Prep-A-Gene® DNA Purification Systems

Instruction Manual

Catalog Numbers

DNA Purification Kits
732-6010, 732-6011

Master Kits
732-6009, 732-6016

BIO-RAD

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Section 1
Introduction to Prep-A-Gene DNA Purification Systems

1 .1 General Information

The Prep-A-Gene* silica-based DNA purification matrix provides a versatile and effective method for fast, efficient purification and concentration of DNA. There are many applications of the Prep-A-Gene DNA purification matrix including

- Purification of DNA from agarose gels
- Purification of PCR** amplified DNA fragments from PCR reaction mixtures
- Purification of single stranded M 13 or phagemid DNA
- Purification of cosmid DNA
- Purification of lambda phage DNA
- Desalting or concentration of previously purified DNA

In addition, the size-selective binding properties of the Prep-A-Gene matrix can be used to remove unreacted, labeled nucleotide triphosphates or small pieces of DNA such as linkers and oligonucleotide primers from reaction mixtures. RNA, protein, organic solvents, SDS, and other enzyme-inhibiting impurities are washed from the DNA/Prep-A-Gene matrix complex.

While it is possible to use the Prep-A-Gene master kit reagents for plasmid minipreps, best results are obtained with the Quantum Prep" miniprep and Quantum Prep midiprep kits.

Prep-A-Gene DNA purification matrix has a higher DNA binding capacity than glass-based matrices (>0.2 µg of supercoiled DNA per pi of suspended matrix). Prep-A-Gene based protocols eliminate phenol/chloroform extractions and time consuming alcohol precipitation of DNA. CsCl density gradients, dialysis procedures, and crush-and-soak techniques are replaced by Prep-A-Gene DNA purification methods, allowing most preparations to be performed in less than 20 minutes.

The Prep-A-Gene DNA purification kit contains buffers specifically designed for the purification of DNA from agarose gels. The Prep-A-Gene master kit conveniently packages all the buffers and components from both the Prep-A-Gene DNA purification kit and reagents for DNA minipreps so that all applications using the Prep-A-Gene matrix can be performed with just one kit.

* US Patent number 5,075,430 issued to Bio-Rad Laboratories.

** The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-LaRoche. Use of the PCR process requires a license.

1.2 Kit Components

DNA Purification Kit

C&log Number	Product Description
732-6010	Prep-A-Gene DNA Purification Kit, includes Prep-A-Gene matrix, 2 ml; DNA purification kit binding buffer, 100 ml; wash buffer, 63 ml; elution buffer, 50 ml: instruction manual. Contains sufficient reagents for DNA purifications from 100 agarose gel slices approximately 250 mg in size.
732-6011	Prep-A-Gene DNA Purification Kit, includes Prep-A-Gene matrix, 12 ml; DNA purification kit binding buffer, 500 ml; wash buffer, 250 ml; elution buffer, 50 ml, instruction manual. Contains sufficient reagents for DNA purifications from 500 agarose gel slices approximately 250 mg in size.
732-6009	Prep-A-Gene Master Kit, includes Prep-A-Gene matrix, 2 ml; DNA purification kit binding buffer (for gel extractions), 100 ml; DNA miniprep kit binding buffer, 20 ml; wash buffer, 63 ml; elution buffer, 50 ml; spin filters, 50; instruction manual
732-6016	Prep-A-Gene Master Kit, large, includes Prep-A-Gene matrix, 12 ml; DNA purification kit binding buffer (for gel extractions), 500 ml; DNA miniprep kit binding buffer, 200 ml; wash buffer, 250 ml, elution buffer, 50 ml; spin filters, 400; instruction manual

Prep-A-Gene Reagent Components

Prep-A-Gene DNA Purification Kit Binding Buffer: 6 M Sodium Perchlorate; 50 mM Tris (pH 8.0); 10 mM EDTA (pH 8.0)

Prep-A-Gene DNA Miniprep Kit Biding Buffer: 6 M Guanidine Hydrochloride; 100 mM Tris (pH 7.5)

PrepA-Gene Wash Buffer: 800 mM NaCl; 40 mM Tris (pH 7.5); 4 mM EDTA (pH 7.5). Final concentrations after the addition of a lx volume. of 95–100% EtOH: 400 mM NaCl; 20 mM Tris (pH 7.5); 2 mM EDTA (pH 7.5); 50% EtOH (v/v)

1.3 Additional items Required but Not Provided

95–100% ethanol	Used to reconstitute wash buffer
Centrifuge tubes	1.5 ml polypropylene microcentrifuge tubes are sufficient for most purposes (catalog number 223-9480). Larger sample volumes require the use of larger tubes.
Centrifuge	A standard benchtop microcentrifuge is sufficient for most applications.
Vortex mixer	For matrix suspension purposes.
Water bath	37–60 °C for incubation of matrix/DNA complex mixtures.

1.4 Buffer, Prep-A-Gene Matrix, and Sample Preparation

The Prep-A-Gene buffers can be stored at room temperature. For long term storage, place at 4 °C. The binding and elution buffers are supplied ready-to-use and require no preparation.

Wash Buffer Preparation

Dilute Prep-A-Gene wash buffer 1:1 with 95–100% ethanol before use. The pH of this wash buffer should be approximately 7.45 +/- 0.15 at room temperature.

Warning: Prep-A-Gene binding buffers contain either sodium perchlorate (DNA purification binding buffer) or guanidine hydrochloride (DNA miniprep binding buffer). Both are strong oxidizers and can be harmful. Avoid contact and inhalation. Store these buffers away from reducing agents and **strong** acids. Wear gloves and safety glasses. See material safety data sheets (MSDS) for proper binding buffer disposal.

