

# Ask Frank

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## Questions about RNA capping and *in vitro* transcription

**Q.** What is RNA capping?

**A.** Capping is the first step in the maturation of eukaryotic mRNA. Shortly after the start of transcription, the 5'-end of the growing RNA molecule is capped by the addition of a guanosine monophosphate residue (from GTP) via a 5'-5' pyrophosphate linkage. After addition of the cap nucleotide, a methyl group is added to the N7 position of the guanine base producing a Cap 0 structure.

**Q.** What modifications to *in vitro* transcribed RNA are required for efficient translation of the RNA *in vivo*?

**A.** For *in vitro*-transcribed RNA to be translated efficiently *in vivo*, the mRNA requires two things:

- A poly(A)-tail at the 3'-end of the transcript.
- A correctly orientated N7-methylated cap (Cap 0) at the 5'-end of the molecule.

However, the addition of another methyl group onto the penultimate nucleotide from the 5'-end of the mRNA (producing a Cap 1 structure) will further boost translation.

**Q.** How much better are the translation efficiencies if I use RNA with a Cap 1 structure instead of a Cap 0 structure?

**A.** It has been reported that methylation at the 2'-O position of the penultimate nucleotide of capped RNA (to give a Cap 1 structure) improves translation by 20% to 50% over RNA with a Cap 0 structure.<sup>1,2</sup>

**Q.** Which process has better capping efficiency, EPICENTRE's new mScript™ mRNA Production System or cap analog-based co-transcriptional systems?

**A.** The mScript mRNA Production System is the first commercially available kit that produces capped mRNA with nearly 100% efficiency, and with all of the caps in the correct orientation.<sup>3</sup> Direct incorporation of

cap analog dinucleotides during traditional *in vitro* co-transcriptional systems is considerably less efficient, with capping efficiencies approaching only 75-80%. In addition, with some cap analogs, 30% or more of the caps are incorporated into mRNA in the wrong orientation, rendering such mRNA untranslatable.

**Q.** Why is there such a large improvement in RNA capping efficiency using the mScript mRNA Production System when compared to cap analog-based co-transcriptional reactions?

**A.** Unlike traditional cap analog-based co-transcriptional reactions that are both costly and inefficient, the mScript System uses an enzymatic process to build cap structures onto the RNA transcripts. Direct incorporation of cap analog during *in vitro* transcription is inconsistent in its capping efficiency and cap orientation. There is also an inherent trade-off between capping efficiency and total RNA yield from the transcription reaction; samples with high capping efficiencies produce lower overall RNA yields. The mScript System includes the Vaccinia virus-derived capping enzyme, which contains all three enzymatic activities (mRNA triphosphatase, guanylyltransferase, and guanine-7-methyltransferase) necessary to build 5'-Cap 0 structures *in vitro*, ensuring nearly 100% capping with 100% proper cap orientation. Furthermore, the mScript System contains the Vaccinia 2'-O-Methyltransferase enzyme, allowing the natural, translation-boosting Cap 1 structure to be built.

**Q.** Will EPICENTRE's ScriptCap™ system work with all *in vitro* transcribed RNA?

**A.** Yes. ScriptCap Capping Enzyme will work on any *in vitro* transcribed RNA provided that the RNA has a 5' di- or tri-phosphate.

**Q.** What is the main benefit of using Anti-Reverse Cap Analog (ARCA) dinucleotides as opposed to standard cap analog dinucleotides for capping of RNA by co-transcriptional incorporation?

**A.** Standard cap analogs can be incorporated at the 5'-end of the RNA in both the forward [m<sup>7</sup>G(5')ppp(5')G(pN)...] and the reverse orientation [G(5')ppp(5')m<sup>7</sup>G(pN)...]; RNA molecules with the cap in the reverse orientation are not efficiently translated. To overcome this problem, EPICENTRE offers an Anti-Reverse Cap Analog. This cap analog has a 3'-OCH<sub>3</sub> group on one of the nucleotides, so the ARCA will be incorporated by the RNA polymerase to produce RNA transcripts capped exclusively in the correct orientation.

**Q.** Which capping system is right for my needs?

**A.** Generally, mRNA produced using the mScript mRNA Production System will outperform the RNA produced using a cap analog during *in vitro* transcription. However, cap analog systems such as the AmpliCap™ and MessageMAX™ kits may have some advantages for certain applications, see the table below.

	AmpliCap-MAX™ T7 and T3 High Yield Message Maker Kits	MessageMAX™ T7 Capped Message Transcription Kit - ARCA-Capped	mScript™ mRNA Production System
Reaction time	30 min	30 min	2 hrs
Capping efficiency	~50*	~80%	~100%
Reverse incorporation of cap	Yes	No	No
Yield per reaction	60 µg	60 µg	60 µg
Capping of difficult-to-cap transcripts†	Yes	Yes	Varies
Poly(A) Polymerase included	No‡	No‡	Yes
2'-O-Methyltransferase included	No‡	No‡	Yes

\*Using a typical 4:1 ratio of dinucleotide cap analog to GTP, only about 80% of the transcripts are capped, of which about 60% are in the correct orientation.

†These include transcripts with extremely strong 5' hairpin structures. Contact Technical Services for more details.

‡The A-Plus™ Poly(A) Polymerase Tailing Kit can be purchased separately.

‡The ScriptCap™ 2'-O-Methyltransferase can be purchased separately.

## References

1. Kuge, H. *et al.*, (1998) *Nucl. Acids Res.* **26**(13), 3208.
2. Meis, R. and Meis, J.E. (2006) *EPICENTRE Forum* **13**(4), 5.
3. Meis, J.E. and Meis, R. (2007) *EPICENTRE Forum* **14**(1), 4.