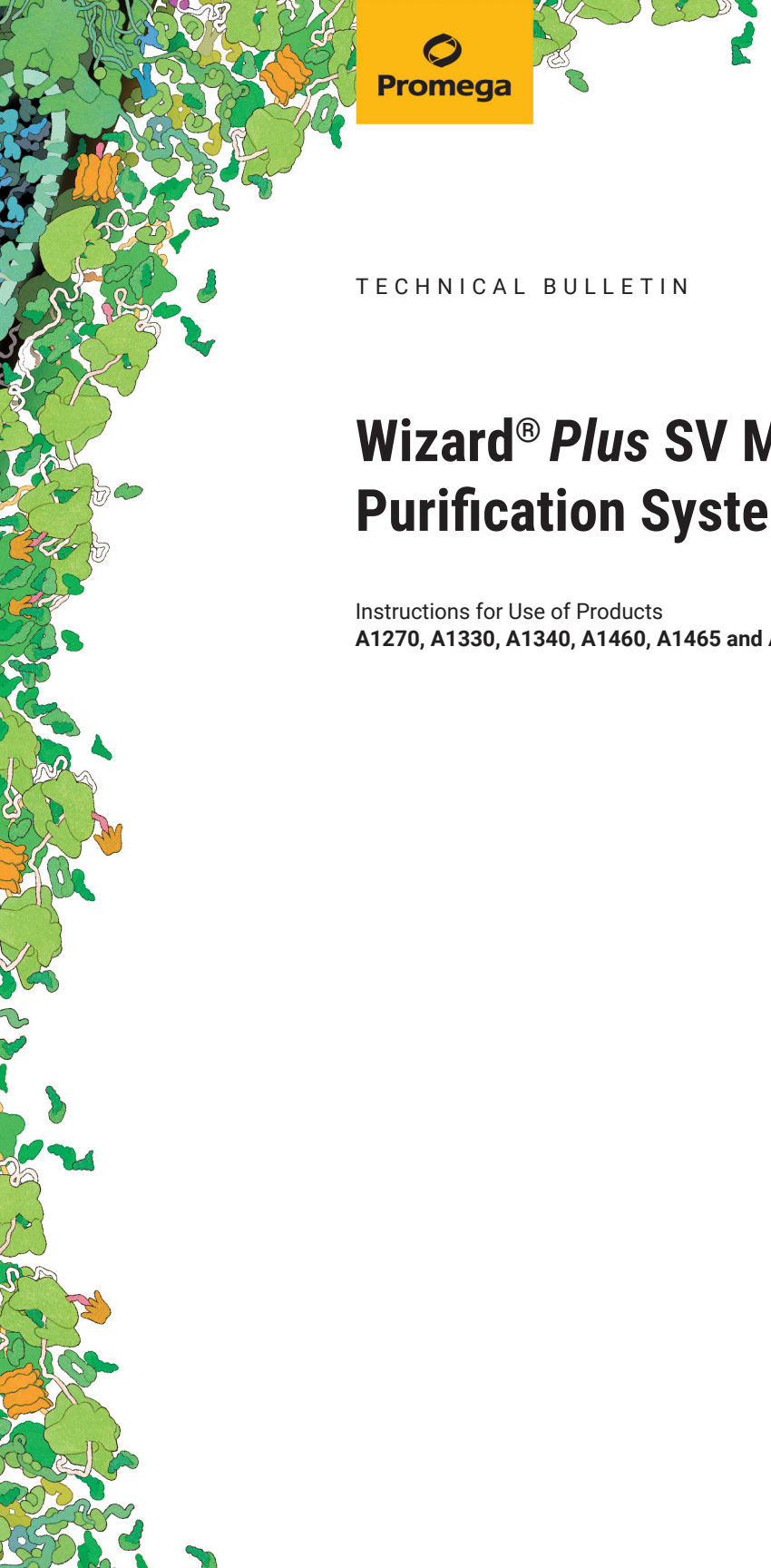

TECHNICAL BULLETIN

Wizard® *Plus* SV Minipreps DNA Purification System

Instructions for Use of Products
A1270, A1330, A1340, A1460, A1465 and A1470

Revised 4/24
TB225

Promega

Wizard® *Plus* SV Minipreps 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

