

DNA extraction Nematodes

Nucleic acid Extraction using Lysis buffer I (Holterman *et al.* 2006):

- Prepare Lysis buffer I with the following composition: 200 mM NaCl, 200 mM Tris-HCl (pH8.0), 1% (v/v) β -mercaptoethanol and 800 μ g/ml proteinase K. Add the β -mercaptoethanol and proteinase K only shortly before use.
- Pipette the DESS solution containing the nematode in a glass well. Transfer the nematode to a PCR tube containing 25 μ l of MGW and add 25 μ l of Lysis buffer I.
- Incubate the PCR tube for 1:30 h at 65°C in a thermostaker at 300rpm, followed by 5 min. at 99°C to deactivate the proteinase K. Alternatively, the incubation can be performed in a thermocycler.
- After DNA extraction, no DNA clean-up is required.
- Either use extracted DNA immediately or store it at -20°C or below until use.

Nucleic acid extraction and purification using the GenElute Mammalian DNA extraction kit (SIGMA):

- Add 180 μ l of Lysis Solution T and 20 μ l Proteinase K (20mg/ml) solution to a reaction tube.
- Pipette the DESS solution containing the nematode in a glass well. Transfer the nematode to the tube containing the lysis solution.
- Place the tube in a thermomixer (e.g. Eppendorf) for 2-4 hours at 55°C and 1 minute intervals of 300 rpm.
- Add 500 μ l of Column Preparation Solution (SIGMA) to preassembled tubes with columns.
- Centrifuge for 1 minute at 12,000 g and discard the flow-through liquid.
- Add 200 μ l of ethanol (95-100%) to the lysate and vortex for 5-10 seconds.
- Transfer the lysate to the prepared binding columns. Centrifuge for 1 minute at 6500 g. Discard the collection tube with flow-through liquid and place the binding column in a new 2 ml collection tube.
- Add 500 μ l of Wash Solution to the column and centrifuge for 1 minute at 6500 g. Place the binding column in a new collection tube.
- Add another 500 μ l of Wash Solution to the column and centrifuge for 3 minutes at 12,000- 16,000 g. Check if the columns are absolutely free of any residual ethanol. If not, centrifuge for another minute and check again. Place the binding column in a new collection tube.
- Add 100 μ l of Elution Solution to the column and centrifuge for 1 minute at 6500 g. Add another 100 μ l of Elution Solution to the column (do not discard the flow-through!) and centrifuge again for 1 minute at 6500 g. Discard the binding column, the flow through liquid contains your purified DNA.
- After DNA extraction, no DNA clean-up is required.
- Either use extracted DNA immediately or store it at -20°C or below until use.