Sample Preparation

No sample preparation is required before using the Prep-A-Gene kits. DNA is bound to the Prep-A-Gene matrix by the binding buffer even in the presence of salts, organic molecules, excess protein, RNA, and dissolved agarose.

Use of the Prep-A-Gene Matrix Suspension

Before using the Prep-A-Gene matrix, resuspend the matrix thoroughly by vigorous vortexing or inversion. This will insure a uniform concentration of matrix and will remove any aggregated matrix particles. Inverting the bottle after vortexing will insure that all matrix has been resuspended. **Warning:** See Section 8 for Health Hazard Information regarding the Prep-A-Gene matrix.

1.5 Scale-Up of Prep-A-Gene Matrix DNA Purifications

Increasing the amount of DNA purified using Prep-A-Gene matrix can be accomplished using the amounts of reagents given in Table 1.

Table 1. Prep-A-Gene Scale-Up for DNA Purification

DNA w	Prep-A-Gene matrix (µl)	Binding buffer (ml)*	Wash buffer (ml)	Elution buffer (PO	Tube needed
2	10	(*)	0.25	20	0.5 ml
5	25	(*)	0.625	50	1.5 ml
20	100	(*)	2.5	200	15.0 ml
200	1,000	(*)	10.0	2,000	15.0 ml
1,000	5,000	(*)	50.0	10,000	50.0 ml

* Binding buffer quantities wed for DNA isolation depend on the volume of the solution containing the DNA. If isolating DNA from solution using the DNA purification kit binding buffer (sodium perchlorate) a 3x volume of binding buffer is used. If isolating DNA from solution using the DNA miniprep kit binding buffer (guanidine hydrochloride) a lx volume of binding buffer is used.

Note: These volumes are provided only as a general guideline for the scale-up of the Prep-A-Gene procedure in the purification of plasmids from solution. Volumes most effective for each circumstance are best determined empirically.

1.6 Prep-A-Gene Matrix Specifications

DNA binding capacity	0.2 µg supercoiled DNA/µl Prep-A-Gene matrix
Size range for purification	0.2–50 Kbp
Operating temperature range	20–60 °C
pH stability	2–12
Shipping solvent	Deionized water
Shelf life	1 year

Note: For DNA fragments ranging from 200–400 bp, the yield of the DNA from the Prep-A-Gene matrix can be variable depending on the fragment. It is recommended that a small quantity of each DNA fragment within the range of 200–400 bp be tested with this kit before attempting to purify the entire sample.

All Prep-A-Gene DNA purification kits, reagents, and buffers are physically and functionally tested before shipment to insure the highest quality and reproducibility.

Section 2
Prep-A-Gene Master Kit Instructions and Protocols

The Prep-A-Gene master kit is a combination of the Prep-A-Gene DNA purification kit and the reagents for DNA minipreps, components, and protocols. To perform the desired DNA purification procedure or application using the Prep-A-Gene master kit, refer to the corresponding protocol in

Section 3 or 4 of this manual. When using the master kit, it is important to note which binding buffer (DNA purification kit binding buffer or DNA miniprep binding buffer) is required for the procedure being performed.

Section 3
DNA Purification Kit Instructions and Protocols

3.1 DNA Isolation from Agarose Gel Slices

Make sure the correct Prep-A-Gene binding buffer is used for this procedure. This procedure requires the use of **the DNA purification kit binding buffer.**

1. Excise the desired DNA band from an ethidium bromide stained agarose gel and place it into a microcentrifuge tube. Estimate the concentration of the DNA in the band by comparison to a band of known concentration on the gel.
2. Centrifuge the sample for several seconds to bring down the gel slice. Estimate the gel slice volume by weighing the gel slice and convert to milliliters. For conversion of grams to milliliters, assume 1 gram of a gel slice is equal to 1 milliliter. Estimate the volume of this slice, to within 20%, by placing a similar tube next to it and dropping liquid from a pipette into the empty tube until the volume is equivalent to that **of the** gel slice.
3. Estimate the amount of Prep-A-Gene matrix required to bind all the DNA present. The capacity of the Prep-A-Gene matrix for supercoiled DNA is 0.2 µg DNA per microliter of completely resuspended matrix. Thus, 5 µl Prep-A-Gene matrix is needed for each microgram of DNA to be adsorbed. For amounts of DNA less than 1 µg, 5 µl of Prep-A-Gene matrix will be required.

4. Based on the volume of the gel slice plus the amount of Prep-A-Gene matrix required for total DNA binding, add 3 volumes of Prep-A-Gene DNA purification kit binding buffer (sodium perchlorate) to the gel slice and agitate gently to dissolve. Note: See Tip number 6, page 26–27. If necessary, heat the tube at 37–55 °C for several minutes to assist in dissolving the agarose. Cutting larger gel slices into 2 mm pieces also will hasten dissolution.

   Note: The Prep-A-Gene DNA miniprep binding buffer (guanidine hydrochloride) cannot be used for this application. Guanidine hydrochloride does not dissolve agarose.

5. Add the predetermined amount of Prep-A-Gene matrix. Mix gently by flicking the tube or vortexing briefly, and incubate 5–10 minutes at room temperature. End-over-end rocking or frequent agitation during the binding step will facilitate binding.

6. Pellet the DNA-containing Prep-A-Gene matrix by centrifuging for 30 seconds in a microcentrifuge. Remove the supernatant either with a pipette tip attached to a vacuum aspirator or with a pipette. Rinse the pellet containing the bound DNA by resuspending it gently in an amount of DNA purification kit binding buffer equivalent to 25 times the amount of added matrix, using brief vortexing or by flicking the centrifuge tube.

7. Centrifuge for 30 seconds to pellet the matrix and dispose of the supernatant from step 6. Wash the PrepA-Gene pellet two times with a 25x matrix volume of prepared wash buffer.

