Promega Notes Magazine **Number 53, 1995, p.12**

Effects of Bacterial Strains Carrying the *endA1* Genotype on DNA Quality Isolated with Wizard(TM) Plasmid Purification Systems

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We investigated the instability of plasmid DNA isolated from endA *positive* E. coli *strains using a number of commercially available DNA purification systems, including the Wizard(TM) Minipreps DNA Purification System. This phenomenon was observed with several of the systems tested when purifying plasmid DNA from* endA *positive hosts; no stability problems were observed with plasmid isolated from* endA *negative host strains. Preliminary data suggests that the presence of endonuclease I, the product of the endA gene, contributes to this inconsistent quality. We describe ways to control this phenomenon through bacterial strain selection, growth media selection, or slight modifications to the protocol supplied with the Wizard(TM) Minipreps DNA Purification System.*

Introduction

Since the introduction of the Wizard(TM) Minipreps DNA Purification System (1), Promega has worked continually to enhance the performance of the product. Over the past 18 months, these enhancements have resulted in increased plasmid yield and modified protocols to improve the quality of the isolated plasmid DNA.

In recent months, we have focused our efforts on improving the quality of plasmid DNA isolated from *endA* positive (*endA*+*) bacterial strains. While high quality DNA was obtained easily from bacterial strains which were *endA* minus (*endA*-*), the quality of plasmid DNA purified from *endA*+ strains appeared inconsistent. The plasmid DNA isolated from *endA*+ hosts has been observed to degrade during long term storage and restriction enzyme digests or produce high backgrounds in automated fluorescent sequencing systems.

*When referring to an *E. coli* genotype, "*endA1*" refers to a mutation in the *endA* gene, producing an inactive form of endonuclease I. This corresponds to our use of *endA*- throughout this article. The absence of *endA1* in an *E. coli* genotype indicates the presence of the wild type gene, which corresponds to our use of *endA*+ in the article.

E. coli endA Gene Product: Endonuclease I

The product of the *endA* gene is endonuclease I, a periplasmic protein of 12kDa. Its activity is magnesium dependent, is inhibited by EDTA, and is partially heat labile. Double-stranded DNA serves as the substrate for the endonuclease. RNA acts as a competitive inhibitor and alters the endonuclease specificity from that of a double-strand nucleolytic enzyme yielding seven base oligonucleotides to a nickase that cleaves an average of one time per substrate (2,3). The function of endonuclease I is not fully understood, and *endA1* mutations have no obvious phenotype other than improved stability of plasmid obtained from them. Table 1 contains a listing of the most commonly used *endA*+ and *endA*- bacterial strains.

Table 1. *endAI* Genotype of Commonly Used *E. coli* Strains.

	Examples
endA positive bacterial strains	NM554, HB101, JM83, JM101, NM522, MC1061, TB1, TG1, BL21(DE3) and Y1088
endA negative bacterial strains	DH5-alpha®, JM103, JM109, MM294, and XL-1 Blue

Note: When referring to an *E. coli* genotype, "*endA1*" refers to a mutation in the *endA* gene, producing an inactive form of endonuclease I. This corresponds to our use of *endA*- throughout this article. The absence of *endA1* in an *E. coli* genotype indicates the presence of the wild type gene, which corresponds to our use of *endA+* in the article.

The expression of endonuclease I has been characterized and is dependent on bacterial growth phase (4). Endonuclease I levels were found to be more than 300 times higher during exponential phase compared to stationary phase. In addition, media compositions that encourage rapid growth (e.g., high glucose levels and addition of amino acids) resulted in high endonuclease I levels.

This article describes strategies to improve consistency in the quality of plasmid DNA isolated from *endA*+ bacterial hosts using the Wizard(TM) Minipreps DNA Purification System. In particular, it evaluates the effects of media and growth conditions on the expression of the *endA* gene and describes protocol modifications which reduce endonuclease contamination and/or activity.

