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Mini-prep plasmid DNA isolation and purification using silica-based resins

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Abstract
It has been common practice in the past to use very large quantities of starting material for experimental molecular biology techniques; however, rapid advancements in subcloning and sequencing procedures on a mini-scale have shown that many of these methods no longer require a substantial amount of purified DNA. Due to the high quality of many commercially available purified enzymes and reagents, and the development of many new mini-isolation techniques and prepackaged kits, high quality mini-prep plasmid DNA can now be had for far less investment of time, labor, and materials. A significant reduction in the time researchers spend on purifying their DNA samples has led to an increase in productivity for experimental biologists, one testimony to this being the rapid appearance and availability of numerous new cloning vectors. In this chapter, I review the different techniques for the isolation and purification of mini-prep plasmid DNA and focus attention on a rapid and inexpensive method utilizing the binding of DNA to diatomaceous earth and other silicates.

Introduction

Isolation of plasmid DNA

The standard cesium chloride - ethidium bromide centrifugation technique (1), has now become the old grey mare of plasmid DNA isolation and purification. Owing to differences in intercalation of the DNA-binding dye ethidium bromide and resulting change in buoyant density, covalently closed circular plasmid DNA (cccDNA) is separated from the nicked, relaxed open and linear forms. The DNA species band at different positions within an ultracentrifuge tube and can be isolated through recovered fractions. Although this technique is still held as the golden shrine of DNA purifications due to the purity of recovered DNA, it relies on a 24 to 48 hour centrifugation step to separate the different DNA forms. More recently, this slow and tedious technique has given way to the more popular rapid mini-isolation methods which can be performed in less than an hour. (2,3) Today, the most widely used plasmid mini-prep purification techniques include quick spin filtration combined with binding to ion exchange resins or silica particles.

The simplest method for isolation of plasmid DNA employs the disruption of bacterial cells with phenol/chloroform. Simultaneous extraction of the crude bacterial lysate with organic substances followed by a quick centrifugation step allows the separation of denatured proteins, cell membrane components, and chromosomal DNA, while the plasmid DNA remains in the aqueous phase. Many singletube procedures have been devised (4-9), some of which have been shown to produce DNA of sequencing quality. (10-14) Unfortunately, this method requires the use of caustic chemicals, prohibiting its routine use without protective clothing, gloves, eyewear, and a fume hood.

Another common method involves mixing of bacterial suspensions with a solution of lysozyme and the non-ionic surfactant Triton^{TM} X-100. Upon boiling, the lysed bacteria spill forth cellular material which forms an insoluble clot of denatured components that can be removed by centrifugation. The pellet of debris is either removed with a toothpick, or the supernatant containing the plasmid DNA is transferred to a clean tube for further manipulations. (15-19)

By far, the most commonly used technique is the alkaline lysis method. Cells are disrupted with the anionic detergent sodium dodecyl sulfate (SDS) in the presence of the alkali sodium hydroxide, causing the denaturation and precipitation of bacterial proteins and other cellular debris. (20,21) Irreversible denaturation of linear chromosomal DNA occurs under alkaline conditions. Renaturation of plasmid cccDNA by neutralization with acid occurs more efficiently than renaturation of linear chromosomal DNA due to the close association of the supercoiled strands, allowing the plasmids to remain soluble in the supernatant. The lysate may then be cleared of debris by centrifugation. Many different miniprep protocols have been developed, all of which are modifications of this method. (22-26) While alkaline lysis has been extensively used for Gram-negative bacteria, purification of plasmid DNA from Gram-positive species, including *Bacillus subtilis* and *Staphylococcus aureus*, has also been done. (27)

Some techniques are based on the selective precipitation or salting out of bacterial proteins, chromosomal DNA, and plasmids from cell lysates (28), while others utilize detergents which allow proteins and larger polysaccharides to remain in solution during the precipitation of lower molecular weight nucleic acids. For example, cationic alkyltrimethylammonium halides such as cetyltrimethylammonium bromine (CTAB), cetyltrimethylammonium chloride (CTAC), and cetylpyridinium chloride (CTC) precipitate nucleic acids and acid polysaccharides under low salt conditions. The DNA is pelleted by centrifugation and the detergent washed out with a high salt buffer. (29-31)

