UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN
DEPARTMENT OF MICROBIOLOGY

THIS IS TO CERTIFY THAT THE SENIOR THESIS PREPARED UNDER MY SUPERVISION BY ROBERT D. BARBER

ENTITLED CLONING AND CHARACTERIZATION OF A CONJUGAL Tc^r ELEMENT FROM BACTEROIDES.

IS APPROVED.

Abigail A Salyers
In charge of Thesis

RECEIVED BY THE DEPARTMENT: APRIL 4 19 88

Head, Department of Microbiology

CLONING AND CHARACTERIZATION OF A CONJUGAL T_c^r ELEMENT FROM *BACTEROIDES*

BY

ROBERT D. BARBER

THESIS

for the

DEGREE OF BACHELOR OF SCIENCE

IN

MICROBIOLOGY

College of Liberal Arts and Sciences

University of Illinois

Urbana, Illinois

1988

TABLE OF CONTENTS

ABBREVIATIONS

To^r	Tetracycline resistance
Amp^r	Ampicillin resistance
Em^r	Erythromycin resistance
Gen^s	Gentamicin sensitive
Gen^r	Gentamicin resistance
Kn^r	Kanamycin resistance
Tp^r	Trimethoprim resistance
Tra^+	Transfer positive
TYG	Trypticase - yeast extract - glucose broth
To	Tetracycline
Tn	Transposon
kb	kilobase pairs

INTRODUCTION

Bacteroides are obligately anaerobic gram negative rods which account for approximately 30% of the bacteria found in the human colon (1). Although a variety of genera are present in high numbers in the gastrointestinal tract, Bacteroides is the genus of colonic anaerobes that is most commonly responsible for opportunistic human infections. These infections range from abscesses to bloodstream infections (2). Bacteroides infections are difficult to treat because of their resistance to so many antibiotics. Bacteroides strains are universally resistant to aminoglycosides. Approximately two thirds of the strains are resistant to tetracycline. Many strains are penicillin resistant with some also exhibiting resistance to ampicillin (3).

Since, most strains are susceptible to clindamycin, clindamycin is frequently chosen for treatment of Bacteroides infections. However, the incidence of resistance to clindamycin has been increasing. This increase in incidence of clindamycin resistance is believed to be due to conjugal transfer of the resistance gene (3). Some investigators have also reported that ampicillin is transfer. by conjugation also (4).

Two distinct types of conjugal elements have been found in Bacteroides. The first type of conjugal element consists of plasmids, both large self transmissible plasmids (eg. pBF4) and smaller mobilizable ones (eg. pBFTM10) (See Table 1). The only resistance known to be conferred on Bacteroides by these plasmids

is clindamycin resistance. The clindamycin resistance gene also confers resistance to erythromycin. Since erythromycin is commonly used instead of clindamycin in laboratory research, this gene will be designated the Em^r gene throughout this paper. Several Bacteroides Em^r plasmids have been characterized (5,6,7,8). In all cases so far studied, the Em^r gene has been found to be part of a transposon (6,7,9).

The second type of conjugal antibiotic resistance element consists of the elements responsible for transfer of Tc^r or cotransfer of Tc^r and Em^r in Bacteroides-Bacteroides matings (10,11,12,13). Since no plasmid is consistently associated with this transfer, these elements are believed to be chromosomal (See Table 1). Although Tc^r itself is not considered a clinical problem, because tetracycline is not used to treat Bacteroides infections, these Tc^r elements are clinically significant because they facilitate transfer of Em^r.

Tetracycline has been found to regulate both the level of tetracycline resistance and the frequency of transfer of the element. Some characterization of the transfer properties of proposed chromosomal resistances has been done (10,11,12), but more detailed characterization of these elements has been hindered by the fact that none of them has been cloned. Previous attempts to clone a Bacteroides Tc^r gene by selecting for Tc^r in E.coli have failed. This could be due to failure of the Tc^r gene to be expressed in E.coli or the gene product could be nonfunctional or unstable in E.coli. It has been shown that the Em^r gene from Bacteroides does not confer resistance on E.coli.

Nor do the Tc^r or Amp^r genes of E.coli confer resistance on Bacteroides (14). To date, no resistance gene has been found that works both in E.coli and Bacteroides.

Because the Bacteroides Tc^r gene might not confer resistance on E.coli, I decided to try a cloning strategy in which a plasmid or cosmid library of DNA from a Tc^r Bacteroides strain was constructed in E.coli then mobilized to Bacteroides for selection of Tc^r clones. This was made possible by a newly developed shuttle cosmid vector constructed by Nadja Shoemaker. The shuttle cosmid vector is known as pNJR-1 (see Figure 1) and has the following components: replication origins for Bacteroides and for E. coli, a mobilization (mob) region that allows pNJR-1 to be mobilized out of E. coli into Bacteroides by Inc P plasmids such as R751 (Tp^r[Tn 4351], Tra^+), a cos site that allows in vitro packaging by phage lambda, and antibiotic resistance genes that are expressed in E. coli. Since pNJR-1 contains no resistance genes that work in Bacteroides, the vector can be used to clone any of the Bacteroides resistance genes (eg. Tc^r, Em^r, and Amp^r).

A clinical strain called B. thetaiotamicron DOT was used as the source of DNA. This strain was chosen because it is Tc^r, Em^r, and Amp^r. The Tc^r and Em^r are cotransferred to other Bacteroides strains by conjugation. The Amp^r is not transferred by conjugation (N. Shoemaker, unpublished). However, I also tried to clone Amp^r gene because this gene would provide another screening tool in Bacteroides. In this paper, I report the cloning of Tc^r and Em^r genes from B.thetaiotamicron DOT.

METHODS

Bacterial strains and media. B. thetaiotamicron DOT (Amp^r, Tc^r, Em^r) was used as the source of DNA for cloning. This strain was obtained from Dr. T. England, Mercy Hospital, Urbana IL. B. uniformis 1001 (Amp^s, Tc^s, Em^s, Gen^r) was utilized as a recipient in mating experiments. B. uniformis 1006 DOT was a transconjugant from a mating in which B. thetaiotamicron DOT Tc^rEm^r was the donor and B.uniformis 1001 was the recipient. This strain was used as a control in experiments involving cloned Tc^r gene in B.uniformis 1001. These strains were grown in prereduced Trypticase (BBL Microbiology Systems)-yeast extract-glucose broth (15) under an atmosphere of 80% N_2-20% CO_2, or on TYG agar plates incubated in Gas Pak jars. Concentrations of antibiotics used for Bacteroides selection were ampicillin, 50 ug/ml; tetracycline, 2 ug/ml; and erythromycin, 10 ug/ml.

The E.coli strains used in this study were: HB101 (Rec A^-, Gen^s), SF8 (Rec B^-, Rec C^-, Gen^s), EM-24(Rec A^-, Gen^s), and S17(Rec A^-, Gen^s) (16). These strains were grown in Luria broth or on Luria broth agar plates. Concentrations for antibiotic resistances used were ampicillin, 50 ug/ml; tetracycline, 10 ug/ml; and kanamycin 50-75 ug/ml.

