DNA EXTRACTION FROM CELLS

DNA Purification

- Spin 500,000 cells at 1,200 rpm, discard the cell culture medium and wash the pellet with PBS (the pellet can be stored at -20 degrees or directed used). If a fresh cell pellet is used for DNA extraction, it has to be stored in ice until the protocol is started.
- Clean the work surface and the micropipettes with 70% ethanol.
- If for DNA extraction a frozen cell pellet is used, before to start with the protocol thaw the cell pellet for 10-15 minutes in ice, so proceed with the assay.

TRIZOL PROTOCOL (Cells grown in suspension):

https://tools.thermofisher.com/content/sfs/manuals/trizol_reagent.pdf

Briefly:

- Add 0.75 mL of TRIzol Reagent per 5–10 × 10^6 cells.
- Pipet the lysate up and down several times to homogenize.
- STOPPING POINT Samples can be stored at 4°C overnight or at –20°C for up to a year.
- Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
- Add 0.2 mL of chloroform per 1 mL of TRIzol Reagent used for lysis, then securely cap the tube.
- Incubate for 2–3 minutes.
- Centrifuge the sample for 15 minutes at 12,000 × g at 4°C (the mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase).
- Transfer the interphase and the lower phenol-chloroform phase containing the DNA to a new tube (Remove any remaining aqueous phase overlying the interphase. This is critical for the quality of the isolated DNA).
- Add 0.3 mL of 100% ethanol per 1 mL of TRIzol Reagent used for lysis AND mix by inverting the tube several times.
- Incubate for 2–3 minutes and centrifuge for 5 minutes at 2000 × g at 4°C to pellet the DNA.
- Transfer the phenol-ethanol supernatant to a new tube.
- Resuspend the pellet in 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 1 mL of TRIzol Reagent used for lysis.
- Incubate for 30 minutes, mixing occasionally by gentle inversion.
- STOPPING POINT The DNA can be stored in sodium citrate/ethanol for at least 2 hours.
- Centrifuge for 5 minutes at 2000 × g at 4°C.
- Discard the supernatant.
- Resuspend the pellet in 1.5–2 mL of 75% ethanol per 1 mL of TRIzol Reagent used for lysis.
- Incubate for 10–20 minutes, mixing occasionally by gentle inversion.
- STOPPING POINT The DNA can be stored in 75% ethanol at several months at 4°C.
- Centrifuge for 5 minutes at 2000 × g at 4°C.

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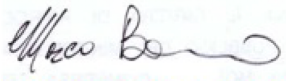


- Discard the supernatant.
- Air dry the DNA pellet for 5–10 minutes.
- Resuspend the pellet in 0.3–0.6 mL of 8 mM NaOH by pipetting up and down.
- Centrifuge for 10 minutes at 12,000 × g at 4°C to remove insoluble materials.
- Transfer the supernatant to a new tube, then adjust pH as needed with HEPES.
- Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C

Application: PCR, Restriction Enzyme digestion and Southern Blots.

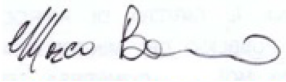
Time: 1 hour.

DNeasy Blood & Tissue Kit (from Blood, Tissue and Cells):

https://www.qiagen.com/us/shop/sample-technologies/dna/genomic-dna/dneasy-blood-and-tissue-kit/#technicalspecification

Briefly:

- For blood with non-nucleated erythrocytes, pipet 20 µl proteinase K into a 1.5 ml microcentrifuge tube and adjust the volume to 220 µl with PBS.
- For cultured cells: Centrifuge the appropriate number of cells (maximum 5 x 106) for 5 min at 300 x g and resuspend the pellet in 200 µl PBS. Add 20 µl proteinase K. If for DNA extraction a frozen cell pellet is used, before to start with the protocol thaw the cell pellet for 10-15 minutes in ice, so proceed with the assay.
- Add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature.
- Add 200 µl Buffer AL (without added ethanol). Mix thoroughly by vortexing, and incubate at 56°C for 10 min.
- Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing.
- Pipet the mixture into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at 10000 rpm) for 1 min. Discard flow-through.
- Add 500 µl Buffer AW1, and centrifuge for 1 min at 10000 rpm. Discard flow-through.
- Add 500 µl Buffer AW2, and centrifuge for 1 min at 10000 rpm. Discard flow-through and collection tube.
- Place the DNeasy Mini spin column in a new 2 ml collection tube and centrifuge for 4 min at 13000 rpm. Discard flow-through and collection tube.
- Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube and pipet 100 µl Nuclease-Free Water onto the DNeasy membrane.
- Incubate at room temperature for 1 min, and then centrifuge for 1 min at 10000 rpm to elute.



DNA EXTRACTION FROM CELLS

- For animal tissue: Cut up to 25 mg tissue (up to 10 mg spleen) into small pieces, and place in a 1.5
  ml microcentrifuge tube. Add 180 µl Buffer ATL. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer.
- Add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature.
- Vortex for 15 s. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.
- Pipet the mixture into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at 10000 rpm) for 1 min. Discard flow-through.
- Add 500 µl Buffer AW1, and centrifuge for 1 min at 10000 rpm. Discard flow-through.
- Add 500 µl Buffer AW2, and centrifuge for 1 min at 10000 rpm. Discard flow-through and collection tube.
- Place the DNeasy Mini spin column in a new 2 ml collection tube and centrifuge for 4 min at 13000 rpm. Discard flow-through and collection tube.
- Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube and pipet 100 µl Nuclease-Free Water onto the DNeasy membrane.
- Incubate at room temperature for 1 min, and then centrifuge for 1 min at 10000 rpm to elute.

Application: PCR, STR Analysis, Sanger Sequencing, NGS.

Time: 20 min -1 hour.

For the isolation of DNA from formalin-fixed, paraffin-embedded tissue sections it is recommended to use the **QIAamp DNA FFPE Tissue Kit:**

https://www.qiagen.com/us/resources/resourcedetail?id=63a84dc7-d904-418e-b71a-1521cf318e82&lang=en

Application: PCR, STR Analysis, Sanger Sequencing, NGS.

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