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I. Introduction

In today's world of DNA analysis by multiplex and real-time PCR, the importance of high-quality, purified DNA cannot be underestimated. Finding a suitable DNA isolation system to satisfy your downstream application needs is vital for the successful completion of experiments. This DNA purification chapter addresses general information on the basics of DNA isolation, plasmid growth and DNA quantitation as well as how purification by silica can help increase your productivity so you spend less time purifying DNA and more time developing experiments and analyzing data. In addition, this chapter covers the wide variety of Promega products available for plasmid, genomic and fragment/PCR product purification and includes a sample protocol for each type of isolation system. Along with the discussion of Promega's DNA purification systems, we also consider the issues of scalability, downstream applications and yield to assist in finding the best system for your needs.

A. Basic Isolation Procedure

The basic steps of DNA isolation are disruption of the cellular structure to create a lysate, separation of the soluble DNA from cell debris and other insoluble material and purification of the DNA of interest from soluble proteins and other nucleic acids. Historically, this was done using organic extraction (e.g., phenol:chloroform) followed by ethanol precipitation. In the case of plasmid preparations, the multiple-day protocol typically involved cesium chloride banding followed by dialysis of the plasmid DNA. These methods were time consuming and used a variety of hazardous reagents.

For ease-of-use, Promega offers an array of conveniently packaged DNA purification products that can isolate DNA in less than an hour using much safer methods. Disruption of most cells is done by chaotropic salts, detergents or alkaline denaturation, and the resulting lysate is cleared by centrifugation, filtration or magnetic clearing. DNA is purified from the soluble portion of the lysate. When silica matrices are used, the DNA is eluted in an aqueous buffer such as TE or nuclease-free water. The purified, high-quality DNA is ready-to-use for a wide variety of demanding downstream applications such as multiplex PCR, coupled in vitro transcription/translation systems, transfection and sequencing reactions. Eluting and storing the DNA in TE buffer is helpful if the EDTA does not affect downstream applications. EDTA chelates or binds magnesium present in the purified DNA and can help inhibit possible contaminating nuclease activity.

DNA fragment purification from an amplification reaction or restriction enzyme digestion involves a direct treatment of the solution to remove the enzyme and reaction buffer and for PCR products, reduce the amount of nucleotides and primers present. Historically, this was done with phenol:chloroform extraction followed by precipitation. However, safety issues and the expense associated make organic extraction a less convenient DNA purification method. Promega's option is adding chaotropic salt to the reaction volume and purifying the PCR products by silica chemistry. This method is quick and results in pure DNA ready for sequencing and cloning.

B. Basis for Purification by Silica

The majority of Promega's DNA isolation systems for genomic, plasmid and PCR product purification are based on purification by silica. Regardless of the method used to create a cleared lysate, the DNA of interest can be isolated by virtue of its ability to bind silica in the presence of high concentrations of chaotropic salts (Chen and Thomas, 1980; Marko *et al.* 1982; Boom *et al.* 1990). These salts are then removed with an alcohol-based wash and the DNA eluted in a low-ionic-strength solution such as TE buffer or water. The binding of DNA to silica seems to be driven by dehydration and hydrogen bond formation, which competes against weak electrostatic repulsion (Melzak *et al.* 1996). Hence, a high concentration of salt will help drive DNA adsorption onto silica, and a low concentration will release the DNA.

Promega has sold and supported silica-based DNA purification systems for nearly two decades. The first technology available was silica resin, exemplified by the Wizard® *Plus* Minipreps DNA Purification System. The protocol for purification by silica resin involves combining the cleared lysate with a resin slurry and using vacuum filtration to wash the bound DNA, followed by centrifugation to elute the purified DNA.

More recent purification systems consist of two different formats: silica membrane column (e.g., the PureYield ™ Plasmid Midiprep System) and silica-coated MagneSil® Paramagnetic Particles (PMPs; e.g., Wizard® Magnetic 96 DNA Plant System). While both methods yield high-quality DNA, the silica membrane column is more convenient. For automated purification, either the 96-well silica membrane plates or the MagneSil® PMPs are easily adapted to a variety of robotic platforms. In order to process the DNA samples, the MagneSil® PMPs require a magnet for particle capture rather than centrifugation or vacuum filtration. The MagneSil® PMPs are considered a "mobile solid phase" with binding of nucleic acids occurring in solution. Particles can also be completely resuspended during the wash steps of a purification protocol, thus enhancing the removal of contaminants. See Figure 9.1 for images of a silica membrane column and the MagneSil® PMPs.

A.

B.

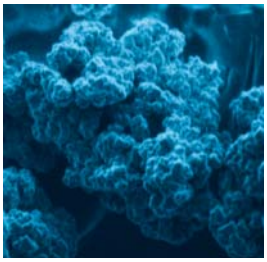

Figure 9.1. Images of two Promega silica purification matrices. Panel A. A PureYield™ Midiprep binding column. The membrane is present at the bottom of the column. **Panel B.** An electron micrograph of MagneSil® PMPs.

Additional Resources for Silica Purification

Promega Publications

Transfection-quality plasmid DNA in as little as ten minutes using the PureYield™ Plasmid Miniprep System

Promega's SV membrane technology: The evolution of an indispensable laboratory tool

C. Overview of Plasmid DNA Purification

The primary consideration for plasmid purification is separation of plasmid DNA from the chromosomal DNA and cellular RNA of the host bacteria. A number of methods have been developed to generate a cleared lysate that not only removes protein and lipids but also efficiently removes contaminating chromosomal DNA while leaving plasmid DNA free in solution. Methods for the preparation of cleared lysates that enrich for plasmid DNA include SDS-alkaline denaturation (Birnboim and Doly, 1979; Birnboim, 1983), salt-SDS precipitation (Hirt, 1967) and rapid boiling (Holmes and Quigley, 1981).

The SDS-alkaline denaturation method, which is used in all Promega plasmid isolation systems, is a popular procedure for purifying plasmid DNA because of its overall versatility and consistency. This technique exploits the difference in denaturation and renaturation characteristics of covalently closed circular plasmid DNA and chromosomal DNA fragments. Under alkaline conditions (at pH 11), both plasmid and chromosomal DNA are efficiently denatured. Rapid neutralization with a high-salt buffer such as potassium acetate in the presence of SDS has two effects that contribute to the overall effectiveness of the method. First, rapid neutralization causes the chromosomal DNA to base-pair in an intrastrand manner, forming an insoluble aggregate that precipitates out of solution. The covalently closed nature of the circular plasmid DNA promotes interstrand rehybridization, allowing the plasmid to remain in solution. Second, the potassium salt of SDS is insoluble, so the protein and detergent precipitate and aggregate, which assists in the entrapment of the high-molecular-weight chromosomal DNA. Separation of soluble and insoluble material is accomplished by a clearing method (e.g., filtration, magnetic clearing or centrifugation). The soluble plasmid DNA is ready to be further purified.

There are several methods available to purify plasmid DNA from cleared lysate. These include:

- binding plasmid to silica in the presence of high concentrations of chaotropic salts (Chen and Thomas, 1980; Marko *et al.* 1982; Boom *et al*. 1990)
- differential precipitation of plasmid DNA from aqueous chaotropic salt/ethanol solutions (Hamaguchi and Geiduschek,1962; Wilcockson, 1973; Wilcockson, 1975)
- ion exchange chromatography over DEAE-modified cellulose membranes (van Huynh *et al*. 1993)
- precipitation with polyethylene glycol (Lis, 1980; Paithankar and Prasad, 1991)
- organic extraction using phenol (Wang and Rossman, 1994)

Promega products like the Wizard® *Plus* SV Minipreps DNA Purification System and the PureYield™ Plasmid Systems combine the benefits of alkaline lysis with the rapid and easy purification by silica. This is done by using a silica-based membrane in a column format to bind the plasmid DNA contained in the cleared alkaline lysates. Purification is based on selective adsorption of DNA to the silica membrane in the presence of high concentrations of chaotropic salts, washes to efficiently remove contaminants, and elution of the DNA with low-salt solutions such as TE buffer or water. See *Promega Notes* 82 for additional discussion of the SV membrane.

Ideal for use with automated platforms, the silica-coated MagneSil® PMP systems are also easily scalable for larger volumes or multiwell format. For plasmid miniprep purification, the MagneSil® PMPs are used for both lysate clearing and DNA binding, eliminating the need for centrifugation or vacuum filtration, as the binding of nucleic acids occurs in solution. The particles are also completely resuspended during the wash steps of a purification protocol, enhancing the removal of impurities from the DNA. The Wizard® MagneSil® Plasmid DNA Purification System uses these PMPs for the purification of plasmid DNA in a 96-well format. This plasmid purification system can be used on automated workstations such as the

Beckman Coulter Biomek® FX or the Tecan Genesis® RSP. See our web site for further information on compatibility of Promega DNA isolation products with various liquid-handling platforms at the Automated Methods web page.

Purified plasmid DNA is used in many applications from preparing vectors for cloning to generating templates for transcription or coupled transcription/translation reactions. The silica-based purification systems from Promega minimize the amount of salts and other impurities carried over during isolation, which can negatively affect downstream applications, lower yield or prevent enzyme systems from synthesizing the product of interest.

Additional Resources for Plasmid DNA Purification

Promega Publications

DNA Analysis Notebook

Subcloning Notebook

D. Overview of Genomic DNA Isolation

Promega provides several systems designed to isolate genomic DNA from a variety of sources. One method, the solution-based Wizard® Genomic DNA Purification Kit, is the most versatile system available from Promega. This purification system relies on a series of precipitation steps to purify high-molecular-weight DNA from a prepared lysate. It is an excellent choice when a pure population of dsDNA molecules is required for downstream applications such as Southern blotting, real-time PCR and restriction digestion. Alternatively, Promega offers genomic DNA isolation systems based on sample lysis by detergents and purification by silica (see Basis for Purification by Silica and Overview of Plasmid DNA Purification for more details). These include both membrane-based systems (e.g., the single-column Wizard® SV Genomic DNA Purification Kit or the high-throughput, 96-well Wizard® SV 96 Genomic DNA Purification System) and the easily automated paramagnetic silica systems (e.g., MagneSil® Genomic, Large Volume System or the MagneSil® Blood Genomic, Max Yield System). All of these systems purify genomic DNA that is amenable for use in many downstream applications. We also offer cellulose-based purification systems (e.g., ReliaPrep™ gDNA Tissue Miniprep System) that come with ready-to-use solutions and offer increased binding capacity compared to silica.

Although techniques like Southern blotting, which require microgram amounts of DNA, are still performed in molecular biology laboratories, most assessment of chromosomal DNA is done by PCR technology including monoplex or multiplex PCR, SNP analysis and real-time PCR. These latter techniques use nanogram amounts of DNA per reaction. Regardless of the system chosen, Promega genomic DNA purification kits not only yield DNA suitable for a wide range of DNA quantity specifications but provide the required amount of high-quality DNA with minimal contaminants.

Additional Resources for Genomic DNA Purification

Promega Publications

DNA Analysis Notebook

E. Overview of DNA Fragment Purification from Agarose Gels and PCR Amplifications

Applications such as cloning, labeling and sequencing DNA frequently require the purification of DNA fragments from agarose gels or amplification reactions. Promega provides multiple systems for DNA fragment purification, including two based on silica membrane technology (Wizard® SV Gel and PCR Clean-Up System and Wizard® SV 96 PCR Clean-Up System) and one based on MagneSil® PMPs (Wizard® MagneSil® Sequencing Reaction Clean-Up System).

The Wizard® SV Gel and PCR Clean-Up System provides a reliable method to purify double-stranded, PCR-amplified DNA either directly from the reaction or from agarose. The quick protocol is simple to perform, and the PCR products are purified from contaminants, including primer dimers, PCR additives and amplification primers. To purify PCR product from nonspecific amplification products, the reaction products can be separated in an agarose gel prior to purification. The agarose is dissolved by chaotropic buffer, freeing the DNA for binding to the silica SV membrane. After removal of contaminants by alcohol-based washes, the DNA bound to the SV column is eluted in water or TE buffer, free of salt or macromolecular contaminants. The Wizard® SV Gel and PCR Clean-Up System can also be used to purify DNA from enzymatic reactions such as restriction digestion and alkaline phosphatase treatment.

Additional Resources for DNA Fragment Purification from Agarose Gels and PCR Reactions

Promega Publications

DNA Analysis Notebook

Subcloning Notebook

F. Overview of Personal Automation™ Systems for Purification

Automation is increasingly used to improve productivity for research, diagnostics and applied testing. Traditionally, automation refers to the use of large, specialized and costly equipment that requires extensive training to operate and maintain. Promega has developed Personal Automation™ with the Maxwell® 16 System which provides a flexible, reliable, compact and easy-to-use alternative to traditional automated systems.

The Maxwell® 16 System combines instrumentation, automated methods, prefilled reagent cartridges, service and support, providing everything needed for purification from a single source. The Maxwell® 16 System is designed for low- to moderate-throughput automated purification of 1–16 small samples. Currently, there are predispensed reagent cartridges in kits for genomic DNA purification, total RNA purification and recombinant protein

purification. These multiple cartridges make the Maxwell® 16 Instrument flexible for laboratories that may use one or all of these different systems. For genomic DNA purification, add blood, mouse tail, tissue (fresh or preprocessed formalin-fixed, paraffin-embedded [FFPE]), or bacteria samples directly to the prefilled reagent cartridge and press "Start". You avoid the time and hands-on labor of Proteinase K or other preprocessing aside from FFPE, and the purified genomic DNA sample is ready in about 30 minutes. The eluted DNA can be used in PCR and other applications. RNA purification follows a similar process, involving preparation of a DNA-free lysate followed by RNA purification. The eluted RNA can then be used in qRT-PCR and other applications. Recombinant polyhistidine- or HQ-tagged proteins can be purified from multiple sample types, including bacteria, mammalian cells, insect cells and culture medium. Purified protein is compatible with many common downstream applications including polyacrylamide gel electrophoresis and detection, functionality studies, Western blot analysis and mass spectrometry.

There are two versions of the Maxwell® 16 Instrument and kits to accompany these choices. The Maxwell® 16 Instrument (Cat.# AS2000) plus the Maxwell® 16 SEV (standard-elution volume) Hardware Kit (Cat.# AS1200) elutes macromolecules (DNA, RNA and protein) in 300µl of elution buffer. Maxwell® 16 Instrument (Cat.# AS2000) with the Maxwell® 16 LEV (low-elution volume) Hardware Kit (Cat.# AS1250) can elute the purified product in 30–100µl of elution buffer. The lower elution volume is advantageous for some applications that benefit from concentrated DNA or RNA. If you have one version of the Maxwell® 16 Instrument (i.e., LEV), purchasing the other hardware kit (i.e., SEV) will allow you to convert your instrument to use the SEV purification kits.

The Maxwell® 16 Flexi Method Firmware makes the system even more versatile by allowing you to create custom methods when isolating DNA, RNA or recombinant protein with the Maxwell® 16 Instrument. You can optimize purification from challenging sample types or unique applications. The Maxwell® 16 Flexi Method Firmware allows you to set sample processing times for lysis, binding, drying and elution. In addition, the number of passes can be customized for particular samples. The firmware can be installed on a new instrument or can be purchased separately to install on an existing Maxwell® 16 Instrument (Cat.# AS1000 or AS2000).

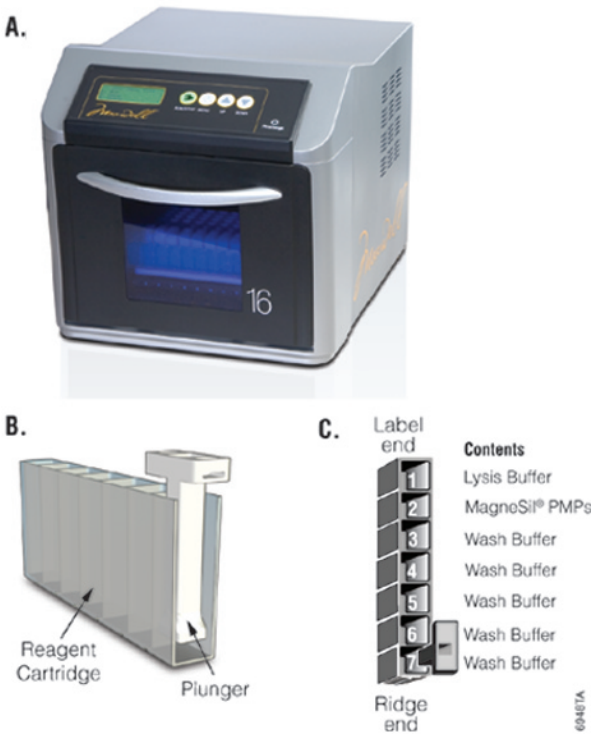

Figure 9.2. The Maxwell® 16 Instrument is used with optimized reagents predispensed into disposable cartridges. Panel A. The Maxwell® 16 Instrument. **Panel B.** A Maxwell® 16 reagent cartridge, cut away to show the plunger. The unique design of the cartridge allows direct processing of a variety of liquid and solid sample types with no need for preprocessing. **Panel C.** Top view of the Maxwell® 16 DNA Purification Cartridge.

G. Methods for Determining DNA Yield and Purity

DNA yield can be assessed using three different methods: absorbance (optical density), agarose gel electrophoresis and fluorescent DNA-binding dyes. Each technique is described and includes information on necessary accessories (e.g., equipment). While all methods are useful, each has caveats to consider when choosing a quantitation system.

