Promega

Technical Bulletin

Wizard® *Plus* Midipreps DNA Purification System

INSTRUCTIONS FOR USE OF PRODUCTS A7640, A7651 AND A7701.

IMPORTANT NOTICE

Please note that the names of the Wizard® Plus Midipreps DNA Purification System components listed below have changed:

Old Name	New Name
Cell Resuspension Solution	Cell Resuspension Solution (CRA)
Cell Lysis Solution	Cell Lysis Solution (CLA)
Neutralization Solution	Neutralization Solution (NSA)
Column Wash Solution	Column Wash Solution (CWB)

This is a name change only. The formulations of these solutions have not changed.

If you have additional questions, please contact Promega Technical Services by phone at 800-356-9526 or by e-mail: techserv@promega.com

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Promega

Wizard® *Plus* Midipreps DNA Purification System

All technical literature is available on the Internet at www.promega.com/tbs Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail techserv@promega.com.

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1. Description

Small-scale purifications of plasmid DNA, known as minipreps, are commonly used in molecular biology procedures. Over the years many miniprep protocols have been used, but few have proven to be consistently reliable (1). The strategy has been improved and adapted for processing larger culture volumes (10–100ml) with the Wizard® *Plus* Midipreps DNA Purification System[(a)].

The Wizard® *Plus* Midipreps DNA Purification System eliminates many of the problems associated with standard midiprep procedures and provides a simple and reliable method for rapid isolation of plasmid DNA. This system can be used to isolate any plasmid but works most efficiently with plasmids <20,000bp. When using the standard protocol, the entire midiprep process can be completed in 90 minutes or less with no organic extractions or ethanol precipitations. Multiple midipreps can be easily processed at one time with the Vac-Man® (20-sample capacity, Cat.# A7231) or Vac-Man® Jr. (2-sample capacity, Cat.# A7660) Laboratory Vacuum Manifold. DNA is eluted from the Wizard Midicolumn in Nuclease-Free Water (Cat.# P1193). The purified plasmid can be used directly for automated fluorescent DNA sequencing or

restriction digestion without further manipulation and also can be used for in vitro transcription reactions supplemented with a ribonuclease inhibitor, such as Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511).

2. Product Components and Storage Conditions

Product	Size	Cat. #
Wizard® *Plus* Midipreps DNA Purification System	25 preps	A7640

Each system contains sufficient reagents and columns for 25 isolations from 10–100ml of bacterial culture (using EndA– strains). Includes:

- 75ml Cell Resuspension Solution (CRA)
- 75ml Cell Lysis Solution (CLA)
- 150ml Neutralization Solution (NSA)
- 250ml Wizard® Midipreps DNA Purification Resin
- 355ml Column Wash Solution* (CWB)
- 25 Midicolumns with Reservoirs

***Column Wash Solution** is provided in two bottles, one containing 125ml and the other 230ml of Column Wash Solution.

Product	Size	Cat.#
Wizard® Midipreps DNA Purification Resin*[(a)]	1,000ml	A7701
Wizard® Midicolumns	100 each	A7651

Storage Conditions and Stability: Store the Wizard® *Plus* Midipreps DNA Purification System at room temperature (15–30°C). No refrigeration is required. **Protect the resin from exposure to direct sunlight.**

3. Production of a Cleared Lysate

Start each Wizard® *Plus* Midiprep with a 10–100ml overnight culture of *E. coli*. DNA yields may vary between 10µg and 200µg depending on the volume of bacterial culture, the plasmid copy number and the bacterial strain used. Up to 200µg of high-copy-number plasmid DNA can be obtained from a 100ml culture. When isolating DNA from low-copy-number plasmids, it is recommended to process 100ml of bacterial culture.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.)

- centrifuge capable of 10,000–14,000 × *g*
- Miracloth™ (Calbiochem Corp. Cat.# 475855, filter paper (Whatman® #1, GFA or GFC) or an autoclaved coffee filter
- ethanol (95%)

Before you begin, dilute both bottles of Column Wash Solution (CWB) as follows:
Add 320ml of 95% ethanol to the large bottle (230ml) for a final volume of 550ml; add 170ml of 95% ethanol to the small bottle (125ml) for a final volume of 295ml.

