Reversible Gels for Electrophoresis and Isolation of DNA

BioTechniques 26:748-756 (April 1999)

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ABSTRACT

Here, the application of the gel-forming carbohydrate polymer, gellan gum, for the electrophoresis and isolation of DNA is detailed. Gellan gum forms gels in the presence of divalent metal cations, and the gels can be converted back to a solution by the addition of a chelating agent such as EDTA. Also, gellan electrophoresis gels can be formed using diamines. These gels are reversible by increasing the pH, which results in the deprotonation of the diamine. Gellan electrophoresis gels were used for separations at concentrations as low as 0.03%. Native gellan electrophoresis gels have significant electroosmosis and were generally run overnight. A gellan electrophoresis gel (0.1%) showed good resolution of DNA from approximately 50-1 kbp. The addition of linear polymers, such as hydroxethyl cellulose, to the gellan gum before casting greatly reduced the electroosmosis. The additional polymer increased the resolution of low-molecular-weight DNA down to approximately 200 bp and allowed gels to be run in a few hours. DNA isolated from gellan electrophoresis gels could be cut by common restriction enzymes and ligated in the presence of the gellan gum. The presence of gellan gum did not significantly inhibit the transformation of competent E. coli cells by plasmid DNA.

INTRODUCTION

The most successful anti-convective materials for gel electrophoresis are polyacrylamide and agarose. Also, the gel functions to separate DNA on the basis of size or shape. The pore size of polyacrylamide makes it suitable for smaller nucleic acids (from about a few base pairs to several thousand). Agarose is more suitable for the electrophoresis of larger nucleic acids. Even purified agarose has varying amounts of negative charge in the form of sulfate and pyruvate groups covalently attached to the carbohydrate polymer. A review of the electrophoretic properties of agarose has been recently published (16). The presence of negative charges on the gel media results in a flow of buffer molecules toward the negative electrode due to electroosmosis. The fixed negative charges in the gel require counterions, and when an electric field is applied, these cations along with waters of hydration migrate to the negative electrode. The result is a net flow of buffer toward the negative electrode. Molecules such as DNA, which are negatively charged and migrate toward the positive electrode, are slowed in their progress because of the opposing buffer flow.

Various methods have been used for the recovery of nucleic acids from isolated bands in agarose and acrylamide gels. Electro-elution consists of cutting the band out of the gel, placing the gel band into a dialysis tube (or a holder) and using an electric field to move the DNA or proteins out of the gel. The DNA or protein is prevented from contacting the electrode by the membrane. Technically, this is a difficult process and suffers from variable losses of material. Crush and soak techniques consist of cutting the band out, physically crushing the gel and allowing the protein or nucleic acid to diffuse out of the gel into a buffer. Problems with these techniques include the slow diffusion process and a variable recovery. A choice with agarose is to use low-melting-point agarose. Low-melting-point agarose will melt when the temperature is greater than 65°C and will remain molten if the temperature is kept above 26° to 30° C. The melting temperature must be carefully controlled because it is close to the temperature in which DNA is denatured (becomes singlestranded). It is possible to do enzymatic manipulation of the DNA in the molten agarose if the agarose is highly pure and screened for the presence of enzyme inhibitors commonly found in agarose. The molten agarose in the DNA sample can also be removed by phenol extraction. The enzyme β agarase will digest molten agarose to smaller oligosaccharides, resulting in the loss of gel-forming property (9). The use of low-melting-point agarose is not suitable for proteins because of the high temperature used. Polymerase chain reaction (PCR) DNA products have been recovered from agarose gel slices using a commercial device that nebulizes the gel slice and filters out the gel particles using an ultrafilter (11).

