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Bulk RNA Isolation (Ding)

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Abstract

Bulk RNA Isolation - Ding Lab



- 1 Add 1 ml Trizol to ~10 mg tissue.
- 2 Add 200 μ L chloroform
- 3 Vortex for 1 min until homogenous.
- 4 Incubate at room temperature for 5 min
- 5 Spin samples in microcentrifuge at 12,000 rpm for 20 min. at 4°C
- 6 Transfer ~500 μ L of clear (aqueous) top phase to a new 1.5 mL tube.
- 7 Add 500 μ L of cold 2-propanol to the 1.5 mL tube.
- 8 Vortex for 30 sec. to mix 2-propanol/sample.
- 9 Put samples in -20°C for 3–24 hrs.
- 10 Spin samples in microcentrifuge at full speed for 20 min. at 4°C
- 11 Remove supernatant from tube.
- 12 Add 1 mL 80% Ethanol and vortex for 10 sec.
- 13 Spin samples in microcentrifuge at full speed for 5 min. at 4°C



- 14 Remove supernatant from tube.
- 15 Let tube sit at room temperature with top open for 5 min. to evaporate excess ethanol.
- 16 Add 87.5 μ L of RNase-free water to the RNA pellet.
- 17 vortex for 5 min. to resuspend RNA, making sure that pellet is completely dissolved.
- 18 Spin in centrifuge briefly (Flash) to collect entire sample at the bottom of the tube.
- 19 Add 10 μ L Buffer RDD and 2.5 μ L DNase I (Qiagen)
- 20 Incubate on the benchtop (20–25°C) for 10 min.
- 21 Add 10 μ L beta-mercaptoethanol per 1 mL buffer RLT
- 22 Add 350 μ L Buffer RLT with beta-mercaptoethanol to the sample and mix well
- 23 Add 250 μ L of 96–100% ethanol to the diluted RNA and mix well by pipetting.
- 24 Transfer the sample (700 μ L) to an RNeasy MinElute spin column placed in a 2 mL collection tube, close the lid gently, and centrifuge for 15 sec. at 10,000 rpm at room temperature. Discard the flow-through.
- 25 Place the RNeasy MinElute spin column in a new 2 mL collection tube. Add 500 μ L buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 sec. at 10,000 rpm at room temperature. Discard the flow-through.
- 26 Add 500 μ L of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min. at 10,000 rpm at room temperature. Discard the flow-through and collection tube.



- 27 Place the RNeasy MinElute spin column in a new 2 mL collection tube. Open the lid of the spin column, and centrifuge at full speed for 5 min. at room temperature. Discard the flow-through and collection tube.
- 28 Place the RNeasy MinElute spin column in a new 1.5 mL collection tube. Add 14 μ L RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min. at full speed at room temperature.