NOTICE:

The copyright law of the United States (Title 17, United States Code) governs the making of reproductions of copyrighted material. One specified condition is that the reproduction is not to be "used for any purpose other than private study, scholarship, or research." If a user makes a request for, or later uses a reproduction for purposes in excess of "fair use," that user may be liable for copyright infringement.

RESTRICTIONS:

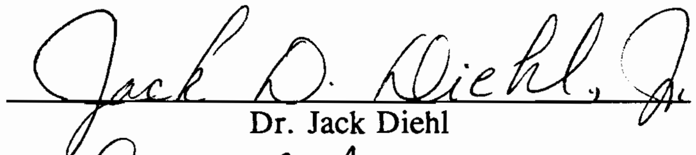
This student work may be read, quoted from, cited, and reproduced for purposes of research. It may not be published in full except by permission of the author.

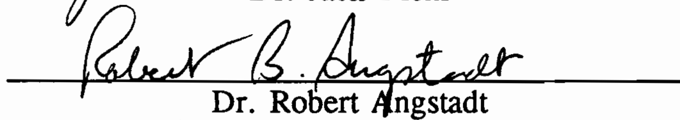
The Use of Pumice to Harvest DNA from Agarose Gels

Presented to the faculty of Lycoming College
in partial fulfillment of the requirements
for departmental Honors in Biology

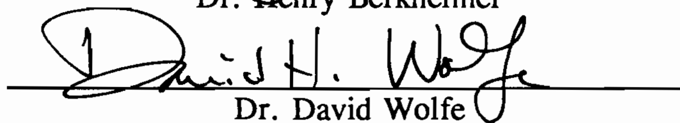
by
Mark L. Heyd
Lycoming College

April 25, 1995


Dr. Jack Diehl


Dr. Robert Angstadt

Dr. Henry Berkheimer


Dr. David Wolfe

The Use of Pumice to Harvest DNA From Agarose Gels

Mark L. Heyd* and Jack D. Diehl
Biology Department, Lycoming College
Williamsport, PA 17701

ABSTRACT

The objective of this research was to determine if pumice could be used for recovering DNA fragments from agarose gels with the retention of biological activity.

Plasmid pBR325, which has a single EcoRI restriction site in the chloramphenicol resistance gene, was electrophoresed after hydrolysis by this enzyme. The linearized plasmid was cut from the agarose gel, dissolved in sodium iodide, adsorbed on pumice, and eluted in water. Half of the eluted sample was ligated with T4 ligase while the remainder was stored on refrigeration. Both ligated and unligated samples were phoresed and transformed into chloramphenicol sensitive *E. coli* strain JF427. The transformed cells were plated on LB/chloramphenicol medium.

Upon electrophoresis the ligated and unligated DNA gave different electrophoretic mobilities, and only cells transformed with ligated DNA grew on medium containing chloramphenicol. This provides positive evidence that pumice can be used to recover DNA fragments from agarose gel with the retention of biological activity.

INTRODUCTION

DNA is commonly recovered in high yield from agarose gels after electrophoresis by a technique that adsorbs DNA to fine particles of glass powder in the presence of sodium iodide. Both DNA and agarose dissolve in high concentrations of sodium iodide; in sodium iodide, DNA binds to glass. This technique, discovered in 1978 by Vogelstein and Gillespie, for recovering DNA from agarose gels is rapid and convenient, but it is also costly for a lab group to use.(Vogelstein & Gillespie 1979) Since the major component of both glass and pumice is silicon dioxide (SiO_2), it was hypothesized that pumice could be used to recover DNA from agarose gels.

The purpose of this study was to determine if pumice could be used as an effective means for recovering biologically active DNA fragments from agarose gels.

Plasmid pBR325 has a single EcoRI cut site in the chloramphenicol resistant gene. This plasmid was digested with this enzyme and electrophoresed. The linearized fragment of DNA was cut from the gel, dissolved in NaI, adsorbed on pumice, and eluted in water. Half of the eluted sample was ligated with T4 DNA ligase at 15^0C, while the remainder was stored on refrigeration overnight(o/n). Both ligated and unligated samples were subjected to electrophoresis and transformation into chloramphenicol sensitive *E. coli* strain JF427. The transformed cells were then plated on LB/chloramphenicol medium.

