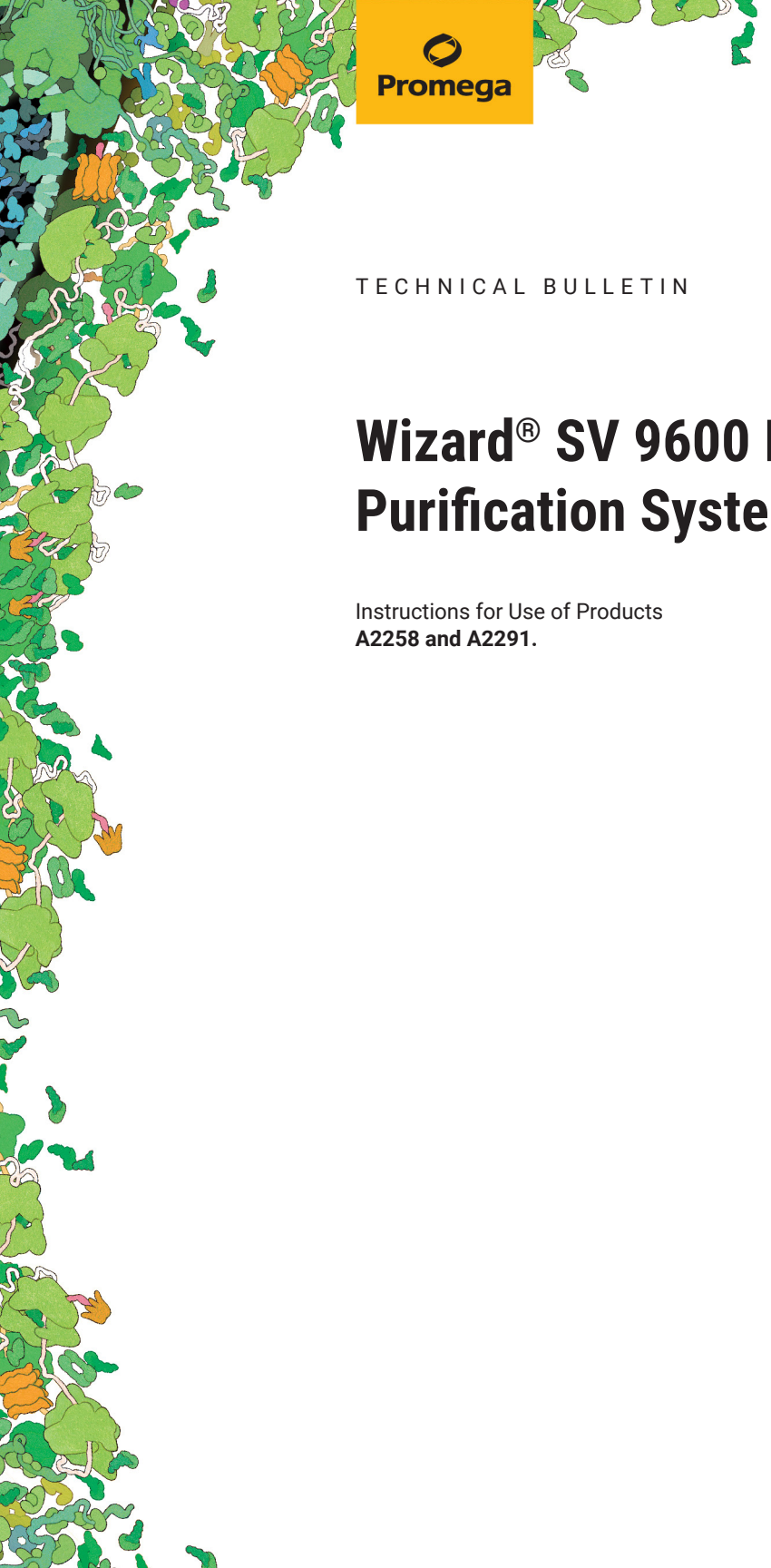

TECHNICAL BULLETIN

Wizard® SV 9600 Plasmid DNA Purification System

Instructions for Use of Products
A2258 and A2291.

