Maximize the Yield of Short RNA Transcripts Using an AmpliScribe™ T7-*Flash*™ or T3-*Flash*™ Transcription Kit

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Obtaining high yields of RNA from an *in vitro* transcription reaction is increasingly important for certain molecular biology techniques and genomics studies, including ribozyme and aptamer synthesis, RNA structure and processing analyses, and RNA interference and antisense RNA techniques. EPICENTRE recently introduced the AmpliScribe™ T7-*Flash*™ and Ampli-Scribe™ T3-*Flash*™ Transcription Kits, allowing researchers to produce the highest RNA yields from an *in vitro* transcription reaction in the shortest reaction time.

Previously we reported that an AmpliScribe T7-*Flash* reaction produces 160 to 180 µg (8 to 9 mg/ml) of a 1.4-kb RNA transcript in 30 minutes[1,2]…more RNA than other commercial kits produce in 2 hours. We further demonstrated that an AmpliScribe T7-*Flash* reaction can be scaled up to produce milligram quantities of RNA.[3] The AmpliScribe *Flash* Transcription Kits also produce the highest yields of short RNA transcripts (<500 bases), and in this report we discuss ways to maximize the yield of short RNA transcripts from an AmpliScribe *Flash* reaction.

General considerations for transcribing short RNAs

Transcription initiation is the rate limiting step in an *in vitro* transcription reaction. When transcribing a long RNA (>500 bases), each transcription initiation event results in the incorporation >500 nucleotides. As a result, long-transcript reactions rapidly deplete the available NTPs and produce the maximum RNA yield in short reaction times (30 minutes with the AmpliScribe *Flash* Kits).

"…using reaction conditions that favor an increase in transcription initiation events will maximize the yield of a short RNA transcript."

However, when transcribing a short RNA (<500 bases), each initiation event incorporates fewer nucleotides, so more initiation events are required to reach the maximum RNA yield. Thus, using reaction conditions that favor an increase in transcription initiation events will maximize the yield of a short RNA transcript. Here we examine the effects that DNA template concentration, reaction time and reaction temperature have on maximizing the yield of short RNA transcripts.

DNA template and template concentration

Typically, *in vitro* transcription reactions producing long RNAs use 1 to 2 µg of linearized plasmid DNA template. However, *in vitro* transcription reactions producing short RNAs may use linearized plasmids, PCR products, or oligonucleotides as templates. The different size templates vary significantly in the number of molecules per microgram of DNA. Therefore, when transcribing short RNAs, it is important to consider the *molar* amount of template added to the reaction. More moles of template in a reaction allow more transcription initiation events, resulting in higher yields of the short RNA in a given reaction time. For example, Figure 1 shows the yield of the same 48-base RNA transcript produced from 1 µg of a 2961-bp linearized plasmid and from 1 µg of a 68-bp double-stranded oligonucleotide. Compared to the linear plasmid template reaction, the oligo template reaction contains many more *moles* of DNA and produces a significantly higher yield of the 48-base RNA transcript in a given reaction time. When equal *molar* amounts of the 2 templates were added to the reactions, the yields of the 48-base RNA were equivalent at each time point (data not shown).

Reaction time

When transcribing a short RNA, increasing the reaction time increases the number of transcription initiation events. Figure 2 shows the effect of reaction time on the yield of a 26-base RNA transcribed from 1 µg of a linearized 2961-bp plasmid. In this example, the reaction produced the maximum yield (~100 µg) of the 26-base RNA in 4 hours.

Figure 1.

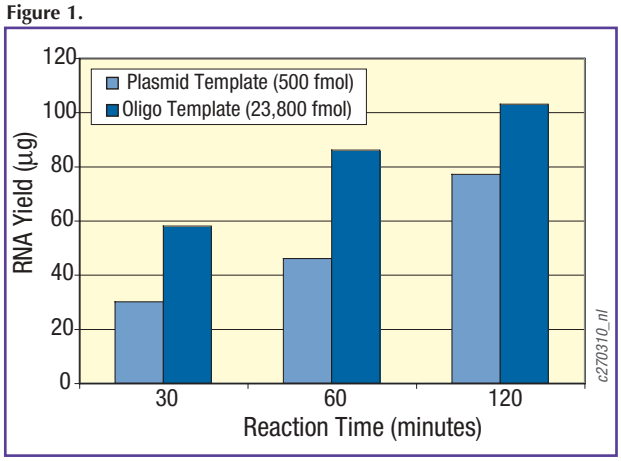

Figure 1. Increasing the number of moles of DNA template in an AmpliScribe™ T7-*Flash*™ *in vitro* transcription reaction, increases the RNA yield in a given reaction time, until the maximum yield is produced. A 48-base RNA was transcribed from 1 µg (511 fmoles; ~25 nM final concentration) of a 2961-bp linearized plasmid template and from 1 µg (23,842 fmoles, ~1200 nM final concentration) of a 68-bp oligonucleotide template. The quantity of the 48-base RNA transcript produced from each template was determined at the indicated time points.

