

nicked circular forms). This gel resolved DNA down to approximately 50 base pairs.

EXAMPLE 3

Demonstration of DNA Recovery, Enzymatic Treatment, and Transformation Using DNA Isolated From Gellan Gum Electrophoresis Gels 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 gels cast with calcium, a concentrated solution (10-fold) of EDTA (pH 8.0) was added to the gel slice so the final concentration was 2 mmol/L. In the case of DNA isolated from gels cast with DAHP, a concentrated solution (10-fold, pH 7.5) of TRIS and EDTA was added to the gel slice so the final concentrations were 10 mmol/L and 1 mmol/L, respectively. Gentle mixing was sufficient to dissolve the gel. The restriction enzyme (10 units) or ligase (1 unit) was added directly to the dissolved gel band and the solution was mixed. The 10x restriction or 5x ligase buffer (Life Technologies, Rockville, Md.) was added, the tubes mixed, and incubated at 37° C. for 2 h to 4 h.

Transformation Efficiency

The effect of the gel on transformation efficiency was determined using chemically competent *E. coli* cells. DNA from gellan gum electrophoresis gels 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 volume of 0.1 mL). Transformation was done according to the supplier's instructions. Briefly, this consisted of incubation on ice for 30 min, heat shock at 37° C. for 45 sec., back on ice for 2 min, and addition of 0.95 ml of LB media. The tubes were then incubated at 37° C. with shaking (225 rpm) 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

DNA isolated from gellan electrophoresis gel was readily cut by a variety of restriction enzymes (Eco RI, Hind III, and Bst EII) in the presence of gellan gum. This was determined by analysis of the restriction fragment using agarose gel electrophoresis. The activity of DNA ligase was not significantly inhibited by gellan gum as shown by the ligation of a test mixture of restriction fragments. Successful ligation of the test mixture was indicated by the formation of higher molecular weight products when analyzed by agarose gel electrophoresis. DNA isolated from gellan gum electrophoresis gels was readily ligated as shown by the formation of higher molecular weight products when analyzed on agarose gel electrophoresis.

DNA isolated from gellan gum electrophoresis gels after dilution could be used in direct transformation of *E. coli* as shown in Table 3 below. DNA isolated from gellan gum electrophoresis gels and ligated to other DNA did not transform with high efficiency unless the gellan gum was removed before or after ligation. This is because of the high concentration of Mg⁺² in the ligation buffer causes the formation of a gel that inhibits transformation. Adding CaCl₂ to the ligation mixture followed by centrifugation precipitates the gellan into a compact pellet and leaves the DNA in solution. The DNA will transform with high efficiency.

These experiments show that enzymatic manipulation of the DNA can be done in the presence of the gel polymer. Restriction enzyme digestion and ligation of DNA are several of the most commonly used steps used in molecular

biology laboratories. An important step in cloning is to use exogenous DNA to transform bacteria. The data Table 3 below shows that gellan gum polymer does not inhibit the transformation of *E. coli* cells by pBR 322 plasmid DNA. The gellan gum polymer was added to a preparation of pBR 322 plasmid DNA and then the mixture was used to transform *E. coli* cells. An aliquot (0.1 mL of a 1:10 dilution) of the transformation reaction was plated on LB plates containing ampicillin and the colonies counted.

The results of this experiment are summarized below in Table 3:

TABLE 3

Effect of Gellan Gum on Transformation of <i>E. coli</i> by pBR Plasmid DNA	
Gel % (before adding cells)	Ampicillin Resistant Colonies per microgram of plasmid DNA (average of two plates)
0	1.9 × 10 ⁶
0.02	2.4 × 10 ⁶
0.05	1.7 × 10 ⁶
0.02 (HEC 250K 0.2%)	1.6 × 10 ⁶
0.05 (HEC 250K 0.5%)	2.4 × 10 ⁶

These results show that the presence of gellan gum in the DNA sample did not inhibit transformation of *E. coli*. The presence of the polymer hydroxethyl cellulose 250,000 molecular weight (HEC 250K) along with gellan did not significantly inhibit transformation. Many of the critical steps in the manipulation and cloning of DNA, including restriction digestion, ligation, and transformation of bacteria by DNA isolated from these gels can be done in the presence of the gellan gum polymer.

EXAMPLE 4

Electrophoresis of Proteins Using Gellan Gum Gels

Gel Formation, Electrophoresis, and Staining of Proteins

Gels were formed as described in Example 1. After electrophoresis the gels were fixed in either 50% ethanol, 2% phosphoric acid or 10 mmol/L HCl for 1 h. The gels were washed in 1 mmol/L HCl and then stained in Coomassie Brilliant Blue G250 (0.015 mg/mL) in 1 mmol/L HCl overnight. The gels were destained in 1 mmol/L HCl for a few hours.

Several standard proteins and crude protein mixtures, such as milk, were used to determine the separation of proteins using gellan gum gels.

These proteins have different isoelectric points and electrophoretic mobilities, shown in Table 4 below.

TABLE 4

Properties of Proteins				
Protein	Abr.	Molecular Mass	Isoelectric Point	Electrophoretic Mobility in TG buffer (cm ² /V · s) × 10 ⁻⁵
Myoglobin (horse heart)	MYO	17,500	7.4	5.8
β Lactoglobulin (mixture of A and B)	BLG	36,700 (dimer)	5.2 and 5.3	27.2 and 25.3
α Lactalbumin	ALA	14,400	4.8	19.4