Revised 12/25
TB292

Promega

Wizard® SV 9600 Plasmid DNA Purification System

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Bulletin.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Wizard® SV 9600 Plasmid DNA Purification System provides a simple and reliable method for the rapid isolation of plasmid DNA from 96 samples at one time. The entire miniprep procedure can be completed in 60 minutes or less. The system may be used with automated robotics (e.g., the Biomek® 2000 Workstation) to yield purified plasmid suitable for automated fluorescent DNA sequencing as well as for other standard molecular biology techniques, including restriction enzyme digestion.

Figure 1 describes plasmid DNA isolation and purification using the Wizard® SV 9600 Plasmid DNA Purification System. This system requires the use of the Vac-Man® 96 Vacuum Manifold or compatible vacuum manifold (Figure 2). Plasmid DNA is purified from bacterial lysates using a 96-well vacuum filtration step to simultaneously clear the bacterial lysate and bind plasmid DNA, eliminating the need for centrifugation. Removal of the Lysate Clearing Plate and subsequent washing of the bound plasmid DNA requires no disassembly of the manifold, and filtrate waste products are delivered directly to a vacuum trap, eliminating the need for emptying waste collection vessels during plasmid DNA recovery. DNA is collected by elution into a 96-well plate.

1. Description (continued)

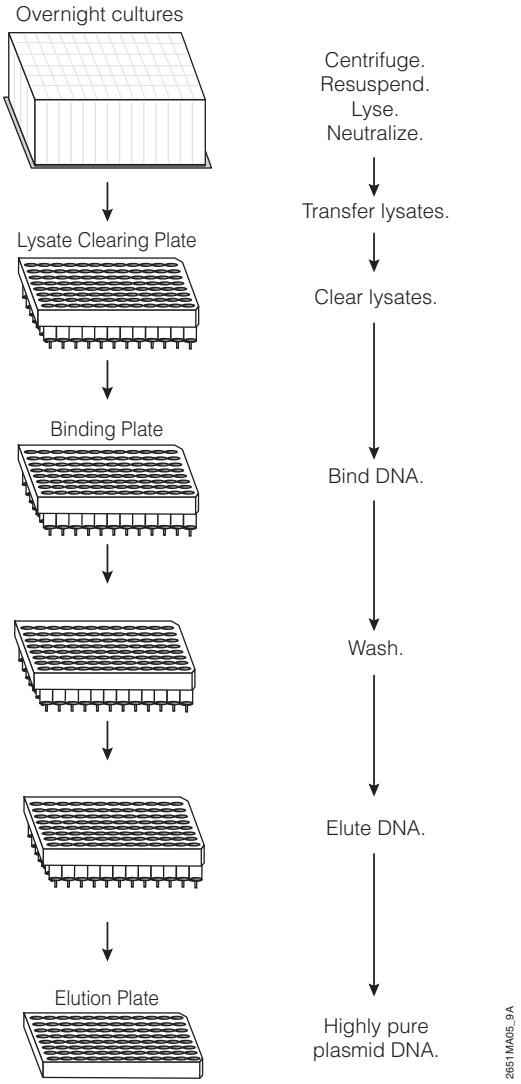

Figure 1. Flow diagram of plasmid DNA isolation and purification using the Wizard® SV 9600 Plasmid DNA Purification System.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wizard® SV 9600 Plasmid DNA Purification System	**100 × 96 preps**	**A2258**

For Laboratory Use. Each system contains sufficient reagents for 9600 isolations. Includes:

- 3.2L Wizard® SV 96 Cell Resuspension Solution (4 × 800ml)
- 3.2L Wizard® SV 96 Cell Lysis Solution (4 × 800ml)
- 8.5L Wizard® SV 96 Neutralization Solution (9 × 950ml)
- 7.4L Column Wash Solution (CWA) (20 × 370ml)
- 100 Lysate Clearing Plates
- 100 Binding Plates

PRODUCT	SIZE	CAT.#
Vac-Man® 96 Vacuum Manifold	**each**	**A2291**

Storage Conditions: All Wizard® SV 9600 Plasmid DNA Purification System components should be stored at 22–25°C.