Purification of plasmid DNA

For subcloning purposes, separations may be done by electrophoresis through an agarose gel and the DNA bands of interest, either the entire cloning vector DNA or a restriction fragment, can be extracted directly from a gel slice. Several clever techniques for the rapid recovery and purification of DNA from agarose gels have recently been developed. However, this method can sometimes be time consuming and the DNA may not be sufficiently pure for subsequent enzyme reactions, some enzymes being sensitive to small contaminating impurities coeluting with the DNA. For example, the use of β-agarase for the enzymatic destruction of agarose polymers and the subsequent precipitation of nucleic acids has resulted in the inability to amplify extracted DNA by the polymerase chain reaction (PCR). (32)

Another molecular size fractionation technique is that of gel filtration through a column of Sephacryl S-1000, a cross-linked co-polymer of allyl dextran and N, N - methylenebis(acrylamide), or Ultrogel A2. This alternative molecular sieve method uses flow through separation based on size exclusion to resolve cccDNA from the other forms. (33-35)

Ion exchange chromatography resins such as RPC-5 or RPC-5 ANALOG, commonly known as the nucleic acid chromatography system (NACS$^{\mathrm{TM}}$) sold by Life Technologies, Inc., Gaithersburg, MD, USA, may be used for analytical and preparative purification of nucleic acids. Due to the attraction of negatively charged phosphate groups to cationic quaternary amines under low salt conditions, nucleic acids bind to the resin particles, usually composed of polychlorotrifluoroethylene coated with alkyltrimethylammonium chloride. The DNA is eluted with increasing salt by charge competition. Many of the commercially available kits used for mini-prep plasmid purifications contain comparable resins. (36,37)

Hydroxyapatite (HAP) chromatography has also been used extensively for the purification of nucleic acids. Interactions of calcium ions on the surface of HAP (calcium phosphate hydroxide) with high molecular weight nucleic acids, trap the DNA onto the matrix while the lower molecular weight extrachromosomal cccDNA flows through. (38)

The use of diatomaceous earth, the fossilized cellular remains of unicellular algae (diatoms), and silica particles from other sources has gained great attention recently. In the presence of a chaotropic buffer composed of highly positively charged molecules, usually guanidine (aminomethanamidine), DNA molecules have very strong affinity for siliceous materials. Fine particles of diatomaceous earth and other amorphous forms of silicon dioxide possess many fine pores and, having a high surface-to-volume ratio, are therefore extremely absorbent. Although the exact nature of the electrostatic interaction between the DNA molecules and the silicon dioxide is not known, the association is strong enough to hold small fragments of DNA during vigorous flow washing. (39-43)

Smaller DNA molecules bond more tightly and are more difficult to elute. Also, larger molecules may bind to more than one particle and be sheared during manipulations of the matrix with bound DNA. Due to this trade-off of properties, it is recommended that the optimal size of DNA to be purified using this method be between approximately 100 base pairs and 6 kilobase-pairs of length, although double-stranded DNA as large as 48 kb has been recovered from clinical specimens with 50% lower efficiency. (40)

Many resins currently available are microporous substances treated with silicifying agents. In addition, paramagnetic particles have been coated with a silicide, making them amenable to magnetic separation techniques after DNA is bound. Others have used crystalline silicon dioxide fragments from other sources such as crushed flint glass, ground glass filters, and silica particles. (44-46)

Diatomaceous earth and silica based mini-prep protocol

Mini-prep method

1. Grow overnight cultures of *E. coli* cells containing the recombinant plasmids to be screened in 3-5 ml of selective broth.

2. Pellet 2-4 ml of the cells in a 2.0 ml eppendorf tube. This can be done by spinning 2.0 ml of culture twice for 1 minute in the eppendorf centrifuge or pelleting the entire culture in a clinical centrifuge for 5 minutes.

