



Control the Length of the 3'-Poly(A)-tail Added to Your RNA Using the A-Plus™ Poly(A) Polymerase Tailing Kit

Ron Meis, EPICENTRE Biotechnologies

EPICENTRE Biotechnologies' new A-Plus™ Poly(A) Polymerase Tailing Kit enables rapid, efficient, and controlled addition of a poly(A)-tail to the 3'-end of any RNA *in vitro*. The presence of a poly(A)-tail at the 3'-end of an RNA molecule can have an important and positive impact on studies requiring RNA including:

- Increased stability and enhanced translation after transfection or microinjection into eukaryotic cells.^{1,2,3}
- Providing a priming site for synthesis of first-strand cDNA using a primer with poly(dT) on its 3'-end portion.
- Cloning of DNA encoding an RNA molecule or a mixture of RNA molecules of unknown or multiple sequences by adding a poly(A)-tail that can anneal to a T-tailed vector.
- 3'-End-labeling of RNA with radioactive ATP.⁴
- Quantifying mRNA.⁵

The A-Plus Poly(A) Polymerase uses ATP as a substrate for template-independent addition of adenosine monophosphate to the 3'-hydroxyl termini of RNA molecules.⁶ In this report, we demonstrate that the length of the poly(A)-tail added to the 3'-end of an *in vitro* transcribed RNA can be easily controlled using the A-Plus Poly(A) Polymerase.

Methods and Results

In vitro transcription and purification of a 5'-capped RNA template

A 1760-base, 5'-capped RNA analog (m⁷G[5']ppp[5']G) transcript was produced using a standard 20 µl AmpliCap-MAX™ T7 High Yield Message Maker Kit reaction (EPICENTRE; see p. 20) from a linearized DNA template. The completed AmpliCap-MAX reaction was treated with 1 U RNase-Free DNase I (EPICENTRE) to remove the DNA template, and the 5'-capped RNA was purified by addition of 20 µl 5M ammonium acetate followed by incubation on ice for 15 minutes and centrifugation at 10,000 x g for 15 minutes. The ammonium acetate selectively precipitates RNA while leaving most of the DNA, protein and unincorporated NTPs in the supernatant. The resulting pellet containing the capped-RNA tran-

script was washed with cold 70% ethanol, dried, and resuspended in 40 µl of Sterile RNase-Free Water (supplied). The yield of the RNA was measured at A₂₆₀ and the quality of the 5'-capped RNA was confirmed by 1% denaturing agarose gel electrophoresis.

Poly(A)-tailing of RNA using the A-Plus Poly(A) Polymerase Kit

The standard 100 µl A-Plus Poly(A) Polymerase reaction containing 60 µg of the 5'-capped RNA, 1X Reaction Buffer (provided in the A-Plus Kit), 1 mM ATP and eight units of A-Plus Poly(A) Polymerase was incubated at 37°C. One microliter aliquots of the reaction were removed at 0, 10, 30 and 60 minutes, and 0.1 µg of RNA from each time point was loaded and run on a 1% denaturing agarose gel with RNA markers, followed by gel staining with ethidium bromide.

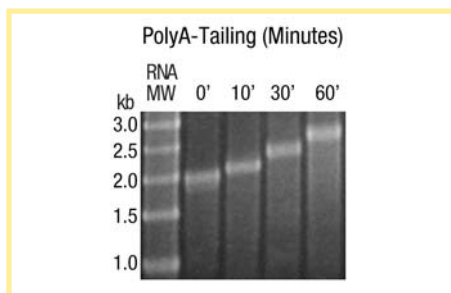


FIG 1. The length of the 3'-poly(A)-tail added to an RNA molecule can be readily controlled by altering the reaction time using the A-Plus™ Poly(A) Polymerase Tailing Kit. A 1760-base, 5'-capped RNA was incubated with 8 U of A-Plus Poly(A) Polymerase under reaction conditions described in the text.

FIG 1 shows the length of the 3'-poly(A)-tail added to the 1760 base RNA under standard reaction conditions after 10, 30, and 60 minutes. The un-tailed 1760 base RNA is shown in the zero minute gel lane. Incubation for 10 minutes yielded RNA with a poly(A)-tail length of approximately 200 bases; after 30 minutes the poly(A)-tail length was approximately 350 - 400 bases and after 60 minutes the reaction produced a poly(A)-tail length of >500 bases.

Other reaction conditions for the A-Plus Poly(A) Polymerase were investigated (data not shown). It was found that:

- The length of the poly(A)-tail was directly proportional to the amount of Poly(A) Polymerase in the reaction. The more Poly(A) Polymerase in the reaction, the longer the poly(A)-tail length.
- The length of the poly(A)-tail was inversely proportional to the molar amount of RNA in the reaction. The less RNA in the reaction, the longer the poly(A)-tail length.
- The length of the poly(A)-tail was inversely proportional to the reaction volume. Reducing the reaction volume produced RNA with longer poly(A)-tails.

Conclusion

We have demonstrated that the A-Plus Poly(A) Polymerase Tailing Kit can be used to rapidly and efficiently add a poly(A)-tail to the 3'-end of an *in vitro* transcribed RNA. The length of the poly(A)-tail can be controlled by altering the:

- Length of reaction time.
- Molar amount of RNA added to the reaction.
- Reaction volume.
- Amount of A-Plus Poly(A) Polymerase added to the reaction.

References

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4. Lingner, J. and Keller, W. (1993) *Nucleic Acids Res.* **21**(12), 2917.
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A-Plus™ Poly(A) Polymerase Tailing Kit

PAP5104H 400 Units

Contents: A-Plus™ Poly(A) Polymerase, A-Plus™ 10X Reaction Buffer, 10 mM ATP, Sterile RNase-Free Water.

RNase-Free DNase I

D9902K 2,500 Units

D9905K 5,000 Units

D9910K 10,000 Units

Supplied at a concentration of 1 U/µl.