DNA Extraction

Reagents:

- Re-suspension Buffer
    1) 150mM NaCl
    2) 10mM EDTA
    3) 50mM Tris 7.5
- 10% Sarkosyl (L-loril sarcosil)
- 10mg/mL RNAse A
- 10mg/mL Proteinase K
- Phenol
- Chloroform
- Phenol/Chloroform/Isoamyl Alcohol (25:24:1)
- 3M Sodium Acetate
- 100% EtOH or 100% Isopropanol

Protocol:

Extraction:

1) Re-suspend parasite in 150mM NaCl; 10mM EDTA and 50mM Tris 7.5 to a final volume of ~500µL.
2) Add 5µL of 10% Sarkosyl (L-loril sarcosil).
3) Add 5µL RNAse A (10mg/mL) for 1hr @ 37°C.
4) Add 5µL proteinase K (10mg/mL). Incubate overnight @ 4°C.
5) Extract x1 w/ phenol.
6) Extract x1 w/ phenol/chloroform/isoamyl alcohol (25:24:1).
7) Extract x1 w/ chloroform.

Note: For each extraction add an equal volume of phenol or chloroform, invert gently to avoid shearing genomic DNA. Spin 15,000xg 5'. Transfer top aqueous layer to new tube (avoid the interface with the white proteins) and add equal volume of phenol or chloroform…

Precipitation:

8) Add 1/10 the volume 3M Sodium Acetate pH 7.
9) Add two volumes of 100% EtOH or 0.8 volume of isopropanol.
10) Mix and leave -20°C overnight or leave @ RT 10'.
11) Centrifuge @ 13,000 rpm 30' @ 4°C.
12) Remove supernatent being careful not to disturb pellet. Air dry.
13) Re-suspend in ddH_2O.
14) Read OD @ A_{260}, store @ -20°C.