3. Resuspend the bacterial cell pellet in 100 μl of 25 mM Tris, 10 mM EDTA, 100 μg/ml RNaseA, pH 8.0

4. Using a micropipette, squirt in 200 μl of 0.15 M NaOH, 1.0% SDS pre-warmed to 65 C on top of the cells without mixing.

5. Add 150 μl of 3 M sodium acetate pH 4.8 to each tube and mix by flicking the tube approximately ten times with your fingernail.

6. Spin 5-10 minutes in the eppendorf centrifuge at 4 C.

7. Mix in 1.0 ml of binding solution containing the binding matrix, composed of either diatomaceous earth or other silica particle resin. While avoiding the white precipitate on the bottom, immediately transfer the solution to a pre-made tip column placed within a vacuum manifold apparatus.(see sections belows for details)

8. Apply the vacuum to concentrate the binding matrix onto the filter within the column.

9. Wash the trapped matrix twice by rinsing with 1.0 ml of 10 mM Tris; 100 mM NaCl; 2.5 mM EDTA; 55% v/v Ethanol; pH 7.5 with the vacuum applied.

10. Allow the packed matrix to completely dry by vacuum for 5 to 10 minutes.

11. Remove each tip and slice off the end just below the filter with a razor blade, leaving no space between the filter and the end. Place the top portion of this, which contains the trapped matrix, inside a 1.6 ml eppendorf centrifuge tube. It is important that a 1.6 ml tube be used at this point since the inserted cutoff tip will leave a 20 μl volume of dead space as a collection reservoir in the bottom of the conical tube.
12. Elute the DNA bound to the resin by pipetting 30 μl of sterile distilled water on top the matrix.

13. The DNA solution is collected by centrifugation for 2 minutes in the eppendorf centrifuge at top speed at room temperature. Remove the plastic tip from the tube with forceps. The liquid still adhering to the tip can be released by smartly tapping the cutoff tip onto the side of the tube.

Preparation of the binding matrix and binding solution

Method 1 - Use of diatomaceous earth as binding matrix

The binding matrix is prepared by mixing 3.0 grams of diatomaceous earth with 30 ml of sterile milliQ water to give a solution of 100 mg/ml. This is allowed to settle for 2 to 3 hours, and the upper liquid phase discarded. The settled particulate matter is resuspended in 6.0 ml of sterile milliQ water to give a final stock solution of approximately 150 mg/ml w/v (density of 1.130 to 1.150 g per ml). To prepare the binding matrix, 2.0 ml of this stock solution is added to 50.0 ml of chaotropic binding solution composed of either 7 M guanidine HCl in 50 mM Tris; 20 mM EDTA; pH 7.0, or 6 M guanidine thiocyanate in 50 mM Tris; 20 mM EDTA; pH 7.0.

An alternative method is to make the chaotropic binding solution separately from the binding matrix and add 30 μl of binding matrix stock to the mixed lysate. In this case, the matrix may be prepared by washing a pellet from 1.0 ml of the diatomaceous earth stock solution (150 mg/ml) several times with TE buffer (25 mM Tris; 10 mM EDTA; pH 8.0), before resuspending it in TE for a final concentration of approximately 10 mg/ml.

Method 2 - Use of ground glass silica particles as binding matrix

Silica powder GLASSMILK$^{\mathrm{TM}}$ may be obtained from BIO101, La Jolla, CA, USA, Celite$^{\mathrm{TM}}$ from Sigma Chemical Co. or Aldrich Chemical Co., St. Louis, MO, USA, or 325 mesh powdered flint glass fines from Cutter Ceramics, Beltsville, MD, USA. The binding matrix is prepared as for the diatomaceous earth by allowing a suspension to settle for approximately 2 to 3 hours. If impure forms of glass are to be used, the settled particles should be mixed with an equal volume of concentrated nitric acid and boiled in a fume hood. After cooling, the centrifuged pellet of glass powder fines is washed extensively with TE buffer and resuspended to give a final concentration of 10 mg/ml. If silica particles are used, the binding solution should be sodium iodide (908 g/l) saturated with sodium sulfite (15 g/l) added as an antioxidant in 20 mM Tris pH 7.5, or 6 M sodium perchlorate, 50 mM Tris, 10 mM EDTA pH 8.0. (47)

