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

Version 2

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

Matus Valach[1]

[1]Université de Montréal, Montreal, Quebec, Canada

dx.doi.org/10.17504/protocols.io.xsyfnfw

Matus Valach
Université de Montréal, Montre...

ABSTRACT

Simple protocol for mapping 3′-P RNA termini by RT-PCR after the addition of a 3′ adaptor using *E. coli* RtcB RNA ligase.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
T4 Polynucleotide Kinase (3' phosphatase minus) - 200 units111212	M0236S	New England Biolabs
RtcB Ligase - 25 rxns111212	M0458S	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, phosphorylating all 5′ RNA ends is useful when using RtcB, because it allows blocking 5′ termini against ligation by RtcB and thus only the 3′ RACE oligo becomes the substrate for the enzyme. In such a case, use T4 polynucleotide kinase without 3′ exnuclease activity (e.g., *NEB* #M0236) and ATP.

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

Mix the following components (9 µL):

1

Component	Amount [µL]	Final concentration
RNA [1 µg]	4	100 ng/µL
5′-hydroxylated 3′ RACE RNA oligo [100 µM]	0.4	
RNase-free water (ddH2O)	4.6	

- *Blocking the 3′ end of the 3′ RACE oligo is not necessary, as 3′-OH ends are not a substrate for EcRtcB RNA ligase. 5′-end has to be hydroxylated. For practical purposes, a 5′ RACE RNA oligo, which usually has both ends hydroxylated, can be used for as the 3′ RACE oligo when mapping 3′-P ends by EcRtcB.*

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)	9	
10× RtcB RNA ligase buffer	2	1×
50% PEG-8000	4	10%
GTP [1 mM]	2	0.1 mM
MnCl2 [10 mM]	2	1 mM
EcRtcB RNA ligase [15 µM]	1	0.75 µM

4 Incubate for 120 min at 37 °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.