RNA extraction from E. coli

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ABSTRACT

RNA extraction is a fundamental step in multiple experiments, for example, qPCR. This protocol helps conduct a simple RNA extraction procedure.

MATERIALS

Buffer LY (added 1% volume of dithiothreitol), Buffer RB, RNA Wash Buffer, DEPC-Treated ddH₂O, RNA Columns, DNA Clearance Column, Collection Tubes, 1.5 mL RNase-free microfuge tube, Lysozyme buffer (0.4 mg/mL)
1. Grow an overnight bacterial culture in the appropriate media at an appropriate temperature.

2. In the following day, take 1 mL from overnight culture and add into 10 mL LB media. Grow until the OD600 reads at 0.6-1.0.

3. Harvest 1.5 mL culture (< 5x10%) by centrifugation at 3,000 rpm, 00:10:00 for 10 min in a 1.5 mL microcentrifuge tube.

4. Discard all supernatant.

   **Note**
   
   You may use a pipette to remove the remaining liquid at the bottom of the tube.

5. Resuspend the pellet in 100 µL freshly prepared Elution Buffer (10mM Tris-HCL pH 8.5) containing lysozyme (0.4 mg/mL lysozyme for Gram negative bacteria). Mix by tapping gently.

6. Incubate the resuspended pellet at room temperature for 3-5 min for Gram-negative bacteria.

7. Add 400 µL Buffer LY. Mix gently.
8. Transfer the cleared lysate to a DNA Clearance column pre-inserted in a 2 mL Collection Tube. Centrifuge at **13,000 rpm, 00:02:00**. Discard the DNA Clearance column and save the flow-through.

9. Transfer flow-through to the RNA binding column. Add 0.5 volume 100% ethanol to the lysate.
   
   **Note**
   
   For example: 250 μL 100% ethanol for 500 μL.

10. Centrifuge at **13000 rpm, 00:01:00**. Discard the collection tube with the flow through and put the column back to a new collection tube.

11. Add **500 µL** Buffer RB to the column and centrifuge at **13,000 rpm, 00:00:30**. Discard the flow-through.

12. Add another **500 µL** RNA Wash Buffer to the column and centrifuge at **13000 rpm, 00:00:30**. Discard the flow-through.
   
   **Note**
   
   Ethanol should be first added into RNA Wash Buffer before use.

13. Add another **500 µL** RNA Wash Buffer to the column and centrifuge at **13000 rpm, 00:00:30**. Discard the flow-through and collection tube, put the column into a new collection tube.

14. Centrifuge the column at **13000 rpm**, with the lid open, for another **00:01:00**.
15 Place the column to a RNase-free 1.5 mL tube, add 50-100 μL DEPC treated ddH₂O to the column and centrifuge at 13000 rpm, 00:02:00.

Note

The RNA is in the flow-through.

16 Store the RNA solution at -20 °C.