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Rapid sequencing of cloned DNA using a transposon for bidirectional priming: sequence of the *Escherichia coli* K-12 *avtA* gene

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ABSTRACT

A new approach to determining the sequence of cloned DNA is described. Unique regions near each end of the transposable element gamma-delta provide a pair of "portable" primer-specific sites for bidirectional sequencing by the dideoxy chain termination method. A set of gamma-delta insertions positioned about 200 bp apart over the entire cloned DNA allowed us to determine the sequence of both strands in a single parental plasmid without subcloning. The *avtA* (alanine-valine transaminase) gene of *E. coli* K-12 was sequenced by this approach. Surprisingly, gamma-delta insertions downstream of the coding region were found to significantly reduce *avtA* expression. We suggest that these nondisruptive insertions probably change the DNA topology and thereby alter gene expression.

INTRODUCTION

The dideoxy chain termination method of Sanger (1) is widely used for sequencing of cloned DNA. In it, the target DNA is generally cloned into a single stranded phage vector and a short oligonucleotide specific for a fixed site on the vector molecule is used as the primer to synthesize a complementary copy of the single-stranded target sequence. The Sanger method has been modified to permit the sequencing of both strands in a double stranded plasmid (2). Since only a few hundred base pairs can be sequenced from the primer, several methods have been devised to bring segments of the long target sequence close to the primer site. These methods include subcloning short fragments of the target DNA or isolating a nested set of deletions by controlled digestion with a variety of nucleases (3-5).

An alternative strategy is described here. Rather than isolating deletions, we took advantage of a transposable element, gamma-delta (Tn*1000*)(6), to provide portable sites for bidirectional priming. A primer specific for one end of the element is used to sequence in one direction and another specific for the other end to sequence in the opposing direction. Since the sequence of 200 to 300 bases can be determined in each direction from a single insertion, the insertions need be within 200 to 300 bp of each other to provide the sequence of both strands. The conjugative F plasmid of *Escherichia coli* carries gamma-delta, which transposes readily and fairly randomly from the F factor into plasmids such as pBR322, forming cointegrates of F and the target plasmid as intermediates in transposition (6). Insertions are isolated simply by mating an F+ donor strain containing the nonconjugative target plasmid with an F- recipient, and

selecting for transfer of a drug-resistant marker usually encoded by the target plasmid. Upon transfer to the recipient, the cointegrates are resolved to produce F and target plasmids carrying gamma-delta. Such insertions have been used generally to delimit genes within the cloned DNA (6,7). We extend the use of gamma-delta insertions as convenient substrates for sequencing.

The use of a transposable element to provide portable primer sites as described here is particularly valuable when a number of insertions of the element are first used to delimit the gene in what may be a large cloned fragment (4.6 kb in this instance), and then these same insertions are used to sequence the gene without further *in vitro* manipulations. This approach is not, however, limited to the sequencing of DNA fragments that are expressed in *E. coli*. It should also be valuable for analyzing eukaryotic DNA, especially if the transposon-mutagenized plasmid can be reintroduced into the eukaryote on a shuttle vector to identify gene disruption mutations.

MATERIALS AND METHODS

Bacterial strains, plasmids and culture conditions

The E. coli K-12 strains used in this study are MG1063 (F+ *recA*56) (8) and CBK741 (*ilvE*12 *avtA*23::Tn*5*) (9). pIF005 is an 8.6 kb plasmid that contains *avtA*+ in a 4.6 kb Hin*d*III-Sal I fragment cloned into the Hin*d*III-Sal I sites in the *tet* gene of pBR322 (10). The positions of the gamma-delta insertions in pIF005 have been described (10). Cells were grown in Lennox (L) broth or appropriately supplemented Vogel and Bonner medium E at 37oC (11).

