Addition of the adaptor to RNA substrates for 3′ RACE (mapping OH ends)

Forked from Addition of the adaptor to RNA substrates for 5′ RACE

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ABSTRACT

Simple protocol for mapping 3′-OH RNA termini by RT-PCR after the addition of a 3′ adaptor (pre-adenylated at its 5′ end) using truncated T4 RNA ligase 2.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
T4 RNA Ligase 2, truncated KQ - 2,000 units111212	M0373S	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

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5′-pre-adenylated 3′ RACE DNA oligo [10 µM]	4	4 µM
RNase-free water (ddH2O)	2	

- *For the 5′ pre-adenylation, see the protocol "Preparation of the adenylated oligonucleotide for mapping 3′OH RNA termini". 3′ end of the 3′ RACE oligo should be blocked to avoid self-ligation (e.g., by a 3′ inverted-dT or a 3′ dideoxy modification).*

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%
T4 RNA Ligase 2, truncated KQ [200 U/µL]	2	10 U/µL
RNase-free water (ddH2O)	2	

- *T4 RNA Ligase 2, truncated (NEB #M0242) can also be used, but T4 RNA Ligase 2, truncated KQ (NEB #M0373) is optimal to avoid unwanted by-products.*

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 a primer reverse complementary to the 3′ adaptor. For the PCR, use an upstream (forward) primer binding to the RNA of interest and a downstream (reverse) primer binding to the 3′ adaptor.

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