Upon electrophoresis, the ligated and unligated plasmid gave

different electrophoretic mobilities. This gave positive evidence that the ligation procedure was successful and that there were no inhibitory elements in pumice that would prevent ligation. Only cells transformed with ligated plasmid grew on medium containing chloramphenicol. This gave positive evidence that the DNA recovered on pumice retains its biological activity.

Materials and Methods

BACTERIUM

The bacteria used in this study were *E. coli* strains JF427 and JF428. The JF428 strain harbors the plasmid, pBR325. The JF427 strain was used as a recipient for transformation. JF427 strain is not resistant to chloramphenicol, and hence, it will not grow on media containing this antibiotic. (Hackett, Fuchs, & Messing 1988)

PLASMID

Plasmids are small, autonomously replicating, covalently closed circular pieces of extrachromosomal DNA. The pBR325 plasmid, which has 5995 base pairs, has three genes that code for antibiotic resistance (Figure 1). These genes code for resistance to tetracycline (TET), chloramphenicol (CAP), and ampicillin (AMP). The pBR325 plasmid also has a single EcoRI cut site in the CAP gene. (Sofer 1991)

MEDIA

The media used in this study included: LB/chloramphenicol plates, LB (Luria-Bertani) broth, and SOC. SOC is made of 2% tryptone, 0.5% yeast, 10 mM NaCl, and 2.5 mM KCl at a pH of 7.2. The LB/CAP plates were all made by dissolving 2.5 g LB powder (DIFCO Laboratories Record #0446-17-3 Lot #56264JB) per 100 ml of distilled water. Then, one drop of 10% (2.5M) sodium hydroxide was added per 100 ml medium to bring the pH to 7.2. The solution was divided into Erlenmeyer flask with 30 ml to a 125 ml flask or 60 ml to a 250 ml flask. Agar was added to the medium to make a 1.75% agar solution. Aluminum foil was placed over the Erlenmeyer, and the solution was autoclaved. When the Erlenmeyer cooled to approximately 60^{0}C, the chloramphenicol was added with a micropipet at 1 microliter of CAP (at 35 mg/ml ETOH) per milliliter of broth, swirled, and approximately 10 ml was poured into sterile Petri dishes.

Fifty milliliters of SOC was made. SOC was made as stated above. After the solution was dissolved, the pH was adjusted to 7.2. The mixture was then autoclaved. Before using, 0.5 ml of 1.0 M magnesium chloride and 1.0 ml of 1.0 M glucose were aseptically added to the 50 ml of sterile SOC broth.

ANTIBIOTICS

The three different antibiotics used in this study were: ampicillin, chloramphenicol, and tetracycline. Chloramphenicol was used at a concentration of 37 micrograms per milliliter ETOH,

and ampicillin was used at a concentration of 45 micrograms per milliliter H_2O. Tetracycline was used at a concentration of 12.5 ug/ml ETOH.

PUMICE PREPARATION

The pumice suspension was prepared by combining 10 g of Baker and Adamson pumice powder (code 2157; lot# E175) with 100 ml of distilled water in a beaker in order to make a 10/1 ratio of distilled water/pumice. A magnetic stir bar was put into the beaker, and the pumice and water were mixed until the suspension was homogenous. Once the mixture was homogeneous, it sat for exactly 30 minutes without stirring; then the supernatant was decanted into second beaker. The supernatant in the second beaker sat for another 30 minutes. The second supernatant was transferred to two sterile 250 ml plastic tubes. The mass of each of these tubes with the supernatant was determined on a triple beam balance and was adjusted so that the two tubes had the same mass. The one tube served as a counter-balance during centrifuge. The two tubes were then centrifuged in the cold for five minutes at 9,000 rpm on the IEC model B-20 centrifuge. The supernatant was carefully drawn off using a pipet and discarded. The pumice was, then, suspended in 500 microliters of sterile distilled water. The pumice/water was transferred to a 1.5 ml sterile microfuge tube and centrifuged for five minutes at 14,000 rpm. The water was aspirated. An estimation of the volume of pumice in the tube was made, and an equal volume of sterile

distilled water was added to make a 50% pumice/water preparation.

RESTRICTION ENZYME METHODOLOGY

EcoRI, purchased form SIGMA biological company (Lot #63H0040) with a concentration of 25 units per microliter, was the restriction endonuclease (enzyme) used to cut the pBR325 plasmid in the CAP gene. The enzyme recognizes the base pair sequence GAATTC and cuts between the G and the adjacent A. The 10X concentrated palette buffer used was also provided by SIGMA (Lot #52H6685).