DNA isolation and analysis. Plasmids were isolated from E. coli and Bacteroides strains by the Ish-Horowitz modification of the method of Birnboim and Doly (16). Chromosomal DNA from B. thetaiotamicron DOT was isolated by the protocol of Saito and

Miura (17). Restriction endonuclease digestion and ligation with T4 DNA ligase were performed by standard procedure (16). Restriction digests and plasmid preparations were electrophoresed on agarose gels of 0.1 to [illegible] sodium acetate, and 2 mM EDTA (pH 8.3). Gels were electrophoresed at 40V for 10 to 15 hours or at 180V for approximately 2 hours. Gels were stained with ethidium bromide and photographed.

Construction of libraries The plasmid library was constructed by partially digesting chromosomal DNA of B. thetaiotaomicron DOT with 1:60 diluted Sau 3A. Digestion was stopped with phenol at time points ranging from 10 to 20 minutes. The fragments obtained were then ligated, using T4 ligase, into the Bam HI site of the shuttle cosmid vector, pNJR-1. The vector had been treated with alkaline phosphatase prior to the ligation to prevent religation of the vector. The cosmid library was constructed similarly, except that the insert-vector complex was packaged in lambda phage particles by an in vitro packaging system (18). This packaging step requires at least 40 to 50 kilobases of DNA. Since vector is approximately 14 kb, the inserts must be 30 to 40 kilobases in size. The strategy for mobilization and screening of libraries is shown in Figure 2.

Mating conditions. Aerobic matings between donor E.coli and recipient Bacteroides were used. For aerobic matings, donors and recipients were grown separately to an optical density at 650 nm

of around 0.2 (1×10^8 to 2×10^8 CFU/ml). E. coli strain S17 has RP4 Tra genes integrated in the chromosome. The mobilization region on pNJR-1 is recognized by RP4 (N. Shoemaker, unpublished), so clones can be mobilized directly out of this donor. In the case of the other E.coli strains used as donors, R751 (Inc P, Tp^r[Tn402], Tra^+), which mobilizes pNJR-1, had to be introduced by triparental mating (19). For triparental mating, E.coli carrying the clones and an E.coli strain carrying R751 were mixed and the mixture pelleted by centrifugation prior to addition of B.uniformis 1001 recipient and subsequent centrifugation. The resulting pellet was resuspended in 0.1 ml of TYG broth and spotted on a nitrocellulose filter that had been placed on the surface of a TYG agar plate. The plates were then incubated aerobically at 37 C for 14 to 16 hours. The bacteria were washed from the filters by vortexing in 5.0 ml of TYG broth containing a tetracycline concentration of 0.1 ug/ml used for induction of tetracycline resistance. The bacteria were incubated at room temperature for 1 hour before being plated on selective media. All selective TYG plates contained 200 ug/ml gentamicin to kill the E.coli donor. The selective TYG plates were incubated anaerobically in GasPak jars at 37 C for 48 hours. Possible transconjugants were restreaked on selective media (anaerobic incubation) and on TYG plates (aerobic incubation). The aerobic controls were used to confirm that transconjugants were B. uniformis and not the E.coli donor.

Characterization of clones. To determine tetracycline minimum

inhibitory concentration (MIC). B. uniformis 1006 or B. uniformis 1001 containing a Tc^r clone were grown overnight in TYG broth which contained tetracycline (2 ug/ml) selection. A 0.1 ml inoculum was injected into series of 10 ml tubes of TYG containing various concentrations of tetracycline. These tubes were scored at 24 and 48 hours to evaluate growth.

To assess inducibility of the Tc^r on the clones, a plating efficiency experiment was performed. B. uniformis 1006 was used as the wild type control. These strains were grown overnight in TYG broth with tetracycline selection (2 ug/ml), then inoculated into tubes of fresh TYG broth, one tube contained 2 ug/ml tetracycline and the other contained no tetracycline. When the cultures reached an absorbance (600 nm) of approximately 0.3, an inoculum of 0.1 ml from each tube was diluted and plated onto TYG and onto TYG-Tc (2 ug/ml). The plating efficiency on tetracycline (2 ug/ml) was expressed by the number of colonies on TYG-Tc agar plates divided by the number on TYG agar plates and multiplied by 100%.

Mapping and Subcloning. Restriction digests were done according to manufacturing directions. Two vectors, pVAL-1 and pNJR-6 (Figures 3 & 4), were used for subcloning. Both plasmids carry the Bacteroides Em^r gene. The Em^r gene provides a selection for transfer of the clone. pVAL-1 also provides a replication origin in Bacteroides.

Southern blot analysis. Plasmid and chromosomal preparations

were obtained as previously done (16). Eco RV digestions were performed according to manufacturers guidelines. The digests were resolved on a 1.0% agarose slab gel in 4X GGB (0.16M Tris, 0.08 M sodium acetate, 0.008 M EDTA [pH 8.3]). Following electrophoresis for 10 to 15 hours, the gel was stained with ethidium bromide and photographed. The DNA from the agarose gel was transferred to nitrocellulose paper via capillary blotting (16). pNJRD2-T3 probe was labelled with ^{32}P by nick translation (20) and hybridized to the DNA on the nitrocellulose paper for 48 hours at 42 C in a hybridization solution containing 50% formamide (16). After hybridization, the blot was washed 2X for 30 minutes each with 0.2% sodium dodecyl sulfate in 2X SSC (1X SSC is 0.15 M NaCl with 0.015 M sodium citrate) and twice with 0.2% SDS in 0.5X SSC. The filter was then autoradiographed.

RESULTS

Construction of libraries. Initially, a plasmid library of the B.thetaiotamicron DOT genome was constructed in E.coli. The plasmid library was tried first to make future subcloning minimal. Also, I hoped that smaller inserts might be more stable in E.coli. However, when I screened 18 transformants, I obtained only two inserts, one 6 to 8 kb and the other 8 to 10 kb in size. No antibiotic resistant clones were found in subsequent matings of the plasmid library transformants with B. uniformis 1001. Accordingly, I decided to try using a cosmid library.

A cosmid library provides increased odds of detecting genes due to the larger size (40-50kb) of the insert. However, beginning with such a large insert makes subcloning a necessity. These large inserts may also prove unstable. E.coli strains EM-24 and S-17 were infected with the lambda particles containing the cloned DNA. Upon titering the cosmids, I found that approximately 11 out of 23 cosmids did not nave inserts. This loss of inserts occurred even in a Rec A$^-$ background. Subsequent titers of the transfected E.coli strains showed that S-17 (RP4 transfer genes integrated into the chromosome) gave the highest number of infected cells. Hence, S17 transformants were used in the matings.

Although, my screen of inserts showed that a substantial proportion of the cosmid inserts had been lost, I went ahead with the conjugation and obtained several clones that were Tc^r, Em^r or

Amp^r. The properties of the cosmids clones obtained are given in Table 2. The Amp^r clones were found to be unstable. The cells carrying these clones appeared to be lysing when they were subcultured in TYG broth. All of my other cosmid clones were stable upon subculturing. Therefore, I decided to study the Tc^r clones. I chose my smallest Tc^r clone (pNJRD2-T3, 18 kb insert) for further study.

