

TECH TIP

The Importance of RNase I Treatment in DNA Cloning

RNA contamination of DNA preparations can arise from the genomic DNA source (cells or tissues) in the primary cloning step, or from the host cell when subcloning. Such contaminating RNA can cause problems in DNA cloning.¹⁻⁴ Standard protocols for preparation of DNA for library construction or subcloning may or may not include an RNase treatment step to remove residual RNA. If an RNase treatment is included, the enzyme traditionally used has been RNase A.

Recent work done by Lucigen in collaboration with a major genome center illustrated the importance of including RNase treatment in obtaining optimal cloning results. In this work, DNase-free RNase I was used instead of RNase A. RNase I completely digests RNA to mononucleotides⁶⁻⁸, allowing easy removal from the DNA to be cloned. In contrast, RNase A leaves residual oligoribonucleotides after digestion,⁵ which can co-purify with the DNA. An additional advantage is that RNase I is easily inactivated by incubation at 70°C for 15 minutes.

To illustrate the importance of RNase I treatment, plasmid DNA from a set of 18 clones was prepared using a standard alkaline lysis method either with or without RNase I digestion. As expected, residual RNA was minimized in the RNase I-treated samples (Figure 1, panel A). However, significant RNA contamination was present in the untreated samples (Figure 1, panel B).

RNase I treatment can be performed after standard alkaline lysis methods of plasmid preparation, as follows:

1. Add 1 µl (10 U) of Lucigen's RNase I. Mix by inverting the tube 10 times.
2. Incubate at 37°C for 30 minutes to degrade contaminating RNA.
3. Incubate at 70°C for 15 minutes to inactivate the enzyme.

Order Information

Lucigen's RNase I (recombinant) is greater than 99% pure by SDS-PAGE and is free of detectable exo- and endo-DNase activities. The enzyme is supplied in a storage buffer of 50% glycerol, 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 0.1 mM EDTA. A 10X RNase I Reaction Buffer is also included.

Products	Cat. No.	Size
RNase I, <i>E. coli</i> (recombinant)	30104-1	1,000 U
	30104-2	5,000 U

References

1. J. Sambrook, E.F. Fritsch, and T. Maniatis (1989). *Molecular Cloning: A Laboratory Manual* (2nd edit.), N. Ford (ed.), Cold Spring Harbor Laboratory Press, p. 1.51.
2. Roswell Park Cancer Institute DNA Sequencing Laboratory (on-line), (www.roswellpark.org/Site/Research/Shared_Resources/Biopolymer_Resource/DNA_Sequencing). "Template preparation and purification" section in *Sequencing Basics*.
3. M. Seki (1998). *Plant J.* 15, 707.
4. D. Mead, personal communication.
5. J.N. Davidson (1972). *The Biochemistry of the Nucleic Acids* (7th edit.), Academic Press.
6. V. Shen and D. Schlessinger (1982). *The Enzymes*, Vol. XV, 501.
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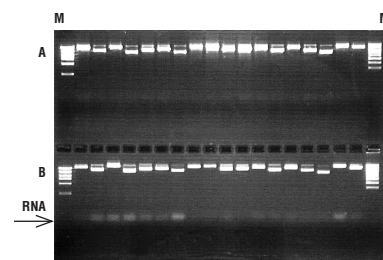


Figure 1. Plasmid mini-preps from a single set of clones were performed with RNase I (Panel A) or no RNase treatment (Panel B). Set B shows RNA contamination. M, mass ladder.