Two sterile 0.5 ml microfuge tubes were labeled #1 and #2. To the #1 tube, the following were added in this order: 23 microliters of sterile distilled water (dH_2O), 3 microliters of 10X concentrated buffer, and 1 microliter of EcoRI enzyme. This tube was mixed by finger flicking and centrifuged for five seconds (s) at 14,000 revolutions per minute (rpm) to pool the mixture in the bottom of the tube. Eighteen microliters from tube #1 were withdrawn and discarded. Three microliters of concentrated pBR325 plasmid (0.5 micrograms per microliter) was added to tube #1. Tube #1 was then mixed by finger flicking, pulsed in the centrifuge for 5 s at 14,000 rpm, and placed in a 37^0C H_2O bath for 1 hr. The ratio of enzyme to DNA was 5 units/ug.

Tube #2 was further labeled "uncut plasmid". To this tube, the following was added: eight microliters of sterile distilled

water, 1 microliter of concentrated pBR325 plasmid (ie. 1 microgram per microliter), and 1 microliter of loading buffer (20% ficol, 25% BPB aqueous solution). One microliter of loading buffer was added to tube #1 after incubation.

ELECTROPHORESIS

After incubation, a gel for electrophoresis was made with 20 ml of 1X TAE buffer (Tris-Acetate-EDTA). This was made by mixing 10 ml 0.5 M EDTA solution in a 100 ml flask with 5.71 ml glacial acetic acid and 2.42 g Tris base and graduated to volume with distilled water. This made a 50X concentrate, and the pH was adjusted to 8.4. Twenty milliliters of Tris-Acetate-EDTA (diluted 50 fold; ie. 1:50) was mixed with 0.16 g (to make a 0.8% agarose gel) of SIGMA agarose powder (lot #63HO487) in a 125 ml Erlenmeyer flask to a rolling boil with a swirling motion.

The mixture was allowed to cool to approximately 60^{0}C, before 2 microliters of 0.5% ethidium bromide was added. The contents of the Erlenmeyer were poured into an agarose gel mold tray, and an eight tooth comb was put in the tray and allowed to solidify. The gel lanes were loaded as follows:

lane 1 and 2 - empty

lane 3 - uncut pBR325

lane 4 - pBR325 cut with EcoRI

lane 5 - Hind III digest lambda DNA (SIGMA Lot #13H6713 610 ug/ml)

lane 6,7,8 - empty.

The electrophoresis was run at 100 volts (approximately 50 volts at the gel) for approximately 1 hr.

PHOTODOCUMENTATION

The gel was put on a transilluminator (Fisher Biotech Systems 312 nm ultraviolet light with variable intensity Model #FBTIV88). A picture was taken with a Fisher Biotech photodocumentation camera model FB-PDC-34. The camera was equipped with a #15 orange Ethidium Bromide stain Filter P.I.D. #616367. The type of film used was polaroid Black and White 667 instant pack film order #617799. The pictures were taken using the following f-stop and exposure time, f=5.6, A=8 (1/8 s), respectively.

DNA HARVESTING (FROM AGAROSE GELS)

The linear (cut) pBR325 fragment was cut out of the agarose gel with a scalpel while under reduced UV illumination. The agar plug (with DNA) was put into a pre-weighed 1.5 ml sterile microfuge tube. Another picture was taken of the gel. The mass of this tube with the gel in it was determined on an analytical balance. After the addition of 2 microliters of concentrated sodium iodide (NaI) per milligram of gel, the tube was placed in the 37^{0}C H_2O bath for approximately 1 hr with occasional finger flicking to dissolve the agarose (Note: The time may be longer or shorter depending on how long it takes the gel to dissolve). The