Regulation of Tc^r on the clones. First, I wanted to assess the regulation of my clones as compared to the wild type. The results of the characterization experiments are seen in Table 3, where we compare the effect of tetracycline on plating efficiency of B. uniformis 1006 DOT, which contains the wild type DOT Tc^{r}Em^r element, and of B.uniformis 1001 containing my smallest clone (pNJRD2-T3) or a larger clone (pNJRD2-T2). The plating efficiency of B.uniformis 1006 DOT on TYG-Tc (2 ug/ml) is increased 100 fold by pregrowth in tetracycline. However, the Tc^r clones did not display this trait. The clones had nearly 100% plating efficiency, whether preexposed to tetracycline or not. An elevated tetracycline minimum inhibitory concentration was seen in the case of the clone pNJRD2-T3, but the increase is only two fold over that of B.uniformis 1006 DOT. The MIC of B.uniformis 1001 carrying pNJRD2-T2 was not determined.

The Tc^r clones obtained from Bacteroides were transformed into E.coli. The E.coli transformants were not Tc^r when plated on tetracycline (10ug/ml) Luria broth agar plates.

Attempts to localize the Tc^r gene within pNJRD2-T3. A restriction digest map of pNJRD2-T3 was made to assist in subcloning (Figure 5). An Eco RV fragment of 0.9 kb, seen in Figure 5, is indigenous to all Tc^r clones and Tc^rEm^r clones isolated (See Figure 6a). The only other common bands are vector bands. This finding indicated that the 0.9 kb Eco RV segment might be associated with the Tc^r gene. The location of the 0.9 kb element within the 18 kb insert was determined and is shown in Figure 5.

I had obtained only one Em^r clone that was not Tc^r, and this clone did not contain the 0.9 kb fragment. In similar experiments independent of mine, Nadja Shoemaker obtained a number of Tc^r and Em^r clones from B. uniformis into which the DOT element had been transferred from the original strain with which I was working. Some of these were Em^r but not Tc^r. To check whether the 0.9 kb fragment was present in any of these clones, I ran EcoRV digests of these clones (Figure 6b). The 0.9 kb Eco RV band does not appear in any of these clones. This finding shows that this fragment is not important for Em^r expression and supports the hypothesis that the fragment is associated with the Tc^r gene.

Eco RI cut my insert into two pieces. I attempted to subclone the 6 kb segment from this clone that contained the 0.9 kb Eco RV fragment by digesting my clone with Eco RI and ligating with Eco RI digested and capped pVAL-1. These subclones were transformed into E.coli strains SF-8 and HB101. The choice of E.coli strains was determined because of instability seen in S17

in the form of deletions and loss of inserts. Again, transformants were titered and the highest count provided the strain used in subsequent matings. Approximately thirty clones were obtained in HB101 and these were screened for inserts. About two-thirds of the subclones had satisfactory inserts. These were mobilized into B.uniformis.

This cloning strategy should provide a 6 kb segment of the insert, which would give me a subclone three fold smaller than my original clone. However, the Eco RI fragment that contains this segment also carries a copy of pB8-51, a cryptic Bacteroides plasmid which provides a replication origin in the vector. pVAL-1 also has a copy of pB8-51. Thus instability could arise in Bacteroides due to recombination of homologous regions. I observed an extremely low rate of transfer into Bacteroides (4×10^2) using Em^r as a marker. Only Tc^s clones were obtained. As mentioned above, this could have been due to recombinatory excision of the insert in Bacteroides. Thus, I cannot conclude yet whether the Tc^r gene is within the 6 kb Eco RI segment. In the future, I plan to subclone the Eco RI fragment into pNJR-6, a Bacteroides suicide vector, which does not contain pB8-51. Since the fragment contains not only 6 kb of my insert but also all of pB8-51, it should provide a replication origin for pNJR-6 in Bacteroides.

Another attempt at subcloning was done using Sau3A partial digestion, similar to the procedure done in the initial chromosomal digestion. However, very few of the clones (approximately 10 percent) exhibited inserts large enough to code

for Tc^r expression. No Tc^r transconjugants were obtained when the clones were mobilized into Bacteroides.

Southern Blot Analysis A hybridization experiment was done to investigate possible homology between various Bacteroides strains which carry conjugal tetracycline resistance elements. The probe used in this experiment was ^{32}P labelled, Eco RV digested pNJRD2-T3. This probe was hybridized to Eco RV chromosomal digests of DNA from various clinical strains and from transconjugants of B. uniformis 0061 containing the same conjugal Tc^r elements. Transconjugants of B. uniformis 0061 were utilized to provide a uniform genetic background in which all of the elements could be compared. An autoradiogram of the hybridization blot is seen in Figure 7. The 0.9 kb Eco RV fragment mentioned above in localization of the Tc^r element is found in all Tc^r strains. This strengthens the argument of its importance to Tc^r expression. A 2.2 kb fragment is also seen to be homologous in all but one clinical strain and its associated transconjugant. These strains also display a high amount of homology outside the proposed Tc^r region.

DISCUSSION

I have utilized a new shuttle cosmid, pNJR-1, for cloning of a *Bacteroides* Tc^r gene. Except for Em^r on some *Bacteroides* plasmids, no *Bacteroides* antibiotic resistances have been previously cloned. The Tc^r clones proved not to confer resistance on *E.coli*. This indicates that the *Bacteroides* Tc^r gene is either not expressed or nonfunctional in *E.coli*. pNJR-1 has further applications then those seen in this paper. Cloning of *Bacteroides* genes by complementation of mutants, using the strategy in Figure 2, is now a possibility. Also, complementation mapping, which was not possible before, may now be feasible in *Bacteroides* if this approach is used. Yet, this new cloning procedure does have flaws. The instability of DNA inserts in plasmids and cosmids in *E.coli* is a serious problem, and it is possible that some *Bacteroides* genes will be uncloneable by this protocol.

The nature of the clones obtained indicates that Tc^r and Em^r genes are linked. Prior to cloning, these Tc^r and Em^r were found to be cotransferred in *Bacteroides*-*Bacteroides* matings, but it was not known whether both were on the same element. The finding that a 25 kb insert contains both the Tc^r and Em^r genes shows that they probably lie within 25 kb of each other. However, because of the instability of *Bacteroides* DNA in *E.coli*, I cannot rule out the possibility that rearrangements occurred. Both the Tc^r and Em^r can be cloned separately and still express. Most of

the clones contained one gene but not the other. Thus, they are probably not adjacent to each other.

Plating efficiency of the wild type element in B. uniformis 1006 was regulated by tetracycline. Yet, the plating efficiency of my clones appeared not to be regulated. There are two possible explanations of this finding. I may have cloned the resistance gene away from a regulatory gene. If so, I can propose a repressor type model for regulation of tetracycline resistance because loss of the regulatory gene gives constitutive expression. Another possiblity is that the apparent loss of regulation is really due to an elevated gene dosage. My clones are probably present at a level of 10 to 20 copies per cell. By contrast, the wild type Tc^r element is presumably a single copy, in the chromosome. Higher basal expression of the Tc^r gene due to higher gene dosage could enable the cells to grow on 2 ug/ml tetracycline without induction. The two fold increase in MIC of the clones shows that increased gene dosage does increase resistance. To determine if the apparent loss of regulation is due to gene dosage, I would have to insert my clones into the Bacteroides chromosome and then check for regulation.

