Extraction of Ribosome-protected Fragments (RPFs) by Electrophoresis

Materials

Small RNA ladder
2× RNA Loading Dye
15% Urea-PAGE gel
SYBR Gold
Gel breaker tubes
Centrifugal filter tubes
10 mM Tris-HCl (pH 7.0)
GlycoBlue
3 M sodium acetate (pH 5.5)
Isopropanol
80% ethanol

Procedure

RPF Separation by Urea-PAGE

1. Mix the RNA sample with 2× RNA Loading Dye at a 1:1 volume ratio.
2. Prepare the small RNA ladder in the same way.
3. Heat the samples at 70 °C for 5 min.
4. Immediately place the samples on ice for at least 1 min.
5. Load the samples onto a 15% Urea-PAGE gel.
6. Run the gel using the following conditions.

Parameter	Condition
Running buffer	1x TBE
Voltage	180 V
Running time	About 1 h

RPF Gel Excision

1. Stain the gel using SYBR Gold.
2. Visualize the RNA bands using a fluorescence transilluminator.
3. Excise fragments between 20 and 40 nt.

RPF Gel Extraction

1. Transfer the excised gel pieces into a gel breaker tube.
2. Centrifuge for 2 min at 17000 × g.
3. Add 0.5 mL 10 mM Tris-HCl, pH 7.0 to the fragmented gel.
4. Incubate at 75 °C for 15 min with agitation.
5. Transfer the sample into a centrifugal filter tube.
6. Centrifuge at 15,000 × g for 2 min at room temperature.
7. Collect the eluate.

RPF Precipitation

1. Add the following reagents to the eluate.

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

2. Mix thoroughly.
3. Incubate at −80 °C overnight.

RPF Recovery

1. Centrifuge at 17,000 × g, 4 °C, for 60 min.
2. Remove the supernatant.
3. Wash the pellet with 80% ethanol.
4. Centrifuge again using 17,000 × g, 4 °C, for 10 min.
5. Remove residual ethanol completely and repeat washing and 10 min-centifuge
6. Briefly air-dry the pellet.