Quality of Plasmid DNA Isolated from *endA*- Strains Using Various Methods

Figure 1 compares the stability of DNA isolated from DH5-alpha® (*endA*-) using the Wizard(TM) Minipreps DNA Purification System and two other commercially available spin column systems. With each system, the plasmid DNA was isolated according to the manufacturer's suggested protocol. To analyze the quality of the DNA isolated, samples of the plasmid DNAs were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining before (Figure 1A) and after (Figure 1B) a 16-hour incubation at 37°C in 1X MULTI-CORE(TM) Buffer (25mM Tris-acetate, pH 7.8, 100mM potassium acetate, 10mM magnesium acetate, 1mM DTT) (DNA stability assay). This assay is a sensitive indicator of nucleases that may be present in the purified DNA. The assay results showed no significant differences in any of the samples; all showed a conversion of nicked plasmid present in small amounts in each sample to linear DNA after incubation with the restriction enzyme buffer.

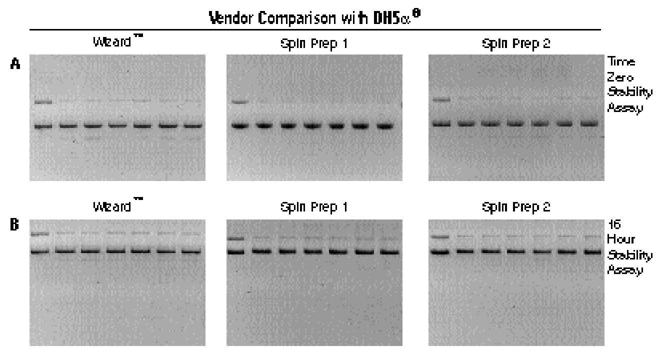

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Figure 1. Comparison of DNA stability isolated from DH5-alpha® (*endA*-). Plasmid DNA (pGEM*®-3Zf(+) plasmid) was isolated from 3ml of an overnight bacterial culture of DH5-alpha® (*endA*-) cells grown in 1X LB medium at 37°C. The isolated plasmid DNAs (0.5µg) were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining before (Panel A) and after (Panel B) a 16-hour incubation at 37°C in 1X MULTI-CORE(TM) Buffer (25mM Tris-Acetate, pH 7.8, 100mM potassium acetate, 10mM magnesium acetate, 1mM DTT) (stability assay). The ethidium bromide stained image was captured using an AMBIS Data Imaging System.

*U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

The DNA quality isolated from *endA*- strains is demonstrated further in Figure 2, which compares the efficiency of restriction enzyme digestion of plasmids isolated using standard phenol:chloroform extraction methods, the Wizard(TM) Minipreps System and two other commercially available spin column plasmid purification systems. The restriction enzyme *Sac* I was used in these experiments. This enzyme is sensitive to a number of potential contaminants, originating from components used in each of the commercial products including NaCl, guanidine hydrochloride and ethanol. Each of the plasmid DNAs isolated from the commercially available products digest with the same efficiency, but required approximately 2-fold more enzyme to completely digest the plasmid than did the phenol:chloroform-treated samples. This is consistent with published observations that DNA isolated by methods which use a phenol:chloroform extraction are easier to digest with restriction enzymes (5).

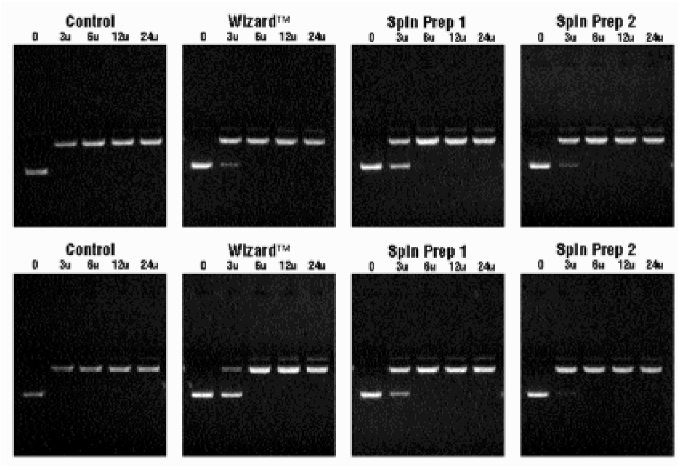
1. 

