FEB 05, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.xspfndn

Protocol Citation: Matus Valach 2019. Preparation of the adenylated oligonucleotide for mapping 3'OH RNA termini.

protocols.io
<https://dx.doi.org/10.17504/protocols.io.xspfndn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Feb 05, 2019

Last Modified: Feb 05, 2019

PROTOCOL integer ID:
 20015

Keywords: RNA, 3' RACE, adenylation, RNA-Seq

Preparation of the adenylated oligonucleotide for mapping 3'OH RNA termini

Matus Valach¹

¹Université de Montréal, Montreal, Quebec, Canada

Matus Valach
 Université de Montréal, Montreal, Quebec, Canada

ABSTRACT

Adapted from the protocol for oligonucleotide 5' adenylation (E2610) of *New England Biolabs (NEB)*.

MATERIALS

MATERIALS

5' DNA Adenylation Kit - 10 rxns **New England Biolabs Catalog**
#E2610S

1 Mix the following components (20 μ L):

Component	Amount [μ L]	Final concentration
5' phosphorylated DNA oligo [50 μ M]	4	10 μ M
10 $\times$ 5' DNA Adenylation buffer	2	1 $\times$
ATP [1 mM]	2	0.1 mM
Mth RNA ligase [50 pmol/ μ L]	4	10 μ M
RNase-free water (ddH ₂ O)	8	

2 Incubate for 60 minutes at 65 °C.

3 Incubate for 5 minutes at 85 °C to inactivate the enzyme.

4 The adenyated oligo can be directly used for RNA ligation with T4 RNA ligase 2 (optimally with T4 RNA Ligase 2, truncated KQ (*NEB* #M0373)).