   (Add one volume of 95–100% ethanol to the Prep-A-Gene wash buffer before first use. See page 4, Section 1.4.)

   After the second wash and centrifugation, carefully remove all traces of liquid in the tube. Re-centrifuge the tube to pellet the matrix firmly and then remove the remaining supernatant. Ethanol and high concentrations of salt can inhibit enzymes, so it is important to

remove the last traces of wash buffer before eluting DNA from the Prep-A-Gene pellet (see Section 5 for suggestions).

8. To elute the bound DNA, resuspend the Prep-A-Gene matrix pellet in at least 1 pellet volume of elution buffer, and incubate at 37–50 °C for 5 minutes. Spin as above to make a solid pellet. Transfer the DNA containing supernatant to a clean tube and centrifuge briefly to check for and, if necessary, to remove any traces of Prep-A-Gene matrix.

At this point the DNA is ready to use for transformation, restriction digestion, ligation, and sequencing procedures. At least 85% of the DNA is recovered in the elution step. However, a second wash with 1 pellet volume of elution buffer will yield an additional 10–15% recovery. This method generally exhibits DNA recovery in excess of 85% and is especially useful for the removal of bound ethidium bromide.

3.2 Purification, Concentration, and Desalting of DNA from Solution

Both single-and double-stranded DNA is purified easily from solution and concentrated using the Prep-A-Gene DNA purification matrix. This procedure may be used, for instance, to further purify DNA resulting from plasmid minipreps or from single-stranded phage preps.

Note: When scaling up the basic protocol to purify from 2 µg to 1 mg of DNA, refer to the quantities of reagents and suggested tube sizes in Table 1.

Make sure the correct Prep-A-Gene binding buffer is used for this procedure. This procedure requires the use of the **DNA purification kit binding buffer.**

1. If using dried nucleic acid pellets, resuspend the pellet with TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), or water. Centrifuge to remove any clumps of undissolved protein. If the DNA is already in solution, proceed to step 2.

2. Determine the amount of Prep-A-Gene matrix you will need, using the capacity figure of 0.2 µg supercoiled DNA/µl Prep-A-Gene matrix. For example, for a cell prep from 3 ml of cultured cells (yielding approximately 5–10 µg of plasmid DNA) add approximately 25 µl of Prep-A-Gene matrix.
3. Add a quantity of DNA Purification Binding Buffer equal to three times the combined volumes of Prep-A-Gene matrix suspension and DNA containing solution to the sample. Add the Prep-A-Gene matrix at this time, and incubate the tube at room temperature for 10 minutes. Frequent agitation of the sample will facilitate Prep-A-Gene matrix/DNA binding.
4. Pellet the DNA-containing Prep-A-Gene matrix by centrifuging for 30 seconds in a microcentrifuge. Remove the supernatant either with a pipette tip attached to a vacuum aspirator or with a pipette. Rinse the pellet containing the bound DNA by resuspending it gently in an amount of DNA purification kit binding buffer equivalent to 25 times the amount of added matrix, using brief vortexing or by flicking the centrifuge tube.
5. Centrifuge for 30 seconds to pellet the matrix and dispose of the supernatant from step 4. Wash the Prep-A-Gene pellet two times with a 25x matrix volume of prepared wash buffer.

   (Add one volume of 95–100% ethanol to the Prep-A-Gene wash buffer before first use. See page 4, Section 1.4.)

   After the second wash and centrifugation, carefully remove all traces of liquid in the tube. Here, you may want to remove most of the supernatant, re-centrifuge the tube to pellet the matrix firmly and then remove the remaining supernatant. Ethanol and high concentrations of salt can inhibit enzymes, so it is important to remove the last traces of wash buffer before eluting DNA from the Prep-A-Gene pellet (see Section 5 for suggestions).

6. To elute the bound DNA, resuspend the Prep-A-Gene matrix pellet in at least 1 pellet volume of elution buffer, and incubate at 37–50 °C for 5 minutes. Spin as above to make a solid pellet. Transfer the DNA containing supernatant to a clean tube and centrifuge briefly to check for and, if necessary, to remove any traces of Prep-A-Gene matrix.

At this point the DNA is ready to use for transformation, restriction digestion, ligation, and sequencing procedures. At least 85% of the DNA is recovered in the elution step. However, a second wash with 1 pellet volume of elution buffer will yield an additional 10–15% recovery. This method generally exhibits DNA recovery in excess of 85% and is especially useful for removal of bound ethidium bromide.

3.3 Removal of Residual Oligonucleotide Primers, Linkers, and Unincorporated Nucleotides from Larger DNA Fragments.

An additional application of the above protocol is the rapid removal of linkers from cloning reactions or of nucleoside triphosphates from radiolabeled reactions. The excess linker fragments or unincorporated nucleotides needed in these reactions must be removed prior to use of the modified DNA in cloning or blotting procedures. The Prep-A-Gene matrix selectively binds DNA larger than 200 base pairs, thereby eliminating smaller oligonucleotides or single nucleotides from the reaction mixture during the washing steps. The use of Prep-A-Gene matrix as described above has been shown to remove up to 99.5% of the unincorporated nucleotides. Follow the protocol in Section 3.2 to achieve removal of small oligonucleotides or nucleotides from large DNA fragments.

3.4 Purification of PCR Amplified DNA Fragments from Reaction Mixtures

This procedure is similar to that used to purify and concentrate DNA from solution (Section 3.2). Additionally, the size selective properties of the Prep-A-Gene matrix remove 99.5% of residual oligonucleotide primers and unincorporated nucleotides. Taq polymerase and other reaction mixture components are completely removed during the wash steps. The resulting purified DNA is suitable for restriction enzyme digestion, ligation, and sequencing.