Note: Throughout the remainder of this document Column Wash Solution (CWB), Cell Resuspension Solution (CRA), Cell Lysis Solution (CLA) and Neutralization Solution (NSA) are referred to simply as Column Wash Solution, Cell Resuspension Solution, Cell Lysis Solution and Neutralization Solution.

1. Pellet 10–100ml of cells by centrifugation at 10,000 × *g* for 10 minutes at 4°C. Pour off the supernatant and blot the tube upside down on a paper towel to remove excess liquid.
2. **Completely** resuspend the cell pellet in 3ml of Cell Resuspension Solution. (To aid resuspension, manually disrupt the pellet with a 12-inch applicator stick or by pipetting until no clumps are visible. Complete resuspension is **critical** for optimal yields.)
3. Add 3ml of Cell Lysis Solution and mix by inverting the tube four times. Do not vortex. The cell suspension should clear immediately.

   Note: Some bacterial cells are more resistant to lysis and may require incubation for 3–5 minutes for efficient lysis. Additionally, culture volumes >50ml will take an extra 3–5 minutes to clear. The lysate may not appear completely clear, but do not extend lysis time beyond 3–5 minutes as this may result in the formation of single-stranded DNA in the preparation.
4. Add 3ml of Neutralization Solution and mix by inverting the tube 4 times.

   Alternatively, if using an EndA+ strain, add 6ml of Neutralization Solution, mix by inverting the tube 4 times, and incubate the lysate at room temperature for 10 minutes. Proceed to Step 5.
5. Centrifuge at 14,000 × *g* for 15 minutes at 4°C. If a tight pellet has not formed by the end of the centrifugation, centrifuge for another 15 minutes.
6. Carefully decant the supernatant to a new centrifuge tube, avoiding the white precipitate. Alternatively, transfer the cleared supernatant by filtering it through Miracloth™ (Calbiochem Corp. Cat.# 475855), filter paper (Whatman® #1, GFA or GFC) or an autoclaved coffee filter into the new centrifuge tube. Proceed immediately to Section 4.

4. Plasmid DNA Purification

Multiple Wizard® *Plus* Midipreps can be easily processed simultaneously with the Vac-Man® or Vac-Man® Jr. Laboratory Vacuum Manifold, which is required for this procedure.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.)

- vacuum pump or vacuum aspirator capable of a vacuum of 15–18 inches of mercury (Hg)
- vacuum manifold (i.e., Vac-Man® (Cat.# A7231) or Vac-Man® Jr. (Cat.# A7660) Laboratory Vacuum Manifold)
- Nuclease-Free Water (Cat.# P1193) preheated to 65–70°C
- **optional:** 40% isopropanol/4.2M guanidine hydrochloride solution (required for EndA+ strains—use only Promega Cat.# H5381)

Comparison of Inches of Hg to Other Pressure Measurements.
15 Inches Hg
50.8kPa
381 Torr
0.501atm
7.37psi
38.1cm Hg
508mbar

Thoroughly mix the Wizard® Midipreps DNA Purification Resin before removing an aliquot.

1. Add 10ml of resuspended Wizard® Midipreps DNA Purification Resin to the DNA solution from Section 3. Swirl to mix.

   Note: Extended incubation of the resin and lysate is not necessary. Do not allow the resin to remain in contact with the lysate for longer than it takes to load the Midicolumns.

2. For each Midiprep, use one Midicolumn. Insert the Midicolumn tip into the vacuum manifold port.

3. Transfer the resin/DNA mixture into the Midicolumn. Apply a vacuum of at least 15 inches of Hg to pull the resin/DNA mix into the Midicolumn. When all of the sample has passed through the column, break the vacuum at the source.

If using an EndA+ strain:

a. The total volume of lysate and resin will exceed the column capacity by 1ml. Therefore, all but 2–4ml of the resin/DNA should be applied to the column. After the vacuum is applied and the column volume drops, the remainder of the lysate and resin can be added.

b. Add 15ml of 40% isopropanol/4.2M guanidine hydrochloride solution (Section 7) to each column. Apply a vacuum continuously until 30 seconds after all of the solution has flowed through the columns. Note that this solution will flow through the column more slowly than the standard Column Wash Solution. Proceed to Step 4.