Conjugal Tc^r elements have been found in different Bactercides strains and species. Some elements transfer Tc^r only and some transfer both Tc^r and Em^r (Table 1). This raises the question of whether the different Tc^r genes and the elements that carry them are related. The homology seen between my clone (used to probe the Southern blot shown in Figure 7) and a series of Tc^r

elements obtained from different clinical isolates presents tentative proof that the cloned Tc^r gene is the same in all the Tc^r strains isolated. That is, the 0.9 kb segment that appears to be associated with the Tc^r gene cross-reacts in all cases. To prove that the Tc^r gene itself is the same in all of these strains, it will be necessary to subclone the 18 kb insert to a fragment that contains only the necessary genetic material for Tc^r expression, so that I can be sure that the homology is due to the Tc^r gene and not to flanking DNA segments or other unimportant DNA due to the size of the clone.

The 0.9 kb Eco RV fragment was not the only fragment that cross-reacted with DNA from other strains. Obviously, these strains share more in common than the Tc^r gene itself. The identity of the cross-hybridizing DNA remains to be established.

SUMMARY

I have tested and succeeded in using a new cloning strategy to obtain clones of a Bacteroides conjugal Tc^r gene that has never been cloned before. This gene does not confer Tc^r on E.coli. I also found that the gene on a multicopy plasmid, unlike the wild type element, appears not to be regulated by tetracycline. But I was not able to determine whether this was an effect of gene dosage or loss of a regulatory element. I made a partial restriction map of my smallest Tc^r clone (18 kb) and attempted to subclone the Tc^r gene. Although no Tc^r subclones have been attained, the data generated has provided a good estimate of the Tc^r gene locale. Also, I used my smallest Tc^r clone as a hybridization probe to show that Tc^r elements from several independent clinical isolates are related to the element I cloned. The clones obtained provide a basis for further study of genetic and physical characteristics of these antibiotic resistances. The success of the cloning strategy indicates that cloning and complementation mapping may now be possible in Bacteroides.

BIBLIOGRAPHY

1. Moore, W E. C., and L. V. Holdeman. 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. 27: 961-971.

2. Finegold, S. M. 1977. Anaerobic Bacteria in Human Disease. Academic Press, Inc., New York.

3. Tally, F. P., Cuchural, G. J., and Malamy, M. H. 1984. Mechanisms of resistance and resistance transfer in anaerobic bacteria: factors influencing antimicrobial therapy. Rev. Infect. Dis. 6(Suppl.): S260-266.

4. Butler, T., Tally F. P., Gorbach, S. L., Malamy, M. Transferable ampicillin resistance in B.fragilis. Clinical Research. 28: 365, 1980.

5. Welch, R. A. and Macrina, F. L. 1981. Physical characterization of Bacteroides R plasmid, pBF4. J. Bacteriol. 145: 867-872.

6. Shoemaker, N. B., E. Guthrie, A. A. Salyers and J. F. Gardner. 1985. Evidence that the clindamycin resistance gene of the Bacteroides plasmid pBF4 is on a transposable element. J.

Bacteriol. 162:626-602.

7. Smith, C. J. 1985. Characterization of Bacteroides ovatus plasmid pBI136 and structure of its clindamycin resistance region. J. Bacteriol. 161: 1069-1075.

8. Tally, F. P., Snydman, D. R., Shimell, M. J. and Malamy, M. H. 1982. Characterization of pBFTM10, a clindamycin-erythromycin resistance transfer factor from Bacteroides fragilis. J. Bacteriol. 151: 686-690.

9. Robillard, N. J., Tally, F. P., and Malamy, M. H. 1985. Tn4400, a compound transposon isolated from Bacteroides fragilis functions in E. coli. J. Bacteriol. 164: 1248-1254.

10. Shoemaker, N. B., C. Getty, E. P. Guthrie, and A. Salyers. 1986. Two Bacteroides plasmids, pBFTM10 and pB8-51, contain transfer regions that are recognized by broad host range IncP plasmids and by a conjugative Bacteroides tetracycline resistance element. J. Bacteriol. 166:959-965.

11. Valentine, P. J., Shoemaker, N. B., and Salyers, A. A. 1988. Mobilization of Bacteroides plasmids by Bacteroides conjugal elements. J. Bacteriol. 170: 1319-1324.

12. Mays, J. D., Smith, C. J., Welch, R. A., Delfine, C., and Macrina, F. L. 1982. Novel antibiotic resistance transfer in

Bacteroides. Antimicrob. Agents Chemother. 21: 110-118.

13. Malamy, M. H., and Tally, F. P. 1981. Mechanisms of drug-resistance transfer in Bacteroides fragilis. J. Antimicrob. Chemother. 8(Suppl.): 59-75.

14. Salyers, A. A., Shoemaker, N. B., and Guthrie, E. P. 1987. Recent advances in Bacteroides genetics. CRC Critical Reviews in Microbiol. 14: 49-71.

15. Holdeman, L. V., E. D. Cato, and W. E. C. Moore (ed.). 1977. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va.

16. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y.

17. Saito, H., and K.-I. Miura. 1963. Preparation of transforming dexyribonucleic acid by phenol treatment. Biochim. Biophys. Acta 72: 619-629.

18. Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y.

19. Meyer, R. J., and J. A. Shapiro. 1980. Genetic organization of the broad-host-range IncP-1 plasmid R751. J. Bacteriol. 143: 1362-1373.

20. Rigby, P. W. J., M. Diechmann, C. Rhodes, and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. 113: 237-251.

Table 1 Types of **Bacteroides** conjugal resistance elements

Plasmids

Plasmid	Size	Resistance transferred	Mobilization	References
pBF4	41 kb	Em^r (Tn 4351)	Self-mobilizing	5,6
pBI136	81 kb	Em^r (Tn 4551)	Self-mobilizing	7
pBFTM10	15 kb	Em^r (Tn 4400)	Mobilized by Tc^r element	8,9,10

Chromosomal (?) elements

Designation	Resistance transferred	Resistance, transfer regulated by Tc	References
Tc^rEm^r DOT	Tc^r,Em^r	Yes	N.Shoemaker, unpublished
Tc^r ERL	Tc^r	Yes	10,11
Tc^rEm^r 12256	Tc^r,Em^r	No	12

Table 2. Cosmid clones obtained.