Note: Components for the Wizard® SV 9600 Plasmid DNA Purification System are available for purchase individually. See **www.promega.com** for ordering information.

Components for the Wizard® SV 9600 Plasmid DNA Purification System should not be exchanged for or replaced with components from Wizard®, Wizard® Plus or Wizard® Plus SV DNA Purification Systems.

3. Protocol for Plasmid DNA Isolation and Purification

Materials to Be Supplied by the User

(Solution compositions are provided in Section 7.A.)

- LB agar plates containing selective antibiotic
- culture medium containing selective antibiotic
- ethanol, 95%
- tabletop centrifuge capable of 1,500 × *g*, fitted with 96-well plate adapters
- vacuum trap for waste collection
- vacuum pump capable of 15–20 inches of Hg (e.g., Fisher Cat.# 01-092-29)
- vacuum tubing
- deep-well culture plates
- elution plates

Before beginning the procedure with a new Wizard® SV 9600 System, dilute the provided Column Wash Solution (CWA):

Add 630ml of 95% ethanol to each bottle of Column Wash Solution (CWA) for a final volume of 1 liter.

The following is a manual protocol that may be used as a guide to develop protocols for automated workstations. We have an on-going effort to develop procedures for different automated platforms. For information about methods for automated workstations contact Technical Services at: **techserv@promega.com**

3.A. Manifold Assembly

Lysate Clearing and DNA Binding (Figure 2, Panel A)

Place the DNA Binding Plate on the Manifold Base. Connect the Vacuum Port in the Manifold Base to a vacuum source, using the Insert (provided with the manifold) and vacuum tubing. Place the Manifold Collar on top of the base and binding plate, aligning the collar with the pins. Finally, place the Lysate Clearing Plate on the Manifold Collar. Note that the vacuum port in the Manifold Collar is sealed because the insert has not been placed in the port.

The Lysate Clearing Plate can be identified by the **blue dot** in the top left-hand corner of the plate.

DNA Washing (Figure 2, Panel B)

Remove the Lysate Clearing Plate and Manifold Collar from the assembly in Figure 2, Panel A. The DNA Binding Plate remains on the Manifold Base during washing.

DNA Elution (Figure 2, Panel C)

Place a 96-well plate (elution plate) onto the Manifold Bed, then place the Manifold Collar on the elution plate. Attach vacuum tubing to the insert and the Insert to the Vacuum Port on the Manifold Collar. Place the Binding Plate on the Manifold Collar for elution of DNA into the elution plate.

Note: The Vac-Man® 96 Vacuum Manifold requires the use of a vacuum trap. The trap can be constructed by connecting a 500–1,000ml sidearm flask between the manifold and the vacuum pump. Alternatively, a vacuum trap assembly including rubber stopper and connector ports can be obtained commercially. The use of a vacuum gauge with the vacuum assembly is also recommended.

3.B. Producing a Cleared Lysate and Binding Plasmid DNA

1. Grow bacteria containing high-copy-number plasmids in a deep-well culture plate. Pellet the culture by centrifugation at 1,500 × *g* in a tabletop centrifuge. As much as 10 $O.D._{600}$ of total cell mass may be processed per well. Using a pipette or a multichannel pipette (8–12 channel) with sterile tips, angle the tips against the side of the well, avoiding direct contact with the cell layer. Gently aspirate the media and discard into a proper waste container.

   Note: The goal is to minimize shear forces that could dislodge cells. Cell pellets can be stored at –30°C to –10°C for later processing; however, storage for more than 30 days is not recommended.