4. Add 15ml of Column Wash Solution to the Midicolumn and apply a vacuum to draw the solution through the Midicolumn.

5. Break the vacuum at the source and add another 15ml of Column Wash Solution to the Midicolumn. Reapply a vacuum to draw the solution through the Midicolumn.

   Note: The Column Wash procedure may take up to 30 minutes.

6. Dry the resin by continuing to draw a vacuum for 30 seconds after the solution has been pulled through the column. **Do not dry the resin for more than 30 seconds.** Remove the Midicolumn from the vacuum source. Separate the Reservoir from the Midicolumn by breaking or cutting with sharp scissors as shown in Figure 1. Transfer the Midicolumn to a 1.5ml microcentrifuge tube. Centrifuge the Midicolumn at 10,000 × *g* in a microcentrifuge for 2 minutes to remove any residual Column Wash Solution. Transfer the Midicolumn to a new microcentrifuge tube.

7. Add 300µl of preheated (65–70°C) Nuclease-Free Water to the Midicolumn and wait 1 minute. Elute the DNA by centrifuging the Midicolumn at 10,000 × *g* for 20 seconds in a microcentrifuge. Remove and discard the Midicolumn.

   For elution of large plasmids (≥20kb), the use of water preheated to 80°C may increase yields.

8. A white pellet of resin fines may be present in the final eluate. Whether visible or not, it is important to separate the fines from the DNA. Centrifuge the sample at 10,000 × *g* in a microcentrifuge for 5 minutes to pellet the fines. Carefully transfer the DNA-containing supernatant to a clean microcentrifuge tube.

9. The plasmid DNA may be stored in the microcentrifuge tube using these storage recommendations: DNA is stable in water without addition of buffer if stored at –20°C or below. DNA is stable at 4°C in TE buffer. To store the DNA in TE buffer, add 30µl of 10X TE buffer to the 300µl of eluted DNA.

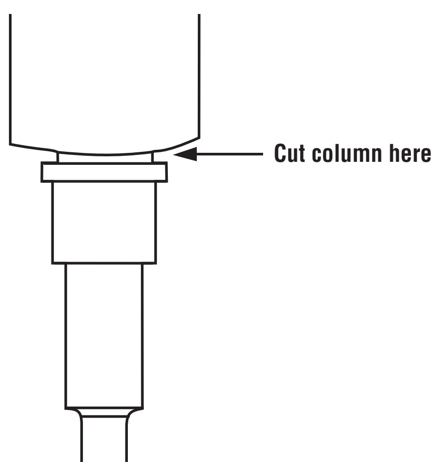

Figure 1. Location of the cut site for separating the Reservoir from the Midicolumn.

5. Supplementary Information

Plasmid DNA can be purified from 10–100ml overnight cultures of *E. coli* with the Wizard® *Plus* Midipreps System. The yield of plasmid will vary depending on a number of factors, including the volume of bacterial culture, plasmid copy number, type of culture medium and the bacterial strain. The protocol presented in this technical bulletin is for the isolation of plasmid DNA from *E. coli*.

5.A. Factors Affecting Plasmid DNA Yield

Plasmid copy number is one of the most important factors affecting yield in a given system. Copy number is determined primarily by the region of DNA surrounding and including the origin of replication in the plasmid. This area, known as the replicon, controls replication of plasmid DNA by bacterial enzyme complexes. Some DNA sequences, when inserted into a particular vector, can lower the copy number of the plasmid. In addition, excessively large DNA inserts can also reduce plasmid copy number.