tube was put in ice and, at the same time, a vortexed pumice slurry (50% pumice/50% water) was also put in ice for five minutes. A microliter of the cold vortexed pumice slurry was added per microgram of DNA in the tube (the amount of DNA was estimated by the previous picture taken during the electrophoresis run). This mixture was kept in ice for 30 minutes with occasional finger flicking. The mixture was centrifuged for 10 s @ 14,000 rpm, and the supernatant was aspirated at 5"Hg (Thomas Scientific USA vacuum pump model DOA-P104D-AA). The remaining precipitate was suspended in 10 microliters of NaI by vortexing, centrifuged for 10 s @ 14,000 rpm, and aspirated on the pump. The precipitate was re-suspended in 10 microliters of wash solution (500 microliters of TNE and 500 microliters of 95% ethanol) by vortexing. TNE is composed of 10 mM Tris-HCl (pH=8), 1 mM EDTA, and 0.1 M NaCl. This was centrifuged and aspirated as described above. This wash step was repeated. The pumice/DNA was suspended in 12 microliters of sterile distilled H_2O by vortexing, put into the 37^0C H_2O bath for 10 min, and centrifuged as above. The supernatant was harvested with a micropipet with sterile tip set at 13 microliters and was put in a sterile 0.5 ml microfuge tube.

LIGATION

The tube containing the eluted DNA was labeled "unligated". Six microliters were drawn out of the unligated tube and added to another sterile 0.5 ml microfuge tube labeled "ligation". To the

ligation tube was added one microliter of 10x ligation buffer (0.66 M Tris pH=7.6, 50 mM $MgCl_2$, 50 mM DTT, and 10 mM ATP) and 1 microliter of T4 ligase (Sigma Lot #102H0840). One unit of the T4 ligase ligates 1 ug of lambda DNA EcoRI fragments in 1 hour in 20 ug reaction volume (1 unit/ug). This tube was mixed, pulsed in the centrifuge for 5 s @ 14,000 rpm, and put into a 15^0C H_2O bath overnight (o/n). The unligated tube was stored at 4^0C.

PREPARATION OF COMPETENT CELLS

A loopful of JF427 *E. coli* cells was put into 2 ml of sterile LB broth which was then put into a 37^0C H_2O shaker o/n (Gyrotory Water bath shaker model 676 manufactured by New Brunswick Scientific). The following day, 1 ml of the o/n culture was transferred to 50 ml of LB broth in a 125 ml Erlenmeyer. The Erlenmeyer was put back into the 37^0C shaker for 3 hrs. When the Erlenmeyer was taken out of the shaker, the culture was swirled and poured into a sterile plastic centrifuge tube, and it's mass was determined on a triple beam balance. The tube was centrifuged in the cold for 5 min @ 5,500 rpm. The supernatant was decanted back into the Erlenmeyer, and the sedimented cells were suspended in 20.0 ml of cold 0.05 M calcium chloride ($CaCl_2$) by finger flicking. The mass of this tube was again determined. The tube was centrifuged in the cold as above, and the supernatant was poured off. The collection of cells were suspended in 2 ml of cold 0.05 M $CaCl_2$ and put in ice for 3 1/2 hrs. (Note: Cells are competent from 20 minutes to 24 hours).

TRANSFORMATION

The JF427 cells were rendered competent for transformation by a modification of the method described by Hanahan (1983). Four sterile 13x100 borosilicate tubes were labeled 1,2,3, and 4 and were put in ice. Using a micropipet with sterile tips, 100 microliters of gently mixed $CaCl_2$-treated cells were added to each of the 4 cold, sterile tubes. DNA was added to the tubes 1-3 as follows:

Tube 1: 1 microliter of ligation DNA mixture

Tube 2: 1 microliter of un-ligated DNA mixture

Tube 3: 1 microliter of 1:100 dilution of pBR325 (at approx. 0.5 microgram/microliter)

Tube 4: nothing added (a control).

Each tube was mixed again by finger flicking. The 4 tubes were kept in ice for exactly 20 min and put immediately in a 43^0C H_2O bath for exactly 90 s without shaking. The tubes then sat at room temperature for 5 min. Nine-hundred microliters of sterile SOC was added aseptically to each tube. The tubes were put into the 37^0C H_2O shaker for 1 hr.

SCREENING OF TRANSFORMANTS

One-hundred microliters of each of the 4 transformed cell cultures were transferred to 4 LB/CAP plates (which were appropriately labeled) and to 4 noninhibitory LB plates with a micropipet with sterile tips. Each culture was spread on the plates with a glass rod that had been submerged in 70% alcohol

and flamed off. One microliter of CAP (37 mg/ml ETOH) was added to each of the 4 tubes before they were put back into the 37^0C shaker for o/n incubation. The four plates were put into a 36^0C incubator in an inverted position. The next day, the colonies were counted by using a colony counter.