Antibiotic resistance conferred	Number	Size (kb)
Tc	3	18, 25
Tc, Em	3	25
Em	1	4
Amp	7	ND

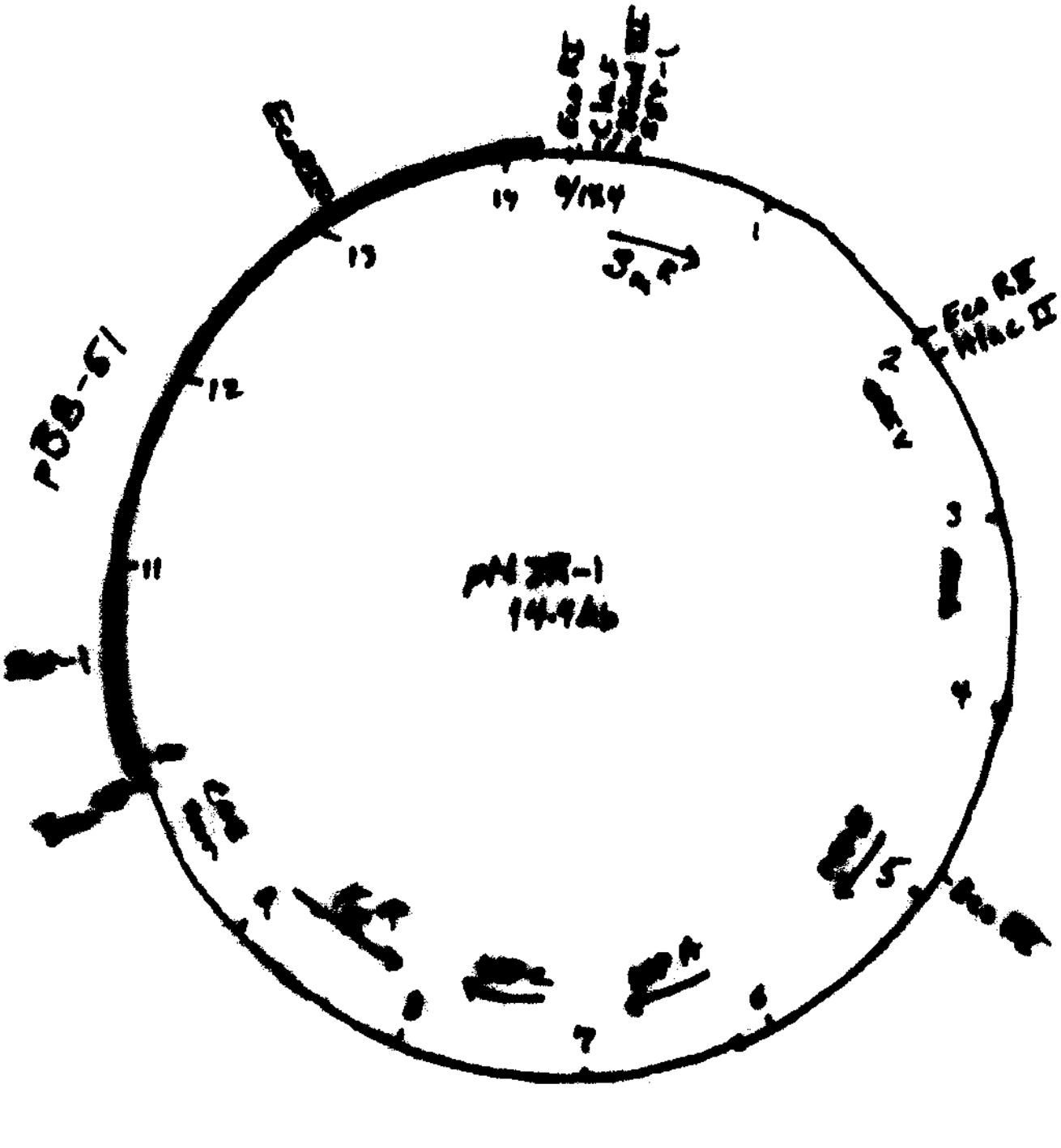
ND indicates "Not Determined."

Table 3. Characterization of clones

	MIC (ug/ml)	Percent viability on TYG (2 ug/ml tet) if pregrown on	
		-Tc	+Tc
B.uniformis 1006 (DOT)	5-10	4	91
B.uniformis 1001 (pNJRD2-T3)	10-20	90	94
B.uniformis 1001 (pNJRD2-T2)	ND	75	85

ND indicates "Not Determined."

Figure 1. Shuttle cosmid vector.



pNJR-1 (shuttle cosmid vector) used in cloning. pB8-51, a cryptic Bacteroides plasmid, provides a replication origin in Bacteroides. RSF 1010 supplies a E.coli origin of replication, a mobilization region, and a cos site for packaging. Also, kanamycin resistance is provided by RSF 1010 and was used for selection in E.coli.

Figure 2 Strategy for cloning.

Transform competent E. coli EM-24 or S17 cells with plasmid library or infect E. coli strains with packaged cosmids

Select for Km^r

↓

Evaluate infectivity and titer cosmids checking for percent with inserts

↓

Grow mixture of clones under Km^r selection and dilute to obtain batches of approximately 25.

↓

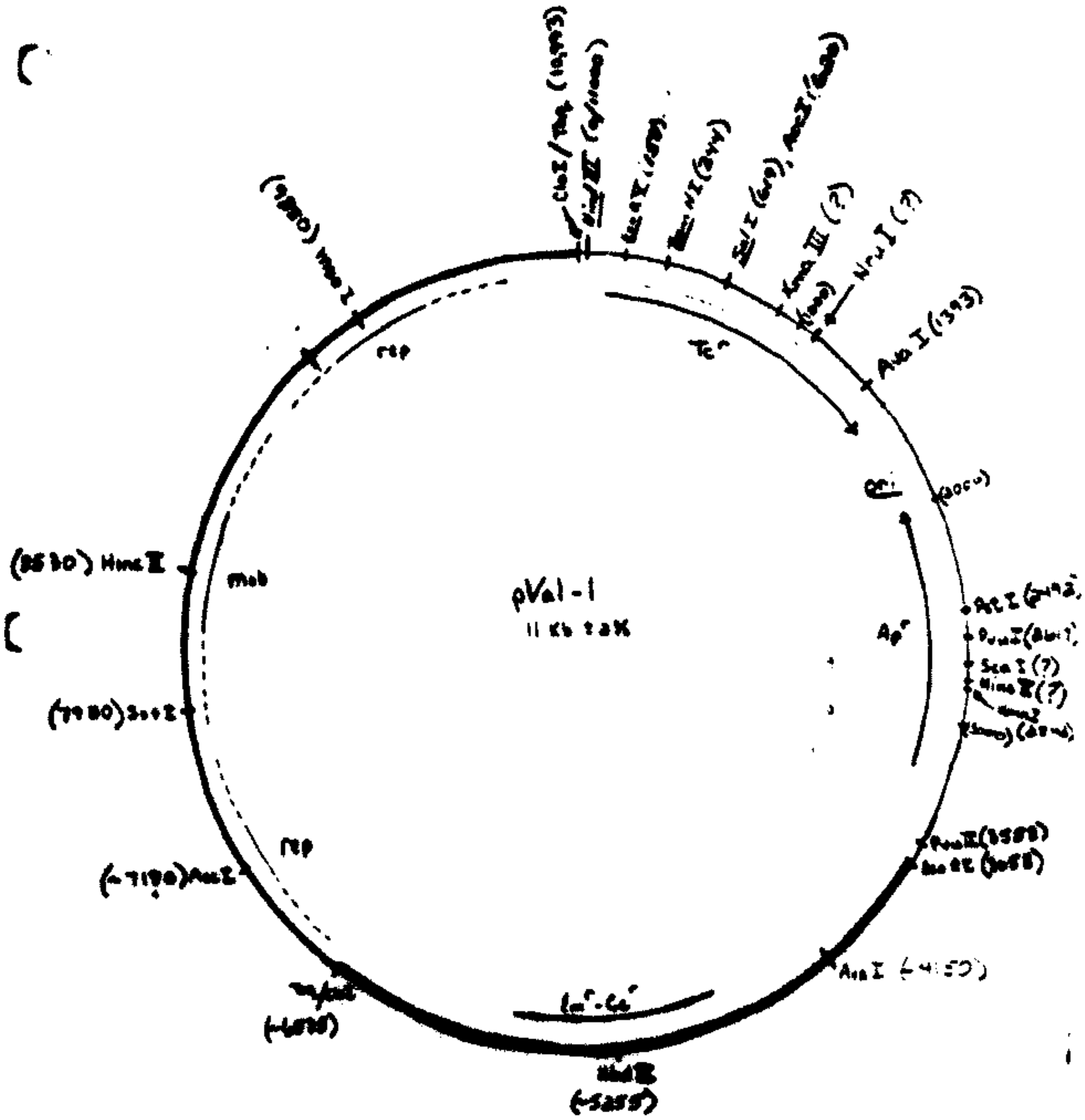
Perform aerobic group matings at 37 C for 14 to 16 hours between E. coli and B. uniformis 1001.

