Standard operating procedure for simultaneous preparation of DNA and RNA from cell culture samples

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Simultaneous preparation of DNA and RNA from cell culture samples

- The protocol is based on the AllPrep DNA/RNA Mini Kit (Qiagen, Cat # 80204), which is designed to extract genomic DNA and total RNA simultaneously from a single biological sample. The AllPrep DNA/RNA procedure integrates selective binding of double-stranded DNA with well-established RNeasy technology (Qiagen).
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Equipment

- Agilent 2100 Bioanalyzer (Agilent Technologies)
- NanoDrop 1000 spectrophotometer (PeqLab, VWR)
- Qubit fluorometer (Invitrogen, Thermo Fisher Scientific)
- Standard microcentrifuge (with rotor for 2 ml tubes)
- Disposable laboratory gloves
- Sterile, RNase-free pipet tips

Kits and Chemicals

- AllPrep DNA/RNA Mini Kit (Qiagen, Cat # 80204)
- RNeasy MinEluteCleanup Kit (Qiagen, Cat # 74204)
- Qubit RNA HS Assay Kit (Invitrogen Cat # Q32852, Thermo Fisher Scientific)
- Agilent RNA 6000 Nano Kit (Agilent Technologies, Cat # 5067-1511)
- Roti nucleic acid free - solution for removal of nucleic acid contaminations from surfaces, (Roth, Cat # HP69.1)
- Freshly prepared 70% Ethanol
- 96% Ethanol
- Nuclease-free water

Samples

- Bovine cells (e.g., kidney (MDBK), mammary (Mac-T), embryonic lung (EBL), trophoblast, luteal cells) grown in a monolayer in cell-culture are trypsinized, washed with PBS and cell number was determined. The cell number should not exceed 10^7. Cell count should be 3 - 4 x 10^6 cells for cells of unknown RNA &DNA quantity.
- Trypsinized cells are centrifuged at 300 x g for 5 min and collected as a cell pellet prior to lysis.

Preparation

- Complete the Buffer RLT Plus before the first use by adding 10 µl β-mercaptoethanol (β-ME) per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25ºC) for 1 month after addition of β-ME. It may form a precipitate during storage. If necessary, redissolve by warming and place at room temperature.
- Buffers RPE, AW1, and AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

Cell Lysis

1. Disrupt the cells by adding Buffer RLT Plus with 1 % β-ME to the cell pellet, dissolve the pellet thoroughly by flicking the tube. For < 5 x 10^6 cells add 350 µl RLT Plus buffer (with 1% β-ME), for <5 x 10^6 - 10^7cells add 600µl RLT Plus buffer (with 1% β-ME).
2. Homogenize the lysate by pipetting it directly into a QIAshredder spin column placed in a 2 ml collection tube and centrifuge for 2 min at maximum speed. If liquid remains on the column membrane, repeat the centrifugation step until all liquid has passed through the membrane.

DNA Binding

3. Transfer the homogenized lysate to an AllPrep DNA spin column placed in a 2 ml collection tube. Close the lid and centrifuge for 30 s at ≥ 8000 x *g* (≥10,000 rpm).
4. Place the AllPrep DNA spin column in a new 2 ml collection tube and store at room temperature (15–25ºC) or at 4°C for later DNA purification in steps 13–17. Use the flow-through for RNA purification in steps 5–12.

Note: Do not store the AllPrep DNA spin column at room temperature or at 4°C for long periods and do not freeze the column.

Total RNA purification

5. Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the flowthrough from step 4, mix well by pipetting up and down. Do not centrifuge.

Note: If some lysate was lost during homogenization and DNA binding to the AllPrep DNA spin column, adjust the volume of ethanol accordingly.

6. Transfer up to 700 µl of the sample, including any possible precipitate, to an RNeasy spin column placed in a 2 ml collection tube. Close the lid and centrifuge for 15 sec at ≥8000 x *g* (≥10,000 rpm). Discard the flowthrough. If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.
7. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 sec at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flowthrough.

Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube. The column should not contact the flow-through. Be sure to empty the collection tube completely.

8. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid and centrifuge for 15 sec at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flowthrough.

Note: Buffer RPE is supplied as a concentrate and ethanol has to be added before use!

9. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. The long centrifugation dries the spin column membrane to ensure that no ethanol is carried over during RNA elution.

Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube. The column should not contact the flowthrough to avoid carryover of ethanol.

10. Transfer the column to a new collection tube and centrifuge dry for 1 min at full speed.
11. Place the RNeasy spin column in a new 1.5 ml collection tube. Add 50 µl RNase-free water directly to the spin column membrane. Close the lid and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.
12. If the expected RNA yield is >30 µg, repeat step 11 using another 50 µl of RNase-free water, or using the eluate from step 11 if high RNA concentration is required. If using the eluate from step 11, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but final RNA concentration will be higher.

Store RNA on ice and/or at -70°C.

Genomic DNA purification

13. Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 4. Close the lid and centrifuge for 15 sec at ≥8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flowthrough.

Note: Buffer AW1 is supplied as a concentrate and ethanol has to be added before use.

14. Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Close the lid and centrifuge 2 min at full speed to wash the spin column membrane. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution.

Note: Buffer AW2 is supplied as a concentrate, ethanol has to be added before use.

15. After centrifugation, carefully remove the AllPrep DNA spin column from the collection tube. If the column contacts the flow-through, empty the collection tube. Centrifuge the spin column again for 1 min at full speed.
16. Place the AllPrep DNA spin column in a new 1.5 ml collection tube. Add 100 µl Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature (15 – 25ºC) for 1 min and centrifuge 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.
17. Repeat step 16 to elute further DNA with another 50 µl EB buffer. To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube to collect the second DNA eluate. Combine the both DNA eluates.

Note: To achieve a higher DNA concentration, elute with 2 x 50 µl Buffer EB. The final DNA yield may be reduced.

Storage of DNA at -20°C

Quantification and Quality check of the total RNA and genomic DNA samples

- RNA samples are tested for remaining traces of genomic DNA via PCR with primers amplifying genomic DNA (according to Weikard et al. 2009, doi: 10.1186/1471-2164-10-186) and, in case of DNA contamination, the RNA sample is further cleansed by additional DNase treatment using the RNeasy MinEluteCleanup Kit (Qiagen, Cat # 74204) .
- RNA concentration and purity are quantified on a NanoDrop 2000 spectrophotometer and on a Qubit 2.0 fluorometer using the Quant-it Qubit RNA HS Assay kit according to the manufactuer's instructions.
- RNA integrity is evaluated by denaturing agarose-electrophoresis and on the Agilent Bioanalyzer 2100 using the RNA 6000 Nano kit according to the manufactuer's instructions.
- DNA concentration and purity is quantified on a NanoDrop 2000 spectrophotometer and DNA integrity is analyzed by agarose-electrophoresis.