Use of filter tips

Although many companies sell filter units that can be used for this mini-prep method, I have found that filtered micropipette tips work very well. To prepare the filter column, pipette 100-200 μl of SigmaCote$^{\mathrm{TM}}$ silanizing agent through the filter of an aerosol resistant tip (ART$^{\mathrm{TM}}$) a few times and allow it to air dry.

Push the filter insert down snugly within the tip by using the small end of another pipette tip. To construct the column, fit the syringe barrel of a Becton-Dickinson 1/2 cc U-100 Insulin syringe less the needle, or a 1.0 ml eppendorf pipette tip or similar into the wide end of the silanized filter tip and place this on a vacuum manifold.

It may be important which type of ART$^{\mathrm{TM}}$ tips can be used since these are typically used to eliminate contaminants for PCR and some, being composed of various hygroscopic materials, are designed to trap moisture. I have routinely used 30-μl Integrity$^{\mathrm{TM}}$ tips purchased from Matrix Technologies Corporation, Lowell, MA, USA, which contain a 4 mm wide hydrophobic filter fitted directly in the middle of the tip. The maximum capacity of the ART$^{\mathrm{TM}}$ filter tip was found to be about 30 to 40 μl of concentrated diatomaceous earth resin matrix, or 10-20 μl of silica particle binding matrix. Any more slowed down the vacuum washing steps considerably and caused blockage of the column.

Construction and use of the vacuum manifold

Each column is placed into a PigLet™ universal vacuum manifold purchased from Molecular Bio-Products, San Diego, CA, USA. Use of a commercial manifold is not necessary, however, and an alternative way is to make a homemade system constructed from a discarded 200 μl pipette tip box. To make the manifold, bore a hole within one side of the box just large enough for a nipple fitting. The entire box can be wrapped in parafilm to seal off any cracks or unused holes and a vacuum line hose attached via the nipple. It is recommended that a collection trap such as a side arm flask be placed in between the manifold and the vacuum source. The tip columns are then inserted by piercing them through the parafilm covering the holes in the vacuum box system.

Purification of DNA without a vacuum manifold

It is possible to do this procedure without the use of a vacuum manifold or filter devices. Instead, the mixture of silica particles and binding solution is transferred to a clean eppendorf tube and centrifuged for 10-15 seconds at top speed. The pellet is then resuspended in 1.0 ml of washing buffer by pipetting and centrifuged again. After two washes, the pellet is dried under vacuum for 5 minutes and the DNA eluted by resuspending it in sterile distilled water. The mixture is again centrifuged and the aqueous DNA solution is pipetted off without disturbing the silica particles. If this method is used, it must be kept in mind that the yield will be lower for larger DNA molecules due to shearing forces.

Scaling-up the protocol

This method can be scaled up for the recovery and purification of larger amounts of plasmid DNA. Larger syringe barrels may be prepared by plugging the hole with a small amount of siliconized polyallomer aquarium fluff. (48) To create a paper filter which is used as the support trap for the binding resin, strips of Whatman™ 3MM filter paper can be soaked in TE buffer and mixed by shaking into a slurry, or macerated within a Waring blendor. (49) A small portion of the paper slurry is spooned into each syringe such that the passage of liquid is not blocked, but that the binding matrix is trapped on top of the newly formed paper filter during the washing steps. Some experimentation may be needed to determine the correct amount of slurry for each type of syringe and binding material. After the drying step, the DNA can be recovered by adding the appropriate amount of sterile distilled water and centrifuging the syringe within a clinical centrifuge tube fitted into a standard swinging bucket rotor to collect the eluate. At this point, the purified plasmid DNA may be concentrated through precipitation by making the DNA solution 2.5 M ammonium acetate (ie. adding $1/4^{th}$ volume of 10 M stock solution) and 2.5 volumes of absolute ethanol at room temperature. The DNA is collected by centrifuging at 16,000 x g for 15 minutes and the pellet washed several times with 70% ethanol to remove any remaining salts. (50)

