Ask Frank

by Fred and Hank


Fred Hyde


Hank Daum

Questions about AmpliScribe™ *Flash* and DuraScribe™ Transcription Kits

A sample of comments from EPICENTRE customers about Fred and Hank

Dr. Dahl,

I wanted to let you know how much I appreciate the technical support of Fred Hyde. I am a professor at a liberal arts college and I am trying to optimize and 'undergrad proof' lab protocols for RNA isolation and RT-PCR reactions to study yeast gene expression in my Cell Biology course. For the past two weeks Fred and I have been exchanging emails. Throughout all of these emails Fred has been exceedingly knowledgeable, prompt, courteous and helpful.

In a day and age where it becomes more difficult to actually contact a person, let alone to get that person to answer your question rather than referring you to a generic script response, I truly appreciate Fred's time and effort.

Thank you for having helpful and knowledgeable employees like Fred.

Karen K Bernd, Ph.D.
Biology Department
Davidson College
Davidson, NC 28035

And this short, and to the point, note sent to Hank:

WOW!!!!

Three words for you:
Excellent Customer Service.

Thank you very much,

Alfredo Lopez De Leon Ph.D.
Postdoctoral Fellow
Molecular Biology and Bioinformatics Department
Novozymes Biotech, Inc.
1445 Drew Avenue
Davis, CA 95616

Q: What is the longest *in vitro* transcript readily made with an AmpliScribe™ *Flash* Transcription Kit?

A:. While we have tested and readily produced 9 kb transcripts at EPICENTRE, our customers report high quality transcripts greater than 11 kb.

Q: I'm using less than the recommended 1 µg of DNA template in an AmpliScribe *Flash* reaction. How can I improve my RNA yield?

A:. With lower concentrations of DNA template, you can increase RNA yields by increasing the reaction time from the recommended 30 minutes up to 2 to 4 hours, depending on the amount of template used. If you are using less than 10 ng of template, allow the reaction to incubate overnight (16 hours). If the desired transcript is 1 kb or smaller, increasing the reaction temperature from 37°C to 42°C also improves the yield.

Q: Why should I set up the AmpliScribe *Flash* reactions at room temperature? Won't that affect the polymerase enzyme activity?

A:. The components of the reagents in a standard 20-µl reaction are very close to their solubility limits. If you set up the reaction on ice, some reaction components (such as the buffer and the nucleotides) will precipitate. After components precipitate, warming the reaction tube only partially resolubulizes the reagents. The AmpliScribe™ *Flash* Enzyme Solution is quite stable and can remain at room temperature for about an hour without affecting the enzyme activity.

Q: How clean does the template DNA need to be for *in vitro* transcription?

A:. Very clean template DNA assures the best performance of an *in vitro* transcription reaction. If using a PCR product for the template, purify the desired product from the PCR reaction. If using a plasmid for the template, in order to transcribe the desired RNA product, completely linearize the plasmid, leaving a blunt or 5′ overhanging end. Uncut plasmid serves as excellent template, but the T7 enzyme will polymerize past the desired transcription stop point and around the plasmid several times.

Q: What is the shortest *in vitro* transcript readily made with an AmpliScribe *Flash* Transcription Kit?

A: We have produced 26-base RNA transcripts with an AmpliScribe *Flash* Transcription Kit. (See page 7 for more information on preparing small transcripts)

Q: Can I use AmpliScribe Kits for RNA interference studies?

A:. Absolutely! We have a number of customer citations using AmpliScribe-synthesized RNA for RNAi.

Q: Can I make nonradioactive, labeled RNA using an AmpliScribe *Flash* Kit (with labeled nucleotides or by end-labeling)?

A: Yes, you can directly incorporate derivatized nucleotides (with moieties like Cy5, biotin, or digoxygenin) into the transcripts or you can do post-transcriptional labeling of purified RNA transcripts at the 5′- or 3′-ends. Please call us for protocols for these methods. (To prepare radioactive RNA, use the RiboScribe™ RNA Probe Synthesis Kit.)

Q: What advantages does an AmpliScribe *Flash* Transcription Kit have over the standard AmpliScribe™ High Yield Transcription Kit?

A:. The two main advantages of the *Flash* Kits are: 1) improved RNA yields, even better than the excellent results obtained with the AmpliScribe™ High Yield Transcription Kits, and 2) a fast, 30 minute procedure.

Q: What is the difference between RNA made with the DuraScribe™ T7 Transcription Kit and RNA made with other *in vitro* transcription kits?

A: The DuraScribe T7 Transcription Kit produces RNA that contains nucleotides with a 2′ fluorine and that is resistant to degradation by A-type RNases (like the RNase found on human skin), while the AmpliScribe *Flash* and other transcription kits make "standard" RNA. DuraScript™ RNA can be reverse transcribed, like regular RNA, and can be digested by RNase III. However, DuraScript RNA cannot be used as a template to produce proteins by *in vitro* translation.