The most common technique to determine DNA yield and purity is also the easiest method—absorbance. All that is needed for measurement is a spectrophotometer equipped with a UV lamp, UV-transparent cuvettes (depending on the instrument) and a solution of purified DNA. Absorbance readings are performed at 260nm (A_{260}) where DNA absorbs light most strongly, and the number generated allows one to estimate the concentration of the solution (see Estimation of DNA Concentration, Yield and Purity by Absorbance for more details). To ensure the numbers are useful, the A_{260} reading should be between 0.1–1.0.

However, DNA is not the only molecule that can absorb UV light at 260nm. Since RNA also has a great absorbance at 260nm, and the aromatic amino acids present in protein absorb at 280nm, both contaminants, if present in the DNA solution, will contribute to the total measurement at 260nm.

Additionally, the presence of guanidine will lead to higher 260nm absorbance. This means that if the A_{260} number is used for calculation of yield, the DNA quantity may be overestimated (Adams, 2003).

To evaluate DNA purity by spectrophotometry, measure absorbance from 230nm to 320nm in order to detect other possible contaminants present in the DNA solution [detailed in the *MagneSil® Genomic, Large Volume System Technical Bulletin*]. The most common purity calculation is determining the ratio of the absorbance at 260nm divided by the reading at 280nm. Good-quality DNA will have an A_{260}/A_{280} ratio of 1.7–2.0. A reading of 1.6 does not render the DNA unsuitable for any application, but lower ratios indicate more contaminants are present. However, the best test of DNA quality is functionality in the application of interest (e.g., real-time PCR).

Strong absorbance around 230nm can indicate that organic compounds or chaotropic salts are present in the purified DNA. A ratio of 260nm to 230nm can help evaluate the level of salt carryover in the purified DNA. The lower the ratio, the greater the amount of thiocyanate salt is present, for example. As a guideline, the A_{260}/A_{230} is best if greater than 1.5. A reading at 320nm will indicate if there is turbidity in the solution, another indication of possible contamination. Therefore, taking a spectrum of readings from 230nm to 320nm is most informative.

Agarose gel electrophoresis of the purified DNA eliminates the issues associated with absorbance readings. To use this method, a horizontal gel electrophoresis tank with an external power supply, analytical-grade agarose, an appropriate running buffer (e.g., 1X TAE) and an intercalating DNA dye along with appropriately sized DNA standards are needed for quantitation. A sample of the isolated DNA is loaded into a well of the agarose gel and then exposed to an electric field. The negatively charged DNA backbone migrates toward the anode. Since small DNA fragments migrate faster, the DNA is separated by size. The percentage of agarose in the gel will determine what size range of DNA will be resolved with the greatest clarity (Sambrook *et al.* 1989). Any RNA, nucleotides and protein in the sample migrate at different rates compared to the DNA so the band(s) containing the DNA will be distinct.

Concentration and yield can be determined after gel eletrophoresis is completed by comparing the sample DNA intensity to that of a DNA quantitation standard. For example, if a 2µl sample of undiluted DNA loaded on the gel has the same approximate intensity as the 100ng standard, then the solution concentration is 50ng/µl (100ng divided by 2µl). Standards used for quantitation should be labeled as such and be the same size as the sample DNA being analyzed. In order to visualize the DNA in the agarose gel, staining with an intercalating dye such as ethidium bromide or SYBR® Green is required. Because ethidium bromide is a known mutagen, precautions need to be taken for its proper use and disposal (Adams, 2003).

DNA-binding dyes compare the unknown sample to a standard curve of DNA, but genomic, fragment and plasmid DNA will each require their own standard curves and cannot be used interchangeably. If the DNA sample has been diluted, you will need to account for the dilution factor when calculating final concentration. Hoechst bisbenzimidazole dyes or PicoGreen® selectively bind double-stranded DNA (dsDNA). To use this method, a fluorometer to detect the dyes, dilution of the DNA solution and appropriate DNA standards are required. However, there are size qualifications: the DNA needs to be at least 1 kilobase in length for Hoechst and at least 200bp for PicoGreen® for successful quantitation. The range of measurement is 10–250ng/ml for Hoechst, 25pg/ml–1µg/ml for PicoGreen®, and the dyes are sensitive to GC content. In addition, the usual caveats for handling fluorescent compounds apply—photobleaching and quenching will affect the signal. While the dyes bind preferentially to dsDNA, RNA and nucleotides may contribute to the signal. [Adams, 2003; The Handbook —A Guide to Fluorescent Probes and Selection Guide Quant-iT™ Nucleic Acid Quantitation Assays accessed October 16, 2008].

Choosing which quantitation method to use is based on many factors including access to equipment or reagents, reliability and consistency of the concentration calculations. Use caution when comparing yields between methods as the level of potential contaminants may cause variable determinations among the different methods.

H. Estimation of DNA Concentration, Yield and Purity by Absorbance

DNA concentration can be estimated by adjusting the A_{260} measurement for turbidity (measured by absorbance at A_{320}), multiplying by the dilution factor, and using the relationship that an A_{260} of 1.0 = 50µg/ml pure DNA.

Concentration (µg/ml) = (A_{260} reading – A_{320} reading) × dilution factor × 50µg/ml

Total yield is obtained by multiplying the DNA concentration by the final total purified sample volume.

DNA Yield (µg) = DNA Concentration × Total Sample Volume (ml)

A_{260}/A_{280} ratio can be used as an estimate of DNA purity [with a number of important limitations (Wilfinger, Mackey and Chanczynski, 1997; Glasel, 1997; Manchester, 1995)]. An A_{260}/A_{280} ratio between 1.7 and 2.0 is generally accepted as representative of a high-quality DNA sample. The ratio can be calculated after subtracting the non-nucleic acid absorbance at A_{320}.

DNA Purity (A_{260}/A_{280}) = (A_{260} reading – A_{320} reading) ÷ (A_{280} reading – A_{320} reading)

Note that the spectrophotometer is most accurate when measurements are between 0.1–1.0.

II. General Considerations for Plasmid DNA Purification

A. Bacterial Growth and Culture Conditions

Successful isolation of quality plasmid DNA begins with culture preparation. A number of factors can influence the growth of bacterial cells. Bacterial growth in liquid culture occurs in three phases: 1) a short lag phase in which the bacteria become acclimated to the media and begin to divide; 2) a log phase, characterized by exponential growth in which most strains of *E. coli* will divide every 20–30 minutes; and 3) a stationary phase in which growth slows and eventually stops in response to the lack of nutrients in the medium. No net increase in biomass will occur in the stationary phase, but plasmid replication will continue for several hours after reaching stationary phase. Most strains of *E. coli* will reach a concentration of 1.0–4.0 × 10^9 cells/ml of culture at this stage, depending on culture media and aeration conditions. Depending on inoculation size and the size of the culture, stationary phase will be reached in 6–8 hours.

Aeration and temperature are of critical importance. The culture volume should be less than or equal to 1/4 the volume of the container (e.g., 250ml medium in a 1 liter flask); using 1/10 the container volume (e.g., 100ml medium in a 1,000ml flask) produces optimal results. The culture tube or flask should be placed in an orbital shaker (approximately 250rpm) to ensure adequate aeration (Ausubel *et al.* 1989). Since most strains of *E. coli* grow best at 37°C, this incubation temperature is recommended unless the strain of interest requires different conditions for optimal growth.

Different culture media will also have a profound effect on the growth of different bacterial strains. Promega plasmid DNA purification systems are appropriate for bacterial cultures grown in 1X Luria-Bertani (LB) medium. However, use of LB-Miller medium containing more NaCl will produce significantly greater yields and is highly recommended. Richer media such as 2X YT, CIRCLEGROW® or Terrific Broth may be used to increase plasmid yields by increasing the biomass for a given volume of culture. Keep the biomass in a range acceptable for the plasmid isolation system used, as overloading may result in poor purity and yield of the plasmid DNA (see Biomass Processed for more information).

Culture incubation time affects both the yield and quality of plasmid DNA isolated. Bacterial cultures grown to insufficient density will yield relatively low amounts of DNA. Overgrown cultures may result in suboptimal yields and excessive chromosomal DNA contamination due to autolysis of bacterial cells after they have reached stationary phase. We do not recommend the use of cultures grown longer than 18–20 hours.

B. Antibiotic Selection

Most plasmids carry a marker gene for a specific antibiotic resistance. By supplementing the growth medium with the antibiotic of choice, only cells containing the plasmid of interest will propagate. Adding antibiotic to the required concentration will help to maximize plasmid yields. Note that adding too much antibiotic can inhibit growth and too little may cause a mixed population of bacteria to grow—both with and without the plasmid of interest. For more information on optimal antibiotic ranges to use in culture as well as the mechanisms of antibiotic action and resistance, see Table 9.1 and the review reference Davies and Smith, 1978.

C. Recommended Inoculation Procedures

1–100ml of Culture

Pick an isolated colony from a freshly streaked plate (less than 5 days old) and inoculate LB medium containing the required antibiotic(s). Incubation with shaking for 8–16 hours at 37°C before harvesting generally results in maximum yields of a high-copy-number plasmid. To achieve a highly reproducible yield, determine the cell density used in a typical experiment, and grow cultures to this density in each subsequent experiment. Typically, after overnight incubation, the absorbance of a tenfold dilution of the culture at a wavelength of 600nm (A_{600}) with a 1cm path length should range from 0.10–0.35.

100–1,000ml of Culture

Using a colony from a freshly streaked plate (less than 5 days old), inoculate 5–50ml of LB medium containing the required antibiotic(s). Grow this starter culture from 8 hours to overnight at 37°C. The following day, use this culture to inoculate the larger culture flask containing antibiotic-supplemented medium by diluting the starter culture between 100- to 500-fold (e.g., adding 10ml overnight culture to 1 liter medium). Incubate this secondary culture for 12–16 hours before harvesting cells. The A_{600} of a tenfold dilution of the culture should be 0.10–0.35. As with smaller cultures, to achieve a highly reproducible yield, determine the cell density used in a typical experiment and grow cultures to this density in each subsequent experiment.

Harvesting

When harvesting bacteria, follow the conditions outlined in either the Wizard® *Plus* SV Miniprep DNA Purification System or the PureYield™ Plasmid Midiprep System protocol. If the recommended centrifugation time or speed is exceeded, the pelleted cells may be more difficult to resuspend. Insufficient centrifugation time or speed may result in incomplete harvesting of cells and loss of starting material. Consult a centrifuge instruction manual for conversion of rpm to *g*-force. Once the bacteria are pelleted, this is a good stopping point in the purification process. Storing the pellet at –20°C results in little loss of plasmid DNA and may enhance lysis.

III. Factors That Affect Plasmid DNA Quality and Yield

A. Bacterial Strain Selection

The choice of host bacterial strain can have a significant impact on the quality and yield of DNA using any purification method. We recommend the use of host strains

Table 9.1. Antibiotic Mode of Action and Mechanism of Resistance.

Antibiotic	Mode of Action	Mechanism of Resistance	Working Concentration	Stock Solution
Ampicillin (Amp)	A derivative of penicillin that kills growing cells by interfering with bacterial cell wall synthesis.	The resistance gene (*bla*) specifies a periplasmic enzyme, β-lactamase, which cleaves the β-lactam ring of the antibiotic.	50–125µg/ml	50mg/ml in water
Chloramphenicol (Cm)	A bacteriostatic agent that interferes with bacterial protein synthesis by binding to the 50S subunit of ribosomes and preventing peptide bond formation.	The resistance gene (*cat*) specifies an acetyltransferase that acetylates, and thereby inactivates, the antibiotic.	20–170µg/ml	34mg/ml in ethanol
Hygromycin (Hygro)	A protein synthesis inhibitor that interferes with 80S ribosome translocation and causes mistranslation.	The resistance gene (*hph*) specifies a phosphotransferase that catalyzes the phosphorylation of the 4-hydroxyl group on the cyclitol ring (hyosamine), thereby producing 7′-O-phosphoryl-hygromycin B, which lacks biological activity both in vivo and in vitro.	20–200µg/ml	100mg/ml in water
Kanamycin (Kan)	A bactericidal agent that binds to 70S ribosomes and causes misreading of messenger RNA.	The resistance gene (*kan*) specifies an enzyme (aminoglycoside phosphotransferase) that modifies the antibiotic and prevents its interaction with ribosomes.	30µg/ml	50mg/m in water
Neomycin (Neo)	A bactericidal agent that blocks protein synthesis by binding to the prokaryotic 70S ribosomal subunit.	Expression of the bacterial APH (aminoglycoside phosphotransferase) gene (derived from Tn5).	50µg/ml	25mg/ml in water
Tetracycline (Tet)	A light-sensitive bacteriostatic agent that prevents bacterial protein synthesis by binding to the 30S subunit of ribosomes.	The resistance gene (*tet*) specifies a protein that modifies the bacterial membrane and prevents transport of the antibiotic into the cell.	10µg/ml in liquid culture; 12.5µg/ml in plates	12.5mg/ml in ethanol

such as DH5α™, JM109 (Cat.# L2001, L1001) and XL1-Blue, which contain mutations in the *endA* gene. *E. coli* strains that are listed as *endA*1 contain such mutations.

The *endA* gene encodes a 12kDa periplasmic protein called endonuclease I. This enzyme is a double-stranded DNase that can copurify with plasmid DNA, thus causing potential degradation. RNA acts as a competitive inhibitor and alters the endonuclease specificity from that of a double-stranded nucleolytic enzyme yielding seven-base oligonucleotides to a nickase that cleaves an average of one time per substrate (Lehman *et al.* 1962; Goebel and Helinski 1970). The function of endonuclease I is not fully understood, and strains bearing *endA*1 mutations have no obvious phenotype other than improved stability and yield of plasmid obtained from them.

The expression of endonuclease I has been characterized and was found to be dependent on bacterial growth phase (Shortman and Lehman, 1964). In this study, endonuclease I levels were found to be more than 300 times higher during exponential phase compared to stationary phase. In addition, media compositions that encouraged rapid growth (e.g., high glucose levels and addition of amino acids) resulted in high endonuclease I levels.

Strains that contain the wildtype endonuclease A (*endA*) gene can yield high-quality, undegraded plasmid DNA if special precautions are used to reduce the probability of nuclease contamination and plasmid degradation (Shortman and Lehman, 1964). Promega has performed a thorough investigation of methods at different points in the purification process to ensure the isolation of high-quality DNA from EndA+ (wildtype) bacterial strains. These include: 1) inclusion of an alkaline protease treatment step that degrades nucleases in the Wizard® *Plus* SV Minipreps DNA Purification System; 2) optimization of culture conditions to limit in vivo expression during bacterial growth; 3) heat inactivation during and after purification; 4) optimization of protocol conditions to limit binding of the nuclease to the resin and 5) post-purification methods to remove endonuclease. These methods and results are summarized in Schoenfeld *et al*. 1995 and the *Wizard®* Plus *SV Plasmid DNA Purification System Technical*

Bulletin . Information on genetic markers in bacterial strains can also be found in Ausubel *et al*. 1989 and Sambrook *et al*. 1989.

B. Plasmid Copy Number

One of the most critical factors affecting the yield of plasmid from a given system is the copy number of the plasmid. 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. Plasmids derived from pBR322 (Cat.# D1511) contain the ColE1 origin of replication from pMB1. This origin of replication is tightly controlled, resulting in approximately 25 copies of the plasmid per bacterial cell (low copy number). Plasmids derived from pUC contain a mutated version of the ColE1 origin of replication, which results in reduced replication control and approximately 200–700 plasmid copies per cell (high copy number).

Some plasmids contain the p15A origin of replication, which is considered a low-copy-number origin. The presence of the p15A origin of replication allows for replication of that particular plasmid in conjunction with a plasmid containing the ColE1 origin of replication. A compatibility group is defined as a set of plasmids whose members are unable to coexist in the same bacterial cell. They are incompatible because they cannot be distinguished from one another by the bacterial cell at a stage that is essential for plasmid maintenance. The introduction of a new origin, in the form of a second plasmid of the same compatibility group, mimics the result of replication of the resident plasmid. Thus, any further replication is prevented until after the two plasmids have been segregated to different cells to create the correct prereplication copy number (Lewin, 2004).

Most plasmids provided by Promega, including the pGEM® Vectors, are considered to be high-copy-number. The only exception is the pALTER®-MAX Vectors.

Some DNA sequences, when inserted into a particular vector, can lower the copy number of the plasmid. Furthermore, large DNA inserts can also reduce plasmid copy number. In many cases, the exact copy number of a particular construct will not be known. However, many of these plasmids are derived from a small number of commonly used parent constructs.

C. Appropriate Sample Size and Throughput

Depending on the volume of the bacterial culture, there are different isolation systems for your needs. For small-volume bacterial cultures of 0.6–3ml, use a system like the PureYield™ Plasmid Miniprep System, which gives a plasmid DNA yield of 1.5–7.5µg with an A_{260}/A_{280} ≥1.8 from a 0.6ml overnight bacterial culture with a total biomass ($O.D._{600}$ of culture × volume of culture in µl) of 1.3–8. For larger cultures with volumes ranging from 50–100ml, the PureYield™ Plasmid Midiprep System is a good choice. With this system, a 50ml culture of a high-copy-number plasmid with a total biomass of 100–200 $O.D._{600}$ units will yield 100–200µg of plasmid. The PureYield™ Plasmid Maxiprep System can isolate plasmid from 100–250ml of culture with yields up to 1mg of plasmid DNA with an A_{260}/A_{280} >1.7 from 250ml of overnight bacterial culture, transformed with a high-copy-number plasmid.