Make sure the correct Prep-A-Gene binding buffer is used for this procedure. This procedure requires the use of the **DNA purification kit binding buffer.**

1. If oil is used in the reaction to prevent evaporation, remove the aqueous phase to a clean tube.
2. Estimate the quantity of Prep-A-Gene matrix required to bind the PCR amplified DNA, using the capacity figure of 0.2 µg supercoiled DNA/µl Prep-A-Gene matrix (if in doubt, use 10 µl).
3. Add a quantity of DNA purification kit binding buffer equal to three times the combined volumes of Prep-A-Gene matrix suspension and DNA containing solution to the sample. Mix briefly. Add the Prep-A-Gene matrix at this time, and incubate the tube at room temperature for 10 minutes. Frequent agitation of the sample will facilitate Prep-A-Gene matrix/DNA binding.
4. Pellet the DNA-containing Prep-A-Gene matrix by centrifuging for 30 seconds in a microcentrifuge. Remove the supernatant either with a pipette tip attached to a vacuum aspirator or with a pipette. Rinse the pellet containing the bound DNA by resuspending it

gently in an amount of DNA purification kit binding buffer equivalent to 25 times the amount of added matrix, using brief vortexing or by flicking the centrifuge tube.

5. Centrifuge for 30 seconds to pellet the matrix and dispose of the supernatant from step 4. Wash the Prep-A-Gene pellet two times with a 25x matrix volume of prepared wash buffer.

   (Add one volume of 95–100% ethanol to the Prep-A-Gene wash buffer before first use. See page 4, Section 1.4.)

   After the second wash and centrifugation, carefully remove all traces of liquid in the tube. Here, you may want to remove most of the supernatant, re-centrifuge the tube to pellet the matrix firmly and then remove the remaining supernatant. Ethanol and high concentrations of salt can inhibit enzymes, so it is important to remove the last traces of wash buffer before eluting DNA from the Prep-A-Gene pellet (see Section 5 for suggestions).

6. To elute the bound DNA, resuspend the Prep-A-Gene matrix pellet in at least 1 pellet volume of elution buffer, and incubate at 37–50 °C for 5 minutes. Spin as above to make a solid pellet. Transfer the DNA containing supernatant to a clean tube and centrifuge briefly to check for and, if necessary, to remove any traces of Prep-A-Gene matrix.

At this point the DNA is ready to use for transformation, restriction digestion, ligation, and sequencing procedures. At least 85% of the DNA is recovered in the elution step. However, a second wash with 1 pellet volume of elution buffer will yield an additional 10–15% recovery. This method generally exhibits DNA recovery in excess of 85% and is especially useful for removal of bound ethidium bromide.

3.5 Replacement for Phenol/Chloroform Extractions

It is frequently necessary to remove proteins or incompatible buffers from DNA solutions so that subsequent enzymatic reactions can take place. The Prep-A-Gene matrix allows the removal of proteins and/or buffers without requiring organic extraction or ethanol precipitation, thus decreasing the time needed for subsequent manipulations, When further purifying a DNA solution that requires the removal of proteins or buffers, the steps used are the same as described in Section 3.2.

3.6 Purification of Genomic DNA for Southern Blots and PCR

DNA fragments greater that 50 Kb in length can be sheared by the Prep-A-Gene matrix particles during purification. It is only recommended to purify genomic DNA using the Prep-A-Gene matrix for PCR or other DNA amplification techniques and in some cases Southern Blot analysis. If interested in using the Prep-A-Gene DNA purification kit for purification of genomic DNA, request bulletins 1848, 1849, and reference number 4 (from Section 6) by contacting your local Bio-Rad representative. The bulletins have been written by independent laboratories and contain protocols for the purification of genomic DNA using the Prep-A-Gene DNA purification kit.

Section 4
Protocols for DNA Miniprep Using the Prep-A-Gene Master Kit Instructions and Protocols

While it is possible to use the Prep-A-Gene master kit reagents for plasmid minipreps, best results are obtained with the Quantum Prep'" miniprep and Quantum Prep midiprep kit.

4.1 Plasmid Miniprep DNA Purification

Additional Equipment Required

See Section 1.3

Sterile loops	To select plasmid-containing cells.

Preparation of Buffers and Solutions

See Section 1.4

Additional buffers not included in Section 1.4

1. Prepare TB Media by dissolving 12 g bacto-tryptone, 24 g yeast extract, and 4 ml glycerol in 900 ml of deionized water. Autoclave, cool to 60 °C, and add 100 ml of a sterile filtered solution containing 2.33 g (0.17 M) potassium dihydrogen phosphate (K&PO,) and 16.4 g (0.72 M) of dipotassium hydrogen phosphate, trihydrate ($K_2HPO_4 \cdot 3H_2O$).

Note: For preparation of the following buffers, it is recommended that deionized H_2O be used. It is also recommended that all buffers be sterilized (except **the alkaline lysis solution)** by autoclaving at 15 psi for 20 minutes.

2. Prepare cell resuspension buffer (50 mM Tris-HCl, 10 mM EDTA, pH 8.0).
3. Prepare elution buffer (TE; 10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
4. Prepare alkaline lysis solution by dissolving 1.0 g SDS in 100 ml of 0.2 M NaOH. Do not autoclave.
5. Prepare 2.55 M potassium acetate, pH 4.8, by dissolving 25.03 g of potassium acetate in sufficient deionized water to yield 80 ml of solution. Adjust the pH to 4.8 with glacial acetic acid and add deionized water to give a final volume of 100 ml.