In this paper, an alternative technique for the recovery of DNA after electrophoresis is presented. Gellan gum is a carbohydrate polymer that forms strong gels in the presence of divalent metal cations (14). Gellan gum is composed of a linear repeating tetrasaccharide unit composed of β -Dglucose, β -D-glucuronic acid, β -D-glucose and α -L-rhamnose. The general properties of gellan gum have recently been reviewed (14). Gellan gum is produced by bacteria in a fermentation process. Gellan gum undergoes deacylation and precipitation steps, which then produces a product that forms strong brittle gels at low concentrations (14). A recent procedure for the efficient purification of gellan gum produces a product that is pure and chemically defined as a monovalent salt (8). This procedure will increase the utility and applications for this interesting carbohydrate polymer.

Physical studies using X-ray crystallography have shown that crystals of gellan gum are intertwined, threefold, left-handed parallel double helixes (4). Ions are believed to promote the association of the intertwined double-helix molecules in solution, resulting in gel formation (5,13). Many carbohydrate polymers are found in nature, but gellan gum was viewed as promising as a potential candidate because of the low concentrations required for gel formation and its reversibility. For example, alginates can form reversible gels at low concentrations, but they have a much higher relative charge compared to gellan gum (15).

Because of the negative charge, gellan gum would not appear to be a suitable candidate for an electrophoresis gel. The presence of the negatively charged glucuronic acid would result in electroosmosis, which is regarded as a negative property of electrophoretic gels. This paper studies the utility of gellan electrophoresis gels for the separation and isolation of DNA. Here, the conditions for electrophoresis and the recovery of DNA after separation using gellan electrophoresis gels will be examined. Ideal criteria for a reversible gel are: (i) that the conditions for the conversion to a solution are relatively gentle to the DNA and (ii) that the presence of the gel-forming material not interfere with the applications the DNA will be used for. Major advantages of not having to remove the gel-forming material are that additional expensive separation steps are not necessary, and fewer processing steps avoids additional losses of material. In this paper, the use of gellan electrophoresis gels for the separation, isolation and manipulation of DNA will be determined.

MATERIALS AND METHODS

Materials

High-molecular-weight DNA standard, kb DNA ladder, lambda DNA, restriction enzymes, agarose (low electroosmosis) and boric acid were obtained from Life Technologies (Gaithersburg, MD, USA). Phytagel (gellan gum), Dowex-50WX8-40, Tris, Bis-Tris and 1,3-diamino-2-hydroxypropane (DAHP) were obtained from Sigma (St. Louis, MO, USA). Hydroxyethyl cellulose (HEC; 250000 average-molecular-weight) was obtained from Sigma-Aldrich (Milwaukee, WI, USA).

Gel Formation

The potassium salt of gellan gum was prepared from a commercial preparation (Phytagel) by a deionization and precipitation procedure (7,8). The powder was weighed in a 250-mL flask, water was added (80% or 70% of the final vol, depending upon additions, see below), and also a stir bar was added. The solution was heated to boiling with stirring on a hot plate. The solution was stirred for 10 min to ensure that all particles of the polymer were dissolved. A concentrated solution of either CaCl₂ or DAHP (50 mmol/L) was added so that the final concentration was 5 mmol/L. A solution of buffer (10-fold concentrated) was also added. In some cases, a solution of 5% HEC (by weight) was added by weighing the viscous solution so that the final solution concentration was 0.5%. The solution was stirred for an additional 10 min. The temperature of the solution was reduced to approximately 60°C, and the solution was poured into the gel tray and allowed to solidify for 45 min at ambient temperature and 20 min at 4°C.

