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Title of resource
DNA Extraction Protocol for *Simulium sp.* and *Onchocerca volvulus*
Authored by
When using this protocol, the following should be referenced: Samuel Armoo[1], Romain Derelle[2], Daniel Boakye[3], Mike Y. Osei-Atweneboana[1], Charles Brockhouse[4], John Colbourne[2.]. Adapted from Brockhouse *et al (*1993). [1]Council for Scientific and Industrial Research – Water Research Institute, Accra, Ghana. [2]University of Birmingham, Birmingham, UK [3]Noguchi Memorial Institute for Medical Research, Accra, Ghana [4]Creighton University, Omaha, NE, USA
DOI
N/A
Description
This protocol is for extraction of genomic DNA from *Simulium* sp and *Onchocerca volvulus* tissues. This is a CTAB-based protocol, which is adapted from Brockhouse *et al* (1993).
Intended use
Scientific research use and training purposes.
Restrictions on use
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Resource history

GN 24 DNA Extraction Protocol for *Simulium sp.* and *Onchocerca volvulus*

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1.0 Introduction

Due to the high pigmentation of blackflies, and the PCR inhibition properties of the pigments, the CTAB-based protocol used by Brockhouse and co (Brockhouse et al., 1993) remains a viable choice for DNA extraction given the consistency of high quality DNA produced. Therefore, this protocol was used for DNA extraction in this project with slight modifications. More details below.

2.0 Preparing Buffers and Solutions

Buffers and solutions are prepared in advance and stored for downstream applications

Details of buffer and solution preparation steps below.

2.1 CTAB homogenization buffer

How to prepare a 200 mL solution of CTAB homogenization buffer (100nM Tris-HCL pH 8; 1.4M NaCl; 0.2% β-mercaptoethanol; 0.02 Na_2EDTA; 2% CTAB)

- Add the reagents below to160 mL of distilled water. Use a fume hood.

Reagent	Mass / Volume
NaCl	16.36 g
β-mercaptoethanol	400 uL
Tris-HCl*	20 mL
Na_2EDTA (0.5M)*	8 mL
CTAB	4 g

*See appendix for preparation

- Top up to 200 mL with distilled water.
- Filter solution through a Stericup® filter unit or any portable filtration.
- Store at 4 °C

2.2 chloroform: isoamyl alcohol (24:1)

- Mix the following in a fume hood.

Reagent	Volume
Chloroform	192 mL
Isoamyl alcohol	8 mL

- Store at room temperature in a fume hood.

2.3 Sample Preparation

DNA was extracted from alcohol-dehydrated blackfly samples, and air-dried individual *O. volvulus* larvae on glass slides. Blackfly heads, and whole larval worms were used for DNA extractions. To re-hydrate the

preserved samples, blackfly heads and worm larvae were transferred into TE buffer and stored overnight at 4 °C.

2.4 Sample Homogenization and DNA Extraction

1. Transfer rehydrated blackfly or worm tissue into 500 uL of pre-warmed (60 °C) CTAB homogenization buffer in a 1.5 mL tube.
2. The animal tissue was crushed in the CTAB homogenization buffer using BioEcho tissue grinding pestle.
3. Add 1 mg/mL of proteinase K and mix.
4. Incubate at 60 °C overnight in a mixing incubator at low speed.
5. Add 500 uL of chloroform/isoamylalcohol (24:1) solution (in fume hood), and gently mix for 3 mins by inverting the tube.
6. Centrifuge at 14,000 xg for 12 min.
7. After centrifugation the mixture separates into a colorless aqueous upper layer, an interphase, and a lower chloroform phase. Transfer the upper aqueous layer into a new 1.5 mL tube, and discard the old one.
8. Add 1 uL DNase-free RNase to the recovered mixture and incubate at 37 °C for. 30 min.
9. Add 350 uL of isopropanol (or 2/3 of the recovered volume), and gently mix by inverting the tube.
10. Incubate mixture overnight at room temperature.
11. Centrifuge the mixture at 14,000 xg for 15 mins to pellet the DNA.
12. Carefully remove and discard the supernatant, then add 100 uL of cold freshly prepared 70% ethanol.
13. Centrifuge the mixture at 14,000 xg for 15 mins to pellet the DNA.
14. Carefully remove and discard the supernatant, then add 100 uL of cold freshly prepared 70% ethanol.
15. Centrifuge the mixture at 14,000 xg for 15 mins to pellet the DNA.
16. Carefully remove and discard the supernatant, then dry the pellet by leaving tube open at room temperature for 5 minutes.
17. Resuspend the pellet in 100 uL of low TE buffer (pH 8), and store at - 20 °C

Reference

Brockhouse, C.L., Vajime, C.G., Marin, R., and Tanguay, R.M. (1993). Molecular identification of onchocerciasis vector sibling species in black flies (Diptera: Simuliidae). Biochem Biophys Res Commun *194*, 628-634.

Appendix

How to prepare a100 mL of 1M Tris-HCL (pH 8.0) solution

Reagent	Mass / Volume
Tris salt	12.11 g
Distilled water	80 mL

Add 12.11 g of Tris base to 80 mL of distilled water. Adjust to pH 8 with HCl (usually 4.2 mL of HCl will be required).

How to prepare a 100 mL of 0.5M EDTA (pH 8.0) solution

Reagent	Mass / Volume
EDTA disodium salt	18.61 g
Distilled water	80 mL

Add 18.61 g of EDTA disodium salt to 80 mL of distilled water. Adjust pH to 8 with a few pellets of NaOH (usually 2g NaOH pellets will be required). Top up with distilled water to 100 mL.

How to prepare a100 mL solution of TE buffer

Reagent	Volume
0.5M EDTA (pH 8)	0.2 mL
Tris-HCL (pH 8)	1 mL

- Top-up the above mixture with distilled water to 100 mL.
- Sterilize by autoclaving
- Store at 4 °C