Instructions

Make sure the correct Prep-A-Gene binding buffer is used for this procedure. This procedure requires the use of the **DNA miniprep kit binding buffer.** This protocol was developed as a modification of the Birnboim and Doly alkaline lysis miniprep for plasmid DNA.[1]

Place 3 ml aliquots of TB media,[2,3] containing the appropriate antibiotic, into 15 ml sterile culture tubes. Using sterile toothpicks or loops, pick and place individual colonies of plasmid-containing cells into the media-containing tubes. Shake for 20 hours at 37 °C (250 rpm).

2. Pour half of each overnight culture into a 1.5 ml microcentrifuge tube. Centrifuge at 13,000 rpm for 30 seconds to pellet the cells. Using a Pasteur pipette tip attached to a vacuum aspirator, suction off the supernatants, leaving the cell pellets intact. **Note: If** a larger yield of DNA is desired, the second half of each culture may be added to the cell pellet-containing tube and pelleted as above.
3. Resuspend each cell pellet in 200 µl of cell resuspension buffer by vortexing. Be sure that each pellet is completely resuspended before proceeding.
4. Add 200 µl of alkaline lysis solution to each sample and mix by inverting the tubes several times.

5. Add 200 µl of 2.55 M potassium acetate, pH 4.8, to each sample and invert the tubes several times to mix. A visible precipitate should form at this point.

6. Centrifuge the tubes at 13,000 rpm for 5 minutes at room temperature in a microcentrifuge. Transfer each resulting supernatant to a clean tube by carefully decanting from the pellet. Recentrifuge for 5 minutes. This second spin removes any remaining particulates that will clog the spin filters, and is recommended. During the centrifugation step, aliquot 300 µl of Prep-A-Gene DNA miniprep kit binding buffer (guanidine hydrochloride) into the appropriate number of clean 1.5 ml microcentrifuge tubes.

7. Following the second spin, pour each supernatant into a tube containing 300 µl of DNA miniprep kit binding buffer (guanidine hydrochloride).

8. Add 30 µl (60 µl if 3.0 ml of culture was used) of resuspended Prep-A-Gene matrix to each tube. Using a pipette, mix by one or two up and down pipetting motions, then apply the entire amount of each sample to a spin filter which is nested inside a 1.5 or 2.0 ml microcentrifuge tube. The spin filter unit should contain the volume produced in the above steps. If not, the balance of the solution should be applied after the initial spin.

9. Place the spin filter unit into a microcentrifuge and centrifuge for 10–30 seconds. Remove the eluted volume using a vacuum suction device or pipette and discard.

10. Pipette 500 µl of Prep-A-Gene wash buffer into each spin filter unit. **(Add one volume of 95% EtOH to the Prep-A-Gene wash buffer before tint use. See page 4, Section 1.4.)** Centrifuge for 10–30 seconds. Remove and discard the eluted volume as above and repeat this wash buffer step once. Note: A final centrifugation should be performed for 2 minutes to insure that all wash buffer has eluted from the spin filter.

Il. Place the spin filter into a clean tube. The plasmid DNA is eluted by placing 100 µl of TE into each spin filter-unit and centrifuging for 1 minute. The resulting eluted solution contains the DNA in a form suitable for agarose gel analysis and subsequent enzymatic reactions, such as restriction enzyme digestion or DNA sequencing.

4.2 Ml 3 or Single-Stranded Phagemid DNA MiniprepPurification

Additional Equipment Required

See Section 1.3

This protocol is a miniprep procedure used to prepare single-stranded DNA from M13 phage or phagemid infected cell cultures. The protocol begins following the phage amplification step or the phagemid induction and amplification step in cell culture.[3]

Preparation of Buffers and Solutions

See Section 1.4

Instructions

Make sure the correct Prep-A-Gene binding buffer is used for this procedure. This procedure requires **the** use of **the DNA miniprep kit binding buffer.**

1. Pour phage/phagemid infected cultures into 1.5 ml microcentrifuge tubes and centrifuge at 12,000 r-pm for 15 minutes to pellet all cellular debris.
2. During the centrifugation step, label the appropriate number of 1.5 ml microcentrifuge tubes and add 400 µl of DNA miniprep kit binding buffer (guanidine hydrochloride) to each tube.

3. Remove 800 µl of the resulting supernatants to the binding buffer containing tubes. Invert the tubes several times and incubate at room temperature for 5 minutes with intermittent inversion to lyse the phage particles.
4. Add 30 µl of Prep-A-Gene matrix to each tube. Vortex the tubes to resuspend the matrix and place at room temperature for 5 minutes with intermittent mixing.
5. Using a pipette, mix the solution and then apply 700 µl of each sample to a spin filter which is nested inside a 1.5 ml or 2.0 ml microcentrifuge tube.
6. Place the spin filter unit into a microcentrifuge and centrifuge for 10–30 seconds. Remove the eluted volume using a vacuum suction device or pipette and discard. Repeat steps 5 and 6 using the remaining solution from step 3.
7. Pipette 500 µl of Prep-A-Gene wash buffer into each spin filter unit. **(Add one volume of 95% EtOH to the Prep-A-Gene wash buffer before first use. See page 4, Section 1.4.**) Centrifuge for 10–30 seconds. Remove and discard the eluted volume as above and repeat this wash buffer step once. Note: A final centrifugation should be performed for 2 minutes to insure that all wash buffer has eluted from the spin filter.
8. Place the spin filter into a clean marked tube. The DNA is eluted by placing 50 µl of TE or sterile deionized water into each spin filter unit and centrifuging for 10–30 seconds. Repeat this step once to enhance recovery of single-stranded DNA (see Section 5: Tips and Suggestions for Using Prep-A-Gene DNA purification matrix). The resulting eluted solution contains the DNA in a form suitable for DNA sequencing. Analyze 5 µl by agarose gel electrophoresis.

