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Genomic DNA extraction from schistosome intermediate hosts and parasite stages

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DNAzol® GENOMIC DNA ISOLATION REAGENT
Using this reagent, the isolated DNA can be used without additional purification for Southern analysis, dot blot hybridization, molecular cloning, polymerase chain reaction (PCR) and other molecular biology and biotechnology applications of most of the intermediate host snails and parasites*

NOTE: Genomic DNA extraction from schistosome worms and *B. glabrata* with this reagent is not recommend for Southern analysis, dot blot hybridization or molecular cloning. Our analysis has found that there is incomplete DNA restriction enzyme digestion (unpublished).

Materials

- **Homogenizer**
- **Centrifuge**

Procedure
Homogenization

- homogenize the snail, parasite or related tissues using a homogenizer under liquid nitrogen
- measure the dry weight of homogenized tissues
- add 10 volumes DNAzol into dried pack homogenate
- mix by inversion or repeated pipetting

Centrifugation (optional)*

- sediment the homogenate for 10 min at 10,000 rpm at RT
- transfer the aqueous to a new tube
  * This step removes insoluble tissue fragments, partially hydrolyzed RNA and excess polysaccharides from the lysate/homogenate. It is required only for the isolation of DNA from a whole body snail.

DNA precipitation

- add 0.5 volumes of 200 proof Ethanol into homogenate and mix by inversion 5-8 times
- incubate at RT, 3 min
- Swirl the DNA onto the tip and transfer into a new tube

DNA wash

- wash DNA with 1 mL 70% DNAzol in EtOH to remove contaminants
- 75% EtOH with 1,000 rpm for 2 min, 4°C
- Solubilize the DNA by adding sterile water or low salt buffer in TE (pH 8.0)

Procedure

Genomic DNA extraction using CTAB Buffer/Phenol Chloroform/Iso-Amyl precipitation

This protocol is used for genomic DNA extraction from schistosome worms and *Biomphalaria glabrata* Winnepennickx et al., 1993

Homogenization

- homogenize the snail, parasite or related tissues using homogenizer under liquid nitrogen
- measure the dry weight of homogenized tissues

Lysis

- add 10 mL pre-warmed *CTAB/Proteinase K (0.1 mg/ml) buffer to 1 ml homogenized tissues
- mix by inversion and incubate at 60oC for 1 hour (mix by inversion every 15 min)

Protein/RNA precipitation

- add equal volume of phenol/chloroform/IAA (25:24:1 vol/vol)
- mix by inversion for 3 min
- spin down 10,00 rpm, 4°C, 15 min
- transfer the aqueous to a new tube
- add equal volume of chloroform/IAA (24:1 vol/vol)
- mix and spin down as above
- transfer the top aqueous to a new tube

RNA decontamination

- add Rnase A to the final concentration of 20 μg/mL
- incubate at 37°C, 30 min
- add equal volume of chloroform/IAA (24:1 vol/vol)
- mix and spin down as above
- transfer the top aqueous to a new tube

DNA precipitation

- add 2.5 volume of cold 200 proof EtOH (-20°C) and mix gently
- precipitate at -20°C, overnight
- spin down as above

DNA washes

- wash pellet with 1 mL 70% ethanol with 10,000 rpm centrifugation for 3 min
- repeat washing twice
- air dry pellet at RT

Solubilize the DNA with nuclease free water

References

Winnepenninckx B, Backeljau T, De Wachter R. Extraction of high molecular weight DNA from molluscs. Trends Genet 1993: 9: 407.

* CTAB buffer: 2% Cethy Trimethyl Ammonium Bromide, 1.4 M NaCl, 0.2% beta-mercaptoethanol, 20 mM EDTA, 100 mM Tris/HCl

For more information, contact André Miller at **amiller@afbr-bri.com**