Buffers, Electrophoresis and Staining

Gels were run using either a buffer containing 45 mmol/L Tris, 45 mmol/L boric acid and 1 mmol/L CaCl₂ (TBC) with a pH of 8.5 or 22 mmol/L Bis-Tris, 75 mmol/L boric acid, 1 mmol/L EDTA and 1 mmol/L DAHP (BBED) with a pH of 6.8. In some cases, the running buffer contained the indicated amount of HEC. A flatbed submarine gel electrophoresis apparatus (CBS Scientific, San Diego, CA, USA) was used and maintained at 20°C by the use of a circulating water bath. The electrode buffer chambers were circulated by means of a peristaltic pump. The gel slab anchors at the ends of the gel tray $(14 \times 20 \text{ cm})$ were enlarged, using a small rotary grinder. For some gels, strips of porous polyethylene (1.5-mm thick and 5-mm wide) were taped along the edge of the ends of the gel tray. These strips stabilized the gel into position and allowed the free passage of buffer and the electric field. A comb with 16 teeth (2-mm thick) was suspended in the gel to form the sample wells. The gel solution was prepared as described above. The solidified gel was flooded with buffer (950 mL), and the comb was removed. The samples were diluted with a solution so that the samples contained Ficoll® (Amersham Pharmacia Biotech, Piscataway, NJ, USA) 400000 molecular weight (2% final concentration) or sucrose (5% final concentration), a trace of bromophenol blue and the electrophoresis buffer. The samples were loaded into wells, and the electric field applied at 50% of the final value for 20 min. Then the voltage was increased to the final value, and buffer circulation was begun.

For staining and imaging, the gels were supported with the use of the acrylic gel casting trays (UV transparent). Acrylic boxes with inner dimensions that closely matched the outer dimensions of the gel casting trays were used for staining. The gels were stained with ethidium bromide (1 μ g/mL) in 0.1 mol/L KCl with gentle mixing for 30–60 min. The gels were destained for 20 min in a solution of 0.1 mol/L KCl and followed by 20 min in deionized water.

Restriction Digestion and Ligation

Bands containing DNA were excised with a blunt spatula, placed in a microfuge tube and weighed. In the case of DNA isolated from gellan electrophoresis gels cast with calcium, a concentrated solution (10-fold) of EDTA (pH 8.0) was added to the gel slice such that the final concentration was 2 mmol/L. In the case of DNA

isolated from gellan electrophoresis gels cast with DAHP, a concentrated solution (10-fold) of Tris and EDTA (pH 8.0) was added to the gel slice so that the final concentrations were 10 mmol/L and 1 mmol/L, respectively. Gentle mixing was sufficient to dissolve the gel. The restriction enzyme (10 U) or ligase (1 U) was added directly to the dissolved gel band, and the solution was mixed. The 10× restriction or 5× ligase buffer (Life Technologies) was added, the tubes mixed and then incubated at 37°C for 2–4 h.

Transformation Efficiency

The effect of the gel on transformation efficiency was determined using chemically competent Escherichia coli cells [subcloning efficiency DH5 α^{TM} (Life Technologies)]. DNA was isolated as described above. The dissolved gel solution (0.05 mL) was placed on ice, and competent cells (0.05 mL) were added to tubes containing the dissolved gel or buffer (final vol of 0.1 mL). Transformation was done according to the supplier's instructions. Briefly, this consists of incubation on ice for 30 min, heat shock at 37°C for 45 s, back on ice for 2 min and the addition of 0.95 mL of LB media. The tubes were then incubated at 37°C with shaking (225 rpm, Model G24 Shaker; New Brunswick Scientific, Edison, NJ, USA) for 1 h. The cells were diluted and plated out on LB plates containing 0.1 mg/mL ampicillin. Colonies were counted the next day after incubation at 37°C.

RESULTS AND DISCUSSION

Electrophoresis of DNA Using Gellan Formed by Divalent Cations

Gellan gum was used to make electrophoresis gels in a range of polymer concentrations and buffer compositions. Take care to ensure that all of the gellan gum particles are in solution before adding the divalent cation. Adding a solution containing divalent cation to the gellan gum particles before they are dissolved makes it difficult to get the polymer into solution. Most of the gels used were cast using calcium, but gels made with magnesium gave similar results.