Figure 2. Restriction enzyme digestion efficiency of plasmids isolated from the host strain DH5-alpha® (*endA*-) with 3 commercially available miniprep kits. pGEM®-3Zf(+) DNA (0.5µg) isolated from DH5-alpha® (*endA*-) using other commercial systems available were digested with increasing amount of the restriction enzyme *Sac* I (indicated above lane) for 1 hour at 37°C. The digested samples were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining and photography.

Effects of *endA*+ Strains On Plasmid Quality and Yield

To examine the effects of *endA*+ strains on isolated plasmid DNA, plasmid DNA was prepared from both NM554 (*endA*+) and DH5-alpha® (*endA*-) bacterial strains. Whereas plasmid DNA isolated from DH5-alpha® was stable for at least 16 hours at 37°C when incubated in the presence of MULTI-CORE(TM) Buffer (Figure 3A), plasmid isolated from the host strain NM554 was completely degraded within the first two hours of incubation (Figure 3B). These samples were isolated using the Wizard(TM) Minipreps DNA Purification System, and similar results would be expected with the Wizard(TM) Midipreps, Maxipreps and Megapreps Systems.

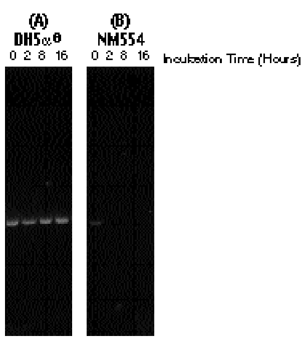
1. 

Figure 3. Stability of DNA isolated from the bacterial strains DH5-alpha® (*endA*-) and NM554 (*endA*+). For Panels A and B, bacteria were cultured in 2X YT medium supplemented with 1% glucose and grown for 16 hours at 37°C. Triplicate samples of pGEM®-3Zf(+) DNA were isolated from 3ml cultures using the Wizard(TM) Minipreps DNA Purification System. Each of the DNAs (0.5µg) was incubated for indicated times at 37°C in 1X MULTI-CORE(TM) Buffer (25mM Tris-acetate, pH 7.8, 100mM potassium acetate, 10mM magnesium acetate, 1mM DTT). The DNA was analyzed on a 1% agarose gel in 1X TBE and the DNA was visualized by ethidium bromide staining and photography. **Panel A:** pGEM®-3Zf(+) isolated from DH5-alpha® (*endA*-). **Panel B:** pGEM®-3Zf(+) DNA isolated from NM554 (*endA*+).

Figure 4 shows that not all *endA*+ strains are as problematic as NM554. Even during mid-log phase when endonuclease I levels are reported to be maximal, the plasmid DNA isolated from HB101 (*endA*+) at this time showed only minimal degradation in the DNA stability assay. These results suggest that the concentration of an endonuclease in the preparation is dependent on the *endA*+ host strain and may influence the extent of degradation observed in the stability assay. These results also show that plasmid DNA isolated at mid-log phase produces a smear after agarose gel electrophoresis, extending from the well to the plasmid band. After the stability assay, a more prominent plasmid band appears in these samples. This result is consistently observed with plasmid DNA isolations from mid-log phase cultures (see also Figure 7) and is not fully understood at this time.

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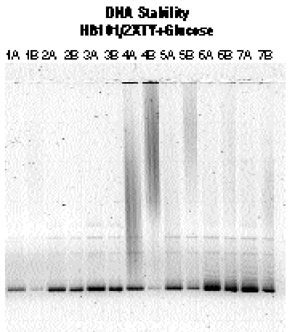

Figure 4. DNA stability of plasmid isolated from HB101 (*endA*+). pGEM®-3Zf(+) DNA was isolated from 3ml cultures of HB101 (*endA*+) at 1 hour intervals (indicated above individual lanes) during the growth of the bacteria. Plasmid DNA samples were tested in the stability assay described in Figure 1 and the image was captured by the AMBIS Data Imaging System. Lanes designated "A" contain untreated plasmid samples. The image was captured using the AMBIS Data Imaging System. Lanes designated "B" contain plasmid DNA samples tested in the stability assay and analyzed as described in Figure 1.

