Addition of the adaptor to RNA substrates for 5′ RACE

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ABSTRACT

Simple protocol for mapping 5′-P RNA termini by RT-PCR after the addition of a 5′ adaptor using T4 RNA ligase 1.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
T4 RNA Ligase 1 (ssRNA Ligase) - 1,000 units111212	M0204S	New England Biolabs

SAFETY WARNINGS

BEFORE STARTING

Prepare samples, including controls, according to the aim of the experiment. Use the chart below to decide which enzyme is appropriate for pre-treatments of the RNA. For example, if only interested in mapping 5′ RNA ends, use T4 polynucleotide kinase with ATP and without ATP to infer the original phosphorylation state of the end of interest.
Setup of the phosphorylation reaction can be found here: dx.doi.org/10.17504/protocols.io.cpdvi5 However, avoid heat denaturation of the enzyme and rather purify the RNA using a trizol extraction (e.g., http://dx.doi.org/10.17504/protocols.io.eiebcbe) or a column clean-up (e.g., Monarch RNA Cleanup Kits from *NEB* is optimal when interested in small RNA molecules <200 nt, which is the usual exclusion limit in other products).

Original	Original	T4PNK (+ATP)	T4PNK (+ATP)	T4PNK (-ATP)	T4PNK (-ATP)	T4PNK—3′Pase⊖ (+ATP)	T4PNK—3′Pase⊖ (+ATP)	T4PNK—3′Pase⊖ (-ATP)	T4PNK—3′Pase⊖ (-ATP)
5′	3′	5′	3′	5′	3′	5′	3′	5′	3′
P	P	P	OH	P	OH	P	P	P	P
P	OH	P	OH	P	OH	P	OH	P	OH
OH	P	P	OH	OH	OH	P	P	OH	P
OH	OH	P	OH	OH	OH	P	OH	OH	OH

1 Mix the following components (10 µL):

Component	Amount [µL]	Final concentration
RNA [1 µg]	4	100 ng/µL
5′ RACE RNA oligo [100 µM]	2	20 µM
RNase-free water (ddH2O)	4	

- *5′ and 3′ ends of the 5′ RACE oligo are hydroxylated.*

2 Denature for 2 min at 70 °C, place on ice.

3 Mix the following components (20 µL):

Component	Amount [µL]	Final concentration
RNA + oligo mix (step 2)	10	
10× RNA ligase buffer	2	1×
50% PEG-8000	6	15%
ATP [10 mM]	2	1 mM
T4 RNA ligase 1 [10 U/µL]	2	1 U/µL

4 Incubate using the following program: 60 min at 25 °C, then 60 min at 16 °C, and then for 120 min at 4 °C.

5 Purify the RNA from the RNA ligase reaction (e.g., trizol extraction or column clean-up).

6 Proceed to RT-PCR. For the RT reaction, use 250-500 ng of the purified adaptor-ligated RNA and random hexamers (or a primer of choice). For the PCR, use an upstream (forward) primer binding to the 5′ adaptor and a downstream (reverse) primer binding to the RNA of interest.