# Adenylator DNA/RNA 5′-Adenylation Kit: clean, efficient enzymatic adenylation of DNA and RNA

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**Adenylation of 5′-monophosphorylated oligonucleotides is a critical enzymatic step in the preparation of sequencing libraries and adapter-linked constructs, particularly in small RNA sequencing (small RNA-seq) and native elongating transcript sequencing (NET-seq) workflows. This modification enables subsequent ligation steps by generating a pre-adenylated intermediate, which is required for efficient and directional ligation. Traditionally, this is achieved using thermostable RNA ligases such as Mth RNA Ligase, which requires an elevated reaction temperature of 65 °C and can produce unwanted ligation side products, such as concatemers or circularized oligos.**

**The Adenylator™ DNA/RNA 5′-Adenylation Kit offers a novel alternative by utilizing a non-ligating, high-efficiency enzyme that specifically catalyzes the 5′-adenylation of both DNA and RNA oligonucleotides under mild conditions (37 °C) and without formation of circularized oligo and concatemerization byproducts. This poster evaluates the enzymatic activity and substrate specificity of the Adenylator Enzyme and provides a direct performance comparison with Mth RNA Ligase under standardized assay conditions.**

## COMPARING ADENYLATOR ENZYME WITH Mth RNA LIGASE

Adenylator Enzyme was compared with Mth RNA Ligase, a widely used enzyme for adenylating 5′-monophosphorylated DNA oligonucleotides (Figure 1). Adenylation reactions were performed using 300 picomole input of 5′-monophosphate DNA oligonucleotides (23-nt DNA) and incubated at 37 °C for one hour. For benchmarking, parallel reactions were carried out using Mth RNA Ligase under its standard protocol, including incubation at 65 °C for one hour. After incubations, all reactions were quenched with loading dye for direct analysis via PAGE. Products were resolved on 20% acrylamide, 8 M urea, 1×TBE gels, and with a band shift indicative of successful 5′-adenylation. SYBR®-Gold staining was used for detection.

In the Mth RNA Ligase reaction (Figure 1, Lane 5), a downward nucleotide shift (blue arrow) reveals an artifact caused by oligonucleotide self-circularization, while DNA oligonucleotide concatemers (red arrows) formed due to the enzyme's non-specific ligase activity. These side products decrease ligation efficiency and introduce contaminants into downstream workflows. In contrast, Adenylator Enzyme avoids these issues entirely due to its high specificity and lack of ligase activity (Figure 1, Lane 3).

## EVALUATING LIGATION OF ADENYLATED OLIGONUCLEOTIDES

In another experiment, the compatibility of Adenylator Enzyme-modified oligonucleotides for downstream ligation and library preparation using T4 RNA Ligase 2, truncated KQ (NEB) was tested. An unadenylated DNA and 5′-OH RNA mixture treated with the T4 RNA Ligase 2 truncated KQ failed to ligate (Figure 2, Lane 4). In contrast, Adenylator Enzyme-treated DNA oligonucleotide and 5′-OH RNA together with the T4 RNA Ligase 2 truncated KQ resulted in a novel 50-nt ligation product (Figure 2, Lane 5, green arrow). These data demonstrate the ability of Adenylator Enzyme to efficiently generate ligation-ready DNA oligos.

## SUMMARY

These data demonstrate that the Adenylator DNA/RNA 5′-Adenylation Kit provides a superior alternative to traditional adenylation methods. Compared to Mth RNA Ligase, the Adenylator kit offers cleaner reaction profiles with no side products, milder reaction conditions (37 °C versus 65 °C), improved capability for downstream ligation workflows, and enhanced exonuclease resistance via 5′- capping.

These advantages translate into higher reliability, reduced reagent waste, and greater efficiency in molecular biology workflows. The absence of unwanted ligation artifacts simplifies downstream processing and improves the fidelity of applications such as small RNA-seq and synthetic biology constructs.

In conclusion, the Adenylator DNA/RNA 5′-Adenylation Kit provides a robust method to generate 5′-adenylated DNA or RNA oligonucleotides. Compared to Mth RNA Ligase, Adenylator enables superior workflow control and reaction cleanliness, making it highly suitable for sensitive applications such as small RNA library construction.

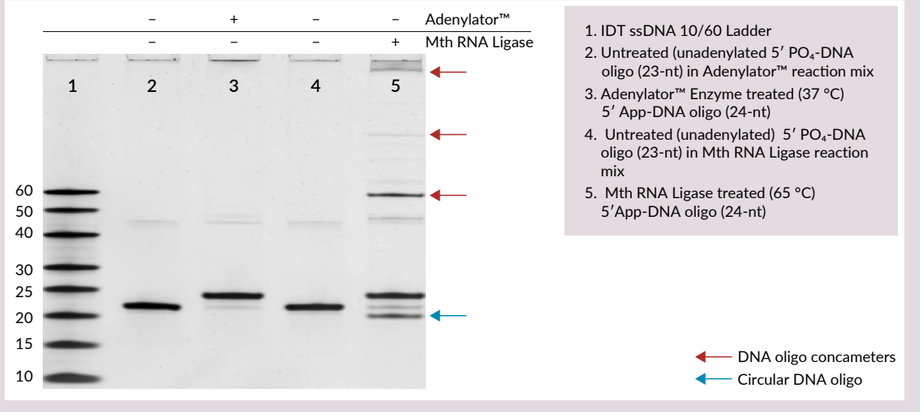


Figure 1 (far left). Comparison of Adenylator™ Enzyme and Mth RNA Ligase for DNA adenylation.

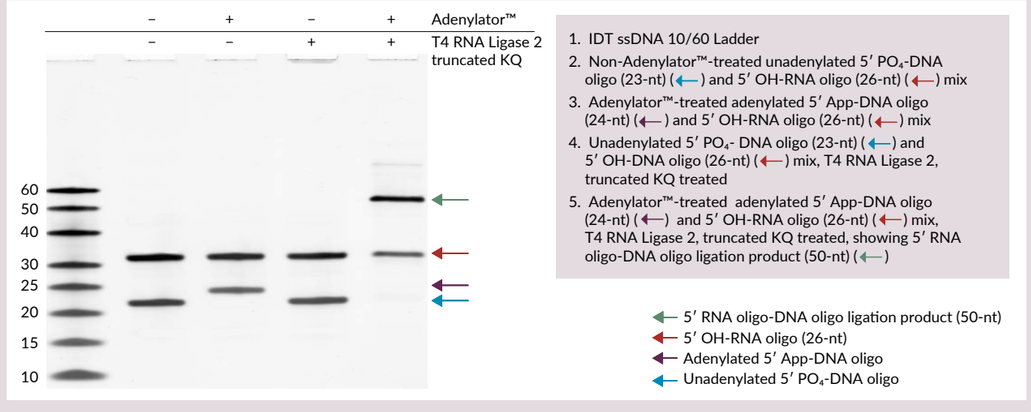


Figure 2 (left). Ligation of Adenylator™ Enzyme-adenylated oligonucleotides.

**Lucas Onder PhD** is a Senior R&D Scientist at CELLSCRIPT™ where he develops next-generation molecular biology tools. Prior to joining CELLSCRIPT™, he worked as an Amplitude Operations Specialist for Thermo Fisher Scientific supporting high-throughput COVID-19 diagnostics. Lucas earned his PhD in Genetics from the University of Wisconsin–Madison, Madison, WI, USA where he studied transcriptional quality control and nutrient stress response in *E. coli*.