The inconsistent stability of plasmid DNA isolated from *endA*+ strains was also observed with other commercially available products. Figure 5 shows the results of the DNA stability assay from 6 individual isolates from 2 different spin column miniprep kits. Each of these samples were isolated at the same time according to the manufacturer's suggested protocol from the same bacterial culture. Comparison of the results before (Figure 5A) and after the stability assay (Figure 5B) suggest that an endonuclease co-purifies with plasmid DNAs isolated using other commercially available systems.

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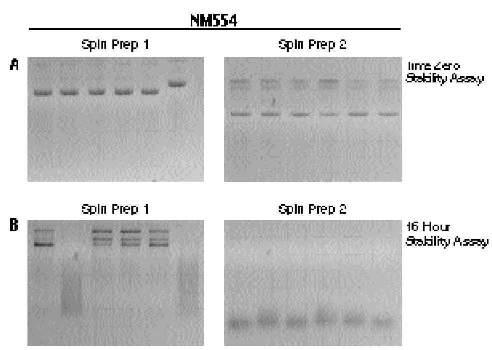

Figure 5. Stability of plasmid DNA isolated from the host strain NM554 (*endA*+) with two spin column miniprep products. Bacteria were cultured in 2X YT medium supplemented with 1% glucose and grown for 16 hours at 37°C. Samples of pGEM®-3Zf(+) DNA were isolated from 3ml cultures. **Panel A:** Plasmid DNA isolated before stability assay. **Panel B:** Plasmid DNA samples as in Panel A except not subjected to the stability assay.

In addition to plasmid quality, the plasmid yield also can be affected by the bacterial host strain. Table 2 compares the yield of plasmid DNA from DH5-alpha® (*endA*-) and NM554 (*endA*+) isolated using Wizard(TM) Minipreps and two other commercial products. The difference in yield of pGEM® DNA was greater than two-fold higher in the *endA*- host for all three of the products used in the comparison. The Wizard(TM) Minipreps System produced higher yields than either of the other kits with both of the cell lines. This result highlights one of the key performance advantages of the Wizard(TM) Minipreps DNA Purification System.

Table 2. Plasmid DNA Yields from *endA*+ and *endA*- Bacterial Strains Using Several Commercially Available Plasmid DNA Purification Systems.

Strains/Isolate	Wizard(TM) Minipreps System	Commercial Spin Prep #1	Commercial Spin Prep #2
DH5-alpha®/Day 1	17µg	11µg	8µg
DH5-alpha®/Day 2	16µg	14µg	8µg
NM554/Day1	6µg	4µg	2µg
NM554/Day 2	7µg	3µg	2µg

DH5-alpha® was purified from 12 samples from two separate

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 users on two different days (24 isolates total).
NM554 was purified from 6 samples
 on two different days (12 isolates total).
Plasmid DNA (pGEM®-3Zf(+) DNA)
 was purified from both DH5-alpha® (endA-) and NM554 (endA+) using
 the manufacturers' recommended procedures.
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Kinetics of Nuclease Binding to the Wizard(TM) Resin

To better understand the dynamics of nuclease co-purification, we performed a series of experiments to determine endonuclease binding kinetics to the Wizard(TM) Resin. Figure 6 illustrates the effects of extended incubation of a cleared lysate with Wizard(TM) Resin on the degradation of DNA. In this experiment, cleared lysate from an overnight culture of NM554 (*endA*+) cells containing a plasmid was incubated in the presence of the Wizard(TM) Resin for 1, 5 or 15 minutes before processing on the Vac-Man(TM) Manifold. The presence of endonuclease was tested by the DNA stability assay. These results show that the longer the lysate was in contact with the resin, the greater the extent of degradation observed. These data suggest that a slow direct or indirect binding of endonuclease I to the Wizard(TM) Resin occurs and that endonuclease I then co-elutes with the DNA during purification. This phenomenon is dependent on the volume of culture processed and the level of endonuclease I expression during the growth of culture (see below). Because of these kinetics, we recommend that contact of the resin with cleared lysates derived from *endA*+ strains be kept to one minute. No significant yield of plasmid DNA is obtained with longer incubations.