The gels are mechanically strong but brittle, and because of this, the gels will crack when not supported. For this reason, the gels were supported by use of a gel tray. Gellan electrophoresis gels as low as 0.03% were used for DNA separations. These low-percentage gels can be cast in a frame composed of higher percentage gellan gum. Lower concentration gels are preferable, because when converted back to solution, there are lower concentrations of gellan gum present in the resulting DNA solutions. A typical gellan electrophoresis gel concentration was 0.1%. These gels have sufficient mechanical strength and resolved DNA in the range of approximately 50-1 kb (Figure 1). Higher concentrations of gellan gum did not



Figure 1. Gel electrophoresis of DNA using 0.1% gellan gum formed with 5 mmol/L CaCl₂. The buffer was TBC, and electrophoresis was at 140 V (4.7 V/cm) for 16 h at 20°C. Samples are: lanes 1 and 3, the kb ladder; lane 2, the high-molecular-weight ladder; lanes 4 and 5, the *Bst*EII restriction digest of λ DNA; lane 6, the *Bst*EII restriction digest of λ DNA; lane 7–9, the pBR322 DNA (containing nicked circular and supercoiled forms); lane 10, the pBR322 DNA restriction digestion by *Hin*dIII; and lane 11, the λ DNA (48.5 kbp). Numbers on the side indicate the size of DNA standards (lanes 1 and 2) in kbp.

improve the resolution of lower-molecular-weight DNA, and the electrophoresis was slower compared to 0.1% gellan electrophoresis gels. The gels were formed in the presence of 5 mmol/L CaCl₂, because lower concentrations of CaCl₂ did not form strong gels. The electrophoresis buffer contained a low concentration of CaCl₂ (1 mmol/L) to prevent depletion in the gel of divalent cations during electrophoresis. Circulation of the buffer is also necessary to prevent depletion of the calcium in the gel.

Figure 2 shows gels that are a comparison of gellan gum and agarose. The electric field used for the gellan electrophoresis gels was 3.5-fold higher than the agarose gel. This reflects the high electroosmosis in the gellan gum. Also, Figure 2 illustrates that staining with ethidium bromide is approximately 4-fold less sensitive with gellan gum compared to staining in agarose. The gellan electrophoresis gels have low background fluorescence allowing long exposures, but the fluorescence intensity of the DNA was lower compared to agarose. When DNA was purposely overloaded (Figure 2, lanes 4 and 5), the capacity of gellan electrophoresis gels appears good, as judged by the lack of smearing between bands, even when the lower sensitivity of staining is taken into account. SYBR® Green I staining (Molecular Probes, Eugene, OR, USA) can be used to detect DNA at low concentrations in gellan electrophoresis gels (unpublished data).

Modification of Electrophoretic Properties of Gellan Electrophoresis Gels Using Additional Polymers

The addition of linear polymers to gellan gum before casting had two effects on the electrophoresis of DNA using gellan electrophoresis gels: (i) the additional linear polymers increased the resolution of lower-molecular-mass DNA, and (ii) the electroosmosis was reduced. Figure 3 shows the results of adding 0.5% HEC (250 000 mol wt) to a 0.1% gellan electrophoresis gel. The additional polymer had the greatest effect on increasing the mobility and resolution of the low-molecular-weight DNA. Using these conditions, the fractionation range was down to approximately 200 bp. Figure 3 also notes the difference in mobility of the supercoiled and nicked circular forms of the plasmid pBR322 (compare lane P in Figure 3, A and C). The addition of the polymer had a greater effect on decreasing the mobility of the nicked circular form (the slower moving band in both gels).

Bode was a pioneer in the addition of linear polymers to gels and membranes to improve their electrophoretic performance (2,3). Perlman et al. (12) performed a comparative study of adding carbohydrate polymers (HEC, methylcellulose and galactomannan) to agarose gels to determine the effect on the resolution of DNA fragments. They found that HEC increased the resolution of lower-molecular-weight DNA (from ca. 50–1000 bp). A variety of neutral polymers have been added to agarose gels to reduce the electroosmosis to perform isoelectric focusing (6) and electroimmunodiffusion (10).