1. Description

The Wizard® *Plus* SV Minipreps DNA Purification System provides a simple, reliable method for rapid isolation of plasmid DNA (Figure 1). The entire miniprep procedure can be completed in 30 minutes or less, depending on the number of samples processed. This system can be used to isolate any plasmid from *E. coli* hosts but works most efficiently when the plasmid is less than 20,000bp in size. Purified plasmids can be used without further manipulation for automated fluorescent DNA sequencing as well as for other standard molecular biology techniques. When used for in vitro transcription reactions, the isolated plasmid DNA should be supplemented with a ribonuclease inhibitor such as Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511).

The protocol presented in this Technical Bulletin is for isolation of plasmid DNA from *E. coli*. Plasmid DNA can be purified from 1–10ml overnight cultures with the Wizard® *Plus* SV Minipreps DNA Purification System. Plasmid yield will vary depending on a number of factors, including culture volume, plasmid copy number, type of culture medium and the bacterial strain used.

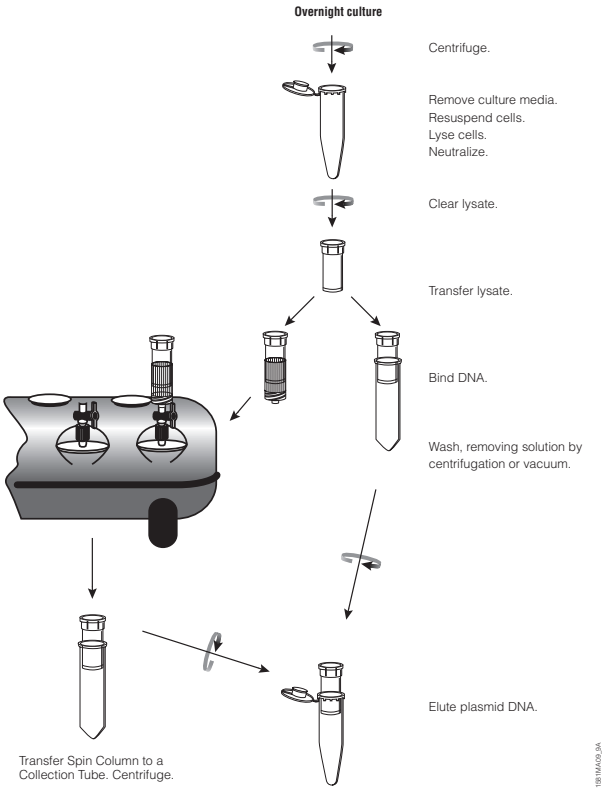

Figure 1. Flow diagram of plasmid DNA isolation and purification using the Wizard® *Plus* SV Minipreps DNA Purification System.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wizard® *Plus* SV Minipreps DNA Purification System (with Vacuum Adapters)	**50 preps**	**A1340**

Each system contains sufficient reagents for 50 isolations from 1–10ml of culture. Includes:

- 20ml Cell Resuspension Solution (CRA)
- 20ml Cell Lysis Solution (CLA)
- 30ml Neutralization Solution (NSB)
- 20ml Column Wash Solution (CWA)
- 50 Wizard® SV Minicolumns
- 50 Collection Tubes (2ml)
- 550µl Alkaline Protease Solution
- 13ml Nuclease-Free Water
- 20 Vacuum Adapters

PRODUCT	SIZE	CAT.#
Wizard® *Plus* SV Minipreps DNA Purification System (with Vacuum Adapters)	**250 preps**	**A1470**

Each system contains sufficient reagents for 250 isolations from 1–10ml of culture. Includes:

- 75ml Cell Resuspension Solution (CRA)
- 75ml Cell Lysis Solution (CLA)
- 100ml Neutralization Solution (NSB)
- 100ml Column Wash Solution (CWA)
- 250 Wizard® SV Minicolumns
- 250 Collection Tubes (2ml)
- 2,700µl Alkaline Protease Solution
- 25ml Nuclease-Free Water
- 20 Vacuum Adapters

PRODUCT	SIZE	CAT.#
Wizard® *Plus* SV Minipreps DNA Purification System, Trial Size	**10 preps**	**A1270**

Each system contains sufficient reagents for 10 isolations from 1–10ml of culture (includes Vacuum Adapters).