For high-throughput processing, systems based on a 96-well format can be performed manually with a vacuum manifold (e.g., Vac-Man® 96 Vacuum Manifold; Figure 9.3) using silica membrane technology such as the Wizard® SV 96 Plasmid DNA Purification System. Alternatively, an automated liquid-handling workstation can process multiwell plates with MagneSil® PMPs and a 96-well magnet (e.g., MagnaBot® 96 Magnetic Separation Device; Figure 9.4) using the Wizard® MagneSil® Plasmid Purification System. Yields for these systems using high-copy-number plasmid range from 3–5µg for the Wizard® SV 96 Plasmid DNA Purification System and up to 6µg for the Wizard® MagneSil® Plasmid Purification System. For more information on plasmid DNA automation, go to the Automated Methods web site.

Figure 9.3. The Vac-Man® 96 Vacuum Manifold. This 96-well vacuum manifold is used for processing SV 96 plates for plasmid, genomic and PCR product purification.

Figure 9.4. The MagnaBot® 96 Magnetic Separation Device. This 96-well magnet is used for capturing MagneSil® PMPs for DNA purification.

Smaller plasmid amounts are helpful for assessing the success of a cloning experiment by PCR or restriction digestion or for use in a coupled transcription/translation system like the TNT® Coupled Reticulocyte Lysate Systems (Cat.# L1170, L2080).

D. Biomass Processed

Optical density (O.D.) is the measure of how much light is blocked by the biomass of the bacterial culture in a path length of 1cm. The density of the culture is measured at a wavelength of 600nm and can have a great effect on plasmid isolation success. For example, the Wizard® SV 96 Plasmid Purification System has a maximum biomass recommendation of 4.0 $O.D._{600}$ to avoid clogging of the SV 96 Lysate Clearing Plate, so calculating the O.D. of the culture is necessary.

O.D./ml culture = 600nm absorbance reading × dilution factor

For O.D. measurement, a 1:10 dilution is typically used (e.g., 0.1ml culture in 0.9ml culture medium) to keep the reading in the range of 0.1–1.0, where the spectrophotometer is most accurate. For the example above, if the 1:10 dilution reading is 0.15, meaning that each milliliter of culture is 1.5 O.D., no more than 2.67ml culture can be processed (4 O.D. divided by 1.5 O.D./ml = 2.67ml). Exceeding the recommendations of the plasmid purification system may cause clogging or contamination of the system.

E. Plasmid Purification Method and Transfection

Many plasmid isolation systems indicate they are transfection-quality (e.g., the PureYield™ Plasmid Systems or the Wizard MagneSil Tfx™ System). This may be important, as some cultured cells are sensitive to the amount of endotoxin and other contaminants present in the plasmid preparation. Endotoxin is a lipopolysaccharide cell wall component of the outer membrane of Gram-negative bacteria (i.e., all *E. coli* strains) that can copurify with the plasmid DNA regardless of the purification system used. The amount of this molecule varies by bacterial strain, growth conditions and isolation method. In the PureYield™ Plasmid Systems, there is an Endotoxin Removal Wash solution that reduces the amount of endotoxin, proteins and other contaminants eluted with the plasmid DNA. For many common cell lines like 293 and HeLa, the amount of endotoxin present for routine transfections has a minimal effect on the efficiency of transfection (Butash *et al.* 2000).

Many factors influence transfection efficiency and/or cellular death including the type and amount of transfection reagent, cell confluency, DNA amount and incubation time with the reagent:DNA complex. Each of these factors will need to be optimized for each cell line-plasmid combination transfected in order to minimize cell death and maximize transfection efficiency. In our experience, transfection experiments with HeLa and NIH/3T3 cells demonstrated that there was little DNA preparation difference with four different plasmid isolation systems used (based on silica membrane, anion exchange and silica resin) when comparing efficiencies using the same transfection reagent. However, the transfection reagent used for DNA uptake had a significant effect on transfection efficiency and cell death. For general considerations for optimization, consult the Protocols and Applications Guide chapter on Transfection.

Additional Resources about Endotoxin

Online Tools

eNotes FAQspeak: What methods exist to remove endotoxin contamination of plasmid DNA?

IV. Plasmid DNA Purification Systems

A. Silica Column-Based Systems

PureYield™ Plasmid Systems

The PureYield™ Plasmid Systems isolates high-quality plasmid DNA for use in eukaryotic transfection and in vitro expression experiments. The unique reagents, proprietary matrix and silica membrane-based design of the PureYield™ Systems greatly reduces the amount of time spent on purification compared to silica resin or other membrane-column methods. While the unique Endotoxin Removal Wash removes protein, RNA and endotoxin contaminants from the bound DNA, the Column Wash Solution followed by membrane drying eliminates salts and alcohols from the plasmid prep, allowing the purified plasmid to be used for highly sensitive applications such as transfection, in vitro transcription and coupled in vitro transcription/translation. An additional benefit is that the same degree of purification can be obtained even with low-copy-number plasmids. Although the system works best for plasmids less than 10kb, plasmids as large as 18kb have been purified.

The unique combination of reagents in the PureYield™ Plasmid Miniprep System (Cat.# A1222, A1223) purifies plasmid either directly from 0.6ml of bacterial culture or cell pellets from up to 3ml of cell culture (Figure 9.5). A typical overnight culture is grown in LB medium for 16–18 hours. If the cell pellet method is chosen, cells are harvested by centrifugation, then resuspended in 600µl of TE buffer or water. Purifying DNA directly from bacterial culture takes less than 10 minutes with elution volumes as low as 30µl, resulting in more concentrated plasmid DNA. The low elution volume is possible because the column design retains virtually no buffer. A transfection comparison of plasmid isolated using the PureYield™ Plasmid Miniprep in various cell lines can be found in Figure 9.6.

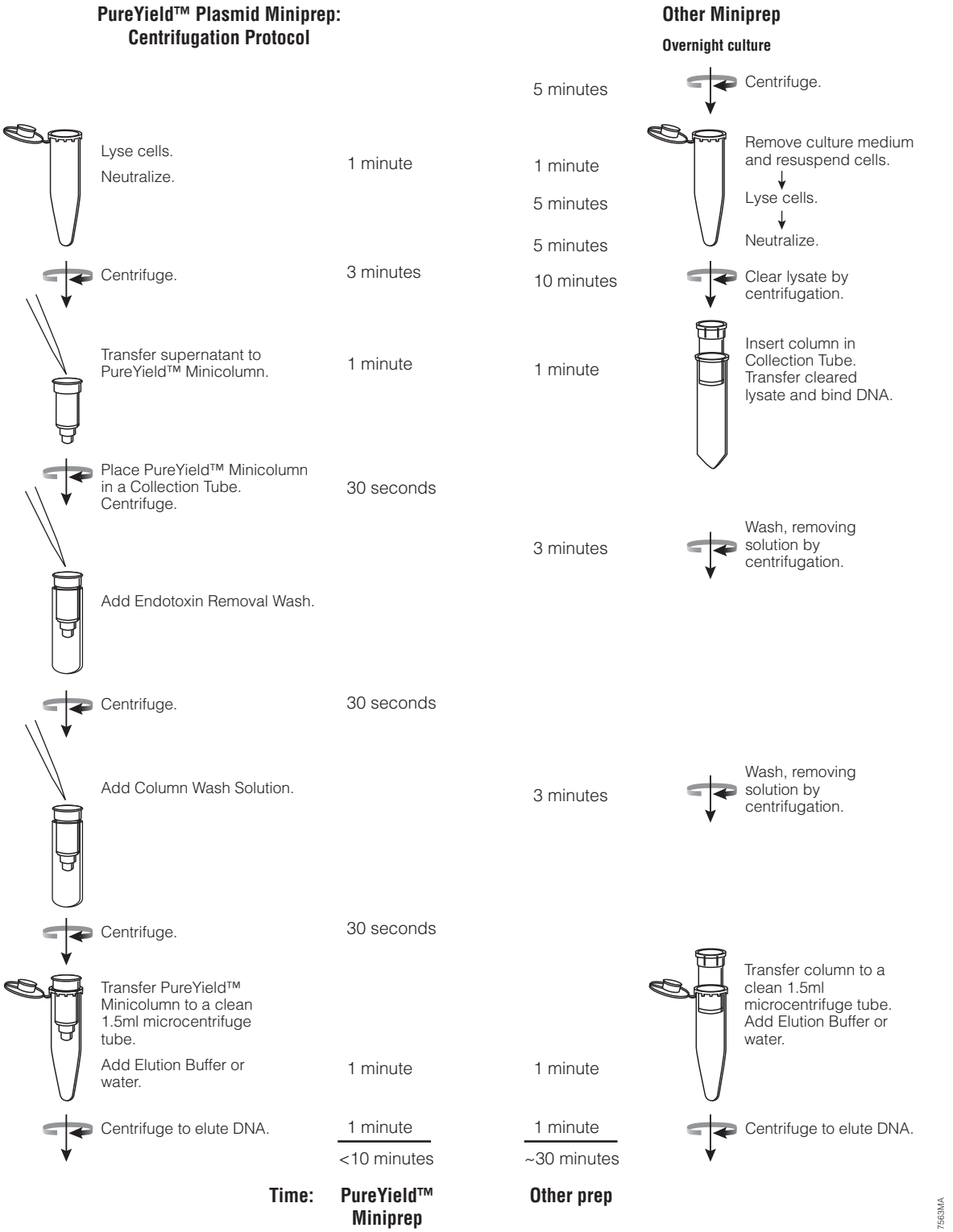

Figure 9.5. The PureYield™ Plasmid Miniprep System yields transfection-quality DNA in approximately 10 minutes.

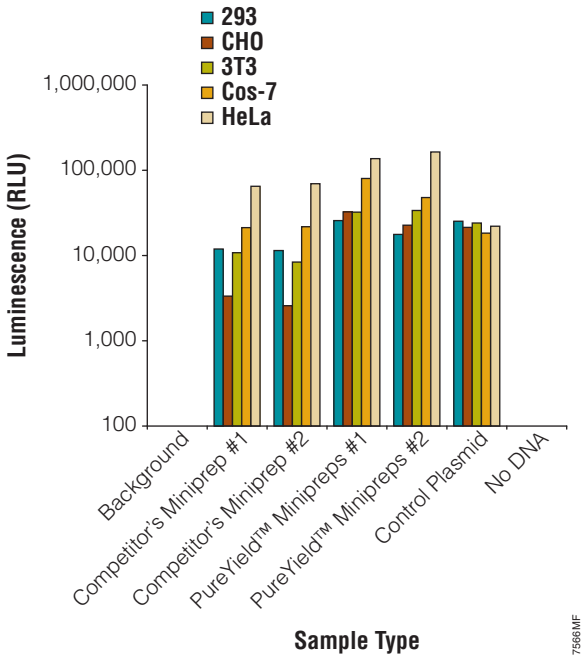

Figure 9.6. Plasmid DNA prepared using the PureYield™ Plasmid Miniprep System consistently works well in transfection experiments. The pGL4.13[*luc2*/SV40] Vector (Cat.# E6681) was prepared using a competing system or the PureYield™ Plasmid Miniprep System. Five different commonly used mammalian cell lines were transfected with the plasmid, and transfection efficiency was assessed by measuring the luciferase activity using the ONE-Glo™ Luciferase Assay System (Cat.# E6110; n = 6).

To isolate larger quantities of high-quality plasmid DNA, use the the PureYield™ Plasmid Midiprep System (Cat.# A2492, A2495). This plasmid midiprep system is designed to purify 100–200µg of plasmid DNA with an A_{260}/A_{280} >1.7 from a 50ml overnight culture of bacteria in as little as 30 minutes, if the culture is grown with a high-copy-number plasmid, reaching a total optical density ($O.D._{600}$ of culture × volume of culture) of 100–200. Larger volumes up to 250ml can be processed, but require greater volumes of solutions than that supplied with the PureYield™ Plasmid Midiprep System.

The PureYield™ Plasmid Midiprep System is designed for purification by vacuum using a manifold such as the Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231), but there are alternative protocols that use all centrifugation or both vacuum and centrifugation. All protocols generate high-quality purified plasmid DNA. A swinging-bucket tabletop centrifuge or the Eluator™ Vacuum Elution Device (Cat.# A1071) is required for the final elution step regardless of the protocol chosen.

For a larger plasmid isolation capacity, the PureYield™ Plasmid Maxiprep System (Cat.# A2392, A2393) is able to purify up to 1mg of plasmid DNA with an A_{260}/A_{280} >1.7 from 250ml of overnight bacterial culture, transformed with a high-copy-number plasmid in approximately 60 minutes. As with the midiprep system, the protocol requires a vacuum pump and manifold (e.g., the Vac-Man® Laboratory Vacuum Manifold, 20-sample [Cat.# A7231]), a centrifuge with a fixed-angle rotor for lysate clearing and either a tabletop centrifuge with a swinging bucket rotor or the Eluator™ Vacuum Elution Device (Cat.# A1071) for the final elution step.

Additional Resources for the PureYield™ Plasmid Systems

Technical Bulletins and Manuals

TB374	*PureYield™ Plasmid Miniprep System Technical Bulletin*
TM253	*PureYield™ Plasmid Midiprep System Technical Manual*
TM280	*PureYield™ Plasmid Maxiprep System Technical Manual*

Promega Publications

Transfection-quality plasmid DNA in as little as ten minutes using the PureYield™ Plasmid Miniprep System

Fast, reliable, high-quality midiprep plasmid purification using the PureYield™ Plasmid Midiprep System

Remove the high-speed spin from PureYield™ Plasmid Preps

Online Tools

PureYield™ Plasmid Miniprep System Video Podcast Protocol

Citations

Gubaev, A., Hilbert, M. and Klostermeier, D. (2009) The DNA-gate of *Bacillus subtilis* gyrase is predominantly in the closed conformation during the DNA supercoiling reaction. *Proc. Natl. Acad. Sci. USA* **106**, 13278–83.

These authors examined conformation of DNA bound to the DNA-gate of *Bacillus subtiliss* gyrase as well as the conformation of the DNA-gate itself. Negatively supercoiled pUC18 plasmid was purified using the PureYield™ Plasmid Midiprep System and used in single-molecule FRET experiments.

PubMed Number: 19666507

Wizard® SV Column-Based Systems

High-quality, purified plasmids are used for automated fluorescent DNA sequencing as well as for other standard molecular biology techniques including restriction enzyme digestion and PCR. Whether you are isolating a few samples or a 96-well plate, there is a silica membrane-based system available.

For manual purification, the Wizard® *Plus* SV Minipreps DNA Purification System (Cat.# A1330, A1340, A1460, A1470) provides a simple and reliable method for rapid isolation of plasmid DNA using a column-based silica membrane (see Figure 9.7 for overview of method). The entire miniprep procedure can be completed in 30 minutes or less, depending on the number of samples processed. The plasmid DNA from 1–10ml of overnight *E. coli* culture can be purified by using either a vacuum manifold like the Vac-Man® Laboratory Vacuum Manifold (process up to 20 samples) or a microcentrifuge (number of samples processed depends on rotor size). This system can be used to isolate any plasmid hosted in *E. coli* but works most efficiently when the plasmid is less than 20,000bp in size. 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 used as discussed in Factors that Affect Plasmid DNA Quality and Yield. The DNA binding capacity of the SV membrane is up to 20µg of high-quality plasmid DNA. An alkaline protease treatment step in the isolation procedure improves plasmid quality by digesting proteins like endonuclease I.

To process more samples at once, consider using the 96-well format of the Wizard® SV 96 (Cat.# A2250, A2255) and SV 9600 (Cat.# A2258) Plasmid DNA Purification Systems. These high-throughput systems provide a simple and reliable method for the rapid isolation of plasmid DNA using a silica-membrane 96-well plate. A single plate can be processed in 60 minutes or less. The Wizard® SV 96 and SV 9600 Systems are designed for use either in a manual format or with automated instruments. Methods to support automated plasmid DNA purification can be found online at: Automated Methods.

To use the Wizard® SV 96 and SV 9600 Systems, a vacuum manifold [e.g., Vac-Man® 96 Vacuum Manifold (Cat.# A2291)] and a vacuum pump capable of generating 15–20 inches of mercury or equivalent with a vacuum trap is needed for sample processing. Figure 9.3 shows the Vac-Man® 96 Manifold set up for purification.

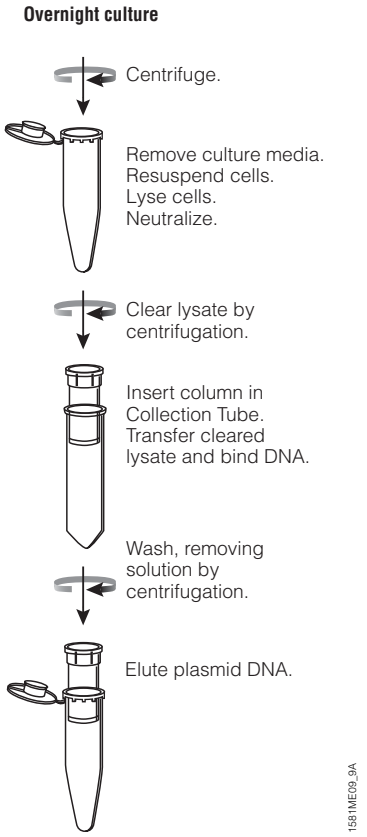

Figure 9.7. Overview of the Wizard® *Plus* SV Minipreps DNA Purification System centrifugation protocol.