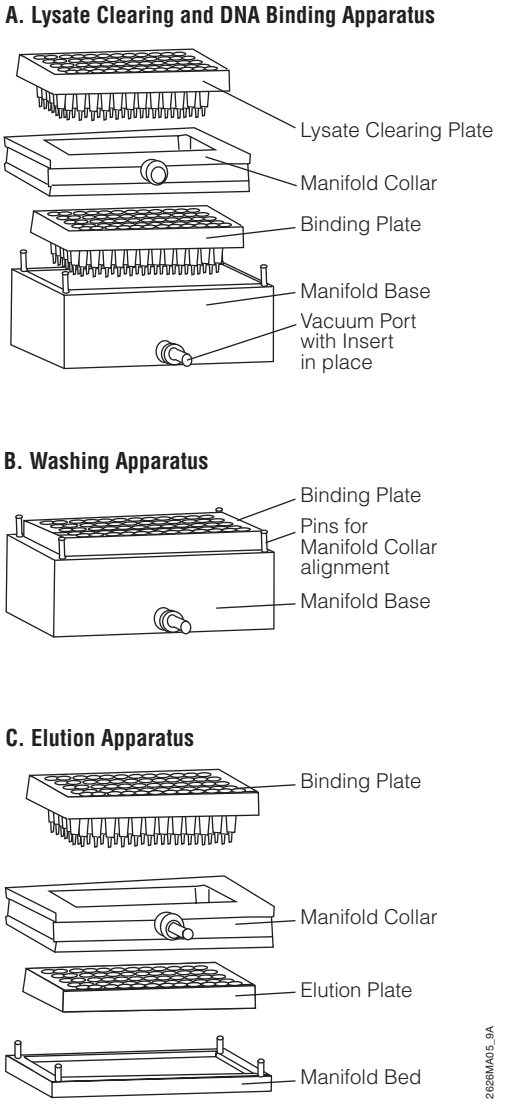

Figure 2. Diagram of the Vac-Man® 96 Vacuum Manifold with the Wizard® SV 9600 Plasmid DNA Purification System components. Panels A, B and **C** show the manifold and plate combinations necessary to accomplish DNA binding, washing and elution, respectively.

3.B. Producing a Cleared Lysate and Binding Plasmid DNA (continued)

2. Thoroughly resuspend each cell pellet by adding 250µl of Cell Resuspension Solution.

   Note: Avoid cross-contaminating samples by using a fresh pipette tip for each sample or sample set during resuspension.

3. Add 250µl of Cell Lysis Solution to each sample. Incubate for 3 minutes at room temperature.

   Note: Allow a minimum of 3 minutes for clearing of the lysate before proceeding to Step 4. Do not incubate longer than 5 minutes.

4. During this incubation, prepare the vacuum manifold as shown in Figure 2 and described in Section 3.A. To ensure that samples and well numbers correspond on both plates, orient the plates with the numerical column headers toward the vacuum port. Attach the vacuum line to the Vacuum Port on the Manifold Base.

5. Add 350µl of Neutralization Solution to each sample. Mixing is not necessary. Transfer the bacterial lysates to the Lysate Clearing Plate assembled on the Vacuum Manifold (Figure 2, Panel A). Allow one minute for the filtration disks to wet uniformly, then apply a vacuum to the manifold (15–20 inches of Hg or the equivalent; see Section 7.B for unit conversion information) using a vacuum pump fitted with a control valve. Allow 3–5 minutes under vacuum for the lysates to pass through both the Lysate Clearing Plate and the Binding Plate.

6 Release the vacuum. Check that the lysate has cleared both the Lysate Clearing and Binding Plates. If not, reapply the vacuum until all lysate is pulled through both plates. Remove the Clearing Plate and collar (as in washing configuration, Figure 2, Panel B).

7. Add 500µl of the Neutralization Solution to each well of the Binding Plate. Apply a vacuum for 1 minute, then turn off the pump.

3.C. Washing

8. With the Binding Plate and Manifold Base configured as in Figure 2, Panel B, add 1.0ml of Column Wash Solution (CWA) containing ethanol to each well of the Binding Plate. Apply a vacuum for 1 minute.