Purification of plasmids from nonviable glycerol stocks or bacterial colonies

There has been some interest in the recovery of plasmid DNA from nonviable bacteria. Transforming competent *E. coli* cells with the DNA may allow the recovery of a lost recombinant clone in times of desperation. (51) Using the method outlined here with a few modifications, I have successfully purified plasmid DNA from glycerol stocks containing nonviable bacteria and was able to recover several seemingly lost clones. The bacteria are pelleted and washed with 1.0 ml of 1 M NaCl and resuspended in TE buffer prior to the lysis step. After addition of the lysis solution the suspensions appear to be more completely lysed. The reason for this is unclear; however, it has been noted that bacterial colonies stored on agar plates at 4 C for some time produce a substantial amount of exopolysaccharides which may interfere with lysis. The protective coating may be reduced or removed, or that the cells become more sensitive to osmotic shock. (52) In any case, this wash step has been demonstrated to be an effective means of lysing bacteria which are normally recalcitrant to this method. (53,54) The entire DNA sample recovered should be added to highly competent cells and the standard procedure for transformation previously described followed. (55)

Discussion

Using the protocol outlined here, approximately 5-10 μg of purified plasmid DNA in 10-15 μl of solution has routinely been recovered from 12 to 36 mini-preps within 30 minutes, excluding the time needed for bacterial growth. Restriction digestions and agarose gel electrophoresis showed the DNA recovered to be as clean as that obtained by other mini-prep procedures using binding resins and the DNA should be of adequate quality for sequence analysis by the dideoxy termination method. Some modifications to previous methods have been made. These include i.) the lowering of NaOH concentration from 0.2 to 0.15 M which may reduce damage incurred onto the DNA during exposure to alkaline conditions, and ii.) the use of a hot lysis solution allowing for complete lysis upon contact. This allows the immediate neutralization of the alkali and a significant reduction of the time DNA is exposed to denaturing conditions. Other hot alkaline methods require a longer incubation time of 30 minutes. (56)

Each of the methods for isolation of plasmid DNA from bacteria reviewed here has its advantages and drawbacks. The method described within this chapter avoids some of the other problems found with DNA purified by other single-tube isolation procedures which utilize organic extractions with phenol and chloroform to denature proteins. While the added expense involved with transferring solutions between tubes is avoided by single-tube mini-prep protocols, the handling of caustic, hazardous, and toxic materials such as phenol and chloroform is required.

On the other hand, there have been reports that omitting the organic extraction step can yield lesser quality DNA. It has been suggested that degradation of recovered DNA is most likely due to endonucleases that remain active during the purification steps. It has been recommended that *end*A- strains of *E. coli* be used for maintaining the plasmid constructs. (57) Since this purification protocol optionally includes guanidine thiocyanate, a nuclease inhibitor, this extra precaution may not be necessary.

Future trends in DNA purification

The use of high-end equipment such as a capillary electrophoresis system or HPLC for the purification of cccDNA is gaining popularity. Due to the low quantity of cccDNA recovered, and the expense and time required for setting up and maintaining a system in a low-funded lab, however, these methods are not widely used in molecular biology laboratories. This has probably prohibited the exploration of many potentially useful techniques. Simple procedures requiring less expensive materials allow the average lab worker to complete many plasmid isolations in a single day. The future therefore looks quite bright for the further development of faster methods and new binding resins to be used in miniprep procedures.

The trend of DNA purification techniques toward commercial kit reagents does have its consequences. Many companies now sell excellent quality controlled binding resins and the materials provided are becoming more reliable and quality controlled. Unfortunately, problems may be encountered if the proprietary binding matrix is changed or removed from the market. For example, many scientists were faced with changing their experimental conditions when the undisclosed binding matrix in a widely used mini-prep kit was recently discontinued. (58,59) In the future, companies providing these materials may consider revealing more details concerning the manufacturing of their products, or they may suffer setbacks within the competitive biotechnology market place.