Figure 2. Comparison of loading and staining intensity of gellan gum and agarose electrophoresis gels. The gellan gel (0.1%) was electrophoresed at 140 V for 16 h using TBC. The agarose gel (0.5%) was electrophoresed at 40 V for 16 h using the TB with EDTA (1 mmol/L). Lanes 1–4 are the kb ladder at 0.04, 0.16, 0.8 and 4.0 μ g total DNA, respectively. Lanes 5–7 contain λ DNA digested with *Hind*III at 5.5, 1.1 and 0.6 μ g total DNA, respectively. The gellan gum gel was exposed for 8 s, and the agarose gel was exposed for 1 s. The exposure for both gels was with an *f*-stop of 8. Numbers on the side are the size of the *Hind*III fragments of λ DNA in kb.

Many types of linear polymer solutions (including HEC) have been used to successfully separate DNA and oligonucleotides using capillary electrophoresis (1). These polymer solutions can be pumped into open tube capillaries and are easily renewed.

The increased resolution due to HEC addition is probably the result of several factors. Besides the increased sieving of DNA, the viscous HEC solution decreases the diffusion of lowmolecular-weight DNA. The decreased time for electrophoresis would also result in the decreased diffusion of lowmolecular-weight DNA. HEC is just one example of a number of polymers with different chemical and physical properties that can be added to gellan electrophoresis gels. We are in the process of systematically studying the influence of different polymer additives and the electrophoresis conditions on the separation of DNA by size and shape in gellan electrophoresis gels.

Formation of Gellan Electrophoresis Gels Using Diamines

It was discovered that diamines could be used to form gels with gellan gum. Gels can be formed using a variety of diamines (unpublished data). The diamine used in this study was DAHP. For diamines to form a gel, both amino groups must be protonated. A practical consequence of this is that the pH of the buffer used must be below 7. The buffer used was BBED, pH 6.8. It is important that the pH of the buffer does not exceed 7.0, because otherwise, the gel will dissolve. It is important to circulate the buffer to prevent pH changes and depletion of the diamine in the gel.

EDTA can be added to gels formed using diamines because divalent metal cations are not required to form the gel. The separations obtained with gels formed with DAHP were very similar to gels formed with calcium. The DNA can be recovered from these gels by increasing the pH above 7.0. EDTA should be added to the gels when the DNA is likely to contain nucleases. The importance of the inactivation of nucleases by chelation was illustrated by the ligation of DNA isolated from gellan electrophoresis gels made with calcium. DNA isolated from gels that had been cast with calcium and run



Figure 3. Modification of electrokinetic properties of gellan electrophoresis gels by incorporating an additional polymer. The gels were run for either 4.8 or 20.4 h (as indicated above the gel) at 140 V using TBC. The gel in Panel A had HEC (0.5%) added to the gel and to the running buffer. Samples are: L, λ DNA; K, kb DNA ladder; H, high-molecular-weight DNA standard; and P, pBR322 plasmid DNA (containing both supercoiled and nicked circular forms).



Figure 4. Restriction digestion of λ DNA cut by restriction enzymes. The λ DNA isolated from gellan electrophoresis gels dissolved, as described in the text, had 10 U of *Eco*RI (R), *Hind*-III (H) or *Bst*EII (B) added and the restriction buffer. The samples were kept at 37°C for 2 h and then analyzed by agarose gel electrophoresis (0.75%). The first two lanes are standards that contain λ DNA, not gel-purified, and digested with the indicated enzymes. Electrophoresis was at 120 V (4 V/cm) for 2 h at 20°C.

overnight did not appear degraded (shown by the lack of smearing below the DNA when analyzed on agarose gel electrophoresis). Variable results were obtained when this DNA was tested for self-ligation. The failure of self-ligation of this DNA indicates damage to the ends of the DNA by exonucleases. When the buffer and staining solutions used for these were boiled for 20 min to inactivate any nucleases, the DNA was readily ligated (Figure 5). Figure 5 shows that the DNA samples isolated from gellan electrophoresis gels containing EDTA were also readily ligated.