9. Turn off the pump and repeat the wash procedure (Step 8). After the wells have emptied, continue for an additional 10 minutes under vacuum to allow the binding matrix to dry.

10. Turn off the vacuum. Release the vacuum line from the Manifold Base and snap it into the vacuum port in the Vacuum Manifold Collar. Remove the Binding Plate from the Manifold Base. Blot by tapping onto a clean paper towel to remove residual ethanol; repeat if necessary to remove all residual ethanol.

11. Place a 96-well plate (elution plate) in the Manifold Bed and position the Vacuum Manifold Collar on top. Orient the plate with the numerical column headers toward the vacuum port.

3.D. Eluting DNA

12. Position the Binding Plate on top of the Manifold Collar and elution plate as shown in Figure 2, Panel C. The Binding Plate tips must be centered over the elution plate wells, and both plates must be in the same orientation. Add 100µl of Nuclease-Free Water to each well of the Binding Plate and incubate 1 minute at room temperature. Apply a vacuum for 1 minute as previously described.

    Note: Add the Nuclease-Free Water to the center of the plate wells.

13. Release the vacuum and remove the Binding Plate. Carefully remove the Manifold Collar, making sure that the elution plate remains positioned in the Manifold Bed. If droplets are present on the walls of the elution plate wells, centrifuge the plate briefly to collect the droplets on the bottom of the wells. Eluate volumes may vary, but are generally 60–70µl. (Samples can be stored by covering the plate tightly with a plate sealer and placing at +2°C to +10°C or –30°C to –10°C.)

    Release the vacuum before removing the Vacuum Manifold Collar from the elution plate.

4. Supplementary Information

4.A. Selecting and Preparing Plasmids and *E. coli* Strains

Plasmid DNA can be purified from overnight cultures of *E. coli* with the Wizard® SV 9600 Plasmid DNA Purification System. The yield of plasmid will vary depending on a number of factors, including the plasmid copy number, cell density of bacterial culture, type of culture medium and the bacterial strain used.

Plasmid copy number is one of the most important factors affecting plasmid DNA yield. Copy number is determined primarily by the region of DNA surrounding and including the origin of replication. This region, known as the replicon, controls replication of plasmid DNA by bacterial enzyme complexes. Some DNA sequences, when inserted into a particular plasmid, can lower the copy number of the plasmid by interfering with replication.

Dispense 1–1.2ml of culture medium containing antibiotic used for selection into the wells of the 96-well culture plate. Choose a single, well-isolated colony from a fresh agar plate (with antibiotic) to inoculate each plate well. Cover the plate with aluminum foil or with a plate sealer that has been pierced to allow aeration of the cells. Agitate the plate at low to moderate speeds to allow aeration without causing cross contamination. The inoculated medium should be incubated overnight (16–24 hours) at 37°C. An $O.D._{600}$ of 1.0–4.0 for high-copy plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation. Using cells at $O.D._{600}$ readings >10.0 may lead to incomplete processing of the bacterial lysate or plugging of the Clearing Plate. This may decrease yields as well as increase contaminant levels in the isolated plasmid DNA.

Note: The culture volume may vary to equal a maximum $O.D._{600}$ of 4.0 per well. It is not critical to determine the $O.D._{600}$ unless there is a possibility that the total cell mass may exceed an $O.D._{600}$ value of 4.0 per well. Cultures grown in LB medium containing antibiotics for up to 24 hours in a 96-well culture plate generally do not exceed an $O.D._{600}$ of 4.0 per well and do not need to be measured. The recommended minimum total cell mass to process per well is an $O.D._{600}$ value of 1.0.

Calculations:

$$\frac{4.0\ O.D._{600}}{O.D._{600}\ \text{per 1ml}} = \text{ml of culture to obtain a total cell mass of 4.0 } O.D._{600}$$

where $O.D._{600}$ per ml = $O.D._{600}$ of culture (diluted 1:10 in medium) measured in a 1cm pathlength cuvette.