Figure 2.

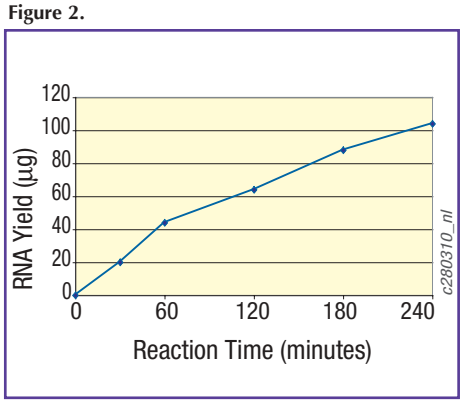

Figure 2. Increasing the reaction time increases the yield of a short RNA transcript from 1 µg of a linearized plasmid template. A 26-base RNA was transcribed from 1 µg of a linearized 2961-bp plasmid template in an AmpliScribe™ T7-*Flash*™ transcription reaction. The quantity of the 26-base RNA produced was determined at the indicated time points. Maximum yield (~100 µg) of the 26-base RNA was achieved in 4 hours.

Reaction temperature

In vitro transcription reactions are usually performed at 37ºC. We examined the effect of performing an AmpliScribe T7-*Flash* reaction at 42ºC. As shown in Figure 3, increasing the reaction temperature from 37°C to 42ºC reduced the time required to produce the maximum yield of the 26-base RNA (~100 µg) from 4 hours to 2 hours, respectively.

AmpliScribe Flash Transcription Kits produce higher yields of short RNAs in less time compared to a short RNA kit from another company.

As shown in Table 1, an AmpliScribe T7-*Flash* transcription reaction produces more short RNA transcript in less time and from a lower DNA template concentration than another supplier's kit, which is specifically designed to transcribe short RNAs.

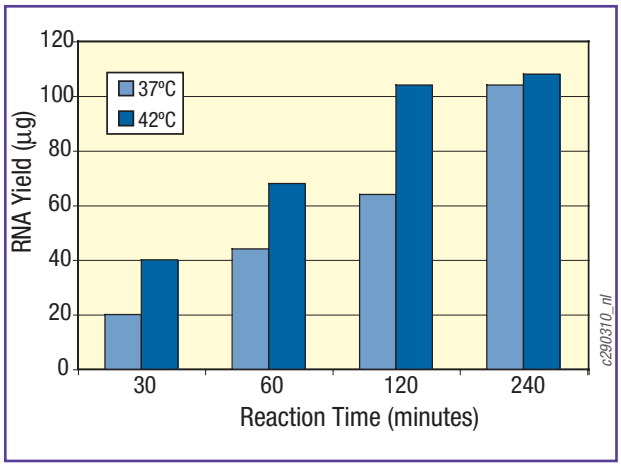

Figure 3. Increasing the *in vitro* transcription reaction temperature to 42ºC significantly reduces the time needed to produce the maximum yield of a 26-base RNA transcript from 1 µg of a linearized plasmid template. A 26-base RNA was transcribed from 1 µg of a linearized 2961-bp plasmid in an AmpliScribe™ T7-*Flash*™ transcription reaction. Maximum yield of the 26-base transcript (~100 µg) was achieved in 2 hours at 42ºC and in 4 hours at 37ºC.

Table 1. An AmpliScribe™ T7-*Flash*™ transcription reaction produces more short RNA transcripts in less time and from a lower DNA template concentration than a competitor's kit, which is specifically designed to transcribe short RNAs. The competitor's data presented is from the company's published literature. (N/R = data not reported).

In Vitro Transcription Kit	Template Concentration	Transcript Size	Reaction time and RNA Yield			
			30′	60′	120′	240′
AmpliScribe™ T7-*Flash*™ Kit	25 nM	96 bases	36 µg	60 µg	92 µg	144 µg
Competitor	50 nM	100 bases	N/R	N/R	~30 µg	~60 µg

Discussion

The key to maximizing the yield of a short RNA (<500 bases) from an *in vitro* transcription reaction is to maximize the number of transcription initiation events. This can be accomplished by (in order of greatest impact on RNA yield):

1.) Increasing the number of moles of DNA template in the reaction. With a small double-stranded oligo or PCR product template, it is easy to significantly scale-up the template molarity in the reaction. If a linearized plasmid template is used, increase the amount of the template to 2 or 3 (or more) µg per reaction.

2.) Increasing the reaction time. This is especially true when transcribing the short RNA from a large DNA template, for example a linearized plasmid.

3.) Increasing the reaction temperature to 42ºC.

References

1. Meis, R. and Pease, J. (2003) *EPICENTRE Forum* **10**(2), 6.
2. Meis, R., *et al.* (2003) *Bioscience Technology* **28**(9), 8.
3. *EPICENTRE Forum* **10**(3), 8.