Additional Resources for the Wizard® SV Column-Based Plasmid DNA Systems

Technical Bulletins and Manuals

TB225	*Wizard® Plus SV Minipreps DNA Purification System Technical Bulletin*
TB272	*Wizard® SV 96 Plasmid DNA Purification System Technical Bulletin*
TB292	*Wizard® SV 9600 Plasmid DNA Purification System Technical Bulletin*

Promega Publications

Wizard® *Plus* SV Minipreps DNA Purification System: The next generation in miniprep purification

Isolation of DNA from *Bacillus subtilis* using the Wizard® *Plus* SV Miniprep DNA Purification System

A simple and reproducible method to isolate plasmid DNA from yeast after a two-hybrid screening

Wizard® SV 96 Plasmid DNA Purification System: High quality plasmid DNA for use in fluorescent sequencing methods

Citations

Nocchi, L. *et al.* (2011) Thrombomodulin is silenced in malignant mesothelioma by a poly(ADP-ribose) polymerase-1-mediated epigenetic mechanism. *J. Biol. Chem.* **286**, 19478–88.

Thrombomodulin (TM) expression was examined by isolating genomic DNA from biopies of human malignant mesothelioma and normal mesothelial tissue, and cultured cell lines with or without PARP1 silencing treated with 5-aza-2′-deoxycytidine and trichostatin alone or in combination and then subjected to biosulfide modification. To analyze methylation of TM, a CpG island in the promoter, 5′ UTR and an exon region containing 44 CpG dinucleotides were PCR amplified, cloned into the pGEM®-T Easy Vector, transformed and positive clones selected using IPTG/X-Gal and analyzed by PCR. Colonies were cultured, the plasmids isolated using the Wizard® *Plus* SV Minipreps DNA Purification System then 10 clones from each sample type were sequenced.

PubMed Number: 21489980

Stressmann, F.A. *et al.* (2011) Analysis of the bacterial communities present in lungs of patients with cystic fibrosis from American and British centers. *J. Clin. Microbiol.* **49**, 281–91.

Sputum samples were collected from cystic fibrosis patients and 16S rRNA sequences amplified by PCR. These products were cloned into a T vector, transformed into competent cells and the resulting colonies grown in 2ml LB broth in 96-deep-well plate for 20 hours. Of this culture, 1.9ml was pelleted and the clones isolated using the Wizard® SV 96 Plasmid Purification System. The purified plasmid DNA was subjected to agarose gel electrophoresis and sequenced.

PubMed Number: 21068277

B. Paramagnetic Particle-Based Systems

For automated, high-throughput plasmid purification, use our MagneSil® paramagnetic particle (PMP)-based systems that yield purified plasmid, which can be used directly for automated fluorescent DNA sequencing, as well as for other standard molecular biology techniques including restriction enzyme digestion and PCR. The Wizard® MagneSil® Plasmid DNA Purification System (Cat.# A1630, A1631, A1635) provides a simple and reliable method for the rapid isolation of plasmid DNA in a multiwell format. The purification procedure uses MagneSil® PMPs for lysate clearing as well as DNA capture, circumventing the need for centrifugation or vacuum filtration. The MagnaBot® 96 Magnetic Separation Device (Cat.# V8151; Figure 9.4) is needed for plasmid purification. The protocol also requires a multiwell plate shaker. This protocol has been optimized using the Micro Mix 5 shaker on the Beckman Coulter Biomek® 2000. To see workstations on which the Wizard® MagneSil® Plasmid Purification System has been automated, visit the Automated Methods page on our web site.

The Wizard MagneSil Tfx™ System (Cat.# A2380, A2381) provides a simple and reliable method for the rapid isolation of transfection-quality plasmid DNA in a multiwell format. DNA purified with using this system is greatly reduced in chemical contaminants as well as RNA, protein, and endotoxin, providing high-quality plasmid DNA suitable for transfection, as well as for other standard molecular biology techniques. Like the Wizard® MagneSil® Plasmid DNA Purification System, the Wizard MagneSil Tfx™ System uses MagneSil® PMPs for lysate clearing as well as DNA capture. In addition, a proprietary paramagnetic endotoxin removal resin reduces the level of endotoxin present in the purified plasmid DNA. By avoiding the need for centrifugation or vacuum filtration, DNA purification with the Wizard MagneSil Tfx™ System can be completely automated, requiring the MagnaBot® 96 Magnetic Separation Device (Cat.# V8151) and Heat Transfer Block (Cat.# Z3271) for the protocol.

An automated method for the Wizard MagneSil Tfx™ System has been developed for the Biomek® FX robotic workstation. The procedure requires no manual intervention and takes approximately 45 minutes to process a single 96-well plate. This automated protocol also can be adapted to other robotic workstations. Visit our web site for information on an automated protocol for your platform. An Automation Support Team member will contact you regarding a method for use with your particular system.

Additional Resources for Paramagnetic Particle-Based Systems

Technical Bulletins and Manuals

TB286	*Wizard® MagneSil® Plasmid Purification System Technical Bulletin*
TB314	*Wizard MagneSil Tfx™ System Technical Bulletin*

Promega Publications

Wizard MagneSil Tfx™ System for the purification of transfection-grade DNA

Automated plasmid purification using MagneSil® Paramagnetic Particles

Isolation of genomic DNA from agricultural bacteria using the Wizard® MagneSil® Plasmid Purification System

Citations

Helms, M.W. *et al.* (2009) TOB1 is regulated by EGF-dependent HER2 and EGFR signaling, is highly phosphorylated, and indicates poor prognosis in node-negative breast cancer. *Cancer Res.* **69**, 5049–56.

To identify molecules that affect metastasis signaling pathways downstream of HER2-Y1248 phosphorylation, suppression subtractive hybridization assays (SSH) were performed using MDA-MB-468 cells overexpressing HER2 and control MDA-MB-468 cells expressing HER2 without the Y1248 phosphorylation site. Reactions were cloned using a T-vector system, transformed and plated. Positive clones from each assay were selected and grown overnight in 2ml deep-well plates. The Wizard® Magnesil® Plasmid Purification System was used to isolate plasmids for BigDye™ sequencing.

PubMed Number: 19491269

V. Plasmid DNA Purification Protocol Featuring the PureYield™ Plasmid Midiprep System

Materials Required:

- PureYield™ Plasmid Midiprep System (Cat.# A2492; 25 preps)
- Eluator™ Vacuum Elution Device (Cat.# A1071) **or** swinging bucket rotor
- isopropanol
- ethanol, 95%
- tabletop centrifuge at room temperature (22–25°C)
- 50ml disposable plastic screw-cap tubes (e.g., Corning® or Falcon® brand)
- high-speed centrifuge capable of at least 15,000 × *g* and appropriate tubes
- vacuum pump, single- or double-stage, producing a pressure of approx. 650mm Hg
- vacuum manifold (e.g., Vac-Man® Laboratory Vacuum Manifold)

Endotoxin Removal Wash and Column Wash must be prepared as described below before lysing cells and purifying DNA (close cap tightly after additions):

Endotoxin Removal Wash
25 preps system: Add 57ml of isopropanol to the Endotoxin Removal Wash bottle.

Column Wash
25 preps system: Add 350ml of 95% ethanol to the Column Wash bottle.

Regardless of the purification method used, **keep these important protocol points in mind:**

- To differentiate the PureYield™ Clearing and PureYield™ Binding columns, note that the clearing columns are blue, while the binding columns are white.
- Perform all purification steps at room temperature (22–25°C).
- The concentration of the plasmid is dependent on copy number and elution volume. If a higher concentration is desired for subsequent applications, perform an ethanol precipitation after plasmid isolation. Add 1/10 volume 3M sodium acetate (pH 5.2), 2.5 volumes 95% ethanol. Place on ice for 15 minutes. Pellet the DNA by centrifugation at 14,000 × *g* for 10 minutes in a microcentrifuge. Wash pellet with 70% ethanol and centrifuge at 14,000 × *g* for 10 minutes. Resuspend DNA pellet in desired volume of nuclease-free water.

A. Standard DNA Purification Protocol

1. Grow 50–250ml of transformed *E. coli* bacterial cell culture overnight (16–21 hours) at optimal culture conditions.

   Note: This protocol is optimized for 50–250ml of culture at an $O.D._{600}$ = 2–4.

2. Pellet the cells using centrifugation at 5,000 × *g* for 10 minutes and discard supernatant. Drain tubes on a paper towel to remove excess media.

3. Resuspend pellet in Cell Resuspension Solution (see Table 9.2 for appropriate volumes).

Table 9.2. Solution Volumes Required to Generate Lysate.

	Bacterial Culture Volume	
Solution Volume	**50–100ml**	**101–250ml**
Cell Resuspension	3ml	6ml[1]
Cell Lysis Solution	3ml	6ml[1]
Neutralization Solution	5ml	10ml[1]

[1] Additional solutions will need to be purchased or made for processing 101–250ml culture volumes.

4. Add Cell Lysis Solution. Invert 3–5 times to mix. Incubate 3 minutes at room temperature (22–25°C).

5. Add Neutralization Solution. Invert 5–10 times to mix.

6. Centrifuge lysate at 15,000 × *g* for 15 minutes at room temperature.

7. Assemble a column stack by placing a blue PureYield™ Clearing Column into the top of a white PureYield™ Binding Column. Place the assembled column stack onto a vacuum manifold as shown in Figure 9.8.

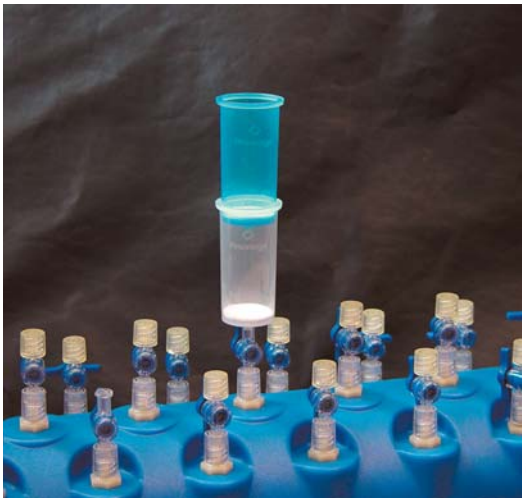

Figure 9.8. The PureYield™ Vacuum Purification Configuration. This image shows a blue PureYield™ Clearing Column nested on top of a white PureYield™ Binding Column. Both columns are sitting on the vacuum manifold port.

8. Pour the supernatant into the clearing column. Apply maximum vacuum, continuing until all the liquid has passed through both the clearing and binding columns.

9. **Slowly release** the vacuum from the filtration device before proceeding. Remove the clearing column, leaving the binding column on the vacuum manifold.

   Note: If the binding membrane has been dislodged from the bottom of the column, tap it back into place using a sterile pipette tip.

Wash

10. Add 5.0ml of Endotoxin Removal Wash to the binding column, and allow the vacuum to pull the solution through the column.

11. Add 20ml of Column Wash Solution to the binding column, and allow the vacuum to draw the solution through the column.

12. Dry the membrane by applying a vacuum for 30–60 seconds. Repeat this step if the top of the binding membrane appears wet or there is a detectable ethanol odor.

13. Remove the binding column from the vacuum manifold, and tap it on a paper towel to remove excess ethanol.

Elute by Vacuum (alternatively, see Elute by Centrifugation below)

14. Place a 1.5ml microcentrifuge tube into the base of the Eluator™ Vacuum Elution Device, securing the tube cap as shown in Figure 9.9, Panel A.

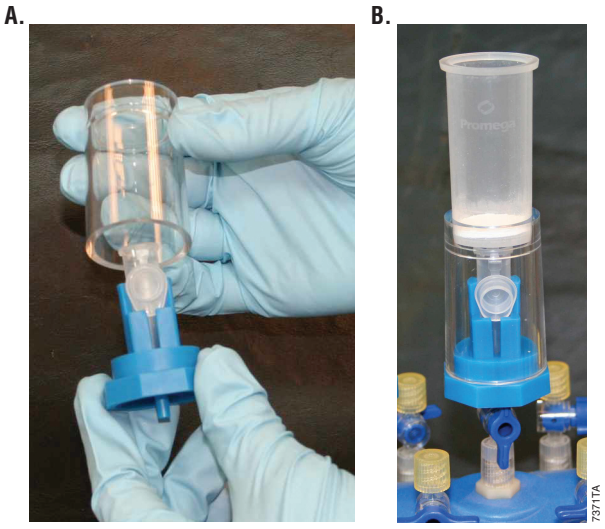

Figure 9.9. The Eluator™ Vacuum Elution Device for elution by vacuum. Panel A. A 1.5ml microcentrifuge tube is placed into the base of the Eluator™ Device, and the tube cap is secured in an open position, as shown. **Panel B.** The Eluator™ Vacuum Elution Device assembly, including the binding column, on a vacuum manifold.

15. Assemble the Eluator™ Vacuum Elution Device, and insert the DNA binding column into the device, making sure that the column is fully seated on the collar.

16. Place the elution device assembly, including the binding column, onto a vacuum manifold (Figure 9.9, Panel B).

17. Add 400–600µl of Nuclease-Free Water to the DNA binding membrane in the binding column. Wait for 1 minute. Apply maximum vacuum for 1 minute or until all liquid has passed through the column.

18. Remove the microcentrifuge tube and save for DNA quantitation and gel analysis.

Elute by Centrifugation

19. Place the binding column into a new 50ml disposable plastic tube.

20. Add 600µl of Nuclease-Free Water to the DNA binding membrane in the binding column. Wait for 1 minute. Centrifuge the binding column at 1,500–2,000 × *g* for 5 minutes using a **swinging bucket rotor**, and collect the filtrate.

    Note: Do not cap the 50ml tube during centrifugation.

For complete protocol information, see the *PureYield™ Plasmid Midiprep System Technical Manual* #TM253 .

VI. Genomic DNA Isolation Systems

Purified genomic DNA is necessary for further analysis of disease states, single nucleotide polymorphisms (SNPs) and for many other multiplex and real-time PCR applications. Many methods exist for isolation of chromosomal DNA, and Promega has genomic purification systems that are both general (able to isolate from many source materials) or specialized (primarily used for one source type). The source types range from bacteria to humans and can encompass tissues from blood to muscle and from leaf to seed.

A. Solution-Based System

The Wizard® Genomic DNA Purification Kit (Cat.# A1120, A1125, A1620) is both a versatile and scalable system for isolating genomic DNA. With this system alone, chromosomal DNA can be isolated from whole blood (Walker *et al*. 2003), plant leaf (Zhang *et al*. 2004), Gram-positive (van Schaik *et al*. 2004) and Gram-negative bacteria (Flashner *et al*. 2004), mouse tail (Lee *et al*. 2005) and yeast (Martinez *et al*. 2004). Additional sample types like fungus (Ahmed *et al*. 2003), infected frog tissues embedded in paraffin (Pereira *et al*. 2005), saliva (Cox *et al*. 2004) and flour beetles (Lorenzen *et al*. 2002) have also been used successfully with the Wizard® Genomic DNA Purification Kit. Not only is this genomic purification system successful with many sample types, it is also easily scaled for the quantity of starting material by adjusting reagent volumes to accommodate your needs. Additional references for the Wizard® Genomic DNA Purification Kit or any of the Promega DNA isolation systems can be found on our Citations web site.

Additional Resources for the Wizard® Genomic DNA Purification Kit

Technical Bulletins and Manuals

TM050 *Wizard® Genomic DNA Purification Kit Technical Manual*

Promega Publications

Isolation of Spirochete DNA using the Wizard® Genomic DNA Purification Kit

Wizard® Genomic DNA Purification Kit provides high-quality genomic DNA template for molecular phylogenetic studies on Copepod crustaceans

Wizard® Genomic DNA Purification Kit and the isolation of plant genomic DNA

Isolation of genomic DNA from small volumes of whole blood using the Wizard® Genomic DNA Purification Kit

Extraction and amplification of DNA from an ancient moss

Online Tools

Sample Types Processed with the Wizard® Genomic DNA Purification Kit

Citations

Nair, N.U. and Zhao, H. (2009) Mutagenic inverted repeat assisted genome engineering (MIRAGE). *Nucleic Acids Res.* **37**, e9.

In this paper, the researchers describe and demonstrate a new method for creating precise genome modifications in *Saccharomyces cerevisiae*. The mutagenic inverted repeat assisted genome engineering (MIRAGE) was tested in *S. cerevisiae* W303a by deleting *gal7* as well as point and frameshift mutations. Genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit, amplified and modifications verified by gel analysis or DNA sequencing.

PubMed Number: 19050015

Gill, M.B. *et al.* (2009) Murid herpesvirus-4 lacking thymidine kinase reveals route-dependent requirements for host colonization. *J. Gen. Virol.* **90**, 1461–70.

The authors examined the role of thymidine kinase (TK) in establishing a herpesvirus infection via the upper respiratory tract. DNA was purified from ex vivo organs of female BALB/c mice infected with a murid herpesvirus-4 (MuHV-4) TK knockout using the Wizard® Genomic DNA Purification Kit. Real-time PCR was used with 50–80ng of purified DNA to determine viral load of the animals.

