Poly(A) Tailing of RNA using *E. coli* Poly(A) Polymerase (NEB #M0276)

Materials Required but not Supplied

E. coli Poly(A) Polymerase
- Nuclease-free Water (NEB #B1500)
- RNase Inhibitor, Murine (NEB #M0314) (optional)

HiScribe® T7 High Yield RNA Synthesis Kit
- Nuclease-free Water (NEB #B1500)

HiScribe® T7 Quick High Yield RNA Synthesis Kit
- Nuclease-free Water (NEB #B1500)

Overview

This protocol is designed to tail up to 10 µg of RNA in a 20 µl reaction. Reaction size can be scaled up as needed. In a poly (A) tailing reaction, the length of the tail depends on the molar concentration of the RNA 3' OH ends, reaction time, amount of enzyme, and ATP concentration. Tail length can be modified by changing one or more of these factors. Please note that the number of adenosines that Poly(A) Polymerase adds to a pool of mRNA molecules has a Gaussian distribution, so it is not possible to plan to add a certain amount of A's to all RNA molecules present in a reaction. As a general guideline, incubation of 5 units of the enzyme with 1-10 µg RNA in a 20 µl reaction at 37°C for 30 minutes (with 1X Reaction buffer and 1mM ATP) will result in a tail length of greater than 100 bases.

Precautions:

1. Wearing gloves and using nuclease-free tubes (microfuge tubes or PCR strip tubes) and reagents is strongly recommended to avoid RNase contamination.

2. RNA used for tailing reactions should be purified prior to use and suspended in nuclease-free water. EDTA should not be present, and the solution should be free of salts. Monarch Spin RNA Cleanup Kits are recommended as an efficient method.

Protocol:

1. Gently vortex the buffer and ATP and gently flick the Poly(A) Polymerase to mix, followed by a brief spin-down.

2. Add the following components in the following order:

COMPONENTS	20 µl REACTION	FINAL AMOUNT
Nuclease-free Water	X µl	-
RNA	X µl	1-10 µg

10X *E. coli* Poly(A) Polymerase Reaction Buffer	2 µl	1X
10 mM ATP	2 µl	1 mM
E. coli Poly(A) Polymerase	1 µl	5 units
Optional - RNase Inhibitor, Murine	0.5 µl	20 units

3. Incubate reaction at 37°C for 30 minutes.

4. Stop the reaction by adding EDTA to a final concentration of 10 mM. Alternatively, directly proceed to cleanup by phenol-chloroform extraction or column-based cleanup. We recommend the 500 µg capacity Monarch® Spin RNA Cleanup Kit (NEB #T2050).

Related Resources

- Minding your caps and Poly A tails – Strategies for synthesizing *in vitro* transcribed (IVT) mRNA
- Avoiding Ribonuclease Contamination