4.3 Purification of PCR Amplified DNA Fragments from PCR Reactions

The size selective binding properties of the Prep-A-Gene matrix (0.2–50 Kbp) allow the removal of unreacted or unincorporated nucleoside triphosphates or small pieces of DNA such as linkers and oligonucleotide primers from reaction mixtures. Thus, the Prep-A-Gene matrix is ideal for purifying PCR amplified fragments (> 200 bp) from the original PCR reaction mixtures after the thermocycling procedure is completed. Note: For PCR amplified fragments ranging from 200–400 bp, yield of the DNA from the Prep-A-Gene matrix can be variable depending on the fragment. It is recommended that a small quantity of each DNA fragment within the range of 200–400 bp be tested with this kit before attempting to purify the entire sample.

Additional Items Required

See Section 1.3

Preparation of Buffers and Solutions

See Section 1.4

Instructions

Make sure the correct Prep-A-Gene binding buffer is used for this procedure. This procedure requires the use of **the DNA miniprep kit binding buffer.**

1. During the PCR thermocycling procedure, mark the appropriate number of 1.5 ml microcentrifuge tubes. Based on the volume of the PCR reaction mixture, place an equal volume of the DNA miniprep kit binding buffer (guanidine hydrochloride) into the tubes.

2. After the PCR thermocycling procedure has ended, remove an aliquot (5 µl) and analyze on an agarose gel. Estimate total DNA yield by comparing the ethidium bromide stain to a DNA band of known concentration on the gel, for example, the DNA Mass Ladder from Bio-Rad, 170-8207.
3. Estimate the **amount** of Prep-A-Gene matrix required to bind all the DNA present. The binding capacity of the Prep-A-Gene matrix is 0.2 µg DNA/µl of completely resuspended matrix. For amounts of DNA less than 1 µg, 5 µl of Prep-A-Gene matrix will be required. If the concentration or total yield of the DNA is uncertain, use 10 µl of the Prep-A-Gene matrix.
4. Add the remaining PCR reaction mixture to the microcentrifuge tubes containing the DNA miniprep kit binding buffer (guanidine hydrochloride). Mix by pipetting up and down several times. Note: Mineral oil is often used to reduce evaporation during the PCR thermocycling procedure. Avoid the mineral oil layer when obtaining the PCR reaction mixture.
5. Add the quantity of Prep-A-Gene matrix that was estimated in step 3. Mix by pipetting. Incubate at room temperature for 5 minutes with intermittent agitation.
6. Remove the entire solution and place into a spin filter which is nested into a 1.5 or 2.0 ml microcentrifuge tube.
7. Place the spin filter unit into a microcentrifuge and centrifuge for 10–30 seconds. Remove the eluted volume from the tube and discard.
8. Pipette 500 µl of Prep-A-Gene wash buffer into each spin filter unit. **(Add one volume of 95-100% Ethanol to the Prep-A-Gene wash buffer before first use. See page 4, Section 1.4.)** Centrifuge for 10–30 seconds. Remove and discard the eluted volume using a vacuum suction device or pipette and discard.

9. Repeat the wash step. Note: A final centrifugation should be performed for 2 minutes to insure that all wash buffer has eluted from the spin filter.
10. Place the spin filter into a clean marked tube. The PCR amplified DNA is eluted by placing 25 µl of TE or deionized water into each spin filter unit and centrifuging for 1 minute. The resulting eluted solution contains the DNA in a form suitable for DNA sequencing and other enzymatic reactions.

4.4 Cosmid Miniprep DNA Purification

When purifying large DNA (e.g., cosmid and lambda phage DNA) it is important to treat the solutions containing the DNA gently to minimize shearing of the DNA. This can be accomplished by using wide bore pipette tips and swirling or inverting the tubes to mix the solution instead of vortexing. Slightly lower recovery efficiencies (75–90%) may occur when purifying larger DNA (>25 Kb) using the Prep-A-Gene matrix versus the recovery of plasmid or phagemid DNA (90–95%).

See Section 4.1

4.5 Lambda DNA Miniprep Purification

This protocol is a miniprep procedure for the purification of lambda DNA from plate or liquid lambda phage lysate stocks. The protocol begins immediately after complete lysis of phage liquid culture or following elution of lambda phage from the top agarose of a confluently lysed plate by diffusion with an appropriate buffer such as SM[3]. The titer of this stock solution should be 1 x 10^{10-11} pfu/ml to obtain the best yield of purified lambda phage DNA using the Prep-A-Gene matrix. Expected yield from 1.0 ml of a 1 x 10^{10-11} pfu/ml stock is 0.5–5.0 pg.

When purifying large DNA (e.g., cosmid and lambda phage DNA) it is important to treat the solutions containing the DNA gently to minimize shearing of the DNA. This can be accomplished by using wide bore pipette tips and swirling or inverting the tubes to mix solution instead of vortexing. Slightly lower recovery efficiencies (75–90%) may occur when purifying larger DNA (>25 Kb) using the Prep-A-Gene matrix versus the recovery of plasmid or phagemid DNA (90–95%).

Additional Items Required

See Section 1.3

Preparation of Buffers and Solutions

See Section 1.4

1. Prepare DNase I stock solution by dissolving 10 mg of lyophilized DNase I into 10 ml of deionized water (1 mg/ml final concentration). Sterilize by passing the solution through a 0.22 micron filter. Aliquot into 1.5 ml microcentrifuge tubes. Store stocks at -20 °C

Instructions

Make sure the correct Prep-A-Gene binding buffer is used for this procedure. This procedure requires **the** use of **the DNA miniprep kit binding buffer.**

1. Add 1.0 ml of lambda phage stock solution into marked 1.5 ml microcentrifuge tubes and centrifuge at 12,000 rpm for 5 minutes to pellet any remaining bacterial cellular debris.
2. Carefully remove 800 µl of the supernatant with a pipette (do not disturb the pellet) and place into a newly marked 1.5 ml microcentrifuge tube.