4.B. Choosing a Bacterial Strain

Endonuclease I is a 12kDa periplasmic protein that degrades double-stranded DNA. This protein is encoded by the endA gene. The *E. coli* genotype endA1 refers to a mutation in the wildtype endA gene, which produces an inactive form of the nuclease. *E. coli* strains with this mutation in the endA gene are referred to as EndA negative (EndA–). Table 1 contains a list of EndA– and EndA+ *E. coli* strains. The absence of an endA1 (or endA) in an *E. coli* genotype denotes the presence of the wildtype gene that expresses an active endonuclease I. The wildtype is indicated as EndA+. Some EndA+ strains can be problematic for a number of applications. In general, we recommend the use of EndA– strains whenever possible, particularly for applications, such as automated fluorescent sequencing.

Table 1. EndA– and EndA+ Strains of *E. coli*.

EndA–		EndA+	
BJ5183	JM108	BL21(DE3)	P2392
DH1	JM109	CJ236	PR700*
DH20	MM294	HB101	Q358
DH21	SK1590	JM83	RR1
DH5α™	SK1592	JM101	TB1
JM103	SK2267	LE392	TG1
JM105	SRB	MC1061	Y1088*
JM106	XL1-Blue	NM522*	BMH71–18
JM107	XLO	NM554*	ES1301

*All NM, PR and Y10 strains are EndA+.

4.C. Special Considerations for Automated Fluorescent Sequencing

For applications such as fluorescent DNA sequencing, special considerations should be given to the selection of plasmid and E. coli strains to optimize yield and plasmid quality. Optimal automated fluorescent sequencing results are obtained by using high-copy plasmids and EndA– strains of *E. coli* for plasmid propagation.

Purified plasmid DNA must be within the proper concentration range for successful automated cycle sequencing (ideally 100ng/µl and not less than 40ng/µl). Concentrations achieved with high-copy plasmid DNA purified using the Wizard® SV 9600 System normally are of sufficient concentration for direct use in these applications; however, plasmid DNAs from low-copy numberplasmids may require concentration. When working with low-copy number plasmids, best results are obtained by ethanol precipitation. We strongly recommend that DNA concentrations be determined by agarose gel/ethidium bromide staining prior to any application, particularly when using low-copy-number plasmids (1). DNA quantitation by spectrophotometric methods is prone to errors and may require a large amount of sample.

The Wizard® SV 9600 System typically results in yields of 3–5µg of plasmid DNA when using a high copy number plasmid such as a pGEM® Vector and DH5α™ cells in 1.2ml of LB (Luria Bertani) medium containing antibiotic.

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.

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5. Troubleshooting

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

Symptoms	Causes and Comments
Poor cell lysis	Too many bacteria cells in the culture medium. All media should contain antibiotics. Process 1.0–4.0 $O.D._{600}$ of cells per well.
	Poor resuspension of bacterial cell pellet. Thoroughly resuspend cell pellets before cell lysis. No cell clumps should be visible after resuspension.
No plasmid DNA purified	Ensure that ethanol was added to the Column Wash Solution (CWA) as instructed in Section 3 before beginning the procedure.
	Inaccurate quantitation of plasmid DNA yield. Quantitate plasmid DNA yield by agarose gel/ ethidium bromide staining.
	DNA floats out of well during loading of gel for quantitation. Be certain to wait the full 10 minutes for drying after the final wash step to allow evaporation of any remaining ethanol. Increase loading dye concentration.
Low plasmid DNA yield	Overgrowth of bacterial culture by nontransformed cells. Make certain that antibiotics are used in all media, both liquid and solid.
	Bacterial culture too old. Inoculate antibiotic containing medium with freshly isolated bacterial colony from an overnight plate.
	Incubate at 37°C for 16–24 hours. Low-copy-number plasmid used. Know the copy number of the plasmid used. We recommend using high-copy-number plasmids.
	Plasmid DNA yield was not accurately quantitated. Use agarose gel/ethidium bromide staining to measure DNA yield.
	Wrong reagents used. Make certain that the Column Wash Solution (CWA) is diluted with ethanol before use. **Note:** Wizard® and Wizard® Plus components should not be used with the Wizard® SV 9600 System.