GROWTH STUDIES OF TRANSFORMANTS IN ANTIBIOTIC MEDIA

To further confirm that the transformed cells had been taken up the manipulated plasmid, colonies that emerged on the LB/CAP plates were also cultured in 3 ml LB/ampicillin, LB/chloramphenicol, LB/tetracycline, and LB. Nontransformed *E. coli* JF427 cells were also cultured in the same types of media. The 13X100 sterile borosilicate tubes with the media and cultures were incubated in the 37°C shaking water bath overnight.

ELECTROPHORESIS OF LIGATED AND UNLIGATED PLASMID

An 0.8% agarose gel with ethidium bromide was prepared as previously described. While the gel was solidifying, a new sterile 0.5 ml microfuge tube was obtained and labeled stock pBR325. The following were added to the stock pBR325 tube: 8.5 microliters of sterile d-H_2O, 3.0 microliters pBR325, and 1.0 microliter of loading buffer. The tube was pulsed in the centrifuge. One microliter of loading buffer was added to the unligated tube and ligation tube. The gel wells were then loaded as follows:

Lane 1-empty

Lane 2-un-ligated

Lane 3-ligation

Lane 4-uncut pBR325

Lane 5-Hind III digest lambda (@ 1/10 dilution)

Lane 6-empty

The electrophoresis was run for 1 hr at 100 V (5 V/cm through the gel).

DNA PLASMID EXTRACTION

To a sterile 13x100 borosilicate tube, 3 ml of SOC and 3 *ul* of chloramphenicol were added. A colony was taken off the LB/CAP plate and put into the tube with the SOC/CAP media. The culture was put into the 37^0C H_2O shaker o/n.

The following day, a plasmid DNA extraction was done on the o/n culture, according to a modification of the method outlined by Birboim and Doly (1979). One and a half ml of cells were added to a sterile microfuge tube and centrifuged for 15 s @ 14,000 rpm. The supernatant was aspirated @ 5"Hg, and another 1.5 ml of cells was added to the same microfuge tube. The above procedure was repeated. The cells were suspended in 100 microliters of GTE (50 mM glucose, 25 mM Tris, and 10 mM EDTA, pH=8) by finger flicking. Two-hundred microliters of NaOH/SDS (0.2 M NaOH/1%SDS) was added and mixed by inverting the tube five times and then put in ice for 5 min. One-hundred and fifty microliters of cold potassium acetate buffer (3 M with respect to potassium and 5 M with respect to acetate) was added, mixed by

inverting the tube five times and returned to ice for 5 min. The tube was centrifuged at 5 min @ 14,000 rpm. Four-hundred microliters of supernatant was transferred to a sterile 1.5 ml microfuge tube, and the tube with the precipitate was discarded. One-hundred and thirty-three microliters of 5 M NaCl solution was added to the tube and vortexed vigorously for 15 s. Five-hundred and fifty-three microliters of 2-propanol was added and mixed by inverting 1.5 min. The tube was centrifuged for 5 min @ 14,000 rpm. The alcohol was decanted, and the tube was inverted on a Kimwipe to drain. The tube was rinsed with 2x50 ml portions of 70% ethanol, drained and inverted on a Kimwipe (being careful not to lose the ppt). The tube was placed in the 36^0C incubator to dry for 1 hr. The dried ppt. was suspended in 25 *ul* of TE (10 mM Tris- 1 mM EDTA, pH=8) and 2.5 *ul* of RNAase and put into the 37^0C water bath. RNAase was made by dissolving pancreatic RNase at a concentration of 10 g/ml in 10 mM Tris HCL and 15 mM NaCl, heated to 100^0C for 15', and allowed to cool slowly. One microliter of proteinase K was added, and the tube was put back into the H_2O bath o/n. Proteinase K is an enzyme that cleaves proteins. It was used at a concentration of 2.5 mg/ml distilled water and filter sterilized. It was used at 1.0 microliter/ 25 microliters of DNA solution which is 1.0 microgram proteinase K per 10 microliters of DNA solution.

RESULTS

Electrophoresis results showed that the EcoRI restriction

enzyme had successfully cut the pBR325 plasmid into linearized form as only one 6 kilobase band was visible upon UV light stimulation (Figure 2). This was compared to a lane of uncut pBR325 plasmid, which was run in an adjacent well next to the cut pBR325, which upon electrophoresis and UV light stimulation showed four bands.

