Simultaneous Purification of Genomic DNA and Total RNA from Animal Tissues

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# Important Points Before Starting

* Do not allow tissue to thaw during weighing or handling prior to homogenization in Buffer RLT Plus/ß-Mercaptoethanol/Reagent DX mixture. To prevent this, excise and handle tissue in a disposable square weight boat positioned on a dry ice-cooled 3”x6” metal cutting block.
* Ensure usage of a small antistatic polystyrene pour boat and closing of scale walls when weighing tissue to limit error in recording of weight.
* The maximum number of samples to be extracted and purified per person is 6 (i.e., 2 researchers x 6 samples = 12 samples for extraction/purification).
* Homogenized tissue lysates from step 4 can be stored at -70˚C for months.
* Buffer RLT Plus may form a precipitate upon storage. If necessary, redissolve by warming, and, then, place at room temperature.
* Preparation of Buffer RLT Plus/ß-Mercaptoethanol/Reagent DX mixture:
  + 10µL of ß-Mercaptoethanol is added to 1mL of Buffer RLT Plus prior to its use.
  + Reagent DX is added to Buffer RLT Plus at a final concentration of 0.05% (v/v)
    - Reduces homogenization-induced foaming.
* Buffer RPE, Buffer AW1, and Buffer AW2 are, each, supplied as a concentrate. Before using for the first time, add the appropriate volume of 100% ethanol, as indicated on the bottle, to obtain a working solution.
* Buffer RLT Plus, Buffer RW1, and Buffer AW1 contain a guanidine salt and are, therefore, not compatible with disinfecting reagents containing bleach.
* Use 70% ethanol to clean gloves and working area periodically, as RNA purification requires proper aseptic technique for high-quality extracts.
* Place Buffer EB in lab coat pocket prior to beginning procedure to ensure gently warming of solution prior to DNA elution.
* All steps of the procedure, following homogenization, should be performed at room temperature (~25˚C). During so, work quickly.

# Required Materials

* Supplied by Qiagen:
  + AllPrep DNA/RNA Kit (Qiagen, [link to product](https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/multianalyte-and-virus/allprep-dna-rna-kits?catno=80204))
  + Reagent DX (Qiagen, [link to product](https://www.qiagen.com/us/products/discovery-and-translational-research/lab-essentials/buffers-reagents/reagent-dx))
    - 1.75μL/sample
* Not supplied by Qiagen:
  + Small Antistatic Polystyrene Pour Boats (VWR, SKU#: [76299-240](https://us.vwr.com/store/catalog/product.jsp?catalog_number=76299-240))

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* + Disposable Square Weigh Boats (100mL) (VWR, SKU#: [10770-406](https://us.vwr.com/store/catalog/product.jsp?catalog_number=10770-406))

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* + Razor Blade (VWR, SKU#: [55411-050](https://us.vwr.com/store/catalog/product.jsp?catalog_number=55411-050))

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* + Lysing Matrix D Tubes (VWR, SKU#: [IC116913100](https://us.vwr.com/store/product?keyword=IC116913100))

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* + Ultrafree® Centrifugal Filter Units (VWR; SKU#: [102970-584](https://us.vwr.com/store/catalog/product.jsp?catalog_number=102970-584))

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* + 100% Ethanol (VWR, SKU#: [89125-172](https://us.vwr.com/store/catalog/product.jsp?catalog_number=89125-172))
  + Spray bottle of 70% Ethanol
  + Forceps
  + Punch Biopsy Tool (Fine Science Tools, SKU#: [18035-03](https://www.finescience.com/en-US/Products/Surgical-Accessories/Sample-Punches/Sample-Corers/18035-03))

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* + 3”x6” Metal Cutting Block

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* + β-Mercaptoethanol (Fisher Scientific, SKU#: [ICN19483425](https://www.fishersci.com/shop/products/2-mercaptoethanol-molecular-biology-mp-biomedicals/ICN19483425))
  + 3x Dry Ice-Filled Containers with Lids

