

Blackfly DNA extraction

First published in Mustapha M, Krüger A, Tambala PAJ & Post RJ (2005) Incrimination of *Simulium thyolense* (Diptera: Simuliidae) as the anthropophilic blackfly in the Thyolo onchocerciasis focus in Malawi. *Ann. Trop. Med. Parasit.* **99**, 181-192.

You will need: 1x Lysis buffer
5% Chelex solution

1. Turn on water bath to 56c
2. Remove body part from Ethanol into fresh empty 1.5ml tube.
3. Place in drying block with top open at 37c to dry. Takes approx 10mins.
4. Mix 100ul lysis buffer (1x) and 1ul proteinase K solution (10mg/ml)
5. Add 2 drops of the lysis buffer solution to the fly at first. Crush for a bit (homogenise) then add the rest. Incubate at 56c for 2 ½ hours.
6. Turn on shaking incubator to 56c.
7. Take 20ul of this lysate and add 200ul Chelex (5%). The remaining lysate can be stored at -20c.
8. Place in shaking incubator at 56c for 1 hour.
9. Spin down for 2mins at 13krpm.
10. Store at -20c long term or +4 short term.
11. Before use shake for 2mins and spin down chelex. Take 3ul of supernatant for PCR.

10x lysis buffer: 1% SDS
10mM Tris HCL pH8.2
1mM EDTA
25mMNaCl

Which is 10g SDS + 0.37224g EDTA + 1.461g NaCl + 5ml TrisHCl (2M) pH8.2 into 1000ml. Store in fridge but reheat before use.