Electrophoresis of the ligated and unligated cut plasmid gave different electrophoretic mobilities. The ligated plasmid and the uncut stock plasmid each had three bands that gave the same electrophoretic mobilities (multimer, relaxed, and supercoiled). (Figure 3)

Results of the transformation experiments were as follows: The plates inoculated with the cells from the transformation procedures that showed growth were the cells receiving ligated plasmid and the stock pBR325. No growth appeared on the control plate, which was *E. coli* cells not transformed with any DNA, and no growth appeared on the plates inoculated with cells transformed with cut, unligated plasmid.(Table 1)

The cut and uncut plasmids extracted from cultures picked off the LB/CAP plate inoculated with cells transformed with ligated plasmid gave the same electrophoretic mobilities as stock pBR325 cut and uncut. (Figure 4)

All the transformants grew in all antibiotic media as well as the noninhibitory LB. The JF427 did not grow in the antibiotic media but did grow in the noninhibitory LB.

DISCUSSION

The single band of DNA revealed by UV illumination of the electrophoresis gel provides evidence that the EcoRI enzyme had successfully cut the pBR325 at one site. The uncut pBR325 revealed four bands under UV illumination representing different topological forms of the DNA: multimer, relaxed, and supercoiled. The single band of the cut (linearized) plasmid aligned slightly beneath the 6.5 kb band of the Hind III digest of phage lambda chromosome; this is the expected result, since pBR325 is a 6 kb plasmid. (Figure 2)

The unligated and ligated plasmid gave different electrophoretic mobilities, and the mobilities of the stock plasmid and the recovered ligated plasmid were the same. The two bands of the ligated plasmid that appear under UV illumination correspond to the multimer and relaxed bands of the stock pBR325. (Figure 3) The results of the electrophoresis experiments gave positive evidence that the DNA recovery from pumice was successful, and that the ligation procedure was successful. It also provides evidence that there were no inhibitory chemicals in the pumice that prevented the cut plasmid from ligating.

The growth on LB/CAP of *E. coli* cells transformed with ligated plasmid gave positive evidence that pumice contains no chemicals that prevent the recovered DNA from expressing its genes. This further proved that the transformation procedure was successful and that the cut plasmid was ligated with retention of the biological function of the CAP gene which was expressed. This also gave supporting evidence that the ligation procedure was successful,

Electrophoresis of the EcoRI restriction digest of the plasmid extracted from cells transformed with the ligated plasmid gave the same electrophoretic mobility as stock cut pBR325. (Figure 4) The electrophoretic mobilities of the uncut extracted plasmid and uncut pBR325 were the same. This results provide supporting evidence that the chloramphenicol resistance observed in the JF427 cells was due to the uptake of the ligated pBR325 plasmid.

Since pBR325 has genes that confer resistance to the three antibiotics tetracycline, ampicillin, and chloramphenicol, the growth of the transformants in all three antibiotic media also provides evidence that the ligated plasmid was taken up by the cells. No growth was present in the unaltered JF427 inoculated in the three antibiotic media, but there was growth in the noninhibitory LB media.

In conclusion, pumice can be used as an effective means of recovering DNA fragments from agarose gels with retention of biological activity. The experimental results from this study showed that: 1.) pumice can effectively recover DNA fragments from agarose gels in high yield, and 2.) the recovered DNA still retains its biological activity as evidenced by the expression of CAP resistance when ligated plasmid is transformed into CAP sensitive cells.

FIGURE 1

Genetic and partial restriction enzyme cleavage map of plasmid pBR325. The plasmid pBR325, a derivative of pBR322, has genes encoding resistance factors to three antibiotics and single sites of cleavage by the following restriction enzymes (the sites are given in parentheses with the *Eco*RI site as a point of reference): *Eco*RI (0/5995), *Hin*dIII (1248), *Bam*HI (1594), *Sal*I (1869), and *Pst*I (4831). The arrows show the direction of transcription of the antibiotic resistance genes. The plasmid is 5995 base pairs, as shown by the inner scale, and has a molecular weight of about 4×10^6.