PubMed Number: 19264614

B. Silica Column-Based Systems

The technology for these genomic DNA purification systems is based on binding of the DNA to silica under high-salt conditions. The key to isolating any nucleic acid with silica is the presence of a chaotropic salt like guanidine hydrochloride. Chaotropic salt present in high quantities is able to disrupt cells, deactivate nucleases and allow nucleic acid to bind to silica. Once the genomic DNA is bound to the silica membrane, the nucleic acid is washed with a salt/ethanol solution. These washes remove contaminating proteins, lipopolysaccharides and small RNAs to increase purity while keeping the DNA bound to the silica membrane column used with the Wizard® SV Genomic DNA Purification Systems. Once the washes are finished, the genomic DNA is eluted under low-salt conditions using either nuclease-free water or TE buffer.

For the single-column isolation, the Wizard® SV Genomic DNA Purification System (Cat.# A2360, A2361) provides a fast, simple technique for the preparation of purified and intact DNA from mouse tails, tissues and cultured cells in as little as 20 minutes, depending on the number of samples processed (up to 24 by centrifugation, depending on the rotor size, or up to 20 by vacuum). A vacuum manifold or a microcentrifuge is used for sample processing. With some modifications, whole blood can also be used with this isolation system (Promega Corporation, 2002). This is a silica membrane-based system, meaning there are limitations to the amount of material that can be loaded onto a single SV column; up to 20mg of tissue (mouse tail or animal tissue) or between 1×10^4 and 5×10^6 tissue culture cells can be processed per purification. With more sample, the prepared lysate may need to be split among two or more columns to avoid clogging the column.

The genomic DNA isolated with the Wizard® SV Genomic DNA Purification System is of high quality and serves as an excellent template for agarose gel analysis, restriction enzyme digestion and PCR analysis as seen in Figure 9.10. Table 9.3 provides typical yields of genomic DNA purified from a variety of sources.

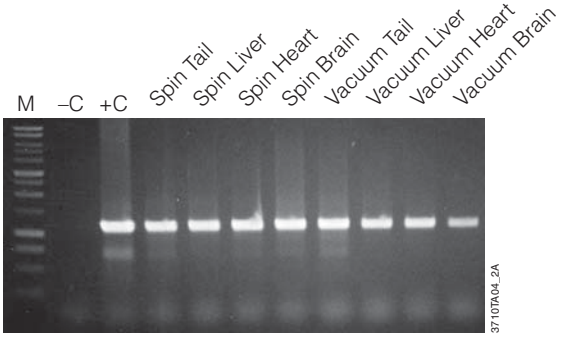

Figure 9.10. Amplification of genomic DNA isolated from various tissue sources using the Wizard® SV Genomic DNA Purification System. One microliter of purified genomic DNA was amplified using PCR Master Mix (Cat.# M7502) and mouse-specific IL-1β primers (1.2kb product). Reactions with Mouse Genomic DNA (Cat.# G3091; +C) and without DNA (–C) were performed as positive and negative controls, respectively. Thermal cycling conditions were: one cycle of 3 minutes at 95°C; followed by 30 cycles of: 95°C for 30 seconds, 60°C for 1 minute, 70°C for 1 minute and 30 seconds; final extension at 70°C for 7 minutes; 4°C soak. All lanes contained 10µl of reaction product separated on a 1% agarose gel. PCR products were visualized by ethidium bromide staining. "Spin" and "Vacuum" designations indicate the protocol used for genomic DNA isolation.

Table 9.3. Typical Genomic DNA Yield From Various Tissues using the Wizard® SV Genomic DNA Purification System.

Sample	Amount	Average Yield
Tail Clipping	20mg	20µg
Liver	20mg	15µg
Heart	20mg	10µg
Brain	20mg	6µg
CHO cells	1×10^6	5µg
NIH/3T3 cells	1×10^6	9µg
293 cells	1×10^6	8µg

Researchers have used this simple and rapid system for many additional sample types and applications including mosquitoes (Stump *et al*. 2005), mammary stem cells followed by STR analysis (Dontu *et al*. 2003), *Bacillus subtilis* (Park *et al*. 2004), *Escherichia coli* (Teresa Pellicer *et al*. 2003), the larval form of the *Schistosoma mansoni* parasite (Smith *et al*. 2004) and viral DNA from Kaposi's sarcoma herpes virus-infected BC3 cells (Ohsaki *et al*. 2004).

For high-throughput, 96-well isolation, the Wizard® SV 96 Genomic DNA Purification System (Cat.# A2370, A2371) is available. Amplifiable genomic DNA can be isolated from up to 5×10^6 cells per prep, from 20mg of tissue or from up to 1.2cm of a mouse tail tip without centrifugation of the lysate prior to purification. This multiwell system requires a vacuum manifold (Vac-Man® 96 Vacuum Manifold) and a vacuum pump capable of generating 15–20 inches of mercury or the equivalent. Genomic DNA was isolated from three different source types then used in a monoplex PCR and run on an agarose gel as shown in Figure 9.11. Figure 9.12 compares the yield from the three Wizard® SV Genomic DNA purification methods (96-well plate, vacuum and centrifugation).

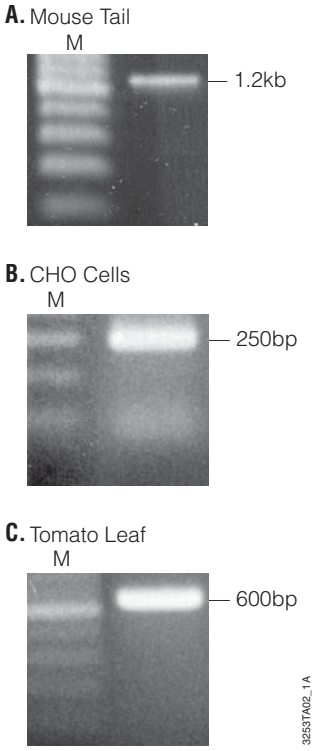

Figure 9.11. Agarose gel electrophoresis of PCR products amplified from 1µl of mouse tail, CHO cells and tomato leaf sample genomic DNA isolated using the Wizard® SV 96 Genomic DNA Purification System. A total of 10µl of PCR product is visualized on a 1.5% agarose gel stained with ethidium bromide. **Panel A.** IL-1β (1.2kb) amplified from mouse tail. **Panel B.** β-actin (250bp) amplified from CHO cells. **Panel C.** Chloroplast DNA (600bp) amplified from tomato leaf. Lane M, 1kb DNA Ladder (Cat.# G5711).

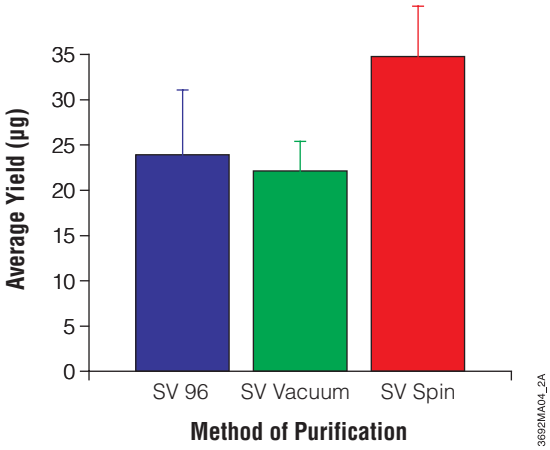

Figure 9.12. Comparison of DNA yields using the Wizard® SV and SV 96 Genomic DNA Purification Systems. Average yield of genomic DNA in micrograms purified from 20mg mouse tail clippings. The average A_{260}/A_{280} ratios are: SV 96, 1.7 ± 0.08; SV vacuum method, 1.7 ± 0.14; SV spin method, 1.7 ± 0.14.

Additional Resources for the Silica Column-Based Systems

Technical Bulletins and Manuals

TB302 *Wizard® SV Genomic DNA Purification System Technical Bulletin*

TB303 *Wizard® SV 96 Genomic DNA Purification System Technical Bulletin*

Promega Publications

Introducing the Wizard® SV and SV 96 Genomic DNA Purification Systems

Automated isolation of genomic DNA using Promega's DNA binding plates on the Beckman Biomek® 2000

A modified Wizard® SV Genomic DNA Purification System protocol to purify genomic DNA from shed reptile skin

Online Tools

Sample Types Processed with the Wizard® SV Genomic DNA Purification System

Citations

Iwuchukwu, O.F. *et al.* (2009) Characterizing the effects of common UDP glucuronosyltransferase (UGT) 1A6 and UGT1A1 polymorphisms on cis- and trans-resveratrol glucuronidation. *Drug Metab. Dispos.* **37**, 1726–32.

This study examined the genotype-phenotype correlation of the two major UGT isoforms, UGT1A1 and UGT1A6, involved in resveratrol metabolism. Genomic DNA was isolated from 30mg human liver tissue samples (normal and metastatic) using the Wizard® SV Genomic DNA Purification System. The purified DNA was eluted with 65°C water and 200–400ng of eluted DNA was used in a PCR-RFLP UGT1A6 genotyping assay. Amplification was carried out using PCR Master Mix in a final volume of 50µl, and the amplimers digested with appropriate restriction enzymes.

PubMed Number: 19406951

Meng, Y. *et al.* (2009) The silkworm mutant *lemon* (*lemon lethal*) is a potential insect model for human sepiapterin reductase deficiency. *J. Biol. Chem.* **284**, 11698–705.

The human sepiapterin reductase (SPR) gene has been mapped at the PARK3 locus, which is related to the onset of Parkinson disease. The silkworm *Bombyx mori* body color mutant *lemon* (*lem*) has been associated with a lack of SPR activity; *lem lethal* is a homozygous lethal allele of *lem*. Genetic linkage analysis was performed with normal silkworm strain p50T, *lem* strain l70, and *lem* ^{l} strain a65 to more closely examine the relationship with SPR. DNA from the F1 and F2 crosses were isolated using the Wizard® SV 96 Genomic DNA Purification System and the genome sequenced.

PubMed Number: 19246455

C. Cellulose Column-Based Systems

We offer two different ReliaPrep™ gDNA Miniprep Systems that purify genomic DNA using a cellulose column-based method: ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081, A5082) and ReliaPrep™ gDNA Tissue Miniprep System (Cat.# A2051, A2052). Both are ready-to-use systems that obtain intact genomic DNA without using ethanol washes or precipitations. The the ReliaPrep™ Blood gDNA Miniprep System processes 200µl of blood or body fluid, either fresh or frozen, in less than 40 minutes, purifying 4–10µg from blood, depending on the white blood cell count. Up to 25mg of tissue, a buccal (cheek) swab or a 1cm mouse tail can be processed with the ReliaPrep™ gDNA Tissue Miniprep System and the eluted DNA recovered in 30 minutes or less. The purified DNA can be eluted in as little as 50µl and is suitable for use in downstream applications such as RT-qPCR.

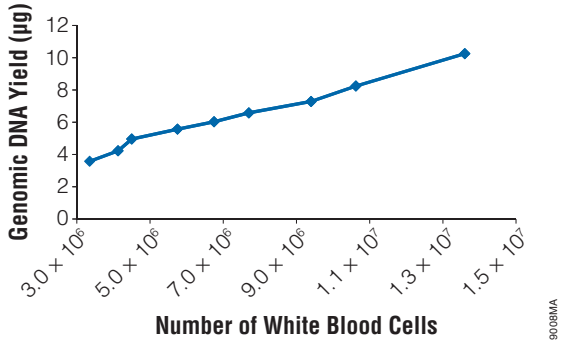

Figure 9.13. The yield of genomic DNA from the ReliaPrep™ Blood gDNA Miniprep System varies with white blood cell count. Whole blood was obtained from several individuals, and white cell counts were determined using a hemocytometer. Two hundred microliters of blood was used for genomic DNA purification (n = 3 or 4), and the amount of isolated gDNA was quantitated by absorbance spectroscopy.

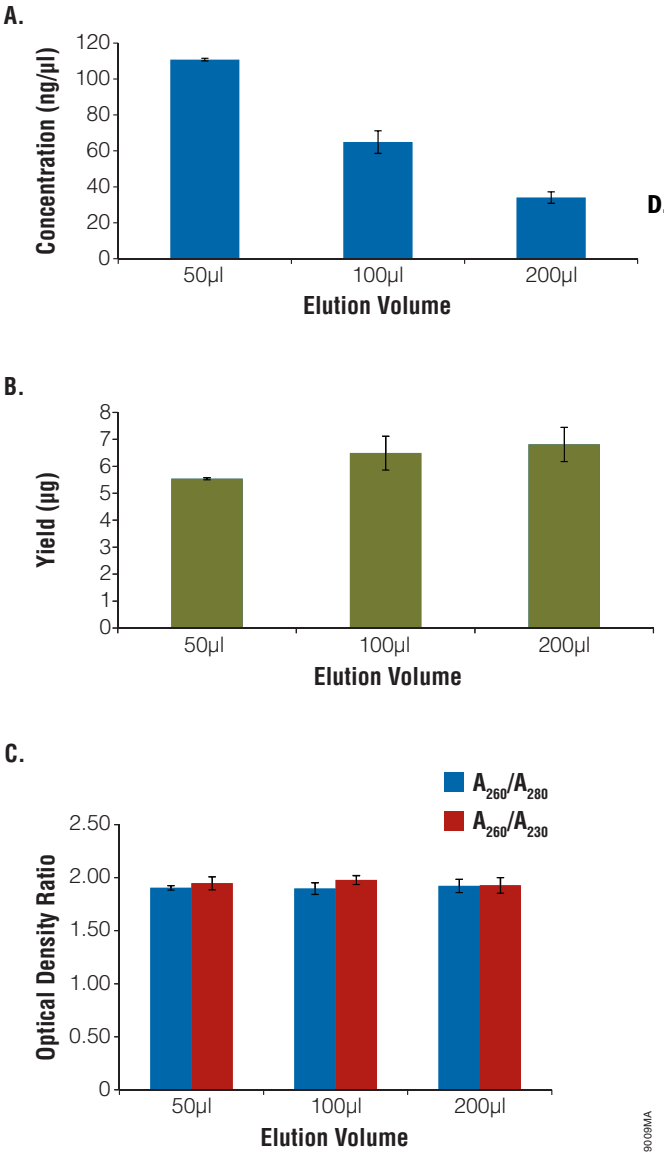

Figure 9.14. Comparison of elution volume with concentration, yield and purity. Aliquots of blood (200µl) were processed using the ReliaPrep™ Blood gDNA Minprep System (n = 4) and eluted with 30–200µl of Nuclease-Free Water. Concentration (**Panel A**), total yield (**Panel B**) and purity (**Panel C**) were assessed using absorbance spectroscopy. Yield decreased slightly with decreases in elution volume, while concentration increased. Purity as measured by optical density ratios remained constant.

Additional Resources for the Cellulose Column-Based Systems

Technical Bulletins and Manuals

TM330 *ReliaPrep™ Blood gDNA Miniprep System Technical Manual*

TM345 *ReliaPrep™ gDNA Tissue Miniprep System Technical Manual*

Promega Publications

ReliaPrep™ Blood gDNA Miniprep System a novel, column-based purification of gDNA from whole blood

ReliaPrep™ Blood gDNA Miniprep System: Low elution volume with high yield

D. High-Throughput Genomic DNA Isolation Systems for Blood

Promega offers several automated high-throughput options to isolate genomic DNA isolation from blood samples. There are three MagneSil® paramagnetic silica-based systems for extracting DNA from whole blood on automated platforms: MagneSil® ONE, Fixed Yield Blood Genomic System; MagneSil® Blood Genomic, Max Yield System and MagneSil® Genomic, Large Volume System. Because these DNA purification systems are for automated use, they require hardware accessories in addition to the instrument workstation. A list of the essential accessories for use with a robotic setup are included on the online catalog pages for each DNA isolation system at: **www.promega.com**. The main differences in these three DNA purification systems are format, the blood volume processed and DNA yield recovered. The MagneSil® ONE, Fixed Yield Blood Genomic System (Cat.# MD1370) purifies 1µg of DNA (±50%) from 60µl of anticoagulated whole blood in a 96-well plate format. Purification of a "fixed yield" of DNA eliminates the need to quantitate and normalize concentrations post-purification.

To maximize the quantity of DNA recovered from 200µl blood, use the MagneSil® Blood Genomic, Max Yield System (Cat.# MD1360). The methodology is the same—lysing the cells and capturing the genomic DNA from the solution—but the recovered yield is more variable, between 4–9µg, depending on the number of white cells. Figure 9.13 shows a multiplex PCR using DNA isolated from both the MagneSil® Blood Genomic, Max Yield System, and the MagneSil® ONE, Fixed Yield Blood Genomic System.

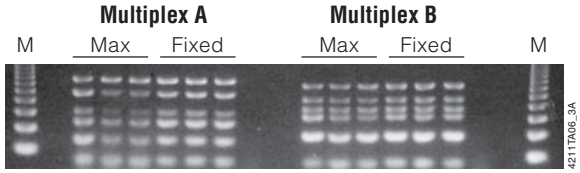

Figure 9.15. Multiplex PCR analysis on genomic DNA purified from blood. Genomic DNA purified with either the MagneSil® Blood Genomic System, Max Yield System (Max) or the MagneSil® ONE, Fixed Yield Blood Genomic System (Fixed) was amplified using the Y Chromosome Deletion Detection System, Version 1.1. Ten microliters of the amplification reactions for Multiplex A and B was run on a gel and visualized by ethidium bromide staining.