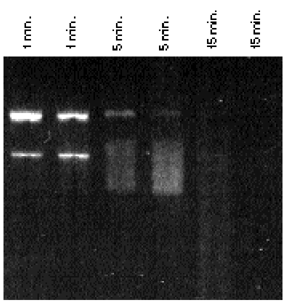
1. 

Figure 6. Effect of incubation time of cleared lysate and Wizard(TM) Resin. In this experiment, duplicate samples of cleared lysate from an overnight culture of NM554 (*endA*+) cells was incubated in the presence of the Wizard(TM) Resin, which contains a final concentration of 4M guanidine hydrochloride, for 1, 5 or 15 minutes before being processed on the Vac-Man(TM) Manifold. Each of the isolated DNAs (0.5µg) was tested in the stability assay and analyzed as described in Figure 1.

Importance of Media Type and Growth Stage on Endonuclease Expression

Based on published results (4), we evaluated the effect of media type and growth stage on endonuclease expression. Figure 7 compares the quality of DNA isolated from NM554 (*endA*+) cells grown in either 2X YT medium supplemented with 1% glucose or 1X LB medium. DNA samples were isolated at one-hour intervals during the growth of the bacteria. As a control, plasmid DNA was isolated from the bacterial strain DH5-alpha® (*endA*-) cultured in the identical media (Figure 7). The data we obtained were consistent with previously reported results. Based on the extent of observed degradation as a measure of endonuclease levels, endonuclease I levels peaked at five hours (mid-log phase, data not shown) and decreased during the stationary phase of growth. Plasmid DNA isolated during the first two hours of growth was stable in the stability assay.

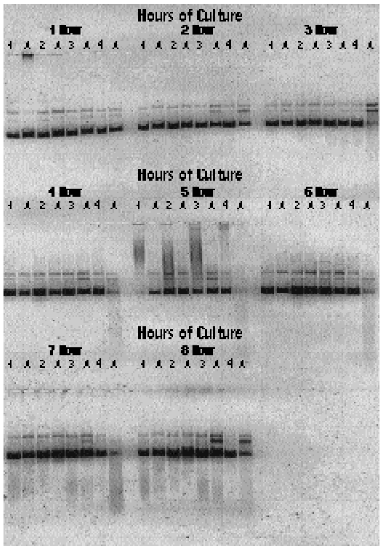
1. 

Figure 7. Effect of media type and growth phase on DNA degradation: A comparison of DH5-alpha® (*endA*-) and NM554 (*endA*+) cultured in 1X LB or 2X YT with 1% glucose. Each of the media type and strain combinations were grown under identical conditions. Fifty milliliter cultures were inoculated with 0.5ml of an overnight growth cultured under identical conditions. Each of the cultures was grown at 37°C with shaking at 200rpm. One milliliter of culture was removed from each sample at one-hour intervals and the plasmid was isolated according to the Wizard(TM) Minipreps DNA Purification minipreps protocol (6). The isolated plasmid DNAs (0.5µg) were analyzed by the stability assay described in Figure 1. Both the unincubated samples (designated by number) as they were isolated from the resin, and the samples that were incubated in 1X MULTI-CORE (TM) Buffer are shown (designated by A) for each of the media and strain combinations. Numbers above lanes correspond to the following strain/medium combinations: 1, DH5-alpha®/2X YT plus glucose; 2, DH5-alpha®/1X LB; 3, NM554/1X LB; 4, NM554/2X YT plus glucose.

