all other samples have been processed. **N.B. It is recommended controls are run in triplicate.**
5. Incubate reactions at 64^{o}C for 80 min. Reactions may be removed from incubation periodically (every 15 to 20 min) and examined by eye as high DNA input may cause reactions to develop more quickly than the recommended 80 min.
6. Once complete, compare to positive and negative controls for interpretation, photograph the reactions and record the results. **N.B. <u>DO NOT</u> open LAMP assay tubes once complete. Opening completed LAMP assay tubes will contaminate the laboratory with DNA product.**