For the ability to isolate large quantities of genomic DNA from large-volume tubes including blood samples (1–10ml), the MagneSil® Genomic, Large Volume System (Cat.# A4080, A4082, A4085) may meet your needs. There are several accessories needed in order to use this system for isolation of genomic DNA, but the MagneSil® Genomic,

Large Volume System, can process even mishandled blood samples and, depending on the white cell count, may yield ~450µg genomic DNA/10ml blood. These DNA isolation systems produce high-quality DNA suitable for use in PCR, multiplex PCR and SNP genotyping applications. As seen in Figure 9.14, genomic DNA isolated using the MagneSil® Genomic, Large Volume System, works well in real-time PCR analysis.

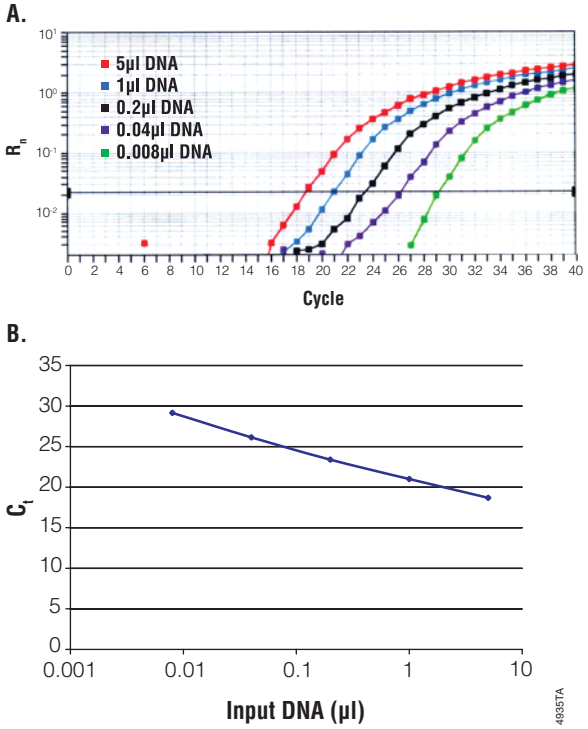

Figure 9.16. Real-time PCR assay for quality of genomic DNA purified by the MagneSil® Genomic, Large Volume System. Decreasing volumes of isolated human DNA were analyzed using β-actin real-time PCR control reagents from Applied Biosystems. **Panel A.** Amplification curve for the DNA volume range tested. **Panel B.** Linear detection for the amplification.

While these MagneSil® Genomic Systems are primarily designed for use with whole blood, other sample types can be used. There are limited specific protocols associated with other sample types (e.g., MagneSil® ONE, Fixed Yield Plant Genomic DNA Purification Automated Protocol). Visit our Citations database or contact techserv@promega.com to learn about other possible sample types used for genomic DNA purification.

The ReliaPrep™ Large Volume HT gDNA Isolation System (Cat.# A1751) isolates genomic DNA from 3–10ml samples of blood in a scalable format. There are no tedious centrifugation steps or hazardous chemicals, which are inherently used in precipitation-based chemistries. The system has been automated on a liquid-handling workstation, offering walkaway purification of genomic DNA from whole blood, regardless of sample storage or shipping conditions. There is an option for low-throughput isolation of gDNA from up to 32 samples at one time when the ReliaPrep™ LV 32 Heater Shaker Magnet (HSM) is used in a manual mode, where the user performs the pipetting functions prompted by the LCD screen on the HSM. The ReliaPrep™ LV 32 HSM Instrument (Cat.# A7015) includes all the accessories needed for automation.

Additional Resources for the High-Throughput Genomic DNA Isolation Systems for Blood

Technical Bulletins and Manuals

TB313	*MagneSil® ONE, Fixed Yield Blood Genomic System Technical Bulletin*
TB312	*MagneSil® Blood Genomic, Max Yield System Technical Bulletin*
TB549	*MagneSil® Genomic, Large Volume System Technical Bulletin*
TM341	*ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual*
TM326	*ReliaPrep™ LV 32 HSM Instrument Technical Manual*

Promega Publications

MagneSil® Genomic, Large Volume System, for large-sample genomic DNA isolation

Automated 96-well purification of genomic DNA from whole blood

Expanding the capabilities of plant genomic DNA purification

Citations

Ichimura, S. *et al.* (2007) Evaluation the Invader Assay with the BACTEC MGIT 960 System for prompt isolation and identification of Mycobacteria from clinical specimens. *J. Clin. Microbiol.* **45**, 3316–22.

These authors compared standard culture conditions, DNA isolation and analysis (e.g, sequencing) with a liquid culture, DNA isolation and a homogeneous fluorescent detection system for identifying mycobacterial species. The standard DNA extraction began with a loopful (3mm^3 sphere) of bacterial colony grown on Ogawa slants that used glass beads to mechanically disrupt the cells. The resulting lysate was extracted using phenol/chloroform, and DNA purified from the aqueous phase using a robotic liquid handler AGE-96 (Biotec) and the MagneSil® Blood Genomic, Max Yield System. The DNA extractions were used in PCR and sequencing reactions.

PubMed Number: 17687020

E. Automated Low- to Moderate-Throughput for DNA Purification

As laboratories try to improve productivity, the need has increased for easy-to-use, low- to moderate-throughput automation of purification processes. The Maxwell® 16 Instrument is designed for efficient, automated purification from a wide range of sample types. The instrument is supplied with preprogrammed automated purification methods and is designed for use with prefilled reagent cartridges, maximizing simplicity and convenience. The instrument can process up to 16 samples in approximately

30–40 minutes (depending on sample type and method). Purified concentrated products are high quality and obtained at high yield to be used directly in a variety of downstream applications.

The Maxwell® 16 System purifies samples using paramagnetic particles (PMPs), which provide a mobile solid phase that optimizes capture, washing and elution of the target material. The Maxwell® 16 Instrument is a magnetic-particle-handling instrument that efficiently preprocesses liquid and solid samples, transports the PMPs through purification reagents in the prefilled cartridges, and mixes efficiently during processing. The efficient magnetic particle-based methodology used by the Maxwell® 16 Instrument avoids common problems associated with automated purification systems, such as clogged tips or partial reagent transfers, which can result in suboptimal purification processing. Several Maxwell® 16 reagent kits are available and allow optimal purification from a variety of sample types including blood, FTA® paper, formalin-fixed, paraffin-embedded tissue, bacteria, plant and animal tissue (see Figures 9.15 and 9.16).

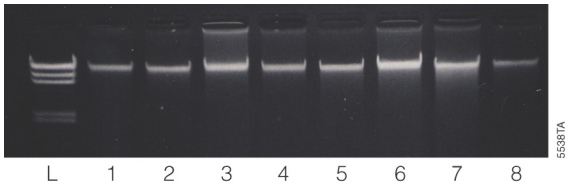

Figure 9.17. A panel of murine tissues purified using the Maxwell® 16 Tissue DNA Purification Kit on the Maxwell® 16 Instrument. Five microliters of genomic DNA was purified from 50mg of the following mouse tissues: Lane 1, brain; lane 2, heart; lane 3, intestine; lane 4, liver; lane 5, pancreas; lane 6, spleen; lane 7, 1cm mouse tail clipping; lane 8, 0.5cm mouse tail clipping; lane L, Lambda DNA/HindIII Marker (Cat.# G1711) All tissue samples were added directly to the reagent cartridge without preprocessing.

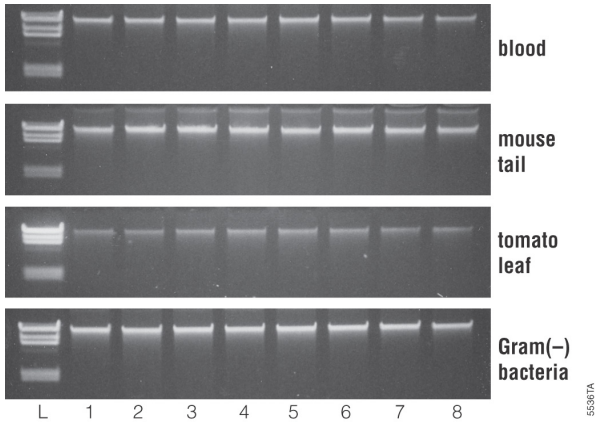

Figure 9.18. Consistent purification using the Maxwell® 16 Instrument. Five microliters of genomic DNA was purified from 400µl of human whole blood, 1cm mouse tail, 25mg tomato leaf or 400µl of an overnight culture of Gram-negative bacteria. Lane L, Lambda DNA/HindIII Marker (Cat.# G1711).

The Maxwell® 16 Instrument is easy to set up—just unpack and begin to use. No training or external computer required, so the instrument is ready for immediate use. Optimized automated methods are preloaded, the prefilled reagent cartridges are snapped into place, your sample is added and you press "Start" to begin the appropriate method. A full list of nucleic acid purification kits is available.

For challenging sample types or more control over the DNA purification application, the Maxwell® 16 Flexi Method Firmware (Cat.# AS6411) allows you to create your own program by optimizing the lysis, binding, drying, elution and paramagnetic particle capture parameters. Parameters are entered using the on-screen prompts on the Maxwell® 16 Instrument. The Maxwell® 16 Flexi Method Firmware is available as an option for new Maxwell® 16 Instruments and can be installed on existing instruments.

In addition to kits and methods developed by Promega [e.g., the Maxwell® 16 Cell LEV DNA Purification Kit (Cat.# AS1140) and the Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Cat.# AS1130)], customers and Promega applications scientists are continuously developing new applications for the Maxwell® 16 System. To facilitate sharing information about samples tested by both Promega scientists and Maxwell® 16 users throughout the world, the Maxwell® 16 Applications Database is available. This online resource allows you to search by keyword or browse through folders organized by sample type and applications, and contains information on samples processed, yields achieved and downstream applications tested. You can submit entries to the database using the provided submission form link sent via email and new applications are continuously added.

Automation eliminates the hands-on time and labor of manual purification, giving you the time to focus on your research. In addition, the Maxwell® 16 Instrument design, optimized reagents and automated methods provide consistent yield and purity for your downstream applications. The instrument is benchtop compact and can purify from many sample types (Table 9.4). Future automated method updates can be added through the instrument communications port.

Table 9.4. DNA yield from various sample types after purification using the Maxwell® 16 Instrument and DNA Purification Kits.

Sample Type	Sample Size	Yield
Whole blood	200µl	4–9µg (>3pg/white blood cell)
Whole blood	400µl	8–15µg (>3pg/white blood cell)
Mouse tail	1.2cm	≥20µg
Animal tissue	20–25mg	60–100µg (mouse liver)
Tissue culture cells	5×10^6	15–20µg (HeLa)
Gram– bacteria	2×10^9	25–30µg (*E. coli* BL21)
Gram+ bacteria[1]	2×10^9	15–25µg (*B. cereus*)
Plant leaf (tomato)[1]	25mg	9–13µg
Drosophila melanogaster	1 fly	0.32µg
Drosophila melanogaster	5 flies	1.52µg
Caenorhabditis elegans	~50,000 worms	0.08µg
Danio rerio	50mg	24.8µg
Saccharomyces cerevisiae [2]	1 colony (3mm)	0.72µg
Arabidopsis thaliana	1 leaf	0.13µg

[1]With optional pretreatment.

[2]Includes 3 hour digestion with lyticase prior to DNA isolation.

Additional Resources for Maxwell® 16 System

Technical Bulletins and Manuals

TM295	*Maxwell® 16 Instrument Operating Manual*
TM284	*Maxwell® 16 DNA Purification Kits Technical Manual*
TM309	*Maxwell® 16 Mouse Tail DNA Purification Kit Technical Manual*
TB383	*Maxwell® 16 Cell LEV DNA Purification Kit Technical Bulletin*
TB349	*Maxwell® 16 FFPE Plus LEV DNA Purification Kit Technical Manual*
TB385	*Maxwell® 16 Viral Total Nucleic Acid Purification Kit Technical Bulletin*
TB381	*Maxwell® 16 Flexi Method Firmware Technical Bulletin*

Promega Publications

Purification of *O. volvulus* genomic DNA from *S. ochraceum* s.l. Black Fly head or body pools using the Maxwell® 16 Instrument

Quick and easy isolation of genomic DNA from *Drosophila* using the Maxwell® 16 Instrument

Maxwell® 16 buffy coat genomic DNA application

Automated DNA purification from Oragene•DNA/saliva samples using the Maxwell® 16 System

Purification of genomic DNA from mouse feces using the Maxwell® 16 System

Online Tools

Maxwell™ Applications Database

Introducing Personal Automation™ from Promega (video and web site)

Citations

Kwan, K. *et al.* (2011) Evaluation of procedures for the collection, processing, and analysis of biomolecules from low-biomass surfaces. *Appl. Environ. Microbiol.* **77**, 2943–53.

These authors used the Maxwell® 16 System to extract DNA from multiple sample collection devices containing a model microbial community (MMC) comprised of 11 distinct species of bacterial, archaeal and fungal lineages associated with spacecraft or clean-room surfaces. The authors compared cotton swabs, polyester wipes and biological sampling kits to assess the success of recovering DNA of rRNA genes for species-specific PCR analysis.

PubMed Number: 21398492

Garm Spindler, K.L. *et al.* (2009) The importance of KRAS mutations and EGF61A>G polymorphism to the effect of cetuximab and irinotecan in metastatic colorectal cancer. *Annals of Oncology* **20**, 879–84.

These authors used the Maxwell® 16 System to isolate genomic DNA from whole blood and normal colonic tissue samples. The DNA was used in genotype analysis, testing for wildtype and mutant KRAS genes, and for various EGFR-related polymorphisms. The results were used in a research study testing the relationship between various genotypes and response to different treatment regimens.

PubMed Number: 19179548

F. Plant Genomic DNA Isolation

The Wizard® Magnetic 96 DNA Plant System (Cat.# FF3760, FF3761) is designed for manual or automated 96-well purification of DNA from plant leaf and seed tissue. The Wizard® Magnetic 96 DNA Plant System has been validated with corn and tomato leaf as well as with canola and sunflower seeds. The DNA purified from these samples can be used in PCR and other more demanding applications, such as RAPD analysis. Additional required equipment includes not only a magnet (MagnaBot® 96 Magnetic Separation Device) but a device capable of breaking up seed or leaf material (e.g., Geno/Grinder® 2000 from SPEX CertiPrep, Inc.). The yield depends on the source

material and how well the seeds or leaf disks are pulverized prior to the genomic DNA isolation. Yield may range from 10–100ng from a single 8mm leaf punch. To increase the yield from the Wizard® Magnetic 96 DNA Plant System, a scale up in volume with up to 5 leaf punches can be used [as demonstrated in *Promega Notes* 79]. The potential scale-up is limited by the volume in a deep-well, 96-well plate.

Additional Resources for Plant Genomic DNA Isolation

Technical Bulletins and Manuals

TB289 *Wizard® Magnetic 96 DNA Plant System Technical Bulletin*

Promega Publications

Expanding the capabilities of plant genomic DNA purification

Automated genomic DNA purification using the Wizard® Magnetic 96 DNA Plant System

Citations

Ghandilyan, A. *et al.* (2009) A strong effect of growth medium and organ type on the identification of QTLs for phytate and mineral concentrations in three *Arabidopsis thaliana* RIL populations. *J. Exp. Bot.* **60**, 1409–25.

Mineral accumulation was studied in *Arabidopsis thaliana* comparing loci involved with growing in soil versus hydroponics. An F2 population derived from a cross between Landsberg erecta (Ler; maternal parent) and Eringsboda-1 (Eri-1; paternal parent) was propagated by single seed descent for nine successive generations in soil. The flower buds of three plants per line were collected, and the DNA extracted using the Wizard® Magnetic 96 DNA Plant System and used for genotyping with 90 amplified fragment length polymorphism PCR (AFLP) and 39 single sequence length polymorphisms (SSLP) markers to build a genetic map of quantitative trait loci (QTL).

PubMed Number: 19346258

G. Food DNA Isolation

Another specialized genomic DNA isolation system is the Wizard® Magnetic DNA Purification System for Food (Cat.# FF3750, FF3751). It is designed for manual purification of DNA from a variety of food samples including corn seeds, cornmeal, soybeans, soy flour and soy milk. In addition, DNA can be purified from processed food such as corn chips, chocolate and chocolate-containing foods, lecithin and vegetable oils if used with the appropriate optimized protocols. The DNA purified from many of these samples can be used in PCR-based testing for Genetically Modified Organism (GMO) DNA sequences including quantitative analysis using TaqMan® assays. As with all isolation systems using the MagneSil® PMPs, a magnetic separation stand is needed (can process from 2–12 samples). With samples containing highly processed food, the genomic DNA isolated will be fragmented and better suited for analysis using amplification rather than a Southern blot. The yield of DNA from this system will vary depending on source type and extent of food processing.

Additional Resources for Food DNA Isolation

Technical Bulletins and Manuals

TB284 *Wizard® Magnetic DNA Purification System for Food Technical Bulletin*

Promega Publications

Wizard® Magnetic DNA Purification System for Food: Part I. DNA isolation and analysis of GMO foods by PCR

Citations

Fumière, O. *et al.* (2006) Effective PCR detection of animal species in highly processed animal byproducts and compound feeds. *Anal. Bioanal. Chem.* **385**, 1045–54.

