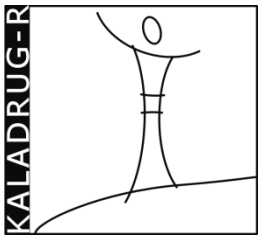
KALADRUG-R: Laboratory SOP #1a



Promastigote DNA extraction at ITMA for sequencing

Date of preparation: April 2nd 2010

Author: ITMA

A. Preparation biological Material

1. Check all cultures for contamination/other irregularities by inverted microscopy before pellet preparation (3 washing steps with PBS).

2. Prepare 1 pellet of promastigotes per flask of 5 mL HOMEM + 20% FCS culture.

3. Two to three stationary cultures should be sufficient for a total yield of 7-10 µg DNA.

4. Harvest cells on the second day of the stationary phase (± 1,3 10^8 promastigotes per flask).

B. DNA isolation from promastigote pellet with QIAamp DNA Mini Kit (#51304)

1. Pipet 20 µL QIAGEN Protease (Proteinase K) to the promastigote pellet of 1 culture in a 1.5 mL microcentrifuge tube.

2. Add 200 µL Buffer AL and mix by pulse-vortexing for 15s.

4. Incubate at 56°C for 10 min.

5. Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.

6. Add 200 µL ethanol (96-100%) to the sample, and mix again by pulse-vortexing for 15s. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.

7. Carefully apply the mixture from step 6 to the QIAamp Spin Column (in a 2 mL collection tube) without wetting the rim, close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 mL collection tube, and discard the tube containing the filtrate.

8. Carefully open the QIAamp Spin Column and add 500 µL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 mL collection tube (provided), and discard the collection tube containing the filtrate.

9. Carefully open the QIAamp Spin Column and add 500 µL of Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed for 3 min.

10. Place the QIAamp Spin Column in a clean 1.5 mL microcentrifuge tube and discard the collection tube containing the filtrate. Carefully open the QIAamp Spin Column and add 200 µL

Buffer AE or distilled water. Incubate at room temperature (15-25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

11. Pool all tubes and measure DNA concentration by Nanodrop.

Amount of DNA required: total min 5 µg (up to 7-10 µg)

DNA stock for shipment: in AE buffer (10 mM Tris-Cl, 0.5 mM EDTA pH 9.0) at a concentration greater than 20 ng/µL.

Obligatory quantity/quality check: run 1 µL DNA sample on a 0,8 % agarose gel (100V for 1 hr) along with 50 ng NEB lambda ladder (New England Biolabs, N3012S)

Check DNA by doing a *L. donovani* specific PCR (ISOP#3)

Dispatching protocol:

1. E-notify Mandy Sanders at, mjs@sanger.ac.uk and Theresa Feltwell at tfelt@sanger.ac.uk of planned shipments. Email her the following information
    - the gel-picture of the DNA-samples along the specified lambda ladder
    - an excel sheet with complete strain information
    - project information: Leishmania diversity projects
    - contact person at Sanger: Matthew Berriman mb4@sanger.ac.uk

2. Ship samples by courier service (no cool shipment required) to:

    Welcome Trust Sanger Institute

    Theresa Feltwell / Mandy Sanders

    Welcome Trust Genome Campus

    Hinxton – Cambridge CB10 1SA

    United Kingdom

    (tel. +441223834244)

!! Make sure the following items are included in the shipment:

- DNA samples in clearly labelled tubes
- print out of matching gel-picture
- print out of matching excel sheet with strain info