RNA EXTRACTION FROM LUNGS

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BACKGROUND

- For isolation of RNA from tissues with Trizol reagent cat# 15596-018, Invitrogen

NOTES

- Perform this procedure in a fume hood, wear goggles, gloves and coat.

EQUIPMENT

- Equipment:
  - Fume Hood in MDCL 4077
  - Bullet Blender for homogenization
  - Vortex
  - Centrifuge
  - Heat-block
  - Tubes for Bullet Blender and Beads (3.2 or 1. mm beads)
  - Pipettes and Tips, Tubes
  - illustra RNAspin Mini kit from GE Healthcare – optional for cleanup and DNase treatment

- Reagents:
  - RNA later
  - Trizol reagent
  - Chloroform
  - Isopropanol
  - 75 % Ethanol (prepared with RNAse free water)
  - RNAse free water

PROTOCOL

(adapted from Invitrogen http://tools.invitrogen.com/content/sfs/manuals/trizol_reagent.pdf)

- Harvest lungs and put in **RNA later overnight at 4°C**, can be frozen after that at -80°C or flash freeze on dry ice – cells must be lysed in order to proceed with Trizol
- Thaw, put lung in **1 ml of Trizol** (per 50-100 mg of tissue)
- Add 5-8 beads and close tube very tightly!!
- **Homogenize** in Bullet Blender for 5 min (option to freeze at this point)
- Take suspension and incubate homogenized sample for around 2 more min at room temp. to permit complete dissociation of nucleoproteins

- **Add 200 ul of chloroform** per 1 ml of Trizol reagent (if frozen proceed with Trizol extraction protocol as soon as thawed by adding chloroform)
- Vortex and incubate at room temp. for 2-3 min.
- Centrifuge at no more than 12.000 x g for 15 min at 2-8°C
- Take aqueous phase that contains RNA and put in new 1.5 ml tube
- Precipitate RNA by adding **500 ul of Isopropyl alcohol** per 1 ml of Trizol reagent
- Incubate sample for **1 hour at -20°C** or for **10 min. at room temp**.
- Centrifuge at no more than 12.000 x g for 10 min at 2-8°C
- Remove supernatant and wash RNA pellet with **1 ml of 75 % ethanol** (prepared with **RNase free water**)
- Vortex and centrifuge at no more than 7.500 x g for 5 min at 2-8°C
- Briefly air-dry pellet, do not over-dry and dissolve RNA in RNase free water, incubate at 55-60°C for 10 min

→ I usually resuspend it in 90 ul

Optional:

Clean-up and DNase treatment on column illustra RNAspin Mini kit from GE Healthcare

- Add 3.5 volumes of **buffer RA1** per 1 volume of sample, vortex (315 ul if pellet resuspended in 90 ul)
- Add **ethanol (95-100 %)** at 3.5 times the volume of sample, vortex
- Load sample directly on the **blue column** (RNAspin Mini column, GE Healthcare) and follow protocol as described in the booklet from the kit including digestion with DNase and at the elution step adding RNase inhibitor.
- Elute the RNA in 60-70 ul of pre-warmed (65 °C) RNase free water. First elution with 30 ul and second elution with 30-40 ul
- Spec RNA
- **Prepare your cDNA:** if not done on same day **- aliquot sample** (avoid freeze-thaw cycles with RNA) for convenient amount for cDNA prep: maximum 2 ug total RNA for cDNA prep in preferably 10 ul total volume

LINKS AND REFERENCES

http://tools.invitrogen.com/content/sfs/manuals/trizol_reagent.pdf