Mutations in *avtA* confer a valine requirement upon isoleucine-requiring *ilvE* strains of *E. coli* and *Salmonella typhimurium* (11, 12). If *avtA*+ is cloned in an *ilvE avtA* strain on a low copy number plasmid, the strain requires isoleucine, but not valine, while if it is cloned on a high copy number plasmid, the strain does not require either isoleucine or valine (10,13). The phenotype of strains with an *avtA*+ plasmid is, therefore, an indicator of moderate (isoleucine-requiring) versus high (prototrophic) transaminase C levels.

Chemicals

Specialized chemicals were obtained from Sigma Chemical Company. Restriction endonucleases and the DNA size standards were obtained from Bethesda Research Laboratories or New England Biolabs. Agarose was obtained from FMC Corporation. DNA polymerase I (Klenow fragment) was obtained from Boehringer-Mannheim or New England Biolabs. 32P dATP and 35S dATP were obtained from Amersham. Dideoxy- and deoxynucleotide triphosphates (Gen Seq ds DNA Reagent Kit) were obtained from Promega Biotech. Acrylamide, bisacrylamide and urea were obtained from IBI. The sequence of the gamma oligonucleotide primer was 5'-TCAATAAGTTATACCAT-3', corresponding to the sequence 53 to 37 bases from the gamma end, while the delta oligonucleotide was 5'-GAATTATCTCCTTAACG-3', corresponding to the sequence 48 to 32 bases from the delta end of this transposon (R. Reed, personal communication).

Preparation of DNA

Plasmid DNA, isolated by alkaline lysis (14), and linearized by digestion with Bam*H*I (which

cuts gamma-delta at one site [8], and does not cut pIF005 [10]), was purified by gel electrophoresis using Seakem GTG agarose.

DNA sequencing

DNA was sequenced by a modification of the dideoxy procedure for double-stranded DNA (2). Linear plasmid DNA was denatured by alkali to expose single-stranded regions that serve as the templates. Two µl of 2M NaOH, 2 mM EDTA was added to 2.5 µg of DNA and the total volume was adjusted to 20 µl with sterile water. After 5 min at room temperature the mixture was neutralized by the addition of 3 µl of 3 M sodium acetate (pH 5.2) and 7 µl of sterile water. DNA was precipitated with 75 µl of ethanol on dry-ice and the DNA pellet was washed by 70% ethanol, dried by vacuum and resuspended in 20 µl sterile water. Six µl of the denatured DNA was mixed with 1 µl TM buffer (70 mM Tris-HCl [pH 7.5], 70 mM $MgCl_2$, 50 mM mercaptoethanol, and 1 mM EDTA), 1.5 ng of an oligonucleotide primer and sterile water to a final volume of 10 µl to give a primer to template molar ration of 3.4. The mixture was incubated at 37oC for 30-60 min. Two units of Klenow fragment and 4 µl 32p-dATP (10 µCi/µl, 740 Ci/mmole) or 35S-dATP (8 µCi/µl, 650 Ci/mmole) was added and 3µl of this mixture dispensed to each of four reaction tubes containing the G, A, T and C nucleotide reaction mixes. After incubation at 37oC for 15 min, 1 µl of chase solution was added to each tube and incubated for another 15 min. Then 5 µl of stop solution was added and incubated at 70oC for 3 min before loading the 5% acrylamide/urea/TBE sequencing gel. Usually, at least 200 bp of target DNA sequence was derived on 40 cm gels with a short and long electrophoresis of the same sample.

RESULTS

Nucleotide Sequence of the avtA region

Eleven gamma-delta insertions that reduced or eliminated the expression of the *E. coli avtA* gene (10) (Fig. 1, top) were used to determine the nucleotide sequence of 1752 bp encompassing *avtA* (Fig. 2). For most of the region both strands were sequenced (Fig. 1, bottom). One long open reading frame, with three possible in-frame translational start codons (at positions 204, 306 and 444) and one stop codon (at position 1122) was detected. The AUG codon at position 444 is preceded by a GAGG ribosome binding site (15) at position 437, while the AUG codon at position 306 is not preceded by an obvious ribosome binding sequence and the GUG codon at position 204 is just upstream of the AvtA+ insertion #35 (Fig. 2). These data suggest that the possible start codon at position 444 is used to initiate translation of *avtA*.