The data in Figure 7 also demonstrated that the use of LB medium, a less rich medium, significantly reduced the levels of endonuclease I at all growth phases compared to the results with 2X YT containing glucose. Plasmid DNA isolated from DH5-alpha® (*endA*-) was stable in the stability assay when the cells were cultured in either media.

We also examined the effect of glucose addition to the 2X YT medium on the plasmid DNA yield and stability. The results in Figure 8 show that no net synthesis of plasmid occurred after four hours of culture in the presence of 1% glucose. In the absence of glucose, however, plasmid DNA synthesis continued well into stationary phase, resulting in a 3-fold greater yield of plasmid DNA even though all growth was identical with or without glucose. In addition, several stability studies indicated a lower level of endonuclease I expression in the absence of glucose (data not shown), which agrees with published observations (4).

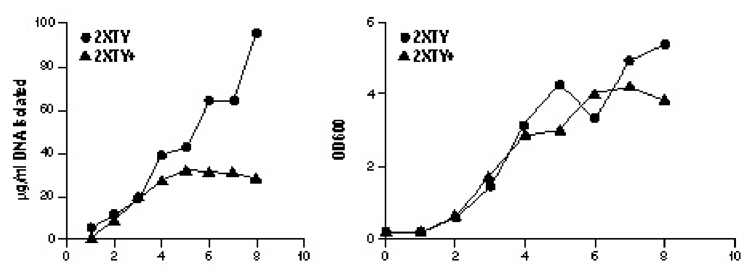
1. 

Figure 8. Effects of 1% glucose supplemented in 2X YT medium on plasmid yield. NM554 (*endA*+) cells were cultured in either the presence or absence of 1% glucose. Plasmid DNA was isolated at the indicated times according to the system protocol from 1.5ml of bacterial culture. Plasmid yields were determined by spectrophotometry at A260. **Panel A:** DNA yields at indicated growth times using different growth media. **Panel B:** Growth curves of NM554 (*endA*+) with and without glucose.

Effect of Heat Treatment on Endonuclease I Activity

We also examined the effects of an additional heat inactivation step to eliminate endonuclease I activity. Heat treatment after cell resuspension, after cell lysis, and after the renaturation step resulted in plasmid DNA that was more stable in the stability assay (Figure

9). However, we also observed a relatively high background of apparent host genomic DNA in the samples that were treated at 65°C. Even though endonuclease I is heat labile, the presence of genomic DNA in the plasmid preparation makes this treatment unacceptable for most applications.

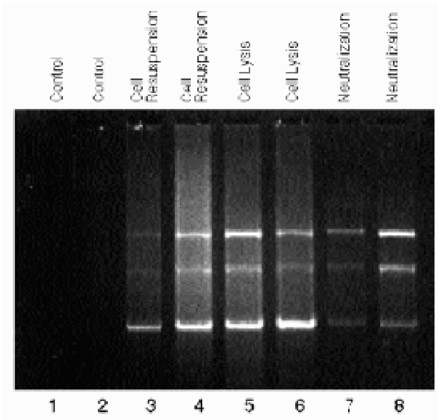
1. 

Figure 9. The effect of heat treatment on the stability of plasmid DNA. Plasmid DNA was prepared from NM554 (*endA*+) cells grown in 2XYT+1% glucose following the standard Wizard(TM) Minipreps protocol with the noted modifications. In lanes 1 and 2, the standard protocol was followed; in lanes 3 and 4, a 10-minute, 65°C incubation step was added after the resuspension of the cells; lanes 5 and 6, a 10-minute, 65°C incubation step was added after the cell lysis step; lanes 7 and 8, a 10-minute, 65°C incubation step was added after the neutralization step. After each step, the sample was allowed to cool to room temperature before completing the standard Wizard(TM) minipreps protocol (6). DNA samples (0.5µg) were analyzed in the stability assay as described in Figure 1.

Removal of the Endonuclease I from Cleared Lysates

Since heat inactivation of the endonuclease resulted in chromosomal DNA contamination of the plasmid DNA, additional experiments were performed to reduce or eliminate endonuclease I from the lysate. Figure 10 shows the effect of including a 10-minute incubation at room temperature at the neutralization step in the protocol. This procedural modification results in more efficient precipitation of contaminating protein, such as endonuclease I, increasing the stability of the plasmid DNA in the stability assay.