amp: ampicillin resistance gene, also designated *bla* (β-lactamase)

tet: tetracycline resistance gene

cap: chloramphenicol resistance gene

ori: origin of replication

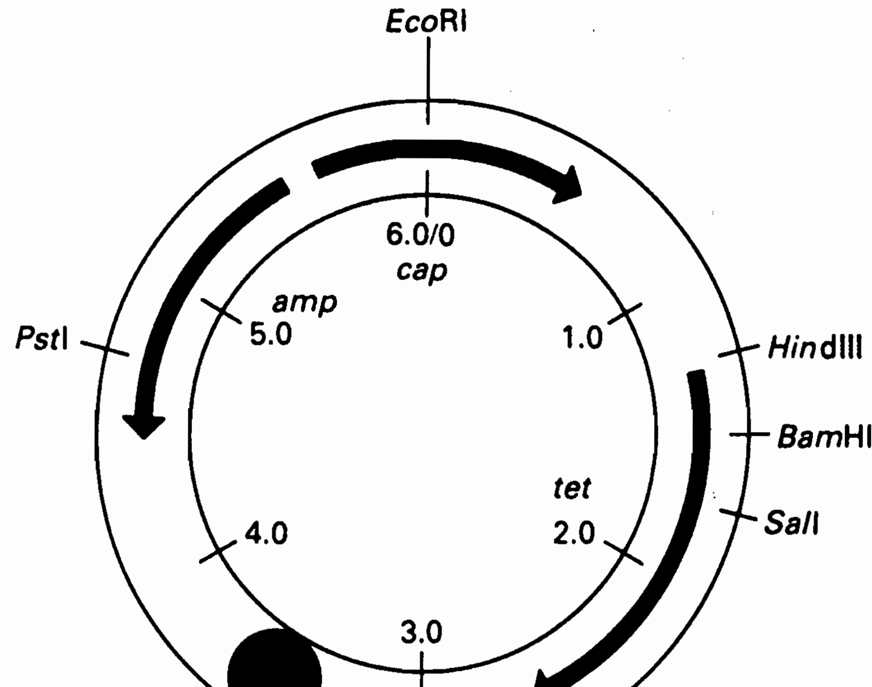
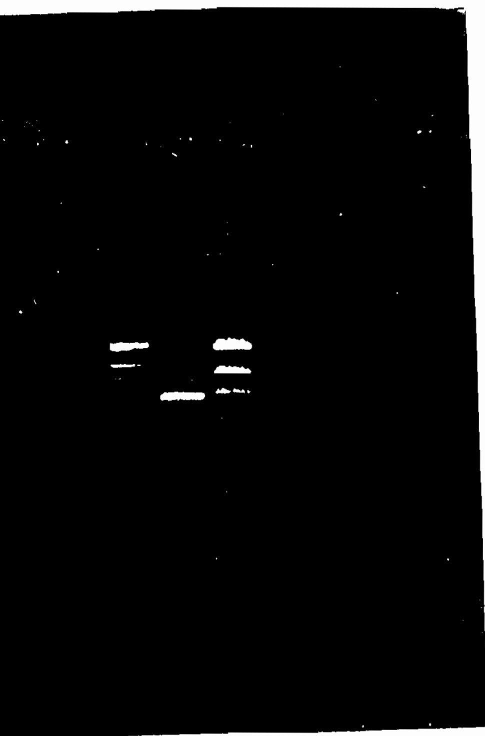


FIGURE 2



2-a

2-b

Figure 2-a shows the picture of the gel before the agar plug containing the EcoRI restriction digest fragment of plasmid pBR325 (in the middle lane). The lanes contain the following: lanes 1,2,6,7,and 8 are empty. Lane 3 contains uncut pBR325, lane 4 contains cut pBR325, and lane 5 contains Hind III digest of phage lambda chromosome.

Figure 2-b shows the gel after the agar plug was removed.