3. Add DNase I to 1.0 µg/ml and incubate for 30 minutes at room temperature.
4. During the incubation, aliquot 400 µl of DNA miniprep kit binding buffer (guanidine hydrochloride) into a newly marked 1.5 ml microcentrifuge tube.
5. Remove 800 µl of the resulting supernatants to the binding buffer-containing tubes. Invert the tubes several times and incubate at room temperature for 5 minutes with intermittent inversion to lyse the phage particles.
6. Add 30 µl of Prep-A-Gene matrix to each tube. Vortex the tubes to resuspend the matrix and place at room temperature for 5 minutes with intermittent mixing.
7. Using a pipette, mix the solution and then apply 700 µl of each sample to a spin filter which is nested inside a 1.5 or 2.0 ml microcentrifuge tube.
8. Place the spin filter units into a microcentrifuge and centrifuge for 10–30 seconds. Remove the eluted volume using a vacuum suction device or pipette and discard. Repeat steps 7 and 8 using the remaining solution from step 5.
9. Pipette 500 µl of Prep-A-Gene wash buffer into each spin filter unit. **(Add one volume of 95-100% Ethanol to the Prep-A-Gene wash buffer before first use. See page 4, Section 1.4.)** Centrifuge for 10–30 seconds. Remove and discard the eluted volume as above and repeat this wash buffer step once. **Note:** A final centrifugation should be performed to insure that all wash buffer has eluted from the spin filter.
10. Place the spin filters into clean marked tubes. The DNA is eluted by placing 50 µl of TE or sterile deionized water into each spin filter unit and centrifuging for 10–30 seconds. The resulting eluted

solution contains the DNA in a form suitable for restriction enzyme digestion and DNA sequencing. Analyze 5 µl by agarose gel electrophoresis.

Note: Phage DNA yield can be increased using the Prep-A-Gene DNA miniprep kit if phage lysate volumes greater than 1.0 ml are used. To use phage lysate volumes greater than 1.0 ml, insert the following instructions between steps 3 and 7 in the protocol above. (i.e., omit steps 4–6 of the previous protocol ifusing the alternative protocol listed below).

Starting after step 3 in the previous protocol:

4. To any volume of the phage lysate add solid NaCl to a final concentration of 1 molar and solid PEG 8000 to a final concentration of 10% (w/v). Dissolve the solids and store this solution on ice for 1 hour to precipitate the phage.
5. Pellet the phage by centrifuging at 1 1,000–12,000 g for 15 minutes at 4 °C. After centrifugation, pour off the supernatant, invert the **tube and allow the** pellet to air dry.
6. Resuspend the pellet in 1.4 ml of DNA miniprep kit binding buffer (guanidine hydrochloride). Incubate at room temperature for 15 minutes. During this incubation, estimate the amount of phage DNA present (assume 0.5 µg of phage DNA per 1.0 ml of a 1 x 10^{10} pfu/ml phage lysate stock); calculate the amount of Prep-A-Gene matrix required to bind the phage DNA (Prep-A-Gene matrix DNA binding capacity is 0.2 µg/µl of Prep-A-Gene matrix). Add the appropriate amount of Prep-A-Gene matrix and incubate at room temperature for 15 minutes with intermittent agitation.

Continue with step 7 of me original lambda phage DNA purification protocol.

Section 5
Tips and Suggestions for Using Prep-A-Gene DNA Purification Matrix

This section provides several helpful hints and suggestions for DNA purification with the Prep-A-Gene matrix.

The Prep-A-Gene matrix should always be added after the binding buffer is mixed with the sample. In the absence of the binding buffer chaotrope, proteins present in the sample will bind to the Prep-A-Gene matrix, thereby decreasing the matrix binding capacity for DNA.

2. One way to insure that all traces of the ethanol-containing wash buffer have been removed is to place the tubes in a Speed-Vat for several minutes before adding the elution buffer. If using the spin filters, recentrifuge the spin filter units for 2 minutes after the last wash buffer application has been eluted from the unit. Such drying does not hinder the release of DNA from the Prep-A-Gene matrix.
3. The wash buffer is used to exchange the salts in the binding buffer for a low salt buffer. Alternatively, 80% ethanol can be substituted in the final wash buffer wash to eliminate Tris and EDTA if these components will be problematic in subsequent manipulations.
4. If a no salt elution is required, (e.g., preceding electroporation), DNA may be eluted from the matrix in sterile double-distilled water.
5. Always be sure to vortex the Prep-A-Gene matrix immediately before pipetting so that the correct amount of matrix is added. Since the slurry is viscous, pipette it slowly so that the correct volume is attained.
6. For the Prep-A-Gene DNA purification kit, it is important to maintain a perchlorate concentration greater than 4 M during the DNA binding

step. A three to one ratio of binding buffer to sample results in a 4.5 M perchlorate final concentration. Therefore, it is better to add more binding buffer than to underestimate the amount needed.

