COLOcATION

Fetal maturity at the feto-maternal interface:
COntribution of fetaL and maternal genOmes and
tissue metAbolism perturbaTIONs

Protocol: purification of total RNA from tissues

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1 Summary

COLOcATION aims to explore the feto-maternal crosstalk associated to pig fetal maturity, using an integrative omics approach (metabolome, lipidome and transcriptome) and by **simultaneously** questioning **the two adjacent tissues (placenta/endometrium)** that are players of feto-maternal interactions.

The project has taken advantage of the unique collection of placenta and endometrium samples already collected in the PORCINET project (ANR-09-GENM-005-01, 2010–2014). This genetic protocol producing pure and reciprocal crossed fetuses by insemination with mixed semen, using two extreme breeds: Large White and Meishan at 90 and 110 days of gestation (dg) (period of fetal maturity acquisition for pigs). The originality and power of this experimental design is to be able to study in a same uterus (one maternal genome) two different fetal genomes in tight interaction.

This is achieved by performing molecular assays on 224 endometrial tissue samples and 224 placental tissue samples. These assays include RNA-seq, ^{1}H NMR metabolomic and l GC/FID lipidomics.

Here we describe the protocol used to purify total RNA from the two tissues.

To perform the purification of total RNA from these tissues, we followed the User manual of the "NucleoSpin RNA Mini kit" from Macherey-Nagel (https://www.mn-net.com/media/pdf/b0/51/ee/Instruction-NucleoSpin-RNA.pdf).

2 Protocol description

2.1 Required reagents and instruments

- NucleoSpin RNA, Mini kit for RNA purification (Macherey-Nagel, Reference 740955.250)
- TRIzol™ RNA Isolation Reagent (Invitrogen™, Reference 15596026)
- Chloroform
- Ethanol
- 2 ml centrifuge tubes
- 1.5 ml centrifuge tubes
- Pipets and tips
- NanoDrop spectrophotometer (ThermoFisher)
- Mixer-Mill MM400 (Retsch) [Tissue Lyser (QIAGEN)]
- Stainless steel beads
- Refrigerated centrifuge
- Weighting scale
- Box of liquid nitrogen
- Cryoprotection gloves

- Box of ice
- Nitrile gloves
- Vortex
- A cleaning spray against RNAse
- Laboratory hood
- Thermo Scientific™ Matrix™ 500µL ScrewTop Tubes (Reference 3745, 2D barcode)
- Thermo Scientific™ Matrix™ ScrewTop Tube Cap Trays (Reference 4477)
- Barcode labels for storage boxes
- 5200 Fragment Analyzer (Agilent)

2.2 Preparatory step

Before starting the purification of total RNA, we weighed the appropriate amount of tissue and performed tissue homogenization. Working above a box of liquid nitrogen, we weighed 80-100 mg of tissue for each sample, placed it in a 2 ml centrifuge tube and stored it at - 80°C before performing the homogenization step. Between each sample, we took care to clean the spatulas with ethanol and to tare the pre-labeled centrifuge tube.

The Mixer-Mill MM400 was used for tissue homogenization. Tissue samples stored at 80°C were placed on ice. We added 1ml of TRIzol™ Reagent to each centrifuge tube containing the tissue samples. We then added stainless-steel beads (5 mm) to each tube and placed the centrifuge tubes in the adapters set (2 x 24, stored for 10 min at -20°C) of the Mixer-Mill MM400 and ran 2 cycles of 2 min at 30 Hz.

After the lysis, the centrifuge tubes containing the homogenate were centrifuged for 10 min at 12,000 x g and 4°C. We transferred 900 µl of the homogenized supernatant to a new 2 ml Eppendorf tube, added 200 µl of chloroform, mixed by vortexing for 15 s, and incubated the mixture for 3 min at 4°C on ice. Then, the mixture was centrifuged for 15 min at 12,000 x g and 4°C.

2.3 Purification of total RNA

1. We transferred 600 µL of the homogenized supernatant to a new 1.5 ml Eppendorf tube containing 600 µL of 70% ethanol and mixed by vortexing.

2. We placed the NucleoSpin RNA Column into a 2 ml collection tube and transferred up to 600 µl of the sample, including any precipitate that may have formed into the column. We gently closed the lid and centrifuged for 30 s at 11,000 x g. We discarded the flow-through and repeated this step.

3. We added 350 µl of MDB Buffer to the column. We gently closed the lid and centrifuged for 1 min at 11,000 x g. We discarded the flow-through and repeated this step.

4. We prepared the DNase reaction mixture in a sterile 1.5 ml microcentrifuge tube: for each isolation, we added 20 µL of reconstituted rDNase to 80 µL of Reaction Buffer for rDNase. Mix by gently flicking the tube.

5. We added the DNase reaction mixture (95 µl) directly onto the center of the silica membrane of the column and incubate at room temperature (20-30°C) for 20 min.

6. Then, we added 200 µl of RAW2 Buffer to the column. We gently closed the lid and centrifuged for 30 s at 11,000 x g. We discarded the flow-through and placed the column into a new 2 ml collection tube.

7. We added 600 µl of RA3 Buffer to the column. We gently closed the lid and centrifuged for 30 s at 11,000 x g. We discarded the flow-through and reused the collection tube.

8. We added 250 µl of RA3 Buffer to the column. We gently closed the lid and centrifuged for 2 min at 11,000 x g to dry the spin column membrane.

9. We placed the RNeasy Mini spin column into a new 1.5 ml collection tube. We added RNase-free water (80 µl for endometrium samples and 40 µl for placenta samples) directly to the spin column membrane. We gently closed the lid and centrifuged for 1 min at 11,000 x g to elute the RNA.

10. We applied the eluate once more onto the column for re-elution. We gently closed the lid and centrifuged for 1 min at 11,000 x g to elute the RNA.

2.4 Storage, quantification, and quality determination of total RNA

Quantification is performed using a Nanodrop to determine the concentration of each purification and the 260/230, 260/280 ratios. RNAs are stored in Matrix™ 500 µL ScrewTop Tubes (2D barcoded) at -80°C.

RNA Integrity Numbers (RINs) were determined using a 5200 Fragment Analyzer.