References

1. Clewell, D. B., and Helinski, D. R. 1969. Supercoiled circular DNA-protein complex in *Escherichia coli:* purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. USA 62: 1159-1166.

2. Griffith, O. M. 1988. Large-scale isolation of plasmid DNA using high speed centrifugation methods. BioTechniques 6: 725-727.

3. Stemmer, W. P. C. 1991. A 20-minute ethidium bromide/high-salt extraction protocol for plasmid DNA. BioTechniques 10: 726.

4. Alter, D. C., and Subramanian, K. N. 1989. A one step, quick step, mini prep. BioTechniques 7: 456-458.

5. Serghini, M. A., Ritzenthaler, C., and Pinck, L. 1989. A rapid and efficient 'miniprep' for isolation of plasmid DNA. Nucl. Acids Res. 17: 3604.

6. He, M., Wilde, A., and Kaderbhai, M. A. 1990. A simple single-step procedure for small-scale preparation of *Escherichia coli* plasmids. Nucl. Acids Res. 18: 1660.

7. Chowdhury, K. 1991. One step 'miniprep' method for the isolation of plasmid DNA. Nucl. Acids Res. 19: 2792.

8. Akada, R. 1994. Quick-check method to test the size of *Escherichia coli* plasmids. BioTechniques 17: 58.

9. Kurien, B. T., and Scofield, R. H. 1994. A short, small-scale preparation of plasmid DNA from *E. coli* using organic solvents. B.R.L. Focus 16: 113.

10. Johnson, K. R. 1990. A small-scale plasmid preparation yielding DNA suitable for double-stranded sequencing and *in vitro* transcription. Anal. Biochem. 190: 170-174.

11. Kibenge, F. S. B., Dybing, J. K., and McKenna, P. 1991. Rapid procedure for large-scale isolation of plasmid DNA. BioTechniques 11: 65-67.

12. Goode, B. L., and Feinstein, S. C. 1992. "Speedprep" purification of template for double-stranded DNA sequencing. BioTechniques 12: 374-375.

13. Yie, Y., Wei, Z., and Tien, P. 1993. A simplified and reliable protocol for plasmid DNA sequencing: fast miniprep and denaturation. Nucl. Acids Res. 21: 361.

14. Tarczynski, M. C., Meyer, W. J., Min, J. J., Wood, K. A., and Hellwig, R. J. 1994. Two-minute miniprep method for plasmid DNA isolation. BioTechniques 16: 514-519.

15. Holmes, D. S., and Quigley, M. 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. 114: 193-197.

16. Wang, L.-M., Weber, D. K., Johnson, T., and Sakaguchi, A. Y. 1988. Supercoil sequencing using unpurified templates produced by rapid boiling. BioTechniques 6: 839-843.

17. Liszewski, M. K., Kumar, V., and Atkinson, J. P. 1989. "Midi-prep" isolation of plasmid DNA in less than two hours for sequencing, subcloning and hybridizations. BioTechniques 7: 1079-1081.

18. Berghammer, H., and Auer, B. 1993. "Easy-Preps": fast and easy plasmid minipreparation for analysis of recombinant clones in *E. coli*. BioTechniques 14: 524-528.

19. Hultner, M. L., and Cleaver, J. E. 1994. A bacterial plasmid DNA miniprep using microwave lysis. BioTechniques 16: 990-994.

20. Birnboim, H. C., and Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res. 7: 1513-1523.

21. Birnboim, H. C. 1983. A rapid alkaline extraction method for the isolation of plasmid DNA. Meth. Enzym. 100: 243-255.