Symptoms	Causes and Comments
Nicking of plasmid DNA	Overincubation during the alkaline lysis step. Total incubation of cell suspension with the Lysis Solution should not exceed 5 minutes.
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 staining. To concentrate the DNA, ethanol precipitate and resuspend the DNA.
	Wrong dilution buffer used with ABI PRISM® BigDye® sequencing chemistry. Use dilution buffer recommended in Section 4.C.
	TE buffer was used for DNA elution. Repurify plasmid DNA and elute in Nuclease-Free Water.
	Plasmid concentration not accurately quantitated. Use agarose gel/ethidium bromide staining to quantitate DNA yield.
No restriction digestion	Concentration of restriction enzyme or length of digestion need to be increased. Increase the amount of restriction enzyme or the length of incubation. Digest at the suggested temperature and in the optimal buffer for the restriction enzyme used.
DNA yields on gel look low compared to spectrophotometer	Traces of contaminants may be present in the eluted DNA, which inflate the spectrophotometer readings. Quantitate DNA by agarose gel/ ethidium bromide staining for the most accurate results.
Clogging of some wells in Lysate Clearing Plate	Too many bacterial cells processed per well. Use a maximum cell density of 4.0 $O.D._{600}$. Grow cells in 1–1.2ml of nonenriched medium (i.e., LB medium). Alternatively, process smaller culture volumes. Increase vacuum to 20 inches of Hg. Extend vacuum time by 10 minutes.

6. Reference

1. Kahn, M. *et al.* (1979) Plasmid cloning vehicles derived from plasmids ColE1, F, R6K and RK2. *Meth. Enzymol.* **68**, 268–80.

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7. Appendix

7.A. Composition of Buffers and Solutions

Wizard® SV 96 Resuspension Solution

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

Wizard® SV 96 Cell Lysis Solution

0.2M NaOH
1% SDS

Wizard® SV 96 Neutralization Solution

4.09M guanidine hydrochloride
0.759M potassium acetate
2.12M glacial acetic acid

Final pH is approximately 4.2.

Column Wash Solution (CWA)

162.8mM potassium acetate
22mM Tris-HCl (pH 7.5)
0.109mM EDTA

Add 95% ethanol as described in Section 3: Add 630ml per bottle. Final concentrations will be approximately 60% ethanol, 60mM potassium acetate, 8.3mM Tris-HCl and 40µM EDTA.

10X TE buffer

100mM Tris-HCl (pH 7.5)
10mM EDTA

Terrific Broth

12g Bacto® tryptone
24g yeast extract
2.31g KH_2PO_4
12.54g K_2HPO_4

Add Bacto® tryptone and yeast extract to 900ml of deionized water; sterilize by autoclaving. Combine salts in 100ml of deionized water; autoclave to sterilize, then add 100ml to the broth.

LB medium

10g casein peptone
5g yeast extract
5g NaCl
15g agar (for plates only)

Dissolve in 1L of distilled water. Autoclave and cool to 55°C before adding antibiotic. **Note:** For LB liquid medium, do not add agar.

7.B. Pressure Measurement Units

Table 3. Comparison of Inches of Hg to Other Pressure Measurements.

15” Hg	50.8kPa	381 Torr	0.501atm	7.37psi	38.1cm Hg	508mbar

8. Summary of Changes

The following changes were made to the 12/25 revision of this document:

1. Document contents were moved to a new template that includes a new cover image and fonts.
2. The protocol in Section 3.B was updated to recommend removing cell medium by pipetting.
3. An expired patent statement was removed.
4. Third party trademarks were updated.
5. Removed Related Products section. See **www.promega.com** for related products.
6. Storage temperatures were updated to modern temperature ranges.