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Addition of the adaptor to RNA substrates for 5′ RACE

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We use this protocol and it's working

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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.

Materials

MATERIALS

T4 RNA Ligase 1 (ssRNA Ligase) - 1,000 units **New England Biolabs Catalog #**M0204S

Before start

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.