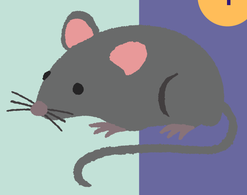
RNA Extraction from Mouse Tissue

Hoffman Lab



1 Prepare your tissue

Perform the dissection on your mouse models and store your tissues of interest in separate 1.5mL centrifuge tubes at -80 °C. Crush and cut 5 mg of your tissue using liquid nitrogen and 3 freeze - thaw cycles.

2 Extracting RNA from tissue

Add 600 µL of Trizol to your sample. Crush the tissue using a pestle, vortex and leave for 10 min on ice. Centrifuge (14000g, 30sec) and extract 550 µL of mixture to new centrifuge tube. Add 550 µL of ethanol.

3 Isolating RNA in spin column

Add 550 µL of the tissue mixture to a spin column with a collection tube. Centrifuge (14000g, 30sec) and repeat with another 550 µL of mixture. RNA is in the spin column now.

4 Prewash and Wash

Add 400 µL of the prewash buffer and centrifuge (14000g, 30 sec, x2). Add 700 µL of wash buffer and centrifuge (14000g, 2 min).Place spin column with RNA in a new centrifuge tube. Add 40 µL of DNase/RNase free water to the spin column and centrifuge (14000g, 30 sec). RNA in centrifuge tube.

5 Purifying RNA

Add 5 µL of DNA digestion buffer and 5 µL of DNase I. Incubate for 15 min at room temperature. Add 150 µL of Trizol and 200 µL of ethanol to centrifuge tube. Extract 400 µL of the mixture to a new spin column and collection tube and centrifuge (14000g, 30 sec). Repeat prewash and wash step.

6 RNA Sequencing Robarts Research Institute

Send off the RNA samples to the Robarts Research Institute for RNA sequencing. Perform analysis on RNA data.

robarts RESEARCH

Protocol and Reagents

Salem Abu Al-Burak
Supervisor: Lisa Hoffman
Lab Coordinator: Andrew McClennan