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Total RNA extraction from frozen placenta tissue

Scott Lindsay-Hewett[1]

[1]University of California, San Diego

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Scott Lindsay-Hewett

This protocol describes the isolation of high-quality total RNA from frozen placenta tissue. Tissue is disrupted using a bead beater, and total RNA is isolated using the *mir*Vana miRNA Isolation Kit from Ambion.

Written steps are adapted from Ambion's manual for the *mir*Vana miRNA Isolation Kit and BioSpec's instructions for the Mini-BeadBeater-16.

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Centrifuge

Benchtop Centrifuge

Eppendorf **5405000441**

Any benchtop centrifuge will suffice

Block heater

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Vortex mixer

Any **xx**

Mini-Beadbeater-16

high-energy cell disrupter

BioSpec **607**

1 speed

Fume hood

Fume hood

Generic **Unknown**

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Set of micropipettes with rack: 100-1000 µl, 20-200 µl, 2-20 µl, and 0.5-10 µl
Pipettor set

Pipetman **QP-1001-07**

Can use equivalent Pipettors

Bioanalyzer
Bioanalyzer

Agilent **G2991AA**

Any bioanalyzer will suffice.

mirVanaTM miRNA isolation kit

Ethanol Pure 200 proof for molecular biology **Sigma Aldrich Catalog #E7023-500mL**

Acid-Phenol:Chloroform, pH 4.5 (with IAA, 125:24:1) **Thermo Fisher Catalog #AM9720**

Dry Ice **Contributed by users**

DNA LoBind Tube 1.5ml **Eppendorf Catalog #022431021**

Nuclease-free water, not DEPC-treated **Life Technologies Catalog #AM9932**

Filter Tips **Contributed by users**

Bioanalyzer RNA 6000 Nano Kit **Agilent Technologies Catalog #5067**

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Preparation 15m

1 Clean workspace, pipettes, and gloves with RNaseZAP.

2 Prepare bucket of ice.

3 Heat nuclease-free water to **95 °C** .

4 Pre-cool microcentrifuge to **4 °C** .

5 Add 200 proof pure ethanol to *miR*Vana wash buffers as instructed.

6 Pull frozen placenta samples (~150-200 mg each) from **-80 °C** and place on dry ice until ready to process.

> Integrity of isolated total RNA will be greater if tissue was preserved in RNA*later* as soon as possible after harvesting.
>
> Typically, tissue is submerged in RNA*later* overnight. The following day, the RNA*later* is removed and the sample moved to **-80 °C** storage.

Cell lysis and tissue disruption 20m

7 Add approximately **1 mL** of 1.0mm zirconia/silica beads to each frozen placenta sample, and **700 µL** *miR*Vana Lysis/Binding Buffer.

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After addition of beads and lysis buffer, tubes should be almost full. Exclude as much air as possible to reduce foaming.

Samples should be in screw-cap microcentrifuge tubes with integral O-rings in the caps. Snap-cap tubes should not be used, unless secured with an adapter.

8 Load samples immediately into Mini-BeadBeater-16 vial holder ring. Up to 16 samples can be accommodated.

IMPORTANT: Rotate the vial holder ring to a position where the small hole in the vial holder ring engages the anti-rotation pin sticking out of the wiggle mechanism. Slide the vial holder down the pin and seat it flat on the wiggle mechanism. Slide the large, black plastic hold-down cap over the stainless steel center bolt, aligning it so that it too slides down the anti-rotation pin. The hold-down cap must make contact with the top of the aluminum wiggle mechanism - not just the tops of the microcentrifuge tubes. Finally, screw on and hand-tighten the black knob firmly. Repeat: Tighten firmly.

9 Switch on the Mini-BeadBeater-16 and run for **00:02:00** . 2m

10 Place samples immediately into pre-chilled microcentrifuge and spin for **00:05:00 at maximum speed** . 5m

11 Remove **500 µL** lysate, being careful not to draw up particulates, and dispense into a fresh labeled microcentrifuge tube.

Organic extraction 40m

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12 Add **50 µL** *miR*Vana miRNA Homogenate Additive (1:10 volume of original lysate) and vortex to mix. Incubate **00:10:00** **On ice** . 10m

13 Add **500 µL** Acid-Phenol:Chloroform (1:1 volume of original lysate) and vortex **00:01:00** to homogenize sample. 1m

Acid-Phenol:Chloroform may appear as a clear, homogeneous phenol phase (lower), overlayed by a small aqueous phase (upper). Pipette from the lower, not the upper, phase.

Phenol is very corrosive and will severely burn the skin. Safety precautions such as gloves, protective eyewear, a lab coat, and working in a fume hood are critical. Discard contaminated pipette tips in appropriate waste container.

14 Spin **00:05:00** **maximum speed** in pre-chilled microcentrifuge. 5m

15 Carefully remove **350 µL** of the top aqueous phase and transfer to a fresh labeled microcentrifuge tube.

It is possible to remove a greater volume to maximize RNA yield, but make sure not to disturb the interphase or organic phase.

Discard phenol liquid waste and contaminated tubes in appropriate waste containers.

Total RNA isolation 30m

16 Add **437.5 µL** (1.25 volumes) 200 proof pure ethanol and mix thoroughly.

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17 Transfer up to **700 µL** lysate/ethanol mixture to *miR*Vana Filter Cartridge (placed into *miR*Vana Collection Tube) and spin **10000 x g, 4°C, 00:00:15** in pre-chilled microcentrifuge. Discard flow-through. Repeat with remaining volume of lysate/ethanol mixture. 15s

*miR*Vana Filter Cartridges can accommodate up to **700 µL** volume - do not overfill. To avoid filter damage, do not spin at speeds greater than **10000 x g** .

18 Add **700 µL** *miR*Vana Wash Solution 1 and spin **10000 x g, 4°C, 00:00:15** in pre-chilled microcentrifuge. Discard flow-through. 15s

Tip: flow-through can be aspirated into an appropriately-labeled waste container using a vacuum.

19 Add **500 µL** *miR*Vana Wash Solution 2/3 and spin **10000 x g, 4°C, 00:00:15** in pre-chilled microcentrifuge. Discard flow-through. Repeat wash step. 15s

20 After discarding flow-through from the last step, spin **10000 x g, 4°C, 00:02:00** in pre-chilled microcentrifuge to remove residual Wash Solution. 2m

21 Transfer *miR*Vana Filter Cartridge into a fresh *miR*Vana Collection Tube. Add **40 µL** pre-heated nuclease-free water to the center of the filter and close the cap. Incubate **00:01:00** and then spin **00:00:30 maximum speed** in pre-chilled centrifuge to elute RNA. 1m 30s

22 Transfer eluate from the *miR*Vana Collection Tube to a low bind microcentrifuge tube and store at **-80 °C** .

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Minimize freeze/thaw cycles.

Tip: Store a small aliquot in a separate tube for QC.

Quality control 1h 30m

23 Quantitate RNA using NanoDrop. Pay attention to A260/A280 ratio. For highly pure RNA, a ratio of 1.8-2.1 is expected. If necessary, repurify by adding 1/10th nuclease-free 5M NaCl and 1.38 volumes 200 proof pure ethanol before repassing the sample over a fresh *mir*Vana Filter Cartridge. Continue the total RNA isolation from Step 17.

RNA can also be quantitated using Qubit RNA Broad Range Assay.

24 Assess RNA quality by running the RNA 6000 Nano Assay on an Agilent 2100 Bioanalyzer. A RIN score of >7.0 is normal for total RNA isolated from placenta.

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