For E.coli EM-24, use triparental mating with E.coli strain carrying R751

↓

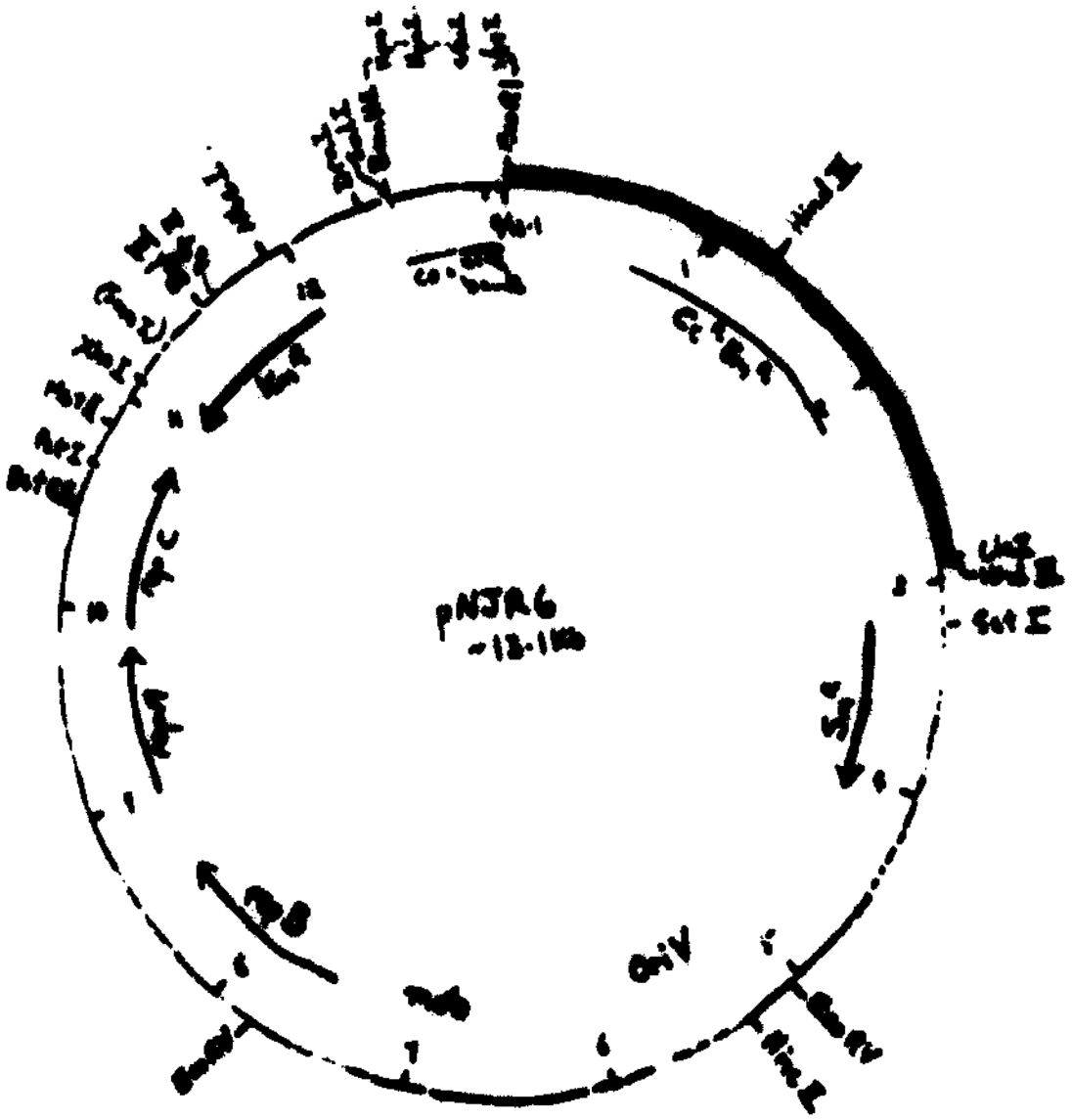
Screen for Tc^r, Em^r, and Amp^r Bacteroides.

Figure 3. pVAL-1.



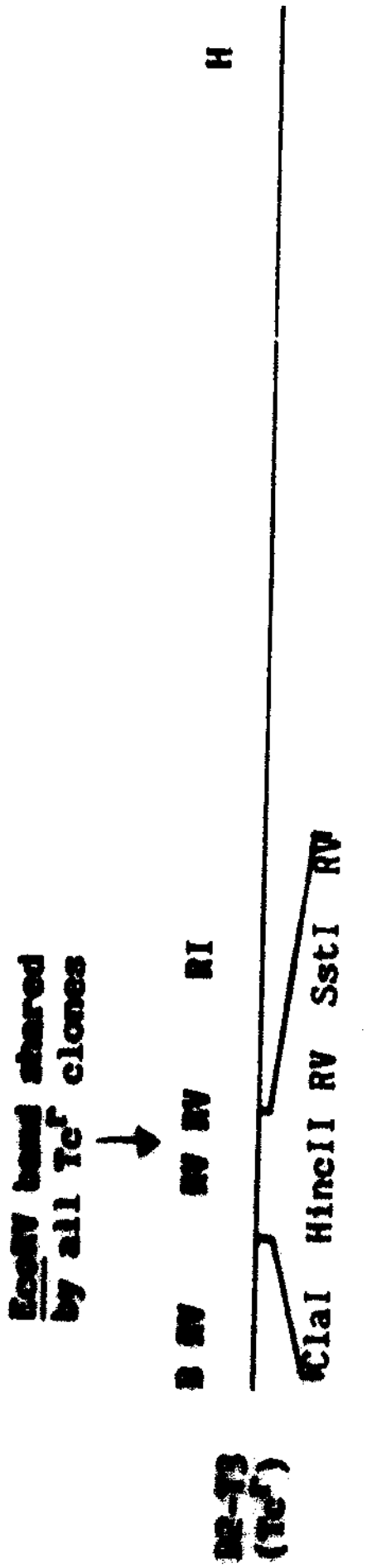
pVAL-1, used in subcloning attempts, has a copy of pB8-51 which provides a Bacteroides origin of replication and the Em^r gene which expresses in Bacteroides.

