tRNA-Adaptor Ligation Efficiency

Materials

E. coli B strain total tRNA (NEB, N6845S)
Stem-loop adaptors A, T, and C
T4 RNA Ligase 2 and 10× reaction buffer
1 M Tris-HCl (pH 9.0)
10× adaptor annealing buffer
3 M sodium acetate
GlycoBlue
100% and 70% ethanol
RNase-free water
RNA loading dye
RNA ladder
Denaturing polyacrylamide gel and RNA staining reagent

Procedure

Preparation of tRNA Samples

1. Prepare two groups of *E. coli* total tRNA samples:
   Untreated group: Total tRNA without deacylation.
   Deacylated group: Total tRNA subjected to deacylation before adaptor ligation.
2. Use equal amounts of tRNA for the subsequent ligation reactions.

tRNA Deacylation

1. Prepare a 50 µL reaction containing total tRNA and 1 µL of 1 M Tris-HCl (pH 9.0). Adjust the volume with RNase-free water to obtain a final Tris-HCl concentration of 20 mM.
2. Incubate at 37 °C for 60 min to remove amino acids attached to the 3′ ends of aminoacylated tRNAs.
3. Add the following reagents:
   5 µL of 3 M sodium acetate
   1 µL of GlycoBlue
   125 µL of 100% ethanol
4. Mix thoroughly and incubate at −20 °C for 1.5 h or overnight.
5. Centrifuge at 14,000 × g for 15 min at 4 °C.
6. Carefully discard the supernatant.
7. Wash the RNA pellet with 500 µL of ice-cold 70% ethanol.
8. Centrifuge at 14,000 × g for 15 min at 4 °C and discard the supernatant.
9. Air-dry the pellet for 5–10 min and resuspend it in 40 µL of RNase-free water.

Stem-Loop Adaptor Annealing

1. Prepare separate reactions for adaptors A, T, and C using either untreated or deacylated tRNA.
2. For each reaction, mix 100 ng of total tRNA with 20 pmol of the corresponding stem-loop adaptor in a final volume of 9 µL.
3. Incubate at 90 °C for 3 min.

4. Add 1 μL of 10× annealing buffer.
5. Incubate at 37 °C for 20 min to allow adaptor hybridization.

Adaptor Ligation

1. Add 10 μL of ligation master mix containing T4 RNA Ligase 2 (1 U per reaction) and its reaction buffer to the annealed tRNA–adaptor mixture, adjusting the buffer to a final concentration of 1×.
2. Mix gently to obtain a final reaction volume of 20 μL.
3. Incubate at 37 °C for 1 h.
4. Continue incubation at 4 °C overnight to promote adaptor ligation.

Gel Electrophoresis

1. Prepare a denaturing polyacrylamide gel suitable for separating small RNAs, such as a 15% polyacrylamide gel containing 8 M urea.
2. Mix equal amounts of each ligation reaction with RNA loading dye.
3. Load the RNA ladder, unligated tRNA control, and the three adaptor ligation products onto the gel.
4. Perform electrophoresis until the unligated tRNA and higher-molecular-weight ligation products are sufficiently separated.
5. Stain the gel using an appropriate RNA staining reagent and visualize the bands using a gel imaging system.
   The samples were organized as follow:
   Group I: Deacylated tRNA
   Lane 1: RNA ladder
   Lane 2: Deacylated total tRNA
   Lane 3: Adaptor A ligation
   Lane 4: Adaptor T ligation
   Lane 5: Adaptor C ligation

Ligation Product Analysis

1. Identify the bands corresponding to unligated tRNA and adaptor-ligated tRNA based on their electrophoretic mobility.
2. Import the gel images into Fiji (ImageJ).
3. Select regions of interest (ROIs) covering the relevant bands and measure their integrated densities.
4. Subtract the local background signal from each measurement.
5. Compare the relative intensities of ligated and unligated tRNA bands to estimate the apparent ligation yield.
6. Compare the band patterns between untreated and deacylated samples to evaluate the effect of deacylation on adaptor ligation.