Restriction Digestion, Ligation and Transformation Using DNA Isolated from Gellan Electrophoresis Gels

DNA isolated from gellan electrophoresis gels was tested for its ability to be cut by common restriction enzymes. The pattern of restriction fragments was determined by agarose gel electrophoresis (Figure 4). For these experiments, the band containing λ DNA was isolated and dissolved as described above. Gels rapidly convert to a solution in a few minutes with gentle mixing. To an aliquot of the λ DNA, a restriction enzyme was added, the solution was mixed, the restriction enzyme buffer was added, and the solution was mixed again. Three commonly used restriction enzymes (EcoRI, HindIII and *Bst*EII) were used to cut the λ DNA isolated from gellan electrophoresis gels formed using calcium or DHAP. The commonly used restriction buffers contain high amounts of magnesium (typically 10 mmol/L) and variable amounts of other salts. This amount of magnesium is enough to cause gellan gum to form a weak gel, but mixing breaks up the gel into a slurry that can be easily pipetted. Figure 4 shows the results of a typical digest of DNA isolated from gellan electrophoresis gels.

Ligation buffers also contain magnesium in sufficient concentrations to cause the dissolved gellan gum to form a weak gel. A similar procedure, as used for restriction enzymes, was used for ligation. A commonly used assay for ligase activity is to measure the self-ligation of a mixture of DNA restriction fragments by analyzing the pattern using agarose gel electrophoresis. Figure 5 (lane 5) shows the self-ligation of the *Hin*dIII digestion of λ DNA. Dissolved gellan gum solutions were added at high concentrations (up to 50% of the ligation reaction) and did not inhibit the self-ligation of the DNA fragments (Figure 5, lane 6). Ligation was also done on DNA fragments isolated from gellan electrophoresis gels. Figure 5 (lanes 2 and 3) show DNA isolated from gellan electrophoresis gels cast with different cations. Figure 5 (lanes 7–11) shows the self-ligation (shift to higher-molecular-weight) of these fragments.

The influence of gellan gum on the transformation of bacteria by plasmid DNA was determined. First, the effect of gellan gum was determined on the transformation of competent *E. coli* cells by added pBR322 plasmid DNA.

Different amounts of dissolved gellan gum were added to a standard preparation of pBR322 and used to transform a preparation of chemically competent E. coli cells (DH5 α). The cells were plated out on duplicate plates, and the colonies were counted. In a typical experiment, a preparation of pBR322 gave 1.9×10^6 ampicillin-resistant colonies/µg of DNA. Adding a solution of dissolved gellan gum gel to the DNA before transformation at concentrations of 0.02% and 0.05% gave 2.4×10^{6} and 1.7×10^6 ampicillin-resistant colonies/ µg DNA, respectively. Addition of dissolved gellan gum gels, at the above concentrations, containing HEC up to 0.5% did not significantly reduce transformation efficiency. Replicate experiments gave similar results.