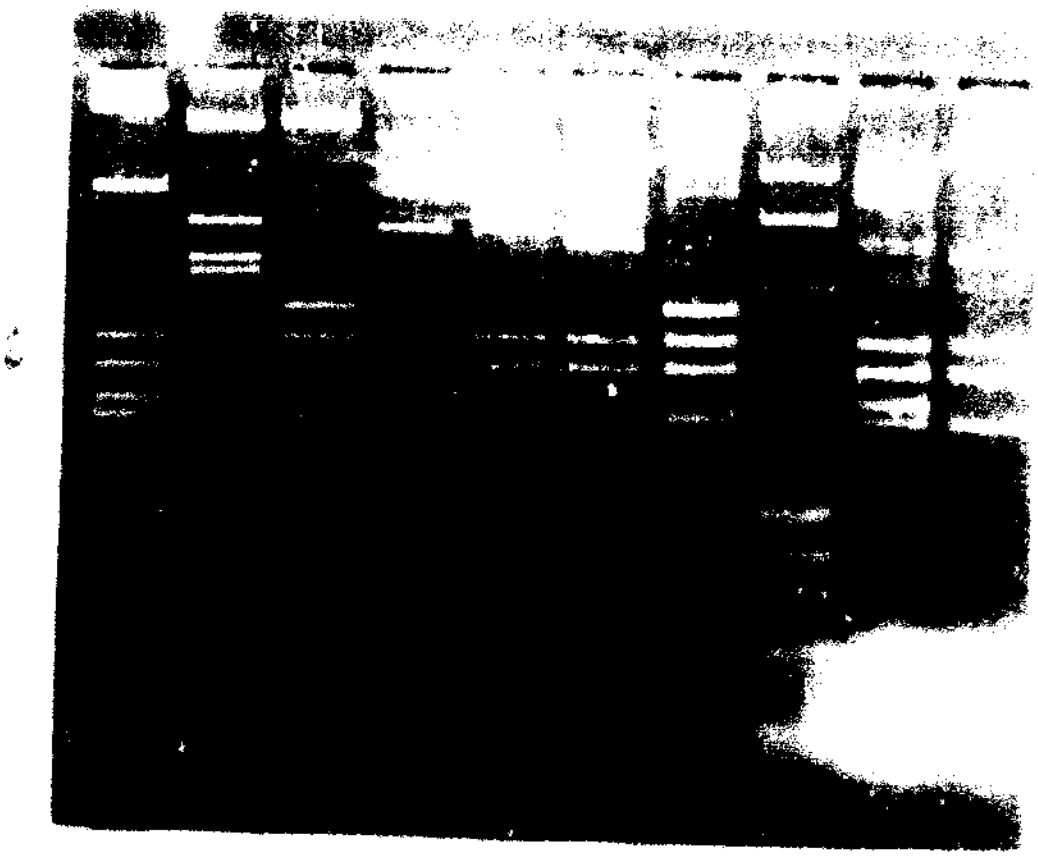
Figure 4. pNJR-6.

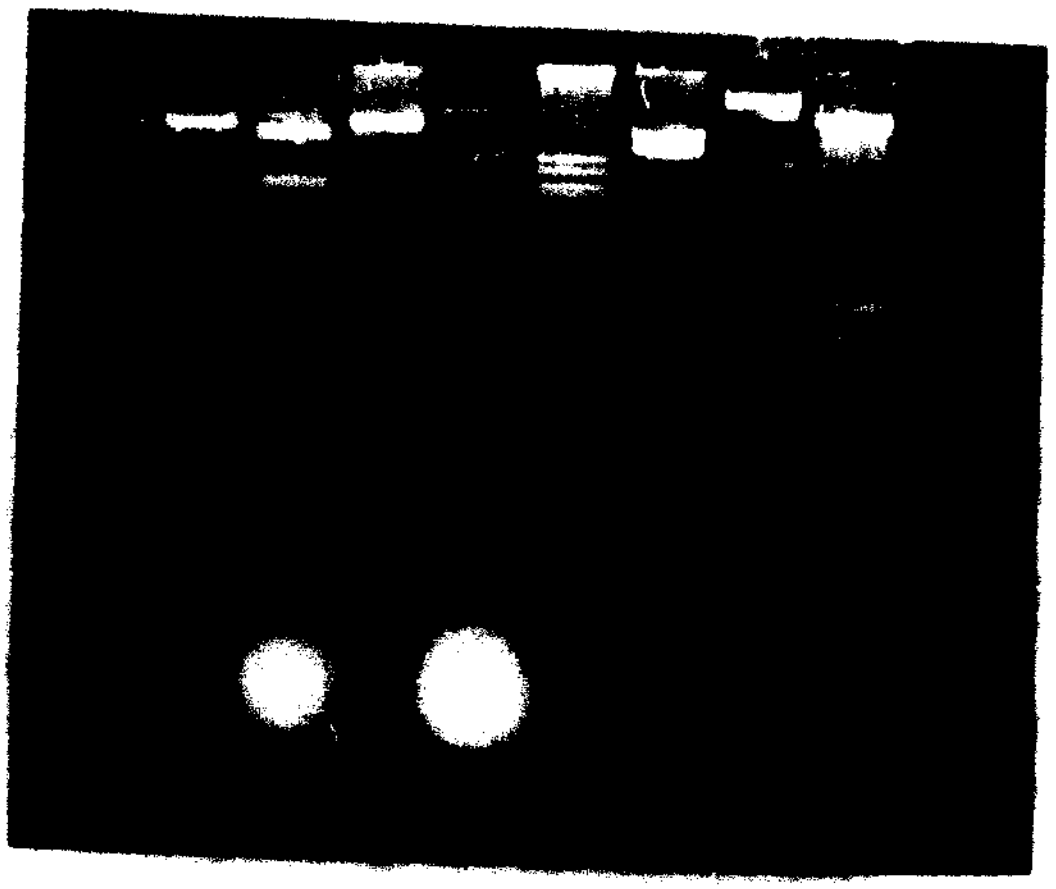


pNJR-6, used in subcloning attempts, has the Em^r gene which express in Bacteroides. Previously used as a suicide vector in Bacteroides, the segment I wish to subclone would provide a Bacteroides origin of replication.