PRODUCT	SIZE	CAT.#
Wizard® *Plus* SV Minipreps DNA Purification System	**50 preps**	**A1330**
	250 preps	**A1460**

Note: Cat.# A1330 and A1460 contain the same components as Cat.# A1340 and A1470, respectively, but do not include Vacuum Adapters.

2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
Wizard® *Plus* SV Minipreps DNA Purification System	**1,000 preps**	**A1465**

Each system contains sufficient reagents for 1,000 isolations from 1–10ml of culture. Includes:

- 300ml Cell Resuspension Solution (CRA)
- 300ml Cell Lysis Solution (CLA)
- 400ml Neutralization Solution (NSB)
- 400ml Column Wash Solution (CWA)
- 1,000 Wizard® SV Minicolumns
- 1,000 Collection Tubes (2ml)
- 12ml Alkaline Protease Solution
- 50ml Nuclease-Free Water

Note: Cat.# A1465 does not include Vacuum Adapters.

Storage Conditions: Store all Wizard® *Plus* SV Minipreps components at room temperature (15–30°C). Reagent expiration dates are listed on the product label.

3. Protocols

Do not exchange or replace components of the Wizard® *Plus* SV Minipreps DNA Purification System with components from any other Wizard® *Plus* System. Components from the Wizard® *Plus* and Wizard® *Plus* SV Systems are **not** interchangeable.

Materials to Be Supplied by the User

(Solution compositions are provided in Section 6.)

- LB agar plates containing antibiotic
- LB medium containing antibiotic
- ethanol (95%)
- microcentrifuge capable of 14,000 × *g*
- sterile 1.5ml microcentrifuge tubes
- centrifuge capable of 10,000 × *g*

Prior to using a new Wizard® *Plus* SV Minipreps DNA Purification System, dilute the provided Column Wash Solution (CWA) as follows:

Add **7ml** of 95% ethanol for a final volume of **11ml** for the 10-prep system (Cat.# A1270).

Add **35ml** of 95% ethanol for a final volume of **55ml** for the 50-prep system (Cat.# A1330 and A1340).

Add **170ml** of 95% ethanol for a final volume of **270ml** for the 250-prep system (Cat.# A1460 and A1470) and 1,000-prep system (Cat.# A1465).

3.A. Preparation of *E. coli*

1. Use a single, well isolated colony from a fresh Luria-Bertani (LB) agar plate (containing antibiotic) to inoculate 1–10ml of LB medium (containing the same antibiotic). We recommend LB culture medium. Rich media, such as Terrific Broth, produce high cell densities that may overload the DNA purification system.
2. Incubate overnight (12–16 hours) at 37°C in a shaking incubator. Incubation time can be optimized to increase the plasmid DNA yield for a given culture volume. However, it has been observed that as a culture ages the DNA yield may begin to decrease due to cell death and lysis within the culture.

Note: An A_{600} reading of 2–4 ensures that cells have reached the proper growth density for harvesting and plasmid DNA isolation.

For high-copy-number plasmids, do not process more than **5ml** of bacterial culture. If more than 5ml of culture is processed, the capacity of the Wizard® SV Minicolumn will be exceeded and no increase in plasmid yield will be obtained.

For low-copy-number plasmids, it may be necessary to process larger volumes of bacterial culture (up to 10ml) for recovery of sufficient DNA. Processing greater than 10ml of culture will lead to insufficient clearing of the bacterial lysate and thus increased contaminants in the plasmid DNA.

3.B. Production of a Cleared Lysate

Note: Throughout the remainder of this document, the supplied Cell Resuspension Solution (CRA), Cell Lysis Solution (CLA), Neutralization Solution (NSB) and Column Wash Solution (CWA) are referred to as Cell Resuspension Solution, Cell Lysis Solution, Neutralization Solution and Column Wash Solution, respectively.