5.B. Choosing a Bacterial Strain

Endonuclease I is a 12kDa periplasmic protein that degrades double-stranded DNA. This protein is encoded by the gene *end*A. The *E. coli* genotype *end*A1 refers to a mutation in the wildtype *end*A gene, which produces an inactive form of the nuclease. *E. coli* strains with this mutation in the *end*A gene are referred to as EndA negative (EndA–). Table 1 contains a list of EndA– and EndA+ *E. coli* strains. The wildtype is indicated as EndA+. Using the Wizard® *Plus* Midipreps System, high-quality DNA is easily obtained from both EndA+ and EndA– strains. Special precautions must be taken when working with EndA+ strains to ensure the isolation of high-quality DNA (2), including the use of several modified protocol steps, as indicated, and the use of a less rich growth medium (e.g., LB). The modified protocol will eliminate most problems associated with these strains. However, the level of endonuclease I produced is strain-dependent, and the modified protocol may not totally exclude endonuclease I from plasmid DNA prepared from very high endonuclease I-producing strains. Also note that the modified protocol requires the use of increased volumes of several of the supplied solutions and, as a result, you will be unable to perform as many isolations. In general, we recommend using EndA– strains whenever possible.

Figure 2 depicts DNA isolated from varying amounts of culture, using high- and low-copy-number plasmids and an EndA– *E. coli* strain with the Wizard® *Plus* Midipreps DNA Purification System.

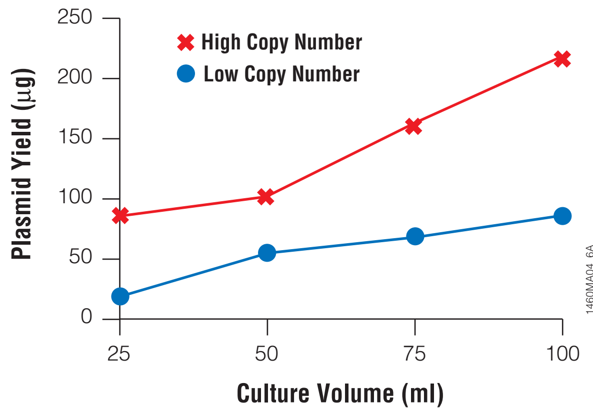

Figure 2. Plasmid DNA yield as a function of culture volume and plasmid copy number, determined by use of the Wizard® *Plus* Midipreps DNA Purification System. Representative yields of a high-copy-number plasmid, pGEM®-3Zf(+) Vector, and a low-copy-number plasmid, pBR322, prepared in *E. coli* strain DH5α™. Cultures were grown overnight at 37°C in 25ml increments of LB medium containing 100µg/ml ampicillin.

Table 1. List of EndA– and EndA+ Strains.

EndA–		EndA+	
BJ5183	JM108	BL21(DE3)	P2392
DH1	JM109	CJ236	PR700 (all PR series strains are EndA+)
DH20	MM294	HB101	RR1
DH21	SK1590	JM83	Q358
DH5α™	SK1592	JM101	TB1
JM103	SK2267	JM110	TG1
JM105	SRB	LE392	Y1088 (all Y10 series strains are EndA+)
JM106	XL1-Blue	MC1061	BMH71-18
JM107	XLO	NM522 (all NM series strains are EndA+)	ES1301

5.C. Special Considerations for Automated Fluorescent Sequencing

For the application of automated fluorescent sequencing, special consideration should be given to the selection of plasmid type and *E. coli* strain to optimize yield and plasmid quality. Optimal automated fluorescent sequencing results are routinely obtained by using high-copy-number plasmids and EndA– strains of *E. coli*.

Note: For fluorescent sequencing applications, elute and store the DNA in nuclease-free water.

Purified plasmid DNA must be within the proper concentration range for successful automated cycle sequencing (ideally 0.2µg/µl, not less than 0.1µg/µl). When working with plasmid DNA from low-copy-number plasmids, we strongly recommend that DNA concentrations be determined by agarose gel/ethidium bromide quantitation prior to any application. DNA quantitation by spectrophotometric methods is prone to errors and requires a large amount of sample.

The Wizard® *Plus* Midipreps System routinely yields 70µg of medium- or high-copy-number plasmid DNA when used with the pGEM® Vector and DH5α™ cells in 25ml of culture. For low-copy-number plasmids, a larger culture volume is required to obtain sufficient DNA for sequencing. In some cases it may be possible to amplify plasmid DNA by growing the bacteria in the presence of antibiotics such as chloramphenicol or spectinomycin (1).