22. Morelle, G. 1989. A plasmid extraction procedure on a miniprep scale. B.R.L. Focus 11: 7-8.

23. Zhou, C., Yang, Y., and Jong, A. Y. 1990. Mini-prep in ten minutes. BioTechniques 8: 172-173.

24. Jones, D. S. C., and Schofield, J. P. 1990. A rapid method for isolating high quality plasmid DNA suitable for DNA sequencing. Nucl. Acids Res. 18: 7463-7464.

25. Xiang, C., Wang, H., Shiel, P., Berger, P., and Guerra, D. J. 1994. A modified alkaline lysis miniprep protocol using a single microcentrifuge tube. BioTechniques 17: 30-32.

26. Wang, L.-F., Voysey, R., and Yu, M. 1994. Simplified large-scale alkaline lysis preparation of plasmid DNA with minimal use of phenol. BioTechniques 17: 26-28.

27. Voskuil, M. I., and Chambliss, G. H. 1993. Rapid isolation and sequencing of purified plasmid DNA from *Bacillus subtilis*. Appl. Environ. Microbiol. 59: 1138-1142.

28. Laitinen, J., Samarut, J., and Hölttä, E. 1994. A nontoxic and versatile protein salting-out method for isolation of DNA. BioTechniques 17: 316-322.

29. Del Sal, G., Manfioletti, G., and Schneider, C. 1989. The CTAB-DNA precipitation method: a common mini-scale preparation of template DNA from phagemids, phages or plasmids suitable for sequencing. BioTechniques 7: 514-520.

30. Ishaq, M., Wolf, B., and Ritter, C. 1990. Large-scale isolation of plasmid DNA using

cetyltrimethylammonium bromine. BioTechniques 9: 19-24.

31. Chang, P. K., and Natori, M. 1994. 'One for all scales' methods for plasmid, phagemid and bacteriophage DNAs preparation. J. Biotechnol. 36: 247-251.

32. Hengen, P. N. 1994. Methods and Reagents - Recovering DNA from agarose gels. Trends in Biochem. Sci. 19: 388-389.

33. Micard, D., Sobrier, M. L., Couderc, J. L., and Dastugue, B. 1985. Purification of RNA-free plasmid DNA using alkaline extraction followed by Ultrogel A2 column chromatography. Anal. Biochem. 148: 121-126.

34. Gómez-Márquez, J., Freire, M., and Segade, F. 1987. A simple procedure for large-scale purification of plasmid DNA. Gene 54: 255-259.

35. Raymond, G. J., Bryant III, P. K., Nelson, A., and Johnson, J. D. 1988. Large-scale isolation of covalently closed circular DNA using gel filtration chromatography. Anal. Biochem. 173: 125-133.

36. Best, A. N., Allison, D. P., and Novelli, G. D. 1981. Purification of supercoiled DNA of plasmid Col E1 by RPC-5 chromatography. Anal. Biochem. 114: 235-243.

37. Thompson, J. A., Blakesley, R. W., Doran, K., Hough, C. J., and Wells, R. D. 1983. Purification of nucleic acids by RPC-5 ANALOG chromatography: peristaltic and gravity-flow applications. Meth. Enzym. 100: 368-399.

38. Shoyab, M., and Sen, A. 1979. The isolation of extrachromosomal DNA by hydroxyapatite chromatography. Meth. Enzym. 68: 199-206.

39. Hall, R. H. 1967. Partition chromatography of nucleic acid components (isolation of the minor nucleosides). Meth. Enzym. 12A: 305-315.

40. Boom, R., Sol, C. J. A., Salimans, M. M. M., Jansen, C. L., Wertheim-van Dillen, P. M. E., and van der Noordaa, J. 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28: 495-503.

41. Carter, M. J., and Milton, I. D. 1993. An inexpensive and simple method for DNA purifications on silica particles. Nucleic Acids Res. 21: 1044.

42. Willis, E. H., Mardis, E. R., Jones, W. L., and Little, M. C. 1990. Prep-AGene^{TM}: A superior matrix for the purification of DNA and DNA fragments. BioTechniques 9: 92-99.

