REFERENCES

1. **Laemmli, U.K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

This work was supported by the Centre National de la Recherche Scientifique, Grant UMR 24.

Pierre Falson
Laboratoire de Biochimie Structurale et Fonctionnelle
Institut de Biologie et Chimie des Protéines
Unité Propre de Recherche n° 412 du Centre National de la Recherche Scientifique
43, Blvd. du 11 Novembre 1918
69622 Villeurbanne Cedex, France

Improved Phenol-Based Method for the Isolation of DNA Fragments from Low Melting Temperature Agarose Gels

The isolation of restriction DNA fragments is often desirable for cloning or labeling purposes. Speed and simplicity have made the phenol-based method of DNA isolation from low melting temperature (LMT) agarose slices to be widely used. However, yield and quality of the isolated DNA are strongly dependent on the quality of both the agarose and the phenol, which can contain various contaminants and may vary from batch to batch (2,3). As a result, extensive washing of the DNA is often required, affecting further the yield of recovered material. Methods obviating the use of phenol have, therefore, been developed that permit the isolation of high-quality DNA. These methods are, however, usually cumbersome or require the use of expensive kits.

In this paper I present a simple and reliable phenol-based protocol that drastically improves the quality of the isolated DNA in contrast to standard phenol-based methods. Results are compared with those obtained with one standard phenol-based and two phenol-free isolation procedures. Since DNA stored at 4°C, or following cycles of freezing and thawing, is often found to be largely nicked after some time, the technique is further evaluated using covalently closed circular (CCC) or open circular (OC) DNA.

In this modified protocol, residual phenol and agarose are centrifugally separated from the DNA solution in the presence of salt following phenol extraction and prior to ethanol precipitation. This extra step in contrast to conventional methods minimizes the time DNA is in contact with phenol and agarose, allowing nearly quantitative and reproducible recovery of high-quality DNA fragments.

Method

1) Run sample on a LMT agarose gel until the band of interest is well separated from any other band.

2) Cut out a slice of gel containing the fragment of interest in the smallest possible volume. Introduce the slice in a tared microcentrifuge tube.

3) Remove any buffer that may have come in with the slice. Estimate the volume of the slice from its weight and add 1 volume of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Place the tube in a 65°C water bath for 5 to 10 min.

4) Make sure that the agarose is fully melted by pipetting the solution up and down a few times. Immediately add 1 volume of Tris-buffered phenol (no chloroform!) at room temperature and mix thoroughly by inversion.

5) Spin for 3 min at 10 000 to 12 000 rpm. Transfer the aqueous phase to a new tube containing 1 volume of Tris-buffered phenol. Mix. Do not be too concerned about picking up small amounts of powdered agarose that formed at the interface because it will be subsequently eliminated.

6) Spin sample as above. Transfer the aqueous phase to a new tube containing 0.1 volume of 4 M LiCl. Mix by inversion. A white precipitate appears immediately. Place the tube on ice for 2 min. Spin as above for 3 min.

7) Transfer supernatant to a new tube, leaving the transparent pellet behind. Since most, if not all, of the phenol and agarose have now been eliminated from the sample, it is a wise precaution to add a carrier molecule at this time, e.g., 1 µl of a 20 mg/ml glycogen (Boehringer Mannheim, Mannheim, FRG) solution, in order to improve the final yield of recovered DNA.

8) Add 2.5 volumes of cold ethanol. Mix by inversion and leave for 5 to 30 min at -70°C. Spin as above for 10 min. Wash pellet with 1 ml of cold 70% ethanol. Dry the DNA pellet under vacuum. Resuspend into 10 to 20 µl water or TE.

I found that for optimal results: a) a single phenol extraction is sometimes, but not always, sufficient; b) further extraction steps, such as using phenol-chloroform and chloroform or re-extraction of the phenol phase, are not required; c) LiCl works better than other salts for the phenol-agarose separation step; and d) TAE or TBE buffers are

Table 1. Electroporation of *E. coli* DH5α Cells with DNA Isolated from LMT Agarose

DNA	cfu/ml		% White Colonies
	Total	White	
Control	9.6×10^4	8.0×10^2	0.8
Standard, phenol-based	1.4×10^4	4.0×10^2	2.9
Modified, phenol-based	2.8×10^4	4.5×10^1	0.2
GeneClean	9.5×10^4	1.0×10^3	1.1
Freeze-squeeze	3.7×10^4	4.0×10^2	1.1

equally suitable.

