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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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Abstract

mRNA extraction and cDNA preparation

- 1 ****Thaw and Resuspend Cells**** - Thaw cells stored in TRIzol (15596026, Invitrogen). - Resuspend cells in 1 mL of TRIzol.
- 2 ****Add Chloroform**** - Add 200 μ L 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 μ L 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.