Cell Lysis and Isolation of Ribosomes with Associated mRNA

Materials

E. coli BL21(DE3) culture
LB medium
RNase-free water
Filtered pipette tips
Liquid nitrogen
Lysis buffer
Sucrose cushion buffer
Resuspension buffer
Murine RNase inhibitor
Micrococcal nuclease (MNase) (NEB, M0247S)
EGTA
TRIzol reagent
Chloroform
GlycoBlue
3 M sodium acetate (pH 5.5)
Isopropanol
80% ethanol

Buffer Preparation

Prepare the following buffers before starting the experiment.

Lysis Buffer

Component	**Final Concentration**
Tris, pH 7.0	20 mM
$MgCl_2$	150 mM
NH_4Cl	100 mM
Triton X-100	0.4%
NP-40	0.1%
$CaCl_2$	5 mM

DNase I	20 U/mL

Sucrose Cushion Buffer

Component	Final Concentration
Sucrose	1.1 M
Tris, pH 7.0	20 mM
NH_4Cl	500 mM
$MgCl_2$	10 mM

Resuspension Buffer

Component	Final Concentration
Tris, pH 7.0	20 mM
$MgCl_2$	15 mM
NH_4Cl	100 mM
$CaCl_2$	5 mM

Keep all prepared buffers on ice before use.

Procedure

Cell Culture and Harvesting

1. Grow BL21(DE3) cells in LB medium until the culture reaches an OD_{600} of 0.8.
2. Transfer 1.5 mL culture into each of six 2 mL microcentrifuge tubes.
3. Centrifuge at 12,000 × g, 4 °C, for 5 min.
4. Remove the supernatant.
5. Resuspend each cell pellet in 100 µL lysis buffer.

Cryogenic Cell Lysis

1. Immediately freeze the six tubes in liquid nitrogen.
2. Grind each frozen cell pellet using a pre-cooled micro-pestle.
3. Continue grinding until the frozen material forms a fine powder.
4. Transfer the samples immediately to −80 °C.

Lysate Clarification

1. Thaw the frozen samples on ice.
2. Immediately centrifuge the thawed lysates at 9,000 × g for 10 min at 4 °C.
3. Collect the supernatants.
4. Pool 90 µL supernatant from each of the six tubes, giving 540 µL clarified lysate in total.
5. Keep the pooled lysate on ice and proceed immediately to ultracentrifugation.

Sucrose Cushion Ultracentrifugation

1. Add 1,000 µL sucrose cushion buffer to a pre-cooled ultracentrifuge tube.
2. Carefully overlay 480 µL of clarified lysate onto the sucrose cushion. Gently add DNase I-free lysis buffer to bring the total volume to 3.5 mL. Place the tube in an SW 55 Ti rotor.
3. Run the ultracentrifugation in an SW 55 Ti rotor at 368,000 × g for 4 °C at 2 h.
4. The ultracentrifuge tube used in this protocol is Beckman Coulter 349623.

Ribosome Pellet Resuspension

1. Carefully remove the supernatant and sucrose cushion after ultracentrifugation.
2. Leave only a minimal amount of residual liquid above the pellet.
3. Add 200 µL resuspension buffer containing RNase inhibitor at a final concentration of 0.5 U/µL.
4. Vortex for 5 min to fully resuspend the ribosome pellet.

MNase Digestion

1. Measure RNA concentration using NanoDrop.
2. Add MNase at a ratio of 30 gel U MNase per 1 µg RNA.
3. Incubate for 30 min at 25 °C.
4. Add EGTA to a final concentration of 6 mM to terminate MNase digestion.
5. Mix thoroughly.
6. To confirm before Wiki publication: specify whether RNA concentration is measured from the clarified lysate or the resuspended ribosome fraction.

TRIzol RNA Extraction

1. Add 750 µL TRIzol reagent to the digested sample.
2. Vortex thoroughly.
3. Add chloroform at 20% of the TRIzol volume.
4. Mix by inversion.
5. Incubate at room temperature for 3 min.
6. Centrifuge at 12,000 × g for 15 min at 4 °C.
7. Carefully transfer the upper aqueous phase into a new RNase-free 1.5 mL tube.

RNA Precipitation

1. Add the following reagents to the recovered aqueous phase.

Reagent	**Amount**
GlycoBlue	2 µL
3 M sodium acetate, pH 5.5	0.1 × aqueous-phase volume
Isopropanol	1 × aqueous-phase volume

2. Mix thoroughly by inversion.
3. Incubate at −20 °C for at least 1 h.
4. Centrifuge at 17,000 × g, 4 °C, for 60 min.

RNA Pellet Washing

1. Carefully remove the supernatant.
2. Add 1 mL ice-cold 80% ethanol.
3. Wash the pellet gently by inversion.
4. Centrifuge at 17,000 × g, 4 °C, for 10 min.
5. Remove the ethanol completely.
6. Air-dry the pellet at room temperature for approximately 5 min.
7. Resuspend the RNA pellet in 12 µL RNase-free water.