The above procedure was evaluated against a standard phenol-based protocol and two phenol-free protocols using a freshly prepared pUC21 DNA (5) present as CCC molecules or an essentially nicked (OC) form of plasmid pK18 (1). For each DNA type, four 400-ng samples of *Sma*I-cut DNA were run on a 0.7% LMT agarose gel. One of the samples was treated as described above. The second sample was processed in a standard manner, i.e., following phenol extraction, the DNA was directly precipitated by addition of 0.1 volume 3 M Na-acetate (pH 5.8) and 2.5 volumes of cold ethanol. The two remaining samples were isolated by phenol-free methods, on the one hand, according to the silica matrix-based method from GeneClean™ (Bio 101, La Jolla, CA), and, on the other hand, by the freeze-squeeze method of Tautz and Renz as described (4). All DNA

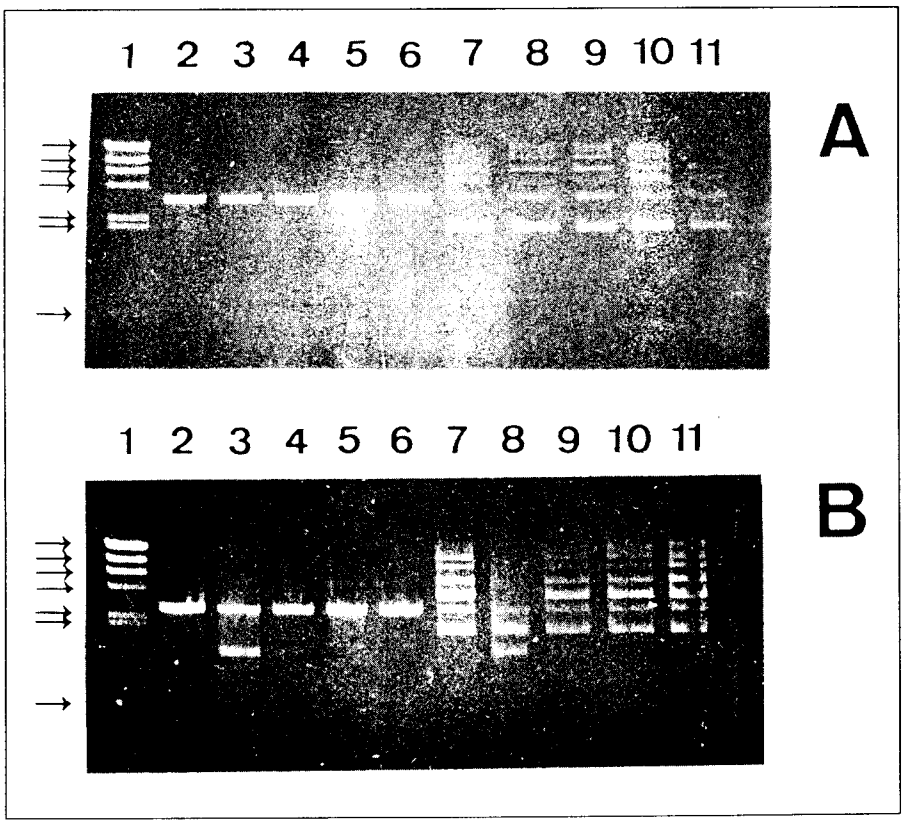

Figure 1. Agarose gel analysis of linear and self-ligated DNAs isolated from LMT agarose. A: pUC21 DNA (stock in CCC form). B: pK18 DNA (stock in OC form). Lane 1, *Hin*dIII-cut λ DNA. Fragment sizes (arrows) are in kb: 23, 9.4, 6.6, 4.4, 2.3, 2.0, 0.56. Lanes 2–6, linear DNA; lanes 7–11, self-ligated DNA; lanes 2 and 7, controls; lanes 3 and 8, standard phenol-based method; lanes 4 and 9, modified phenol-based method; lanes 5 and 10, GeneClean method; and lanes 6 and 11, freeze-squeeze method.

pellets were resuspended in 20 µl H_2O. Of these, 5 µl were run on a 0.7% agarose gel, along with 5 µl (pUC21) or 10 µl (pK18) of the same samples that were previously self-ligated in a 20-µl reaction volume for 4 h at room temperature. A further 1-µl sample was self-ligated in a 50-µl reaction volume, 2 µl of which were electroporated into *E. coli* DH5α™ cells (Life Technologies AG, Basel, Switzerland). Dilutions of the electroporated cells were then plated out onto LB plates containing 100 µg/ml ampicillin (pUC21) or 50 µg/ml kanamycin (pK18) and spread with 50 µl of a 2% 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal) solution. After overnight incubation at 37°C, the colony-forming units/ml (cfu/ml) and the proportion of white-to-blue colonies were scored. As controls, corresponding amounts of *Sma*I-cut DNA were purified by the GeneClean method and subsequently handled like the samples isolated from agarose.