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* + - 1 for lysing matrix D tubes containing excised tissue
    - 1 for cutting block and disposable square weigh boat
    - 1 for master sample tubes and forceps
  + Pipettes with Corresponding Pipette Tips
  + Microcentrifuge Tube Racks

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# Extraction

1. Excise 7.0-9.5mg of tissue, from a master sample tube, using forceps and a tissue biopsy tool for sample handling. Denote the exact weight of the excised tissue.
   1. **NOTE:** If necessary, use a 100% ethanol-cleaned razor blade to cut pieces of tissue obtained by the tissue biopsy tool, in a weigh boat on a dry ice-cooled 3”x6” metal cutting block, to reduce sample weight.
   2. **NOTE:** Instruments (e.g., forceps, punch biopsy tool, razor blade) should be cleaned in 100% ethanol prior to initial usage and between excision of different samples.
2. Use forceps to submerge the excised tissue in beads within a lysing matrix D tube.
3. Add 350µL of Buffer RLT Plus/ß-Mercaptoethanol/Reagent DX mixture to each lysing matrix D tube.
   1. **NOTE:** Ensure Buffer RLT Plus/ß-Mercaptoethanol/Reagent DX mixture does not freeze in dry ice-cooled lysing matrix D tubes prior to homogenization. This is best achieved by indirectly cooling the lysing matrix D tubes in a microcentrifuge tube rack.
4. Disrupt and homogenize the lysate at 6m/s for 40 seconds.
   1. **NOTE:** Settings stored in the homogenizer as the Human Kidney protocol.
   2. **NOTE**: If excessive foaming occurs in lysing matrix D tubes, gently spin down for ~10 seconds prior to proceeding to step 5.
5. Dump the lysate and beads from the lysing matrix D tubes into centrifugal filter units.
6. Centrifuge for 3 minutes at 21,100 x g.
7. Carefully transfer supernatant (avoiding pellet) to an AllPrep DNA spin column placed in a 2mL collection tube (supplied).
8. Centrifuge for 30 seconds at 10,000 x g.
   1. **NOTE**: Make sure that no liquid remains on the column membrane following centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.
9. Place the AllPrep DNA spin column in a new 2mL collection tube (supplied), and store at room temperature for later DNA purification in steps 18-21. Use the flow-through for RNA purification in steps 10-17.
   1. **NOTE**: Do not store the AllPrep DNA spin column at room temperature for long periods. Do not freeze the column.

# Total RNA Purification

1. Add 350µL of 50% ethanol to the flowthrough from step 9, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 11.
   1. **NOTE**: Do not create bubbles/foaming while mixing with the pipette.
2. Transfer up to 700µL of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2mL collection tube (supplied), and centrifuge for 15 seconds at 12,000 x g. Discard the flowthrough, and reuse the collection tube in step 12.
3. Add 700µL of Buffer RW1 to the RNeasy spin column, and centrifuge for 15 seconds at 12,000 x g. Discard the flowthrough, and reuse the collection tube in step 13.
   1. **NOTE**: After centrifugation, ensure the RNeasy spin column does not contact the flowthrough. Be sure to empty the collection tube completely.
4. Add 500µL of Buffer RPE to the RNeasy spin column, and centrifuge for 15 seconds at 8,000 x g. Discard the flowthrough, and reuse the collection tube in step 14.
5. Add 500uL of Buffer RPE to the RNeasy spin column, and centrifuge for 2 minutes at 8,000 x g.
6. Place the RNeasy spin column in a new 2mL collection tube (supplied). Centrifuge for 1 minute at 21,100 x g.
7. Place the RNeasy spin column in a new 1.5mL collection tube (supplied), and add 50µL of RNase-free water directly to the spin column membrane. Allow the RNase-free water to interact with the spin column membrane for 12 minutes, and, then, centrifuge for 1 minute at 8,000 x g.
   1. **NOTE**: Final eluate can be stored at -70˚C for months.