1. Harvest 1–5ml (high-copy-number plasmid) or 10ml (low-copy-number plasmid) of bacterial culture by centrifuging for 5 minutes at 10,000 × *g* in a tabletop centrifuge. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.
2. Add 250µl of Cell Resuspension Solution and completely resuspend the cell pellet by vortexing or pipetting. **It is essential to thoroughly resuspend the cells.** If they are not already in a microcentrifuge tube, transfer the resuspended cells to a sterile 1.5ml microcentrifuge tube(s).

Note: To prevent shearing of chromosomal DNA, **do not** vortex after Step 2. Mix only by inverting the tubes.

3. Add 250µl of Cell Lysis Solution and mix by inverting the tube 4 times (**do not vortex**). Incubate until the cell suspension clears (approximately 1–5 minutes).

   Note: It is important to observe partial clearing of the lysate before adding the Alkaline Protease Solution (Step 4); however, **do not incubate for longer than 5 minutes**.

4. Add 10µl of Alkaline Protease Solution and mix by inverting the tube 4 times. Incubate for **5 minutes** at room temperature.

Alkaline protease inactivates endonucleases and other proteins released during the lysis of the bacterial cells that can adversely affect the quality of the isolated DNA. Do not exceed 5 minutes of incubation with Alkaline Protease Solution at Step 4, as nicking of the plasmid DNA may occur.

5. Add 350µl of Neutralization Solution and immediately mix by inverting the tube 4 times (**do not vortex**).
6. Centrifuge the bacterial lysate at maximum speed (around 14,000 × *g*) in a microcentrifuge for 10 minutes at room temperature.

3.C. Plasmid DNA Isolation and Purification Protocols

The Wizard® *Plus* SV Minipreps DNA Purification System allows a choice of methods for purification of plasmid DNA when systems with Vacuum Adapters are purchased (Cat.# A1340, A1470). Plasmid DNA may be purified from the bacterial lysate using microcentrifugation to force the cleared lysate through the Wizard® SV Minicolumn and wash the plasmid DNA. Alternatively, a vacuum can be used to pull the lysate through the Spin Column and wash the plasmid DNA. Vacuum Adapters allow the use of a vacuum manifold (e.g., a Vac-Man® Laboratory Vacuum Manifold) and vacuum source for DNA purification.

Centrifugation Protocol

Prepare plasmid DNA purification units by inserting one Spin Column into one 2ml Collection Tube for each sample.

1. Transfer the cleared lysate (approximately 850µl, Section 3.B, Step 6) to the prepared Spin Column by decanting. Avoid disturbing or transferring any of the white precipitate with the supernatant.

   Note: If the white precipitate is accidentally transferred to the Spin Column, pour the Spin Column contents back into a sterile 1.5ml microcentrifuge tube and centrifuge for another 5–10 minutes at maximum speed. Transfer the resulting supernatant into the same Spin Column that was used initially for this sample. The Spin Column can be reused but **only** for this sample.
2. Centrifuge the supernatant at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the flowthrough from the Collection Tube. Reinsert the Spin Column into the Collection Tube.
3. Add 750µl of Column Wash Solution, previously diluted with 95% ethanol, to the Spin Column.
4. Centrifuge at maximum speed in a microcentrifuge for 1 minute at room temperature. Remove the Spin Column from the tube and discard the flowthrough. Reinsert the Spin Column into the Collection Tube.
5. Repeat the wash procedure using 250µl of Column Wash Solution.
6. Centrifuge at maximum speed in a microcentrifuge for 2 minutes at room temperature.
7. Transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube, being careful not to transfer any of the Column Wash Solution with the Spin Column. If the Spin Column has Column Wash Solution associated with it, centrifuge again for 1 minute at maximum speed.
8. Transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube.
9. Elute the plasmid DNA by adding 100µl of Nuclease-Free Water to the Spin Column. Centrifuge at maximum speed for 1 minute at room temperature in a microcentrifuge.
10. After eluting the DNA, remove the assembly from the 1.5ml microcentrifuge tube and discard the Spin Column.