Figure 1A shows that the yield (at least 70%, nearly quantitative for the modified phenol-based and GeneClean methods, lanes 3–6) and degree of ligation of DNA (lanes 8–11) are similar to those of the controls (lanes 2 and 7) for all the methods tested using CCC DNA as starting material. By contrast, Figure 1B shows that when OC DNA was used, the standard phenol-based protocol produced a DNA strongly affected by some contaminant activity (smaller band and smear in lane 3) that may in part be responsible for lowering both the yield and ligation efficiency (lanes 3 and 8). Using the modified method (lanes 4 and 9), DNA remained essentially intact, as were those isolated by phenol-free protocols (lanes 5 and 6). Importantly, these differences between phenol-based procedures suggest that impurities may be efficiently eliminated from the DNA sample by the LiCl separation step in the modified method but not by direct ethanol precipitation (such as in the standard protocol).

Table 1 shows that electro-transformation of cells with the ligated pUC21 DNA isolated by the modified phenol-based method generated the lowest proportion of white colonies, 15-fold lower than that found for the standard method. In this respect, the former appears to be even more reliable than phenol-free methods. On the other hand, somewhat higher numbers of cfu/ml were found when phenol-free methods were used. Since the yield of DNA from the modified phenol-based method was at least as good as that from the phenol-free methods, this may simply reflect the presence of a higher number of multimer molecules in the ligation mixture from the former.

Electro-transformation results using pK18, OC DNA, were in agreement with those presented above using CCC DNA (not shown).

I have shown that a simple modification of a standard phenol-based method for isolating DNA from agarose slices affords significant improvement in the quality of the resulting material, whether CCC or OC DNA is used. The modified method is particularly recommended for the latter since it appears to be especially sensitive to contaminants. DNA so obtained is at least as good as that obtained from phenol-free methods, but extraction is both simpler and less expensive. The modified method has proven quick (about an hour from cutting out the gel slice to the purified fragment), efficient and reliable over time. Our lab has used it successfully with fragments ranging from 0.3 to more than 15 kb, although, as is the case with most DNA isolation methods, yield decreases as size increases over approximately 10 kb.

REFERENCES

1. **Pridmore, R.D.** 1987. New and versatile cloning vectors with kanamycin-resistance marker. Gene *56*:309-312.
2. **Sambrook, J., E.F. Fritsch and T. Maniatis.** 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
3. **Selden, R.F. and J. Chory.** 1987. Isolation and purification of large DNA restriction fragments from agarose gels, p. 2.6.1.-2.6.8. *In* F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl (Eds.), Current Protocols in Molecular Biology. John Wiley & Sons, New York.
4. **Tautz, D. and M. Renz.** 1983. An optimized freeze-squeeze method for the recovery of DNA fragments from agarose gels. Anal. Biochem. *132*:14-19.
5. **Vieira, J. and J. Messing.** 1991. New pUC-derived cloning vectors with different selectable markers and DNA replication origins. Gene *100*:189-194.

I am grateful to J.-F. Viret and S.J. Cryz Jr. for critical reading of the manuscript.

Didier Favre
Swiss Serum and Vaccine Inst.
P.O. Box 2707
3001 Berne, Switzerland

ADDENDUM

A production error in the May issue of *BioTechniques* inadvertently deleted one of the authors' names after correction of the galley proofs. The correct citation is "Ried, J.L., J.D. Everard, J. Diani, W.H. Loescher and M.K. Walker-Simmons. 1992. Production of polyclonal antibodies in rabbits is simplified using perforated plastic golf balls. *BioTechniques* 12:660-666." W.H. Loescher, whose name was omitted from the original publication is a faculty member of the Dept. of Horticulture, Michigan State University. Corrected reprints are available from the authors.