Special Considerations for Sequencing Using BigDye® Chemistry

When performing dilutions of BigDye™ terminator ready reaction mix, it is essential to use an appropriate dilution buffer, such as 250mM Tris-HCl (pH 9.0), 10mM $MgCl_2$.

Table 2 outlines the amount of terminator-ready reaction mix and dilution buffer required to obtain the appropriate dilution for BigDye® terminator reactions. For details on running these reactions, please refer to the protocol supplied with the BigDye® terminator system. For each reaction, add the reagents in Table 2 to a separate tube.

Table 2. Appropriate dilutions for BigDye™ Terminator Reactions.

	Amount			
Component	**No Dilution**	**1:2**	**1:4**	**1:6**
terminator-ready reaction mix*	8.0µl	4.0µl	2.0µl	1.3µl
double-stranded plasmid DNA template	200–500ng	200–500ng	200–500ng	200–500ng
primer	3.2pmol	3.2pmol	3.2pmol	3.2pmol
dilution buffer**	0µl	2.0µl	3.0µl	3.4µl
Nuclease-Free Water to a final volume of	20µl	20µl	20µl	20µl

*Terminator-ready reaction mix is a 2.5X solution.
**Dilution buffer is a 5X solution.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Poor cell lysis	Too many bacterial cells in culture medium. Use LB medium to grow bacteria. The use of rich media or excessive culture volumes may lead to a biomass value too high for complete lysis. All media should contain antibiotics at the appropriate concentration.
	Poor resuspension of bacterial cell pellet. The cell pellet must be thoroughly resuspended prior to cell lysis. Pipet or disperse (using an applicator stick) the pellet with Cell Resuspension Solution. No cell clumps should be visible after resuspension.
No plasmid DNA purified	Ethanol not added to the Column Wash Solution. Prepare the Column Wash Solution as instructed before beginning the procedure.
	EndA+ strain of bacteria used. DNA appears degraded or lost upon incubation with Mg^{2+} containing buffer, i.e., restriction enzyme buffer. Follow protocol modifications for EndA+ strains of bacteria.
	Inaccurate quantitation of plasmid DNA yield. Quantitate plasmid DNA yield by agarose gel/ ethidium bromide electrophoresis.
DNA floats out of well during loading of agarose gel	Carryover of residual ethanol from Column Wash Solution. Follow directions for appropriate drying of resin by vacuum and centrifugation. If DNA has already been eluted, precipitate DNA and dry remaining ethanol from the DNA pellet prior to resuspension in Nuclease-Free Water. Increase loading dye concentration by 2X.
Low plasmid DNA yields	Overgrowth of bacterial culture by nontrans-formed bacteria. Make certain that antibiotics were used in all media, both liquid and solid. Do not culture bacteria longer than 24 hours. Optimal culture length is 12–16 hours.
	Bacterial culture too old. Inoculate antibiotic-containing media with freshly isolated bacterial colony from an overnight plate.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low plasmid DNA yields (continued)	Low-copy-number plasmid used. See Section 5.A. Cultures should not exceed the maximum recommended volumes per isolation.
	Precipitate formed in resin. Warm resin in 37°C water bath for 15–20 minutes. Gently swirl bottle to mix and allow to cool to 30°C prior to use.
	Resin fines in eluted DNA. Follow directions for removal of resin fines from eluted DNA, i.e., filtration and centrifugation. If DNA aggregate has formed, heat in the presence of 1M NaCl to redissolve aggregate. Centrifuge to remove resin fines. Precipitate DNA with ethanol and wash with 70% ethanol to remove residual NaCl before using in downstream applications.
	Overdrying of resin on vacuum source. Follow directions for drying on vacuum source. Do not dry for times longer than suggested.
	Wrong reagents used. Make certain that Column Wash Solution is diluted with ethanol before use. Note that Wizard® *Plus* and Wizard® *Plus* SV components are not interchangeable.
	Plasmid DNA yield not accurately quantitated. Use agarose gel/ethidium bromide quantitation.
Nicking of plasmid DNA	Overincubation during the alkaline lysis step. Total incubation of cell suspension with Cell Lysis Solution should not exceed 5 minutes.
No results or poor results with automated fluorescent sequencing	Too little DNA was added to the sequencing reaction. Inoculate fresh LB medium with a newly isolated *E. coli* colony. Purify plasmid DNA and quantitate by agarose gel/ethidium bromide electrophoresis.
	TE buffer was used for DNA elution. Ethanol precipitate and resuspend pellet in Nuclease-Free Water. (The EDTA in TE buffer can interfere with downstream applications by chelating Mg^{2+}.)
	ABI PRISM® BigDye® chemistry was used. Use of this chemistry necessitates ethanol precipitation of eluted DNA prior to sequencing reaction.
	Plasmid concentration not accurately quantitated. Use agarose gel/ethidium bromide electrophoresis to accurately quantitate plasmid DNA.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
No restriction digestion	Increase the amount of restriction enzyme used and/or the length of incubation time. Digest at the recommended temperature and in the optimal buffer for the restriction enzyme used.
	DNA degraded during restriction digestion due to use of EndA+ *E. coli* strain. Repurify DNA from fresh culture containing antibiotics. Follow instructions (Section 3 and 4) for EndA+ strains or use an EndA– strain of *E. coli*.
Genomic DNA contamination	Vortexing or overmixing after addition of the Cell Lysis Solution. Do not vortex samples after addition of Cell Lysis Solution to prevent shearing of genomic DNA.
DNA yields on gel look low compared to spectrophotometer readings	Traces of contaminants may be present in the eluted DNA, inflating the spectrophotometer readings. Phenol:chloroform extract and precipitate DNA, then wash with 70% ethanol before repeating spectrophotometer readings. Alternatively, quantitate DNA by agarose gel/ethidium bromide electrophoresis.

