RNA Circularization using T4 RNA Ligase 1 (ssRNA Ligase) (NEB #M0204)

Materials Required but not Supplied

T4 RNA Ligase 1 (ssRNA Ligase)

- Nuclease-free Water (NEB #B1500)
- RNase Inhibitor, Murine (NEB #M0314) (optional)

Overview

This is a general protocol that serves as a starting point to circularize single-stranded RNA using T4 RNA Ligase 1 (ssRNA Ligase). Increasing the concentration, length of incubation, and adding PEG can increase the number of intramolecular ligations. Blocking one end of the molecule with a dideoxy terminator will prevent the molecule from forming a circle.

Protocol:

1. Set up the following reaction on ice:

COMPONENTS	**20 µl REACTION**
10X T4 RNA Ligase Reaction Buffer	2 µl (1X)
ssRNA with 5'P and 3'OH ends	X µl (200 ng - 1 µg)
10,000 units/ml T4 RNA Ligase 1	1 µl (10 units)
50% PEG 8000	4 µl (10%)
10 mM ATP*	0.1 µl (50 µM)*
Optional - RNase Inhibitor, Murine	0.5 µl (20 units)
Nuclease-free Water	to 20 µl

**Note: A working solution of 1mM ATP can be prepared so that 1 µl ATP [1mM] can be pipetted into a 20 µl reaction volume.*

2. Incubate at 25°C for 1-2 hours. For longer oligos, overnight incubation at 16°C may improve yield.

3. Heat inactivate at 65°C for 15 minutes.

Linked Resources:

- RNA Ligation Overview

- RNA Cloning Overview