

DNA Extraction from Cells or Tissue for mtDNA Quantification

Modified from Tom Getty's lab

Purpose:

To isolate total DNA from cultured cells for use in RT-PCR for mtDNA quantification

Materials:

- 1) RNazol B (Tel-test)
- 2) Back extraction buffer (BEB, 200 ml)
 - a. 4 M guanidine thiocyanate, MW 118.16, 94.53 g in 200 ml (SIGMA-G9277)
 - b. 50 mM sodium citrate, MW 294.1, 2.94 g in 200 ml
 - c. 1 M Tris free base, MW 121.14, 24.23 g in 200 ml
- 3) Polyacryl carrier (Molecular Research Corporation PC152)
- 4) Chloroform
- 5) Isopropanol
- 6) 75% ethanol
- 7) 8 mM NaOH
- 8) 1 M Hepes
- 9) 100 mM EDTA

Prepare:

8mM NaOH	100mM EDTA
320mg NaOH	100mls of 0.5mM EDTA pH=8
QS to 1L	400mls H ₂ O
Sterile filter	Sterile filter

Procedure:

- 1) Add 1 ml RNazol to each well of 6-well plate. Pipette up and down to homogenize cells.
- 2) Add cells to 5ml Falcon tube (2063 tube)
- 3) Wash well with an additional ml of RNazol

OR For Tissue

- 1) Add 2 mls of RNazol to an 8ml Sttarstedt tube and add 50-100mg tissue
- 2) Homogenize 2x10 seconds
- 3) Transfer to a 5ml falcon tube (2063 tube)

- 4) Add 200 ul chloroform per 2 ml of RNazol.
- 5) Vortex tubes vigorously for 15 s and incubate 5 min.
- 6) Centrifuge 10,500 rpm in High-Speed Sorvall or Beckman for 15 min at 4°C.
- 7) Remove the top aqueous phase containing RNA. You can proceed to isolate RNA from this phase per the RNA protocol.
- 8) To remaining interphase and organic phase, add 500 ul BEB per 1 ml RNazol.
- 9) Mix well by inverting several times.
- 10) Let sit at RT for 10 min
- 11) Centrifuge 3000g for 30 min at 4°C in Sorvall or Beckman High speed.
- 12) Transfer upper phase containing DNA to new Eppendorf tubes.

- 13) Add 4ul polyacryl carrier per ml RNAzol used.
- 14) Precipitate DNA by adding 400 ul isopropanol per ml RNAzol.
- 15) Mix by inverting several times and let sit at RT for 5 min.
- 16) Centrifuge 12,000g for 5 min at 4°C in microfuge.
- 17) Remove supernatant and discard.
- 18) Wash pellet with 1 ml 75% ethanol and let sit at RT for 5 minutes. Shake to ensure that pellets are detached from the tubes. (You can freeze after adding EtOH.)
- 19) Centrifuge 12,000 g for 5 min at 4°C.
- 20) Remove ethanol.
- 21) Repeat steps 17-19 for a total of three washes.
- 22) Air dry (or carefully use a Kim-wipe) pellet 3-5 min at RT.
- 23) Add 30 ul 8 mM NaOH to dissolve pellet.
- 24) Vortex and heat at 55°C for 2 min if necessary to dissolve pellet.
- 25) Centrifuge 12,000g, 10 min, 4°C to remove undissolved material.
- 26) Transfer 30 ul to new Eppendorf tubes.
- 27) Add 0.34 ul 1 M Hepes to bring sample to pH 8.
- 28) Add 0.3 ul 100 mM EDTA and vortex.
- 29) If DNA does not go completely into solution, heat again at 55°C for 2-4 min.
- 30) Store at +4 or -20.

Proceed to SYBR green assay for mitochondrial vs nuclear DNA

Materials:

- 1) ND1 (NADH dehydrogenase subunit 1)
 - a. 202 bp fragment primers (10 uM working stock; 100 uM stock; IDT):
 - b. Forward: CCC ATT CGC GTT ATT CTT
 - c. Reverse: AAG TTG ATC GTA ACG GAA GC
- 2) LPL 225 bp fragment primers (10 uM working stock; 100 uM stock; IDT):
 - a. Forward: GGA TGG ACG GTA AGA GTG ATT C
 - b. Reverse: ATC CAA GGG TAG CAG ACA GGT
- 3) Total (mitochondrial + nuclear) DNA template (10 ng or 5 ng/ul usually)
 - a. DNA isolated with RNAzol method above
- 4) 2x Sybr Green master mix from Applied Biosystems, Part number 4309155

Follow QRT-PCR protocol from here using 5ng of DNA per reaction.

To compare samples, divide the amount of mitochondrial DNA by the amount of nuclear DNA within samples.