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JAX-Sen: Extraction of Bulk RNA from Mouse Placenta

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Cellular Senescence Net...

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We use this protocol and it's working

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Abstract

This protocol outlines the process of tissue homogenization and bulk RNA extraction that occurs prior to submission for bulk RNA sequencing. Whole mouse placentas were collected from male (n=4) and female (n=4) fetuses across 8 mouse strains (WSB, A/J, NOD, NZO, 129S1, PWK, CAST, C57Bl/6). Placentas were collected at 4 different timepoints for the C57Bl/6 strain (E9.5, E11.5, E13.5, and E17.5) and 1 timepoint (E13.5) for the other 7 strains for a total of 88 placenta samples. Tissue disruption of the whole mouse placenta is accomplished via the BeadBug 6 homogenizer, after which an aliquot of homogenized lysate is used with the **QIAGEN RNeasy Plus Mini kit** to extract bulk RNA.

Reagents and Materials:

1 Supplies:
- Ethanol/RNase Zap
- Ice/Ice Bucket
- Tissue samples
- BeadBug tubes (2 per tissue sample) with 0.5mm high impact zirconium beads
- Small petri dishes
- Scalpels
- Tweezers
- QIAshredder tubes
- RNeasy plus mini kit
- 70% ethanol
- B-mercaptoethanol
- Microcentrifuge tubes (2uL)

Equipment:
- BeadBug 6
- Benchtop centrifuge
- Nanodrop

Procedure:

2 Clean working area and all equipment with 70% ethanol and RNase zap; continue to use RNase zap periodically throughout the protocol

3 Retrieve tissue samples from -80^{o}C storage and thaw on ice

4 Fill BeadBug tubes with 600uL of Buffer RLT Plus
a. To make Buffer RLT Plus, add β-mercaptoethanol to RLT buffer (10 µl β-ME per 1 ml Buffer RLT)

5 Remove placenta from tube with clean tweezers and place into small petri dish

6 Use tweezers and small scalpel to cut the tissue into small pieces
a. For mouse placenta: cut into quarters
b. Make sure to cut different tissue samples in separate areas of the petri dish. Also make sure to clean tweezers and scalpel between different tissue samples.

7 Use tweezers to place cut tissue pieces into beadbug tubes w/ 600uL RLT buffer

a. For mouse placenta: 2/4 in each tube

8 Run BeadBug 6 on Cycle 2, Speed 3000, for 90 seconds
a. Stop after first 90 seconds. If tissue is well homogenized in the beadbug tube, no need for any additional time.
b. Run one additional BeadBug cycle as necessary.
c. Tubes will be foamy! Spin down in small centrifuge to get a clear look.

9 Transfer liquid (and as much of the foam as possible) from the 2 BeadBug tubes for each tissue sample into the same 2mL microcentrifuge tube
a. Foam can make the transfer process difficult. Spin down as you go to make more space in the tube for the combined lysate.
b. Avoid beads or tissue chunks.
c. Can pause here and place homogenized lysate into storage at -80^{o}C. If so, it's good practice to separate out the amount of homogenized lysate needed for next steps to avoid freeze-thawing the entire lysate unnecessarily (for mouse placenta, separate 1/4 of the total lysate volume, or approximately 250uL). If proceeding with the rest of the protocol, save any unused lysate in -80.

10 Transfer appropriate aliquot of homogenized lysate to a QIAshredder tube
a. For mouse placenta: 1/4 of the total lysate volume, or approximately 250uL

11 Spin QIAshredder tubes in centrifuge on 8000g for 30 seconds, or until liquid flows through the QIAshredder

12 (Step 2 of QIAGEN RNeasy Plus Mini kit) Transfer all flowthrough lysate to gDNA Eliminator spin column placed in a 2mL collection tube
a. For mouse placenta, this should correspond to ~250uL of liquid

13 Continue from here with the **QIAGEN RNeasy Plus Mini kit protocol**, keeping track of the following notes:
a. Step 3–When adding 1 volume of 70% ethanol, add equal amount of lysate used (for mouse placenta, use 250uL of ethanol)
b. After Step 7–Do perform the optional spin to dry out column after last wash with Buffer RPE
c. Step 8–Use 30uL of RNase free water for final elution step. Add RNase free water directly to the spin column membrane.

14 Measure RNA concentration with Nanodrop

15 Store RNA at -80^{o}C until submission for Bulk RNA sequencing