The authors developed a PCR method to detect the presence of meat and bone meal (MBM) in animal feed even if the MBM had been heat-treated, and discern whether the animal component is bovine or porcine in origin. The genomic DNA from 100mg of various feedstuffs with known and unknown amounts of MBM, fishmeal or poultry feed or a combination of these compounds was isolated using the Wizard® Magnetic DNA Purification System for Food with the KingFisher® System. Real-time PCR was performed using 5µl of extracted DNA.

PubMed Number: 16761123

H. Fixed-Tissue Genomic DNA Isolation

The MagneSil® Genomic, Fixed Tissue System (Cat.# MD1490), provides a fast, simple technique for the preparation of genomic DNA from formalin-fixed, paraffin-embedded tissue. After an overnight Proteinase K digestion, genomic DNA can be manually purified from formalin-fixed, paraffin-embedded thin tissue sections in less than an hour. Amplifiable genomic DNA can be isolated from 10µm sections without centrifugation of the lysate prior to purification. Up to 12 samples can be processed in the manual format using the MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5342). One advantage this system has over other purification methods, such as phenol:chloroform extraction, is its ability to remove most inhibitors of amplification, including very small fragments of DNA. Tissue that has been stored in formalin for extended periods of time may be too cross-linked or too degraded to perform well as template for amplification. Figure 9.17 shows an amplification of 16 short tandem repeat (STR) loci and demonstrates how well the isolated DNA can work in multiplex PCR using the PowerPlex® 16 System.

The Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Cat.# AS1130) is an automated method for purifying up to 16 samples of one to ten 5µm sections of FFPE tissue samples on the Maxwell® 16 Instrument. The DNA-binding capacity of the system is limited to a few hundred nanograms of pure DNA, which is suitable for amplification applications including qPCR and multiplex PCR.

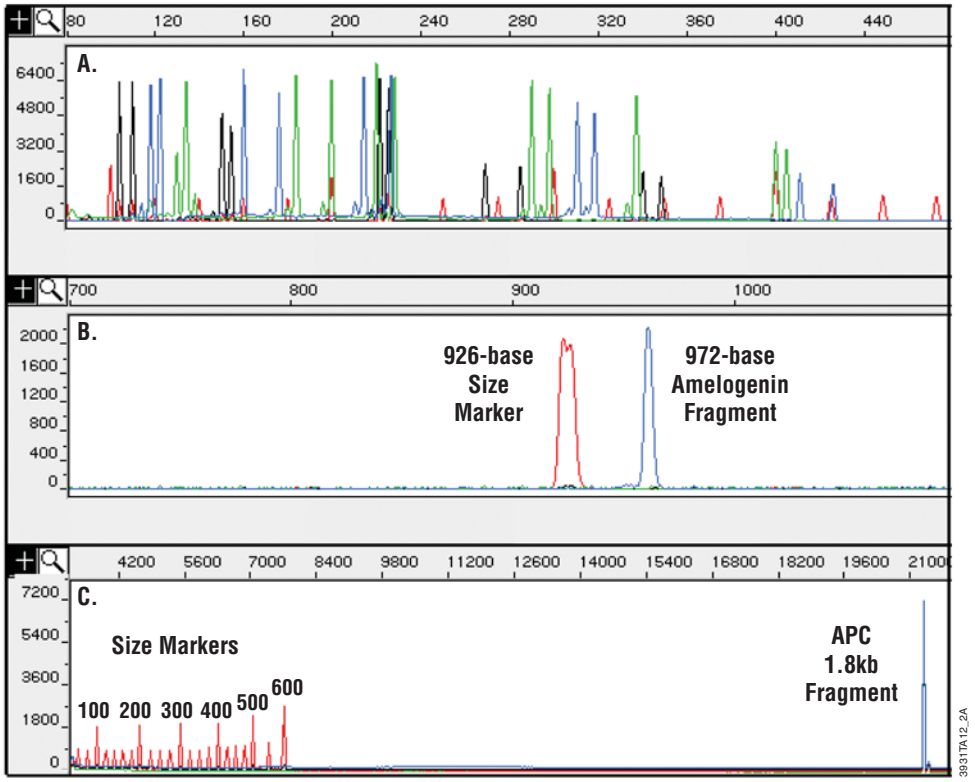

Figure 9.19. Analysis of DNA purified from paraffin-embedded, formalin-fixed 10µm thin sections using the MagneSil® Genomic, Fixed Tissue System. Purified DNA was amplified, and the amplification products were analyzed on an ABI PRISM® 310 or 3100 genetic analyzer. **Panel A.** Amplification with a set of 16 fluorescently labeled primers. Amplification products range in size from 104 to 420 bases. **Panel B.** A 972-base fragment amplified using an amelogenin primer set. **Panel C.** A 1.8kb fragment amplified from the *Adenomatosis polyposis coli* (*APC*) gene. Increasing the extension time during amplification may help to balance yields between small and large amplification products and increase yields for large amplification products. Results will vary depending on the degree of cross-linking due to formalin fixation.

Additional Resources for Fixed-Tissue Genomic DNA Isolation

Technical Bulletins and Manuals

TB319	*MagneSil® Genomic, Fixed Tissue System Technical Bulletin*
TB382	*Maxwell® 16 FFPE Tissue LEV DNA Purification Kit Technical Bulletin*
TB349	*Maxwell® 16 FFPE Plus LEV DNA Purification Kit Technical Manual*

Citations

Halberg, R.B. *et al.* (2009) Long-lived Min mice develop advanced intestinal cancers through a genetically conservative pathway. *Cancer Res.* **69**, 5768–75.

To better understand tumor progression in mice carrying the *Min* allele of *Adenomatous polyposis coli* (*Apc*), a longer lived cross was generated and studied. Intestinal tumors and adjacent normal tissue were microdissected, frozen in liquid nitrogen and genomic DNA isolated using the Magnesil® Genomic, Fixed Tissue System. The purified DNA was then used for microsatellite instability (MSI) analysis.

PubMed Number: 19584276

VII. Genomic DNA Purification Protocols Featuring the Wizard® Genomic DNA Purification Kit

The Wizard® Genomic DNA Purification Kit can isolate genomic DNA from many source types. The four purification protocols detailed below can be used for whole blood, tissues, bacteria, yeast and plants. Table 9.5 lists typical yields for specific source types.

Table 9.5. DNA Yields from Various Starting Materials.

Material	Amount of Starting Material	Typical DNA Yield
Human Whole Blood	300µl	5–15µg
	1ml	25–50µg
	10ml	250–500µg
Mouse Whole Blood	300µl	6–7µg
K562 (human)	3 × 10^6 cells	15–30µg
COS (African green monkey)	1.5 × 10^6 cells	10µg
NIH/3T3 (mouse)	2.25 × 10^6 cells	12.5µg
CHO (Chinese hamster ovary)	1–2 × 10^6 cells	6–7µg
Sf9 Insect	5 × 10^6 cells	16µg
Mouse Liver	11mg	15–20µg
Mouse Tail	0.5–1cm tail	10–30µg
Tomato Leaf	40mg	7–12µg
Escherichia coli JM109, overnight culture, ~2 × 10^9 cells/ml	1ml	20µg
Staphylococcus epidermis, overnight culture, ~3.5 × 10^8 cells/ml	1ml	6–13µg
Saccharomyces cerevisiae, overnight culture, ~1.9 × 10^8 cells/ml	1ml	4.5–6.5µg

A. Isolation of Genomic DNA from Whole Blood

Materials Required:

- Wizard® Genomic DNA Purification Kit (Cat.# A1120)
- sterile 1.5ml microcentrifuge tubes (for 300µl blood samples)
- sterile 15ml centrifuge tubes (for 3ml blood samples)
- sterile 50ml centrifuge tubes (for 10ml blood samples)
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)

Red Blood Cell Lysis

1. Using volumes from Table 9.6, combine the appropriate volumes of Cell Lysis Solution and blood. Mix by inversion.
2. Incubate for 10 minutes at room temperature.
3. Centrifuge:
   ≤300µl sample: 13,000–16,000 × *g* ; 20 seconds
   1–10ml: sample 2,000 × *g*; 10 minutes
4. Discard supernatant. Vortex pellet.

Nuclei Lysis and Protein Precipitation

5. Using volumes from Table 9.6, add Nuclei Lysis Solution and mix by inversion.
6. Add Protein Precipitation Solution; vortex for 20 seconds.
7. Centrifuge:
   ≤300µl sample: 13,000–16,000 × *g* ; 3 minutes
   1–10ml: sample 2,000 × *g*; 10 minutes

DNA Precipitation and Rehydration

8. Transfer supernatant to a new tube containing isopropanol (using volumes from Table 9.6). Mix by inversion.
9. Centrifuge as in Step 7.
10. Discard supernatant. Add 70% ethanol (same volume as isopropanol).
11. Centrifuge as in Step 7.
12. Aspirate the ethanol and air-dry the pellet (10–15 minutes).
13. Rehydrate the DNA in the appropriate volume of DNA Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

Table 9.6. Solution Volumes for Whole Blood Genomic DNA Isolation.

	Lysis Solution				
Sample Size	Cell	Nuclei	Protein Precipitation Solution	Isopropanol	DNA Rehydration Solution
300µl	900µl	300µl	100µl	300µl	100µl
1ml	3ml	1ml	330µl	1ml	150µl
3ml	9ml	3ml	1ml	3ml	250µl
10ml	30ml	10ml	3.3ml	10ml	800µl

B. Isolation of Genomic DNA from Tissue Culture Cells and Animal Tissue

Materials Required:

- Wizard® Genomic DNA Purification Kit (Cat.# A1120)
- 1.5ml microcentrifuge tubes
- 15ml centrifuge tubes
- small homogenizer (Fisher Tissue-Tearor™, Cat.# 15-338-55, or equivalent; for animal tissue)
- trypsin (for adherent tissue culture cells only)
- PBS
- 0.5M EDTA (pH 8.0; for mouse tail)
- Proteinase K [20mg/ml in water (Cat.# V3021); for mouse tail]
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)

Prepare Tissues

Tissue Culture Cells: Centrifuge at 13,000–16,000 × *g* for 10 seconds. Wash the cell pellet with PBS, vortex and then add 600µl of Nuclei Lysis Solution and mix by pipetting.

Animal Tissue: Add 10–20mg of fresh or thawed tissue to 600µl of chilled Nuclei Lysis Solution and homogenize for 10 seconds. Alternatively, use 10–20mg of ground tissue. Incubate at 65°C for 15–30 minutes.

Mouse Tail: Add 600µl of chilled EDTA/Nuclei Lysis Solution to 0.5–1cm of fresh or thawed mouse tail. Add 17.5µl of 20mg/ml Proteinase K and incubate overnight at 55°C with gentle shaking.

Lysis and Protein Precipitation

1. Add 3µl of RNase Solution to the cell or animal tissue nuclei lysate and mix. Incubate for 15–30 minutes at 37°C. Cool to room temperature.
2. Add 200µl of Protein Precipitation Solution. Vortex and chill on ice for 5 minutes.
3. Centrifuge at 13,000–16,000 × *g* for 4 minutes.

DNA Precipitation and Rehydration

4. Transfer supernatant to a fresh tube containing 600µl of room temperature isopropanol.
5. Mix gently by inversion.
6. Centrifuge at 13,000–16,000 × *g* for 1 minute.
7. Remove supernatant and add 600µl of room temperature 70% ethanol. Mix.
8. Centrifuge as in Step 6.
9. Aspirate the ethanol and air-dry the pellet for 15 minutes.
10. Rehydrate the DNA in 100µl of DNA Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

For additional protocol information, see Technical Manual #TM050.

C. Isolation of Genomic DNA from Gram-Positive and Gram-Negative Bacteria

Materials Required:

- Wizard® Genomic DNA Purification Kit (Cat.# A1120)
- 1.5ml microcentrifuge tubes
- water bath, 80°C
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)
- 50mM EDTA (pH 8.0; for Gram-positive bacteria)
- 10mg/ml lysozyme (Sigma Cat.# L7651; for Gram-positive bacteria)
- 10mg/ml lysostaphin (Sigma Cat.# L7386; for Gram-positive bacteria)

Pellet Cells

Centrifuge 1ml of overnight culture for 2 minutes at 13,000–16,000 × *g*. Discard the supernatant.

For Gram-Positive Bacteria

1. Suspend cells in 480µl 50mM EDTA.
2. Add lytic enzyme(s) [120µl (lysozyme and/or lysostaphin)].
3. Incubate at 37°C for 30–60 minutes.
4. Centrifuge for 2 minutes at 13,000–16,000 × *g* and remove supernatant.
5. Go to Step 1, **Lyse Cells** (below).

For Gram-Negative Bacteria

Go to Step 1, **Lyse Cells** (below).

Lyse Cells

1. Add 600µl of Nuclei Lysis Solution. Pipet gently to mix.
2. Incubate for 5 minutes at 80°C, then cool to room temperature.
3. Add 3µl of RNase Solution. Mix, incubate at 37°C for 15–60 minutes, then cool to room temperature.

Protein Precipitation

4. Add 200µl of Protein Precipitation Solution. Vortex.
5. Incubate on ice for 5 minutes.
6. Centrifuge at 13,000–16,000 × *g* for 3 minutes.

DNA Precipitation and Rehydration

7. Transfer the supernatant to a clean tube containing 600µl of room temperature isopropanol. Mix by inversion.
8. Centrifuge as in "Pellet Cells" above, and decant the supernatant.

9. Add 600µl of room temperature 70% ethanol. Mix.
10. Centrifuge for 2 minutes at 13,000–16,000 × *g*.
11. Aspirate the ethanol and air-dry the pellet for 10–15 minutes.
12. Rehydrate the DNA pellet in 100µl of Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

D. Isolation of Genomic DNA from Yeast Cultures or Plant Tissue

Materials Required:

- Wizard® Genomic DNA Purification Kit (Cat.# A1120)
- 1.5ml microcentrifuge tubes
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)
- microcentrifuge tube pestle or mortar and pestle (for plant tissue)
- YPD broth (for yeast)
- 50mM EDTA (pH 8.0; for yeast)
- 20mg/ml lyticase (Sigma Cat.# L2524; for yeast)

Prepare Yeast Lysate

1. Pellet cells from 1ml of culture by centrifugation at 13,000–16,000 × *g* for 2 minutes.
2. Suspend the cell pellet in 293µl of 50mM EDTA.
3. Add 7.5µl of 20mg/ml lyticase and mix gently.
4. Incubate for 30–60 minutes at 37°C. Cool to room temperature.
5. Centrifuge as in Step 1. Discard the supernatant.
6. Add 300µl of Nuclei Lysis Solution. Proceed to **Protein Precipitation and DNA Rehydration** Table 9.7, Step 1.

Prepare Plant Lysate

1. Grind approximately 40mg of leaf tissue in liquid nitrogen.
2. Add 600µl of Nuclei Lysis Solution. Incubate at 65°C for 15 minutes.
3. Add 3µl of RNase Solution. Incubate at 37°C for 15 minutes. Cool sample to room temperature for 5 minutes. Proceed to **Protein Precipitation and DNA Rehydration** Table 9.7, Step 1.

Table 9.7. Protein Precipitation and DNA Rehydration.

	Yeast	Plant
1. Add Protein Precipitation Solution. Vortex. **For yeast only:** Incubate 5 minutes on ice.	100µl	200µl
2. Centrifuge at 13,000–16,000 × *g*.	3 minutes	3 minutes
3. Transfer supernatant to clean tube containing room temperature isopropanol.	300µl	600µl
4. Mix by inversion and centrifuge at 13,000–16,000 × *g*.	2 minutes	1 minute
5. Decant supernatant and add room temperature 70% ethanol.	300µl	600µl
6. Centrifuge at 13,000–16,000 × *g*.	2 minutes	1 minute
7. Aspirate the ethanol and air-dry the pellet.		
8. Add DNA Rehydration Solution.	50µl	100µl
9. **For yeast only:** Add RNase. Incubate at 37°C for 15 minutes.	1.5µl	—
10. Rehydrate at 65°C for 1 hour or overnight at 4°C.		

Additional Resources for the Wizard® Genomic DNA Purification Kit

Technical Bulletins and Manuals

TM050 *Wizard® Genomic DNA Purification Kit Technical Manual*

Online Tools

Sample Types Processed with the Wizard® Genomic DNA Purification Kit

VIII. Specialized Genomic DNA Purification Protocol Featuring the ReliaPrep™ FFPE gDNA Miniprep System

Materials Required:

- ReliaPrep™ FFPE gDNA Miniprep System (Cat.# A2351)
- 95–100% ethanol
- 80°C heat block
- 56°C heat block
- equivalent of ≤100µm tissue sections (see Technical Manual #TM352)

Note: All centrifugations are performed at room temperature.

A. Deparaffinization Using Mineral Oil

1. Add mineral oil to the sample:
   - For sections ≤ 50 microns, add 300µl of mineral oil
   - For sections ≥ 50 microns, add 500µl of mineral oil
2. Incubate at 80°C for 1 minute.
3. Vortex to mix.

B. Sample Lysis

1. Add 200µl of Lysis Buffer to the sample.
2. Spin at 10,000 × *g* for 15 seconds. Two phases will be formed, a lower (aqueous) phase and an upper (oil) phase.
3. Add 20µl of Proteinase K directly to the lower phase; mix the lower phase by pipetting.
4. Incubate at 56°C for 1 hour.
5. Incubate at 80°C for 1 hour.
6. Allow the sample to cool to room temperature. Centrifuge briefly to collect any drops from the inside of the lid.