Gamma-delta target sequences

For each insertion mutation used, the sequence of the 5 bp target duplication generated upon gamma-delta insertion was obtained. Our results from 11 insertions show that gamma-delta has a decided bias towards A/T-rich sequences (Table 1), stronger than what was reported previously (16). We note that there is also a preference with which gamma-delta chooses the

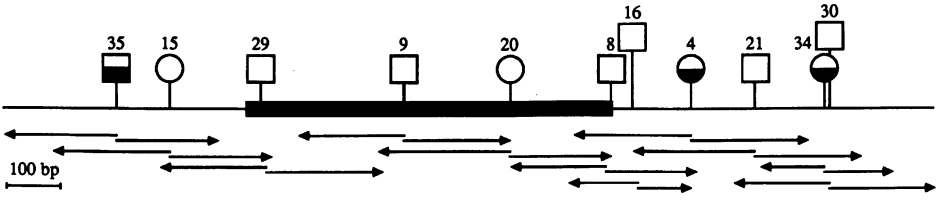

Fig. 1. Map of gamma-delta insertions used in sequencing. A preliminary map, obtained by restriction endonuclease digestion (10), was corrected based on the data presented in Fig. 2. The direction of transcription was determined to be from left to right, as drawn, by the analysis of miniMu-*lac* insertion mutations (10), and confirmed by DNA sequence analysis (Fig. 2). Symbols: Squares, insertions with gamma on the left; Circles, insertions with delta on the left; Open symbols, no detectable enzyme activity (requires both isoleucine and valine); half-filled symbols, low level of transaminase activity (requires isoleucine, but not valine); Closed symbols, high level of transaminase activity (does not require either vaine or isoleucine); Heavy line, extent of *avtA* coding region as determined by DNA sequence analysis (Fig. 2); Arrows, direction and extent of DNA sequenced from each insertion.

target site with respect to the orientation of the insertion: only one duplication is palindromic and there is a weak consensus sequence of 5'-ATATT-3' preceding the gamma end and following the delta end (Table 1).

Possible location of the avtA promoter

The location of the *avtA* promoter was suggested by the effects of insertions #35 and #15 which map roughly 200 and 100 bp, respectively, upstream of the *avtA* coding region (Fig. 2). Whereas insertion #35 reduced *avtA* expression by 4-5 fold, insertion #15 abolished it (Table 2). This suggested that the promoter is located between these two insertion sites and that a sequence upstream of #35 might enhance the promoter activity. A computer search has identified a potential promoter sequence beginning at position 303 (Fig. 2). The putative -35 region of the promoter has a TTGATG sequence and the -10 region a TATCCA sequence, with a spacing of 16 bp. This sequence yields a promoter homology index of -4.1, comparable to that of the pRE promoter of bacteriophage lambda (17).

Gamma-delta insertions downstream of *avtA* affect gene expression

Surprisingly, a number of insertions located downstream of the *avtA* coding region affected *avtA* expression. Three insertions (#16, 21 and 30), located 38, 271 and 431 bp, respectively, downstream of the TAA stop codon, completely blocked *avtA* expression while two others (#4 and 34), located between insertions #16 and 30, have partial activity (Table 2). We have ruled out possible mapping or sequencing errors. Ways in which insertions downstream of a gene might affect its expression are discussed below.