11. DNA is stable in water without addition of a 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 11µl of 10X TE buffer to the 100µl of eluted DNA. **Do not** add TE buffer if the DNA is to be used for automated fluorescent sequencing.
12. Cap the microcentrifuge tube and store the purified plasmid DNA at –20°C or below.

Vacuum Protocol

Attach one Vacuum Adapter with Luer-Lok® fitting to one port of the manifold (e.g., a Vac-Man® Laboratory Vacuum Manifold). Insert a Spin Column into the Vacuum Adapter until snugly in place.

1. Transfer the cleared lysate (approximately 850µl, Section 3.B, Step 6) to the prepared Spin Column by decanting. Avoid disturbing or transferring any of the white precipitate with the supernatant.

   Note: If the white precipitate is accidentally transferred to the Spin Column, pour the Spin Column contents back into a sterile 1.5ml microcentrifuge tube and centrifuge for another 5–10 minutes at maximum speed. Transfer the resulting supernatant into the same Spin Column that was used initially for this sample. The Spin Column can be reused but **only** for this sample.
2. Apply a vacuum of at least 15 inches of mercury (Hg) to pull the liquid through the Spin Column. When all liquid has been pulled through the column, release the vacuum.

   Table Comparing Inches of Hg to Other Pressure Measurements

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

3. Add 750µl of the Column Wash Solution, previously diluted with 95% ethanol, to the Spin Column.
4. Apply a vacuum to pull the Column Wash Solution through the Spin Column. When all the liquid has been pulled through the Spin Column, release the vacuum.
5. Repeat the wash procedure using 250µl of Column Wash Solution. Apply a vacuum to pull the liquid through the Spin Column.
6. Dry the Spin Column by applying a vacuum for 10 minutes.
7. Turn off the vacuum and transfer the Spin Column to a 2ml Collection Tube. Centrifuge at maximum speed for 2 minutes to remove any residual Column Wash Solution. Discard the 2ml Collection Tube and any liquid collected during this step.

8. Transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube.
9. Elute the plasmid DNA by adding 100µl of Nuclease-Free Water to the Spin Column. Centrifuge at maximum speed for 1 minute at room temperature in a microcentrifuge.
10. After eluting the DNA, remove the assembly from the 1.5ml microcentrifuge tube and discard the Spin Column.
11. DNA is stable in water without addition of a 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 11µl of 10X TE buffer to the 100µl of eluted DNA. **Do not** add TE buffer if the DNA is to be used for automated fluorescent sequencing.
12. Cap the microcentrifuge tube and store the purified plasmid DNA at –20°C or below.

4. Supplementary Information

4.A. Selection and Preparation of Plasmids and *E. coli* Strains

Plasmid DNA can be purified from overnight cultures of *E. coli* with the Wizard® *Plus* SV Minipreps 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 bacterial strain used.

Plasmid copy number is an important factor affecting 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.

Choose a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate (containing antibiotic) and use the colony to inoculate 1–10ml of LB media (also containing antibiotic). The inoculated medium should be incubated overnight (12–16 hours) at 37°C. An A_{600} of 2.0–4.0 for high-copy-number plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation.

4.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 are referred to as EndA–. Table 1 contains a list of EndA– and EndA+ *E. coli* strains.

The absence of *end*A1 (or *end*A) in an *E. coli* genotype denotes the presence of the wildtype gene, which expresses an active endonuclease I. The wildtype is indicated as EndA+. Using the Wizard® *Plus* SV Minipreps DNA Purification System, high-quality DNA is easily obtained from both EndA+ and EndA– strains. However, 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.

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-number plasmids and EndA– strains of *E. coli* for plasmid propagation.

