tRNA SL-Adaptor Ligation

Materials

Deacylated total RNA (100 ng)
SL-adaptor (20 pmol)
RNase-free water
10× Annealing Buffer (50 mM Tris-HCl (pH 8.0), 5 mM EDTA, and 100 mM $MgCl_2$)
10× Reaction Buffer (NEB #B0239S)
T4 RNA Ligase 2

Procedure

1. Prepare a 9 µL annealing mixture in a PCR tube:
   100 ng deacylated total RNA
   2 µL SL-adaptor (20 pmol)
   RNase-free water to a final volume of 9 µL.
2. Denature the RNA–adaptor mixture at 90°C for 3 minutes.
3. Add 1 µL of 10× Annealing Buffer to the reaction, bringing the total volume to 10 µL.
4. Incubate at 37°C for 20 minutes to allow adaptor annealing.
5. Prepare the ligation reaction by adding the following to the annealed mixture:
   2 µL 10× Reaction Buffer (NEB #B0239S)
   0.1 µL T4 RNA Ligase 2 (Rnl2)
   7.9 µL RNase-free water
6. Incubate the ligation reaction at 37°C for 1 hour, the reaction contains 20 µL of tRNA–adaptor ligation product, which can be used directly for subsequent downstream applications.