Hot-phenol RNA Extraction

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Abstract

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Protocol

Step 1.

Transfer 12-15 mL of culture to a 50 mL conical tube (10 mL of culture at stationary phase) and add 25 mL of ice to tube. The ice will help to slow transcription.

Step 2.

Immediately pellet at 10,000x g and 4° C for 10 minutes and discard the supernatant.

Step 3.

Flash freeze the pellet in liquid nitrogen and store at -80º C until extraction.

Step 4.

Resuspend the pellet in 600 µL of solution A and vortex to mix completely. Solution A contains 0.5% SDS, 20 mM sodium acetate, 10 mM EDTA in RNase-free water.

Step 5.

Immediately add to 500 µL acid phenol:chloroform preheated to 65º C. Vortex.

Step 6.

Incubate at 65°C for 10 min.

Step 7.

Centrifuge the cells at 21,000 x g (or maximum g in a benchtop microcentrifuge) at room temperature for 10 min.

Step 8.

Carefully remove the supernatant. Extract supernatant again with 500 µL hot acid phenol:chloroform. Incubation is not needed in this extraction.

Step 9.

Remove supernatant and extract with room temperature phenol:chloroform:isoamyl alcohol to remove any remaining protein contamination. One or two extractions may be needed to clear the protein contamination.

Step 10.

Extract twice with 400 µL of chloroform to remove residual phenol.

Step 11.

Place the supernatent into a clean tube and add 2.5 volumes of ice-cold 100% ethanol to the sample. Place in -80°C freezer for at least 15 min. Sample can be stored in this condition as needed.

Step 12.

Centrifuge the samples at 21,000 x g (or maximum g in a benchtop microcentrifuge) at 4ºC for 30 min. Decant liquid.

Step 13.

Add 400 µL of 70% ethanol to wash the RNA pellet. Dislodge the pellet from the centrifuge tube wall but do not attempt to completely resuspend. Centrifuge at maximum for 15 min. Carefully aspirate the liquid and allow the pellet to air dry to remove all ethanol.

Step 14.

Resuspend in 50 µL of RNase-free water. Volume of water will depend on expected RNA recovery and desired dissolved RNA concentration.

DNAse treatment using Turbo DNA-free kit from Ambion

Step 15.

Add 50 µg of RNA to a total volume of 85 µL of RNase-free water. Add 10 µL of Turbo DNA-free 10x buffer. Add 5 µL of Turbo DNase enzyme. Incubate at 37ºC for 20 min. Bring volume to 200 µL using RNase-free water. Add 200 µL of phenol:chlorofform and extract one time as described above. To supernatant, add 20 µL of 3M sodium acetate and 750 µL of 100% ethanol. Precipitate RNA as described above.