C. RNase Treatment

1. Add 10µl of RNase A directly to the lysed sample in the lower phase. Mix the lower phase by pipetting.
2. Incubate at room temperature (20–25°C) for 5 minutes.

D. Nucleic Acid Binding

1. Add 220µl of BL Buffer to the lysed sample.
2. Add 240µl of ethanol (95–100%).
3. Vortex briefly to mix.
4. Spin at 10,000 × *g* for 15 seconds. Two phases will be formed, a lower (aqueous) phase and an upper (oil) phase.
5. For each sample to be processed, place a Binding Column into one of the Collection Tubes provided.

   Note: Wear gloves when handling the columns and tubes.
6. Transfer the entire lower (aqueous) phase of the sample, including any precipitate that may have formed, to the Binding Column/Collection Tube assembly, and cap the column. Discard the remaining mineral oil.

   Note: The mineral oil is inert and will not interfere with the extraction procedure if some of the oil phase is carried over to the Binding Column.
7. Spin the assembly at 10,000 × *g* for 30 seconds.
8. Discard the flowthrough, and reinsert the Binding Column into the Collection Tube.
9. Proceed immediately to Column Washing and Elution.

E. Column Washing and Elution

1. Add 500µl of 1X Wash Solution (with ethanol added) to the Binding Column. Cap the column.
2. Spin at 10,000 × *g* for 30 seconds.
3. Discard the flowthrough, and reinsert the Binding Column into the same Collection Tube.
4. Add 500µl of 1X Wash Solution (with ethanol added, see Section 3) to the Binding Column. Cap the column.
5. Spin at 10,000 × *g* for 30 seconds.
6. Discard the flowthrough, and reinsert the Binding Column into the Collection Tube used for the Nucleic Acid Binding.
7. Open the cap on the Binding Column, and spin the Binding Column/Collection Tube assembly at 16,000 × *g* for 3 minutes to dry the column.

   Note: Centrifuging with the cap open ensures thorough drying of the column. It is important to dry the column to prevent carryover of ethanol to the eluate.
8. Transfer the Binding Column to a clean 1.5ml microcentrifuge tube (not provided), and discard the Collection Tube.
9. Add 30–50µl of Elution Buffer to the column, and cap the column.
10. Spin at 16,000 × *g* for 1 minute. Remove and discard the Binding Column.
11. Cap the microcentrifuge tube, and store the eluted DNA at –20°C.

Additional Resources for the ReliaPrep™ FFPE gDNA Miniprep System

Technical Bulletins and Manuals

TM352 *ReliaPrep™ FFPE gDNA Miniprep System Technical Manual*

IX. Fragment/PCR Product Purification Systems

Generally, purification of DNA fragments or PCR products does not involve disruption of cellular structures but rather separation of DNA from in vitro reactions or agarose gel slices. In many cases, after a PCR amplification or restriction enzyme digestion, the reaction components include protein and salts that may inhibit subsequent applications and will need to be removed from the DNA fragments. An agarose gel may be run to isolate a fragment of the correct size if there is more than one product present. Fragment DNA purification can improve efficiency in subsequent reactions.

For example, PCR products can be used directly in T-vector cloning. However, nonspecific products and primer dimers can compete for ligation with the desired PCR product, resulting in low frequency of positive clones. Also, removing the reaction components prior to sequencing will ensure the right primers are used for sequencing and the fluorescently labeled nucleotides are not competing with the unlabeled dNTPs remaining from the PCR amplification.

A. Wizard® SV Gel and PCR Clean-Up System

The Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281, A9282) is designed to extract and purify DNA fragments of 100bp to 10kb from standard or low-melting point agarose gels in either Tris acetate (TAE) or Tris borate (TBE) buffer, or to purify PCR products directly from an amplification reaction, using the SV silica membrane column. This purification kit is a single column system that can be used with a vacuum manifold [e.g., Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231)] or a standard microcentrifuge. Up to 95% recovery is achieved, depending upon the DNA fragment size (see Table 9.9). PCR products are commonly purified to remove excess nucleotides, primers and PCR additives like DMSO and betaine (Table 9.10). This membrane-based system, which can bind up to 40µg DNA, allows recovery of isolated DNA fragments or PCR products in as little as 20 minutes, depending on the number of samples processed and the protocol used. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

Table 9.8. Percent Recovery Versus Double-Stranded DNA Fragment Size Using the Wizard® SV Gel and PCR Clean-Up System.

DNA Fragment Size	Percent Recovery
55bp	26%
70bp	39%
85bp	55%
100bp	84%
500bp	89%
1,000bp	92%
3,199bp	95%
9,416bp	95%
23,130bp	47%

Table 9.9. Effect of Various PCR Additives on Percent Recovery of a 1,000bp PCR Product Using the Direct Purification Method and the Wizard® SV Gel and PCR Clean-Up System.

PCR Additive	Percent Recovery[1]
no additive	100%
1M betaine	94%
1M Q-Solution	97%
0.1% Triton® X-100	92%
0.1% Tween®-20	87%
0.1% NP-40	82%
5% glycerol	87%
5% formamide	90%
5% DMSO	87%
0.5M tetramethylene sulfoxide	94%
0.4M sulfolane	94%
0.4M 2-pyrollidone	95%
1mM tartrazine	100%
1% Ficoll®-400	100%

[1]Percent recovery shown is relative to the "no additive" recovery.

For direct purification from a reaction, note that any nucleic acid present in solution will be isolated. Therefore, if an amplification reaction has more than one product, all fragments will be present in the eluted DNA. If you are interested in isolating a single amplimer, separate the reaction products on an agarose gel and cut out the band desired prior to purification.

When purifying DNA from an agarose slice, the primary consideration is to melt the agarose so the DNA is available for binding to the silica membrane. The purified DNA can then be used for cloning or sequencing.

Additional Resources for the Wizard® SV Gel and PCR Clean-Up System

Technical Bulletins and Manuals

TB308 *Wizard® SV Gel and PCR Clean-Up System Technical Bulletin*

Promega Publications

Wizard® SV Gel and PCR Clean-Up System

Removal of ethidium bromide and calf intestinal alkaline phosphatase using the Wizard® SV Gel and PCR Clean-Up System

Citations

Mochida, S. *et al.* (2010) A recombinant catalytic domain of matriptase induces detachment and apoptosis of small-intestinal epithelial IEC-6 cells cultured on laminin-coated surface. *J. Biochem.* **148**, 721–32.

The authors determined that a recombinant catalytic domain of rat matriptase (His6t-S-CD) caused detachment of small-intestinal epithelial cells (IEC-6 cells) from laminin-coated plates. To determine if this catalytic domain induced apoptosis, IEC-6 cells were treated with

His6t-S-CD, then harvested, and genomic DNA was purified using the Wizard® SV Gel and PCR Clean-Up System. DNA fragmentation, a hallmark of apoptosis, was assessed by agarose gel electrophoresis and ethidium bromide staining.

PubMed Number: 20855298

B. Wizard® SV 96 PCR Clean-Up System

To purify 96 amplification reactions at once, use the Wizard® SV 96 PCR Clean-Up System (Cat.# A9340, A9341, A9342, A9345) with a 96-well vacuum manifold (Vac-Man® 96 Vacuum Manifold) and a vacuum pump capable of generating 15–20 inches of mercury or the equivalent. This system is designed to purify 100bp to 10kb PCR products directly from a reaction with typical recovery >90% as seen in Figure 9.18.

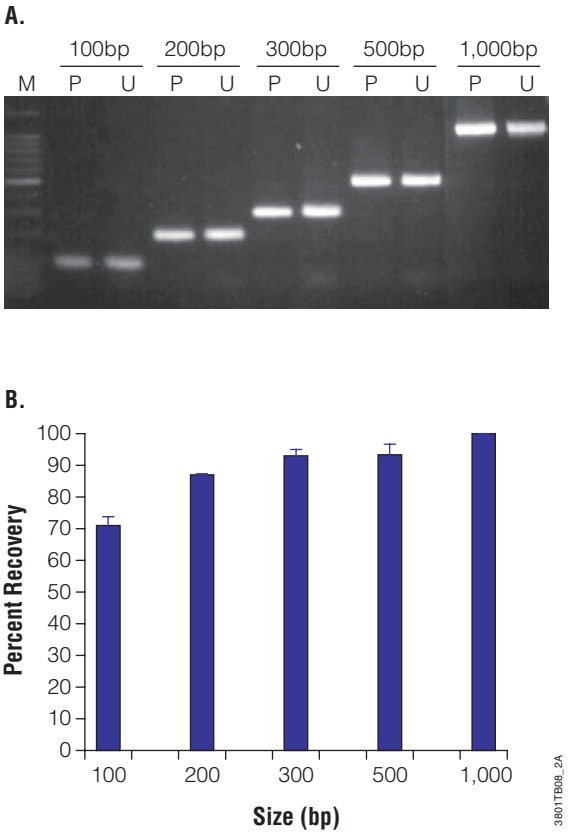

Figure 9.20. Purification and recovery of PCR products using the Wizard® SV 96 PCR Clean-Up System. PCR fragments of 100, 200, 300, 500 and 1,000 base pairs were purified using the Wizard® SV 96 PCR Clean-Up System on the Biomek® 2000 robotic workstation. **Panel A.** Agarose gel analysis. Purified (P) and unpurified (U) fragments were separated on an ethidium bromide-stained, 2% agarose gel. **Panel B.** Percent recovery of purified PCR products. Percent recovery was quantitated using a Hitachi FMBIO® Fluorescent Scanner. Results show the mean and standard deviation for 6 purified fragments of each size. For small PCR fragments (<500bp), optimal recovery requires a 95% ethanol wash. For larger fragments (>500bp), optimal results are achieved using an 80% ethanol wash.

The technology is the same as the single-column system, utilizing the SV silica membrane and chaotropic salts to purify the nucleotides and primers from the PCR product(s). This system allows recovery of 96 PCR fragments in as little as 20 minutes in multiwell plate format. The DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or DNA microarray analysis without further manipulation.

Additional Resources for the Wizard® SV 96 PCR Clean-Up System

Technical Bulletins and Manuals

TB311 *Wizard® SV 96 PCR Clean-Up System Technical Bulletin*

Promega Publications

Introducing the Wizard® SV 96 PCR Clean-Up System

Citations

Nagase, T. *et al.* (2008) Exploration of human ORFeome: High-throughput preparation of ORF clones and efficient characterization of their protein products. *DNA Research* **15**, 137–49.

To clone ORFs into the Flexi® Vector System, 1–4ng of a plasmid containing the ORF was amplified and the Wizard® SV 96 PCR Clean-Up System was used for purifying the PCR products. The cleaned up amplimers were then digested with SgfI and PmeI prior to cloning into the pF1K T7 Flexi® Vector.

PubMed Number: 18316326

C. BigDye® Sequencing Clean-Up

Designed for BigDye® sequencing reaction clean-up, the Wizard® MagneSil® Sequencing Reaction Clean-Up System (Cat.# A1831, A1832, A1835) can be placed on a robotic platform and purified using the MagneSil® PMPs to clean up sequencing reaction products prior to analysis. We have developed procedures for use on several robotic workstations with standard 96- and 384-well amplification plates. The Plate Clamp 96 (Cat.# V8251) is recommended for automated protocols and is designed to ensure PCR plates are uniformly flat for liquid transfer on a robotic platform. No user intervention is required from the time the multiwell plates are placed on the robot deck until the samples are loaded onto the DNA sequencer. For further information on robotic platforms and required hardware, visit: Automated Methods.

Additional Resources for the Wizard® MagneSil® Sequencing Reaction Clean-Up System

Technical Bulletins and Manuals

TB287 *Wizard® MagneSil® Sequencing Reaction Clean-Up System Technical Bulletin*

Promega Publications

High-throughput DNA fragment purification using the MagneSil® Automated 384-Well Clean-Up Systems

Citations

O'Leary, V.B. *et al.* (2005) Screening for new MTHFR polymorphisms and NTD risk. *Am. J. Med. Genet.* **138A**, 99–106.

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) has at least one polymorphism that is a neural tube defect (NTD) risk factor within the Irish population. To survey for common variations in MTHFR, genomic DNA was extracted from blood, and exons 1–11 of MTHFR were amplified and sequenced with BigDye® Terminator mix. The Wizard® MagneSil® Sequencing Reaction Clean-Up System was used to purify the sequencing reactions prior to analysis on an ABI PRISM® 377 DNA sequencer.

PubMed Number: 16145688

X. Fragment/PCR Product Purification Protocol Featuring the Wizard® SV Gel and PCR Clean-Up System

Materials Required:

- Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281)
- 1.5ml microcentrifuge tubes
- ethanol (95%)
- Vacuum Adapters (Cat.# A1331; only for vacuum purification)
- agarose gel (standard or low-melting point; only for gel purification)
- 1X TAE or TBE electrophoresis buffer (only for gel purification)
- 50–65°C heating block (only for gel purification)

A. Preparing the Membrane Wash Solution

Add the indicated volume of 95% ethanol to the Membrane Wash Solution prior to beginning the procedure (see Table 9.11). Mark the bottle label to record that this addition was made. Tightly close the bottle cap after each use to prevent evaporation.

Table 9.10. Volume of 95% Ethanol to Add to Membrane Wash Solution for Each System Size.

System Size	Volume of 95% Ethanol
10 preps	15ml
50 preps	75ml
250 preps	375ml

B. DNA Purification by Centrifugation

Gel Slice and PCR Product Preparation

Dissolving the Gel Slice

1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
2. Add 10µl of Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

Processing PCR Amplifications

1. Add an equal volume of Membrane Binding Solution to the PCR amplification.

Binding of DNA

1. Insert SV Minicolumn into Collection Tube.
2. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
3. Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.

   Note: If the volume of the DNA solution is >700µl, repeat Steps 2 and 3, transferring ≤700µl until all the solution has been processed.

Washing

4. Add 700µl of Membrane Wash Solution (ethanol added). Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
5. Repeat Step 4 with 500µl of Membrane Wash Solution. Centrifuge at 16,000 × *g* for 5 minutes.
6. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

Elution

7. Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
8. Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × *g* for 1 minute.
9. Discard Minicolumn and store DNA at 4°C or –20°C.

C. DNA Purification by Vacuum

Gel Slice and PCR Product Preparation

Dissolving the Gel Slice

1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
2. Add 10µl of Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

Processing PCR Amplifications

1. Add an equal volume of Membrane Binding Solution to the PCR amplification.

Binding of DNA

1. Attach Vacuum Adapter to manifold port and insert SV Minicolumn into Adapter.
2. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn. Incubate at room temperature for 1 minute.
3. Apply vacuum to pull liquid through Minicolumn. Release vacuum when all liquid has passed through Minicolumn.

Washing

4. Add 700µl of Membrane Wash Solution (ethanol added). Apply a vacuum to pull solution through Minicolumn.
5. Turn off vacuum and repeat Step 4 with 500µl of Membrane Wash Solution. Apply a vacuum to pull solution through Minicolumn.
6. Transfer Minicolumn to a Collection Tube. Centrifuge at 16,000 × *g* for 5 minutes.
7. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

Elution

8. Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
9. Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × *g* for 1 minute.
10. Discard Minicolumn and store DNA at 4°C or –20°C.

XI. Composition of Solutions

LB (Luria-Bertani) medium (1 liter)

10g Bacto®-tryptone
5g Bacto®-yeast extract
5g NaCl

Adjust pH to 7.5 with NaOH. Autoclave.

LB-Miller medium (1 liter)

10g Bacto®-tryptone
5g Bacto®-yeast extract
10g NaCl

Adjust pH to 7.0 with NaOH. Autoclave.

Membrane Wash Solution
(Wizard® SV Gel and PCR Clean-Up System)

10mM potassium acetate (pH 5.0)
80% ethanol (after ethanol addition)
16.7µM EDTA (pH 8.0)

To prepare this solution, add 95% ethanol to the supplied Membrane Wash Solution (concentrated) as described in Table 9.11 in the Fragment/PCR Product Purification protocol section.

Membrane Binding Solution
(Wizard® SV Gel and PCR Clean-Up System)

4.5M guanidine isothiocyanate
0.5M potassium acetate (pH 5.0)

1X TE buffer

10mM Tris-HCl (pH 7.5)
1mM EDTA (pH 8.0)

1X TBE buffer

89mM Tris base
89mM boric acid
2mM EDTA (pH 8.0)

1X TAE buffer

40mM Tris base
5mM sodium acetate
1mM EDTA (pH 8.0)

Terrific Broth (1 liter)

12g Bacto®-tryptone
24g Bacto®-yeast extract
4ml glycerol

Add components to 900ml deionized water. Autoclave and allow solution to cool to ~60°C. Add 100ml of a sterile solution of 0.17M KH_2PO_4, 0.72M K_2HPO_4 and mix to disperse evenly.

0.17M KH_2PO_4, 0.72M K_2HPO_4 sterile solution

2.31g KH_2PO_4
12.54g K_2HPO_4

Dissolve in 90ml deionized water. Adjust volume to 100ml and sterilize by autoclaving.

YPD broth (1 liter)

10g yeast extract
20g peptone
20g dextrose

Autoclave. Final pH 6.5±0.2 at 25°C.

2X YT medium (1 liter)

16g Bacto®-tryptone
10g Bacto®-yeast extract
5g NaCl

Adjust pH to 7.0 with NaOH. Autoclave.

XII. References

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