DISCUSSION

A commonly used procedure to determine the boundaries of cloned bacterial genes prior to sequencing is to isolate insertions of the transposable element gamma-delta and map the sites of

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ACAAGCGTCAGTCAGTAGTGTCAAAAGTCAGCCATGCCTAGCCAGCAGCCGTTTGTAAAGCGTTGCTCTCTCTCAACGCCGAACAGTGCGCTTGTTTATG   100

CCAGATCCGTAAAGCGCCTTATCCGGCCTACAAATTCTTCCAAATTCAATATATTGCAGAAATCATGTAGGCCTGATAAGCGTAGCGCATCAGGCAATTT   200

      35
TTAGTGACTTTCAGCCCAGGCTCTTTCTATCTCTTCCGGCCAGAATCTTCACCCCCGCCTCAATTTTCTTCCGGCTCTGGTACGTCAGTTCATGCGCATA   300

 15
CATTGATGCGTATCGGCCACGGTTTATCCAGCCCTGGGAAGAAGCTGTTGCCCCGGCACCATCAGCACGCCGCGTGCTTTCAGGCGCTGATAGAGCTGCT   400

                                                29
TGGTCGTAATGGGCGAAGATCCTTAAACCATAGCCAGAGGAAAATGGCTCTTCCGGTTTATGAATCAGGCAGCCGATTTTCCGGTAACATAGCGGCGAAT   500
                                                 MetAlaLeuProValTyrGluSerGlySerArgPheSerGlyAsnIleAlaAlaAsn

GATGGCGATAGTTTCCTGAACACGCTGGTAGTAAAACGGTTTGATGACTGTTTCAGACAGGCGCACAGATCGTTACGCTTAATCATTTCACACATCATCG   600
AspGlyAspSerPheLeuAsnThrLeuValValLysArgPheAspAspCysPheArgGlnAlaHisArgSerLeuArgLeuIleIleSerHisIleIleA

CCGGACCAATACGCCAGGTGCCAGGCTATAATGCCGTTCATATTGGTACTGGCGGTGATGATTTTTTCATTGGCGATGATAATGCCGCAGCGGGAGCGCA   700
laGlyProIleArgGlnValProGlyTyrAsnAlaValHisIleGlyThrGlyGlyAspAspPhePheIleGlyAspAspAsnAlaAlaAlaGlyAlaGl

                    9
GGTAGACCCAGCTTGGAAAGACTCATGCAGCAGCACGATATTCGGATTCCATAGCGGGCGCGCTTCACTGAAGATGATACCCGGGAACGGACGCCATAAG   800
nValAspProAlaTrpLysAspSerCysSerSerThrIlePheGlyPheHisSerGlyArgAlaSerLeuLysMetIleProGlyAsnGlyArgHisLys

CGTTATCAATCACCAGCGGAATGCCGTGTTGATTCGCCCAGCGCGTCAAGCTTCAGCAACTCTTCGTCAGTAATCACATTGCCTGTTGGATTCGTCGGCC   900
ArgTyrGlnSerProAlaGluCysArgValAspSerProSerAlaSerSerPheSerAsnSerSerSerValIleThrLeuProValGlyPheValGlyA

                   20
GGGAGACGCAAATCATCCCGGTTTCTTCGCCAATATGCAGATGCTCAAAATCCGACGTGGTATTTAAACTGGCCTTCCGGCAGCAGTTCAATATTCGGAC  1000
rgGluThrGlnIleIleProValSerSerProIleCysArgCysSerLysSerAspValValPheLysLeuAlaPheArgGlnGlnPheAsnIleArgTh

GCGCAGAGACAAACAGATCTTCTTCCAGTCCGGCGTCAGCATAGCCAATGTATTCCGGTGCAAGCGGGAACAGCACTTTTTGACCCGACCATCGGCACG  1100
rArgArgAspLysGlnIlePhePheGlnSerGlyValSerIleAlaAsnValPheArgCysLysArgGluGlnHisPhePheAspProThrIleGlyThr

      8                                    16
GCGTCCGGCAAACAGGTTAAATAAGTAGAAAAACGCGCTCTGGCTGCCGTTTGTTAGTGCAATATTCTGTGGTTCGATATCCCAACCCAACTTCTCGCGC  1200
AlaSerGlyLysGlnValLysEnd