# Genomic DNA Purification

1. Add 500µL of Buffer AW1 to the AllPrep DNA spin column from step 9, and centrifuge for 15 seconds at 10,000 x g. Discard the flowthrough, and reuse the spin column in step 18.
2. Add 500µL of Buffer AW2 to the AllPrep DNA spin column, and centrifuge for 2 minutes at 21,100 x g.
   1. **NOTE**: After centrifugation, ensure the AllPrep DNA spin column does not contact the flowthrough. If the column contacts the flowthrough, empty the collection tube, and centrifuge the spin column, again, for 1 minute at 21,100 x g.
3. Place the AllPrep DNA spin column in a new 1.5mL collection tube (supplied), and add 35µL of Buffer EB directly to the spin column membrane. Allow the Buffer EB to interact with the spin column membrane for 5 minutes, and, then, centrifuge for 1 minute at 8,000 x g.
4. Repeat step 19 with 20uL of Buffer EB.
   1. **NOTE**: Final eluate can be stored at -70˚C for months.

# Troubleshooting

* Clogged AllPrep DNA or RNeasy spin column
  + Inefficient disruption and/or homogenization?
    - Increase *g*-force and/or centrifugation time.
    - Reduce the amount of excised tissue and/or increase homogenization time.
  + Too much starting material?
    - Reduce the amount of excised tissue.
* Low nucleic acid yield
  + Insufficient disruption and/or homogenization?
    - Increase *g*-force and/or centrifugation time.
    - Reduce the amount of excised tissue and/or increase homogenization time.
    - Increase the amount of lysis buffer (Buffer RLT Plus/ß-Mercaptoethanol/Reagent DX mixture).
  + Too much starting material?
    - Reduce the amount of excised tissue.
  + RNA still bound to RNeasy spin column membrane?
    - Repeat RNA elution (step 16).
  + DNA still bound to AllPrep DNA spin column membrane?
    - Repeat DNA elution, but incubate the AllPrep DNA spin column on the benchtop for 10 minutes with Buffer EB before centrifugation.
    - Heat Buffer EB to 70˚C prior to DNA elution.
  + Ethanol carryover?
    - During the second wash with Buffer RPE (step 14), increase centrifugation time to dry the RNeasy spin column membrane.
* DNA contaminated with RNA
  + Lysate applied to the AllPrep DNA spin column contains ethanol?
    - Add ethanol to the lysate after passing it through the AllPrep DNA spin column.
  + Excised tissue is affecting pH of eluate?
    - pH eluate to ensure it is neutral (~7).
* RNA contaminated with DNA
  + Excised tissue has high DNA content?
    - Reduce the amount of excised tissue.
* Low A260/A280 value in RNA eluate
  + RNase-free water used to dilute RNA for A260/A280 measurement?
    - Use 10mM Tris-Cl, pH 7.5, instead of RNase-free water to dilute the sample prior to examining purity.
* RNA degraded
  + Inappropriate handling of tissue?
    - Ensure that frozen tissue was flash-frozen immediately and stored in liquid nitrogen.
    - Perform all steps prior to homogenization quickly (steps 1-3) to avoid thawing of excised tissue.
  + RNase contamination?
    - Although the AllPrep buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce RNases during the protocol and/or external handling.
  + Homogenization too vigorous?
    - Reduce homogenization time and/or use a gentler homogenization method.
* Low nucleic acid concentration
  + Elution volume too high?
    - Elute nucleic acids in a smaller volume. Do not use less than 50µL of Buffer EB for the AllPrep DNA spin column, or less than 1x 30µL of RNase-free water for the RNease spin column. Although eluting in a reduced volume will increase nucleic acid concentration, yield may be decreased.
* Poor performance of nucleic acids in downstream experiments
  + Salt carryover during elution?
    - Ensure buffers are at room temperature (~25˚C).
    - Ensure that the correct buffer is used for each step of the protocol.
    - When reusing collection tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels.
  + Ethanol carryover?
    - During the second wash with Buffer RPE (step 14), increase centrifugation time to dry the RNeasy spin column membrane.