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

EndA–	EndA+
BJ5183	BL21(DE3)
DH1	CJ236
DH20	HB101
DH21	JM83
DH5α™	JM101
JM103	JM110
JM105	LE392
JM106	MC1061
JM107	NM522 (all NM series strains are EndA+)
JM108	NM554
JM109	P2392
MM294	PR700 (all PR series strains are EndA+)
SK1590	Q358
SK1592	RR1
SK2267	TB1
SRB	TG1
TOP10	Y1088 (all Y10 series strains are EndA+)
XL1-Blue	BMH 71-18
XLO	ES1301

4.C. Use of Alkaline Protease

To improve the quality of plasmid DNA isolated from both EndA+ and EndA– strains of *E. coli*, the Wizard® *Plus* SV Minipreps DNA Purification System includes an alkaline protease solution. Alkaline protease, originally identified as subtilisin Carlsberg, is isolated from the bacterium *Bacillus licheniformis* (1). Approximately 250µg are added per sample at the end of the lysis step during the preparation of a cleared bacterial lysate to inactivate endonucleases. The alkaline protease also acts to nonspecifically degrade proteins, thus reducing the overall level of protein contaminants in the cleared bacterial lysate (2,3).

Alkaline protease is useful in this procedure, because it is optimally active at pH 9 and above, the conditions present during the alkaline lysis procedure. When the lysate is neutralized, alkaline protease activity is substantially reduced (4,5).

4.C. Use of Alkaline Protease (continued)

The DNA prepared by this procedure has been tested extensively in a range of molecular biology applications including fluorescent sequencing, restriction enzyme digestion and cloning.

4.D. 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.

Note: Optimal automated fluorescent sequencing results are routinely obtained by using high-copy-number plasmids and EndA– strains of *E. coli*.

Purified plasmid DNA must be within the proper concentration range for successful automated cycle sequencing (ideally 0.2µg/µl and not less than 0.1µg/µl). When working with DNA from low-copy-number plasmids, we strongly recommend that DNA concentration be determined by agarose gel/ethidium bromide quantitation prior to any application (6). DNA quantitation by spectrophotometric methods is prone to errors and requires a large amount of sample.

The Wizard® *Plus* SV Minipreps System routinely yields 3.5–5.0µg of plasmid DNA when using a pGEM® Vector and DH5α™ cells in 1.5ml of LB medium. For low-copy-number plasmids, a larger culture volume is required to obtain sufficient DNA for sequencing. Typical low-copy-number plasmid yields are 1.5–3.0µg of plasmid DNA from 10ml of LB culture medium using the pALTER®-1 Vector and DH5α™ cells.

Special Considerations for Sequencing Using BigDye™ Chemistry

The Wizard® *Plus* SV Minipreps System yields template suitable for use in a number of fluorescent dye sequencing methods, including BigDye™ terminator reactions (Applied Biosystems).

When performing dilutions of the BigDye™ terminator-ready reaction mix, it is essential to dilute the reaction mix using the appropriate dilution buffer (250mM Tris-HCl [pH 9.0], 10mM $MgCl_2$).

When using high-copy-number plasmids, over 500 bases of readable sequence can be obtained from terminator-ready reaction mixes diluted as much as sixfold.

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.