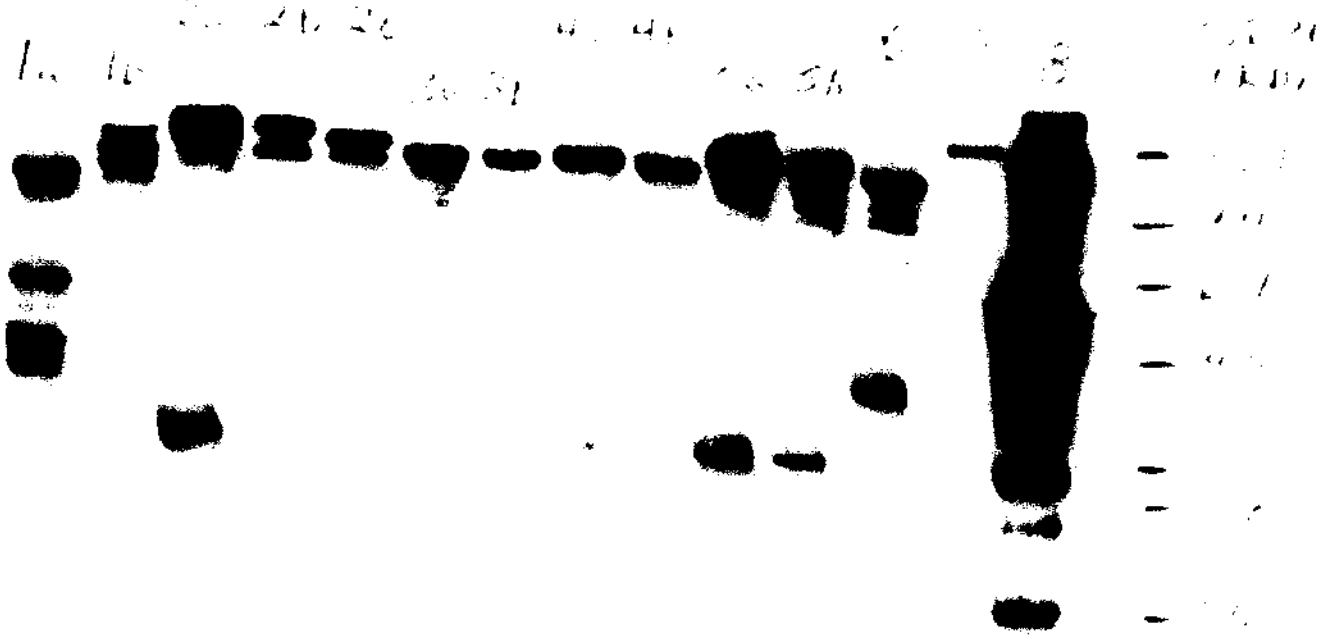
Figure 5. Restriction digest map of the insert in pNJRD2-T3.







A



B

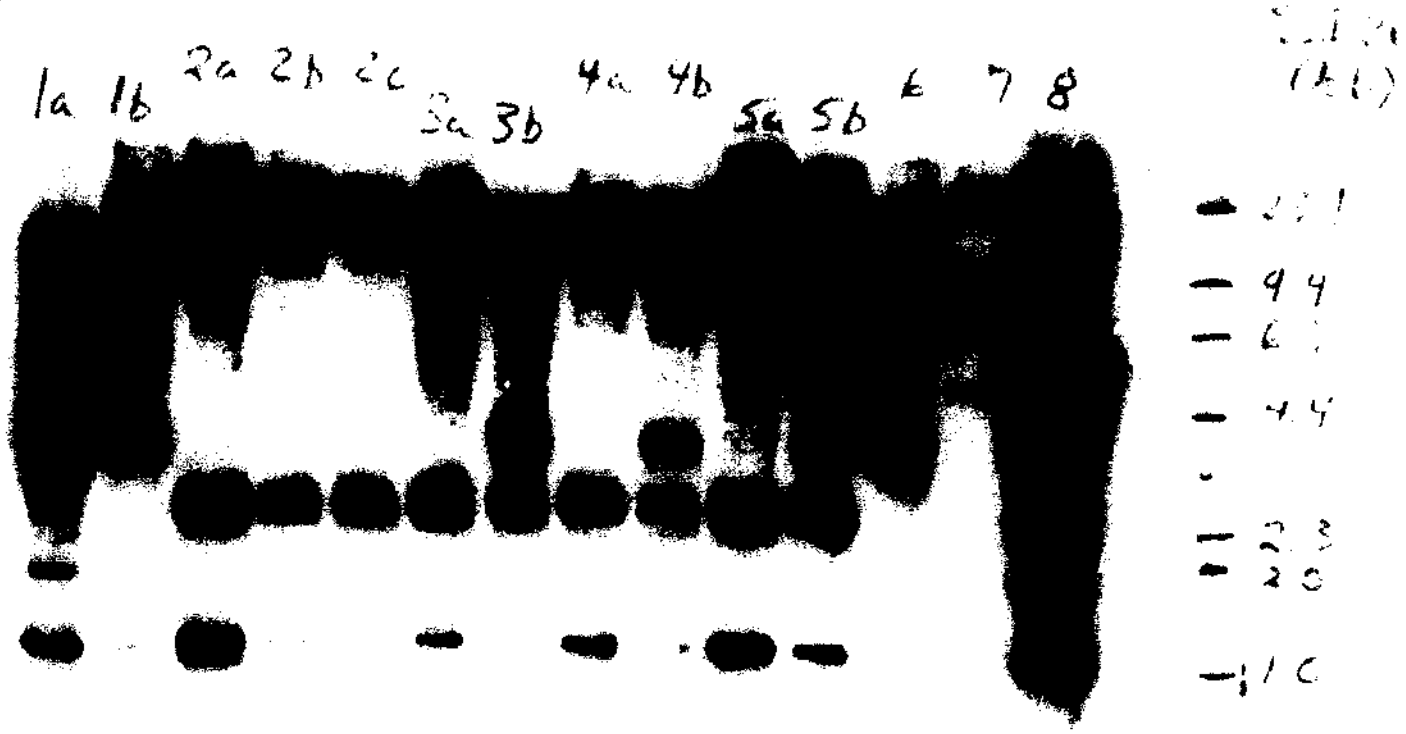


Figure 7. Southern Blot analysis of **Bacteroides** strains containing conjugal Te^r elements. (See facing page for Figures 7A and 7B).

Figure A is a 24 hr exposure, while Figure B is a 48 hr exposure of the same hybridization blot. The total DNA of Bacteroides strains containing conjugal Te^r elements was digested with Eco RV. These digests were electrophoresed on a 1% 4X GeB gel overnight, the DNA was subsequently transferred to a nitrocellulose filter. The probe used in the hybridization blot was ^{32}P labelled pNJRD2-T3. The sample order is:

Clinical str.	B.uniformis transconjugant
1a. B.fragilis V479 (Te^r)	1b. BU 1002 (Te^r)
2a. B.fragilis EHL (Te^rEm^r)	2b. BU 1004 (Te^r)
	2c. BU 1005 (Te^rEm^r)
3a. B.thetaiotaomicron 10T (Te^rEm^r)	3b. BU 1006 (Te^rEm^r)
4a. B.fragilis CEST (Te^rEm^r)	4b. BU 1003 (Te^rEm^r)
5a. B.fragilis 12256 (Te^rEm^r)	5b. BU 1006 (Te^rEm^r)

6. B.uniformis 1001 (w.t.)
7. Hind III digested Lambda DNA
8. Eco RV digested pNJRD2-T3