43. Iyer, K., Crabtree, J., Clifton, S., Chissoe, S., Mardis, E., and Roe, B. A. 1994. Isolation of cosmid, plasmid and P1 DNAs via diatomaceous earth-based maxi, midi and mini-preps, and the non-diatomaceous earth-based mini-prep. Genet. Anal. Tech. Appl. *in press*.

44. Vogelstein, B., and Gillespie, D. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

45. Marko, M. A., Chipperfield, R., and Birnboim, H. C. 1982. A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder. Anal. Biochem. 121: 382-387.

46. Sparks, R. B., and Elder, J. H. 1983. A simple and rapid procedure for the purification of plasmid DNA using reverse-phase C18 silica beads. Anal. Biochem. 135: 345-348.

47. Hengen, P. N. 1994. Frequently Asked Question (FAQ) list for bionet.molbio.methds-reagnts version number 01.13.10.1994 available via anonymous FTP from ftp.ncifcrf.gov as file pub/methods/FAQlist or upon request by e-mail to pnh@ncifcrf.gov

48. Levine, R. A. 1994. Aquarium filter floss: an alternative to silanized glass wool as a porous support matrix. BioTechniques 17: 67.

49. Chuang, S.-E., and Blattner, F. R. 1994. Ultrafast DNA recovery from agarose by centrifugation through a paper slurry. BioTechniques 17: 634-636.

50. Crouse, J., and Amorese, D. 1987. Ethanol precipitation: ammonium acetate as an alternative to sodium acetate. B.R.L. Focus 9: 3-5.

51. Shuldiner, A. R., and Tanner, K. 1992. Recovery of plasmid DNA from nonviable bacterial colonies and cultures. BioTechniques 12: 66.

52. Schwinghamer, E. A. 1980. A method for improved lysis of some Gram-negative bacteria. FEMS Microbiol. Letters 7: 157-162.

53. Dénarié, J., Boistard, P., Casse-Delbart, F., Atherly, A. G., Berry, J. O., and Russell, P. 1981. Indigenous plasmids of *Rhizobium*. In: International Review of Cytology, Supplement 13. *Biology of the Rhizobiaceae*. K. L. Giles and A. G. Atherly, eds. Academic Press, New York. p. 225-246.

54. Kuykendall, L. D., and Hengen, P. N. 1988. Microbial genetics of legume root nodulation and nitrogen fixation. In: Biological Nitrogen Fixation - Recent Developments. N. S. Subba Rao, ed. Oxford and IBH Publishing Co., New Delhi, India. p. 71-111.

55. Hengen, P. N., and Iyer, V. N. 1992. DNA cassettes containing the origin of transfer (*ori*T) of two broad-host-range transfer systems. BioTechniques 13: 57-62.

56. Musich, P. R., and Chu, W. 1993. A hot alkaline plasmid DNA miniprep method for automated DNA sequencing protocols. BioTechniques 14: 958-960.

57. Taylor, R. G., Walker, D. C., and McInnes, R. R. 1993. *E. coli* host strains significantly affect the quality of small scale plasmid DNA preparations used for sequencing. Nucl.

Acids Res. 21: 1677-1678.

58. Hengen, P. N. 1994. Methods and Reagents - kit wars. Trends in Biochem. Sci. 19: 46-47.

59. Hengen, P. N. 1994. Methods and Reagents - on the magic of mini-preps.... Trends in Biochem. Sci. 19: 182-183.

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C.T.J. Chan [cchan@uk.ac.crc]
Ravi R. Iyer [rriyer@unix1.circ.gwu.edu]
Bill Melchior [wmelchior@ntbtox.nctr.fda.gov]
Chia Jin Ngee [mcblab47@.leonis.nus.sg]
Doug Rhoads [drhoads@mercury.uark.edu]
Bruce Roe [broe@aardvark.ucs.uoknor.edu]
Claudia A. Sutton [cas9@cornell.edu]

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Paul N. Hengen
National Cancer Institute
Frederick Cancer Research and Development Center
Frederick, Maryland 21702-1201 USA
e-mail: pnh@ncifcrf.gov