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 bacterial cells. Use cultures grown to an A_{600} of 2–4. All media should contain an antibiotic. Use only recommended culture volumes for low- and high-copy-number plasmids (10ml maximum for low-copy-number and 5ml maximum for high-copy-number plasmids).
	Poor resuspension of bacterial cell pellet. Thoroughly resuspend cell pellets prior to cell lysis. Vortex or pipet pellet with Cell Resuspension Solution. No cell clumps should be visible after resuspension.
No plasmid DNA purified	Ethanol was not added to Column Wash Solution. Prepare the Column Wash Solution as instructed in Section 3 before beginning the procedure.
	Inaccurate quantitation of plasmid DNA yield. Quantitate plasmid DNA yield via agarose gel/ethidium bromide electrophoresis.
	DNA floats out of the wells during gel loading. Be certain to allow the full 10 minutes for drying (with vacuum protocol) after the final wash step to allow evaporation of any remaining ethanol. Increase loading dye concentration.
	Low-copy-number plasmid used. Use a high-copy-number plasmid.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Low plasmid DNA yields	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 media with a freshly isolated bacterial colony from an overnight plate. Incubate at 37°C for 12–16 hours.
	Low-copy-number plasmid used. Know the copy number of plasmid used; we recommend use of high-copy-number plasmids.
	Plasmid DNA yield not accurately quantitated. Use agarose gel ethidium bromide quantitation. Do not rely on spectrophotometric measurement.
Nicking of plasmid DNA	Overincubation during alkaline lysis. Do not exceed 5 minutes of incubation with either Cell Lysis Solution or Alkaline Protease.
Poor results with automated fluorescent sequencing	Too little DNA 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. Repurify plasmid DNA and elute in Nuclease-Free Water.
	Plasmid concentration not accurately quantitated. Use ethidium bromide gel electrophoresis to accurately quantitate plasmid DNA.
No restriction digestion	Concentration of restriction enzyme or digestion time need to be increased. Increase the amount of restriction enzyme or the length of incubation. Digest at the recommended temperature and in the optimal buffer for the restriction enzyme used. Ethanol precipitate the plasmid DNA to remove any salts that may have carried over.
Genomic DNA contamination	Vortexing or overmixing resulted in genomic DNA contamination. Do not vortex samples after addition of Cell Lysis Solution to prevent shearing of genomic DNA.
	Wrong reagents used. Make certain that Column Wash Solution has been diluted with ethanol before use. **Note:** Wizard® *Plus* and Wizard® *Plus* SV components are **not** interchangeable.
DNA yields on gel look low compared spectrophotometric readings	Traces of contaminants may be present in the eluted DNA, which inflate spectrophotometer readings. Phenol:chloroform extract and ethanol precipitate DNA, then wash with 70% ethanol before repeating readings. Quantitate DNA by agarose gel/ethidium bromide electrophoresis for the most accurate results.

6. Composition of Buffers and Solutions

5X dilution buffer

250mM Tris-HCl (pH 9.0)
10mM $MgCl_2$

10X TE buffer

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

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.

Cell Lysis Solution (CLA)

0.2M NaOH
1% SDS

Cell Resuspension Solution (CRA)

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

Neutralization Solution (NSB)

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
22.6mM Tris-HCl (pH 7.5)
0.109mM EDTA (pH 8.0)

Add 35ml of 95% ethanol for the 50-prep system (170ml for the 250-prep system, 7ml for the 10-prep system) as described in Section 3. Final concentrations will be approximately 60% ethanol, 60mM potassium acetate, 8.3mM Tris-HCl, 0.04mM EDTA.

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7. References

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2. Aehle, W. *et al.* (1993) Rational protein engineering and industrial application: Structure prediction by homology and rational design of protein-variants with improved 'washing performance'—the alkaline protease from *Bacillus alcalophilus*. *J. Biotechnol.* **28**, 31–40.
3. von der Osten, C. *et al.* (1993) Protein engineering of subtilisins to improve stability in detergent formulations. *J. Biotechnol.* **28**, 55–68.
4. Vetter, R. *et al.* (1994) Highly alkaline proteases. U.S. Pat. No. 5,352,603. (October 4, 1994).
5. Shetty, J.K., Patel, C.P. and Nicholson, M.A. (1995) Method of preparation of purified alkaline protease. U.S. Pat. No. 5,439,817. (August 8, 1995).
6. Kahn, M. *et al.* (1979) Plasmid cloning vehicles derived from plasmids ColE1, F, R6K, and RK2. *Meth. Enzymol.* **68**, 268–80.

8. Related Products

Product	Size	Cat.#
Alkaline Protease Solution	3ml	A1441
Wizard® SV 96 Plasmid DNA Purification System	1 × 96 preps	A2250
	5 × 96 preps	A2255
Wizard® SV 9600 Plasmid DNA Purification System	100 × 96 preps	A2258
Vac-Man® Laboratory Vacuum Manifold	20-sample capacity	A7231
	2-sample capacity	A7660
Vac-Man® 96 Vacuum Manifold	96-well capacity	A2291
Vacuum Adapters	20 each	A1331
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
	300 preps	A2496

9. Summary of Changes

The following changes were made to the 4/24 revision of this document:

1. Cover image and font were updated.
2. Expired legal disclaimers were removed.
3. Third party trademarks were updated.
4. Cat.# A1465 was added to Section 2.

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