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mRNA extraction and cDNA preparation

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We use this protocol and it's working

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Disclaimer

Abstract

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1 **Thaw and Resuspend Cells** - Thaw cells stored in TRIzol (15596026, Invitrogen). - Resuspend cells in 1 mL of TRIzol.

2 **Add Chloroform** - Add 200 µL of chloroform to the samples.

3 **Centrifuge Samples** - Centrifuge the samples at 12,000 g for 15 minutes at 4°C.

4 **Collect Aqueous Phase** - Carefully collect the aqueous phase.

5 **Precipitate RNA** - Add GlycoBlue Coprecipitant (AM9515, Invitrogen) and isopropanol to precipitate the RNA.

6 **Wash RNA Pellet** - Wash the RNA pellet with 75% ethanol.

7 **Dry and Resuspend RNA** - Air-dry the RNA pellet. - Resuspend the RNA in 40 µL of nuclease-free water.

8 **Isolate RNA** - Isolate the RNA using the Zymo Research RNA Clean & Concentrator-5 Kit (R1014, Zymo).

9 **Quantify RNA** - Quantify the RNA using the Qubit RNA HS Assay Kit (Q32852, Invitrogen).

10 **Equalize RNA Concentrations** - Dilute the RNA to equalize concentrations.

11 **Generate cDNA Libraries** - Use the qScript cDNA SuperMix (101414-102, VWR) for cDNA library preparation. - Follow this temperature profile: - 25°C for 5 minutes - 42°C for 30 minutes - 85°C for 5 minutes

12 **Dilute and Store cDNA** - Dilute the resulting cDNA threefold. - Store the cDNA at −80°C.