7. Additional binding buffer or wash buffer washes can be used to improve the purity of the DNA. These washes remove protein and RNA that are weakly bound to the matrix.
8. The efficiency of elution of DNA from the matrix depends upon the amount of elution buffer added relative to the volume of the pellet. Thus, 5 µl of elution buffer might only elute 50% of the DNA from a 5 µl Prep-A-Gene pellet, while 50 µl of elution buffer would elute >90% of the DNA from the same pellet. Unfortunately, the more elution buffer added, the more dilute the resulting DNA solution will be. Two consecutive elutions using recommended elution volumes will permit maximum recovery with minimal dilution. To maximize yield, elutions may be performed in larger volumes and ethanol precipitated to concentrate the DNA.
9. To help improve the yield of DNA from the Prep-A-Gene matrix, a wash step of one tenth the Prep-A-Gene matrix pellet volume with elution buffer helps to remove any remaining salts or wash buffer contaminants before the recommended elution buffer volume is applied. This small volume elution buffer wash step can be saved or discarded.
10. A general rule of thumb for estimating the amount of Prep-A-Gene matrix to use in a DNA prep from culture is to add 30 µl of matrix for each 1 .0 ml of culture.
11. When purifying previously isolated single-stranded DNA (rather than isolating it from cell culture), it is important that a three to one ratio of binding buffer to sample be used in the binding step.
12. Elution of the DNA from the Prep-A-Gene matrix may be facilitated by pre-warming the elution buffer to 37–60 °C and/or by

incubating the elution bufferPrep-A-Gene matrix solution at 37–60 °C for 5–10 minutes before pelleting the Prep-A-Gene matrix.

13. When binding DNA to the Prep-A-Gene matrix in a large binding buffer solution volume (>2.0 ml) increase the binding time and constantly agitate to insure that the Prep-A-Gene matrix binds all the DNA in solution.

14. When purifying large DNA (e.g., cosmid and lambda phage DNA) it is important to treat the solutions containing the DNA gently to minimize shearing of large DNA. This can be accomplished by using wide bore pipette tips and swirling or inverting the tubes to mix solutions instead of vortexing.

15. If using the Prep-A-Gene DNA purification kits for the first time, it is a good practice to save all the eluates, binding, and wash buffer washes for troubleshooting if problems arise. To all eluates or wash solutions, add 0.1x volume of 3 M sodium acetate (pH 5.2) and a lx volume of isopropanol to precipitate DNA that may be present. Centrifuge at 10,000 g to pellet the precipitate. Discard the supernatant and wash the pellet twice with 70% EtOH. Discard the EtOH washes and air dry the pellets. Dissolve the pellets in TE and analyze on an agarose gel. Visualize the DNA by ethidium bromide staining.

Section 6
References

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Section 7
Reagents and Instruments for DNA Purification and Analysis

Product Description	Catalog Number
Prep-A-Gene ***DNA Purification Kit***	
Prep-A-Gene DNA Purification Matrix, 2 ml	732-6012
Prep-A-Gene DNA Purification Matrix, 12 ml	732-6013
Prep-A-Gene DNA Purification Buffer Kit, includes DNA Purification Kit Binding Buffer, 500 ml; Wash Buffer, 250 ml; Elution Buffer, 50 ml	732-6020
Prep-A-Gene DNA Purification Kit Binding Buffer, 500 ml	732-6022
Prep-A-Gene Wash Buffer, 250 ml	732-6024
Prep-A-Gene Elution Buffer, 50 ml	732-6026
Prep-A-Gene DNA Miniprep ***Kit***	
Prep-A-Gene DNA Miniprep Kit Binding Buffer, 200 ml	732-6023
Quantum Prep Kits	
Quantum Prep Plasmid Miniprep Kit, 100 preps, includes 20 ml cell resuspension solution, 25 ml cell lysis solution, 25 ml neutralization solution, 20 ml Quantum Prep matrix, 63 ml wash buffer, 100 spin filters, and instructions	732-6100
Quantum Prep Plasmid Midiprep Kit, 20 preps, includes 110 ml cell resuspension solution, 110 ml cell lysis solution, 110 ml neutralization solution, 20 ml Quantum Prep matrix, 125 ml wash buffer, 20 spin columns, instructions	732-6120
Accessories	
Quantum Prep Matrix, 20 ml	732-6110
Quantum Prep Neutralization Solution, 25 ml	732-6115
Quantum Prep Neutralization Solution, 100 ml	732-6122
Spin Filters, 100	732-6027
Quantum Spin Columns, 20	732-6116

See the Bio-Rad Catalog or contact your local Sales Representative concerning the full line of the following related products.

Bst® Polymerase Sequencing Kits

Sequi-Gen® GT DNA Sequencing Cell Systems

Agaroses and DNA Size Standards

Sub-Cell@ GT DNA Agarose Electrophoresis Systems

Nucleic Acid Blotting Systems (Membranes, Reagents, and Apparatus)

Power Pat 300 and 3000 Power Supplies

Premixed Electrophoresis Buffers and Reagents

Gene Transfer Systems (Electroporation and Particle Delivery)

Mutagenesis and Molecular Biology Kits

Section 8
Prep-A-Gene Matrix Health Hazard Information

Symptoms of Overexposure (for each potential route of exposure)

Inhaled: The diatomaceous earth in this product is in an aqueous slurry and should not pose an inhalation problem. If spilled and allowed to dry out, this product contains crystalline silica (CS), which is considered a hazard by inhalation of the dust. IRAC has classified powdered CS as probably carcinogenic for humans. This classification is based on the findings of laboratory animal studies that were considered sufficient and data from epidemiological studies that were considered limited for carcinogenicity. CS is also a known cause of silicosis, a non-cancerous lung disease. CS has not been classified as a carcinogen by NTP or OSHA.

Contact with skin or eyes: May irritate eyes causing redness, tearing, and pain.

Absorbed through **skin:** Not expected to be absorbed through the skin due to the nature of the product.

Swallowed: **Not** hazardous when ingested.

Special Handling Information

Ventilation and Engineering Controls: Not required as long as product is maintained as a slurry.

Respiratory Controls: Not required as long a product is maintained as a slurry.

Eye Protection: Safety glasses with splash guards. Maintain eye wash fountain and quick-drench facilities in work area.

Gloves: Chemical resistant gloves such as neoprene.

Protective measures during maintenance of contaminated equipment: Proper protective clothing, eye protection, and respiratory equipment should be worn if working with large amounts that have been allowed to dry out.

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