Figure 5. Effect of gellan gum on self-ligation of DNA isolated from gellan electrophoresis gels. The isolated DNA and ligation products were analyzed by agarose gel (0.75%) electrophoresis using the TB buffer and containing 1 mmol/L EDTA. Electrophoresis was at 120 V (4 V/cm) for 2 h at 20°C. Lanes 1 and 4 are the *Hin*dIII restriction digest of λ DNA. Lane 2 is the 9.4-kb band (*Hin*dIII restriction digest of the λ DNA) isolated from a 0.1% gellan electrophoresis gels (cast with calcium). Lane 3 is the 9.4-kb band (*Hin*dIII restriction digest of the λ DNA) isolated from a 0.1% gellan electrophoresis gels (cast with calcium). Lane 3 is the 9.4-kb band (*Hin*dIII restriction digest of the λ DNA) isolated from a 0.1% gellan electrophoresis gels (cast with calcium). Lane 6 is a test ligation of the *Hin*dIII restriction digest of λ DNA with 20 µL of gellan gum (0.1% concentration and cast with calcium) added to the ligation mixture (total vol 40 µL). The DNA shown in lane 2 (9.4-kb fragment isolated from a calcium gellan electrophoresis gel) was ligated using either 10 µL of DNA (Lane 7) or 20 µL of DNA (Lanes 8 and 9) in a 40-µL ligation mixture. The DNA shown in lane 3 (9.4-kb fragment isolated from a DAHP gellan electrophoresis gel) was ligated using either 10 µL of DNA (Lane 10) or 20 µL of DNA (Lane 11) in a 40-µL ligation mixture. Ligase was added to the ligation mixtures at levels of either 2 U (Lanes 5–8, 10 and 11) or 0.5 U (Lane 9) in a total of 40 µL.

In another set of experiments, a preparation of pBR322 plasmid DNA was electrophoresed in gellan electrophoresis gels, and the region of the gel containing the supercoiled and nicked circular forms (Figure 1, lanes 8-10) was isolated. In a typical experiment, control plasmid DNA (pBR322 DNA) and plasmid DNA isolated from gels was used to transform E. coli cells (DH5 α). The cells were plated out on duplicate plates, and the colonies were counted. Control plasmid DNA (not electrophoresed) gave 5.1×10^6 cells/ µg. DNA isolated from a 0.1% gellan electrophoresis gel (cast with calcium) gave 5.2×10^6 cells/µg. DNA isolated from a 0.1% gel (cast using DAHP and 0.5% HEC) gave 2.3×10^6 cells/µg. The equivalent of 3.6 ng of DNA was used in each transformation. Duplicate experiments gave similar results.

The ionic composition of the DNA used for the transformation had a very strong influence on the success of the transformation. EDTA must be added in sufficient quantities to fully chelate the calcium to free the DNA from the gel (2 mmol/L final concentration). Increasing the EDTA concentration higher than 2 mmol/L had an inhibitory effect on the transformation efficiency. The high concentration of magnesium in ligation buffers (typically 10 mM) results in the formation of a weak gel. The presence of this gel does not allow the transformation of bacteria using ligation mixtures containing DNA isolated from gellan electrophoresis gels. Adding sufficient EDTA to chelate the calcium was inhibitory when added to the chemically competent E. coli cells (prepared in high concentrations of calcium). A simple method was developed to remove the gellan gum from the ligation mixtures. Addition of CaCl₂ (5 mmol/L final concentration) to the ligation mixture, followed by centrifugation (12000× g for 15 min), collapsed the gel to a compact pellet leaving the DNA in the supernatant. Based on this technique, we are currently developing an efficient method to subclone DNA fragments isolated from gellan gum gels (unpublished data).

Gellan gum offers a very flexible platform that can be used to separate and isolate DNA. When additional polymers are added, they will influence

the separation by the effect on electroosmosis in the gel and direct the interaction with the DNA. The polymer chemical structure, the molecular weight and the concentration will all influence the separation. This paper provides a few examples of the separations that are possible using gellan electrophoresis gels. At this point, gellan gum offers an alternative gel material that has some unique properties. The conditions for the recovery of DNA are mild and easily accomplished. As new applications are developed to take advantage of these unique properties, gellan gum gels will become an increasingly important technique to isolate and manipulate DNA.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Landis Donner of the US Department of Agriculture, Agricultural Research Service (ARS) (Philadelphia, PA) for helpful advice and discussions on the purification of gellan gum. Certain commercial equipment, instruments or materials are identified in this paper to specify adequately the experimental procedures. Such identification does not imply recommendation by NIST, nor does it imply that the material or equipment are necessarily the best available for the purpose.

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Received 17 August 1998; accepted 9 December 1998.

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