7. Composition of Buffers and Solutions

Cell Resuspension Solution (CRA)

50mM	Tris-HCl (pH 7.5)
10mM	EDTA
100µg/ml	RNase A

Cell Lysis Solution (CLA)

0.2M	NaOH
1%	SDS

Neutralization Solution (NSA)

1.32M	potassium acetate (pH 4.8)

Column Wash Solution (CWB)

80mM	potassium acetate
8.3mM	Tris-HCl (pH 7.5)
40µM	EDTA

Add 320ml and 170ml of 95% ethanol to the large and small bottles, respectively, of Column Wash Solution (Section 3). Final ethanol concentration will be approximately 55%. (Component concentrations listed are for final solution with ethanol added.)

TE buffer (1X)

10mM	Tris-HCl (pH 7.5)
1mM	EDTA

40% isopropanol/4.2M guanidine HCl

66.9g guanidine hydrochloride (use only Promega Cat.# H5381)

Prepare a 7M solution by dissolving the guanidine hydrochloride in 50–60ml of sterile, distilled water. This reaction is very endothermic; warming the mixture to 37°C (do not exceed 37°C) will speed the process. Bring to a final volume of 100ml with sterile, distilled water.

Prepare the 40% isopropanol/4.2M guanidine HCl solution by combining 30ml of the 7M guanidine HCl solution with 20ml of isopropanol in a 50ml screw-cap tube and mixing thoroughly. Store at room temperature.

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8. Related Products

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
Wizard® *Plus* SV Minipreps DNA Purification System	50 preps	A1330
(does not include Vacuum Adapters)	250 preps	A1460
Cell Resuspension Solution	150ml	A7112
Cell Lysis Solution	150ml	A7122
Neutralization Solution	150ml	A7131
Column Wash Solution	125ml	A8102

Product	Size	Cat.#
Vac-Man® Laboratory Vacuum Manifold	20-sample capacity	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold	2-sample capacity	A7660
One-Way Luer-Lok® Stopcocks	10 each	A7261

9. References

1. Ausubel, F.M. *et al.* (1989) *Current Protocols in Molecular Biology*, Vol. 2, John Wiley & Sons, New York.
2. Schoenfeld, T. *et al.* (1995) DNA purification: Effects of bacterial strains carrying the *end*A1 genotype on DNA quality isolated with Wizard® plasmid purification systems. *Promega Notes* **53**, 12.

[(a)]U.S. Pat. Nos. 5,658,548 and 5,808,041, Australian Pat. No. 689815 and European Pat. No. 0 723 549 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures. Other patents are pending.

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