FIGURE 3

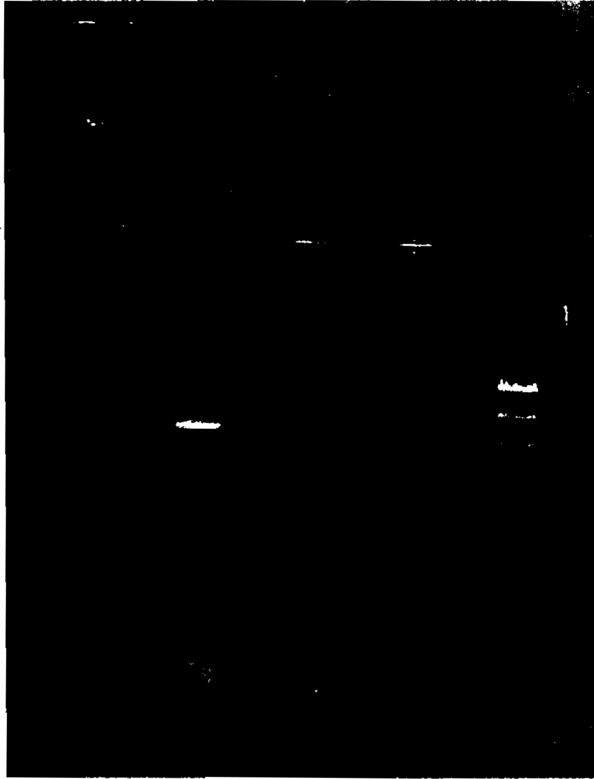


Figure 3 shows the electrophoresis of unligated pBR325 (lane 2), ligated pBR325 (lane 4), unaltered, stock pBR325 (lane 6), and a Hind III digest of phage lambda chromosome (lane 8). As can be seen, the ligated and stock plasmid have 2 bands with the same electrophoretic mobilities.

FIGURE 4

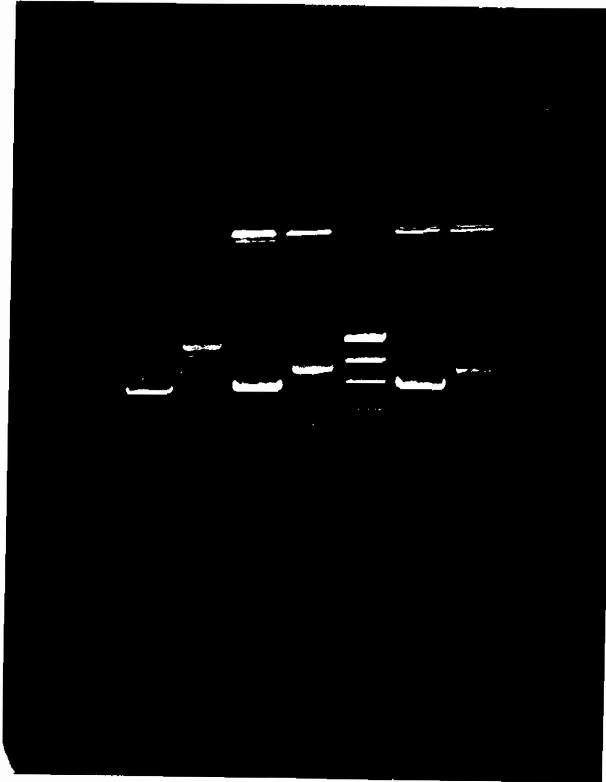


Figure 4 shows cut and uncut stock pBR325 (lanes 1 and 2, respectively), cut and uncut extracted plasmid from the transformants recieving the ligated plasmid (lanes 3 & 6 and lanes 4 & 8, respectively), and a Hind III digest of phage lambda chromosome (lane 6).

TABLE 1

Results of *E. coli* JF427 (CAP sensitive) Transformation Cultures Plated on LB/CAPplates:

Cells Plated	Results
1. Ligated plasmid	Growth
2. Unligated plasmid	No Growth
3. 1:100 Dilution of Stock pBR325	Growth
4. No DNA added	No Growth

LB plates:

All four transformation cultures showed growth.

Bibliography

Birboim, H.C. & Doly, J. A rapid alkaline extraction procedure for screening recombinant DNA. Nucleic Acids Research. 7:1523-1523. 1979.

Hackett, Fuchs, and Messing. An Introduction to Recombinant Techniques. Benjamin/Cummings Publishing Co. 1988.

Hanahan J. Studies on transformation of E. coli with plasmids. Journal of Molecular Biology. 166:557. 1983.

Li, Quanzhi and Ownby, Charlotte L. A Rapid Method for Extraction of DNA from Agarose Gels Using a Syringe. Biotechniques. 16(6):976-978. 1993.

Lu, Zhun, Templer, M., & Nielsen, B. L. Rapid Method for Recovery of DNA from Agarose gels. Biotechniques. 16(3):400. 1994.

Sofer, William. Introduction to Genetic Engineering. Butterworth-Heinemann. 1991.

Vogelstien, Bert and Gillespie, David. Preparative and Analytical Purification of DNA from Agarose. Proceeding of the National Academy of Science of the United States. 76:615-619. 1976.