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

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Protocol status: Working

We use this protocol and it's working

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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 $^{\circ}$ C.
- 3 Incubate for 5 minutes at 85 $^{\circ}$ C to inactivate the enzyme.
- 4 The adenylated oligo can be directly used for RNA ligation with T4 RNA ligase 2 (optimally with T4 RNA Ligase 2, truncated KQ (*NEB* #M0373)).