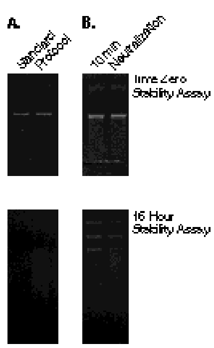
1. 

Figure 10. Modification of the neutralization step. Panel A: Plasmid DNA was isolated according to the standard Wizard(TM) minipreps protocol (6). **Panel B:** Plasmid DNA was isolated according to the standard Wizard(TM) protocol with the following modifications, a 10-minute incubation at room temperature was added following the addition of the neutralization solution.

Summary

We compared the quality of plasmid DNA isolated from *endA*+ and *endA*- bacterial hosts and found that the host strain can affect the overall plasmid quality. In general, *endA*- strains produced DNA of higher quality than *endA*+ strains for all the strains we tested. The concentration and expression of endonuclease I can be controlled easily by strain selection or the use of a less rich growth medium (e.g., LB), providing a means to consistently produce plasmid DNA of sufficient quality. Another alternative to eliminate endonuclease I from a Wizard(TM) plasmid preparation involves adding a short 10-minute room temperature incubation to the neutralization step to efficiently precipitate protein prior to column purification.

While we found that plasmid quality can be improved by the choice of strains and growth media, we understand that this will not always be consistent with the experimental goals of all researchers. Therefore as part of an ongoing effort to improve our products and provide necessary support information, we are currently expanding our analyses of the Wizard(TM) line to include a broader array of bacterial strains (both *endA*+ and *endA*-) and determine the plasmid DNA quality for such applications as automated fluorescent sequencing and transfection. As new enhancements are achieved, we will communicate them through modifications to the supplied protocols and through *Promega Notes*.

Methods to Improve Stability of Plasmid DNA Isolated from *endA*+ Strains

1. If possible, select a *endA*- bacterial host for plasmid DNA isolations. When referring to an *E. coli* genotype, "*endA1*" refers to a mutant in the *endA* gene and produces an inactive form of endonuclease I. This corresponds to our use of *endA*- throughout the associated article. The absence of *endAI* in an *E. coli* genotype indicates the presence of the wild type gene, which corresponds to our use of *endA*+ in the associated article.
2. If you must use an *endA*+ host, use a less rich medium for growth. In our experiments, 2X YT with 1% glucose resulted in the most endonuclease I expression, whereas growth in LB resulted in higher plasmid yields as well as a more stable plasmid preparation. Simply using 2X YT without glucose resulted in an improved yield of plasmid with better stability.
3. Include an incubation at room temperature for 10 minutes to efficiently precipitate protein, such as endonuclease I after the addition of neutralization solution.
4. Keep the amount of time the resin and DNA are in contact to 1 minute. When processing multiple samples on a Vac-Man(TM) Manifold, turn on the vacuum and load columns individually while continuing to pull a vacuum.
5. Phenol:chloroform extract the DNA after the preparation. This is the least desirable option, but may be necessary if none of the other solutions are suitable.

References

1. *Promega Notes* **43**, 1.
2. Lehman, R. *et al*. (1962) *J. Biol. Chem.* **237**, 819.
3. Goebel, W. and Helinski, D.R. (1970) *Biochemistry* **9,** 4793.
4. Shortman, K. and Lehman, R. (1964) *J. Biol. Chem.* **239**, 2964.
5. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
6. *Wizard(TM) Minipreps DNA Purifcation System Technical Bulletin* #TB117, Promega Corporation.

Ordering Information

Product	Size	Cat.#
Wizard(TM) Minipreps DNA Purification System	50 preps	A7100
	100 preps	A7500
	250 preps	A7510
Wizard(TM) Minipreps DNA Purification Resin	250ml	A7141
Wizard(TM) Minicolumns	250 each	A7211