                                                  4
AGCATTCCGGCAAGCAGTGTGAGTAGCTCCGTTTTCCCCTGTGGACCGTCGTAGTTACACAGTGCATCAGTCGCTTTGCCACTTTCCAGCATGTCGGTCA  1300

                                                    21
GTAGCGTCTGGAAGTAGTCCTGCATTTCCGGGATCTGCGCCGGATTACCGCCGCCCAGCATAATCGCGCCAGGCGTGCGTAAACCGTCGTTCAGATCTTC  1400

CATCAACAGCGTAATGCCGGAGTGGCGGGTAAATTTGTCACCAAAAAGGGAGAATGTCATAGCCGGGATATCTGTCGAACTCTGTAGCAAGGAAGGTAAC  1500

          34                  30
AATAACGCTACATTTGCTGGGGTGCAAATCGGTCTGTTGAAGAGTGAGCGTGCTTTATCCTGCCATCAGCTGATTAGGGCTGACATTTTATCCTGGTTGG  1600

CGGCTTCCCGAGGTACTGACGAACGGGAAGCCGGAAAAGTTACTGTTGCCCTGCCCAGACGACCAGCACTTTATCGCTCGCCATGCTCACGAACAAAGCC  1700

GTAGCCCTGCTTCAGCAAAAGTGTCGTTTGTTTGCCGGCAATTGCGGATTGG
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Fig. 2.Nucleotide sequence of the *avtA* coding region and the deduced amino acid sequence of the alaninine-valine transaminase. The overlining designates the 5 bp target site duplications, with the insertion number shown. The underlining designates the possible promoter (-35 region, starting at nucleotide 303 and -10 region, starting at nucleotide 325) and the probable GAGG ribosome binding site (starting at nucleotide 437).

insertions which do, and do not, inactivate the gene (6,7,10). We have demonstrated here the direct use of gamma-delta insertions to sequence the *E. coli avtA* gene by utilizing the ends of the transposon as mobile and unique sites for bidirectional priming. The use of transposon ends is

Table 1. Gamma-delta target site duplications

Insertion #	Duplicationa
35	ACTTT
15	CAATG
29	GTTTA
9	ATATT
20	AGCAT
8	AATAA
16	ATATT
4	GAAAG
21	ATCTT
34	TCAAC
30	TTATC

a. The sequence shown is 5'-3'-gamma end of insertion.

not limited to gamma-delta. Any natural or genetically engineered transposable element (18) that has unique sequences near its ends can be used for bidirectional sequencing, and the transposon Tn*5* (19), has been modified specifically for this purpose to contain unique sequences near the ends of its long inverted repeats (D. Nag, H. Huang and D.E. Berg, personal communication). In addition, transposons Tn9 and Mu have been used for unidirectional sequencing (20,21).

Location and Sequence of avtA

Gamma-delta insertions into a pBR322-*avtA*+ plasmid did not provide a clear picture of the *avtA* boundaries because insertions that permitted a low level of gene expression were found at both ends of the gene, and were intermixed with null-activity insertions at the downstream end (10; Fig. 1). The gamma-delta ends of three of these anomalous insertion mutations and eight null activity insertion mutations were used to determine the nucleotide sequence of 1752 bp, including *avtA* and flanking segments (Fig. 1, 2). One long open reading frame, with three possible in-frame translational start codons (at positions 204, 306, 444), and a single UAA stop codon (at position 1122) was detected. The corresponding polypeptides would be 306, 272 and 226 amino acids long. Only the start codon at position 444 is preceded by a potential ribosome binding site (GAGG at position 437). The other possible start codons lack ribosome binding sites and one of them, at position 204, is upstream of an insertion that does not completely block

Table 2. Alanine-valine transaminase activities and phenotypes of strains with gamma-delta insertions in pIF005

Insertion		Relevant Phenotype		Sp. Ac.[a]
#	Position[b]	Ile	Val	
44	-2050	+	+	367.8
35	212	-	+	177.5
15	306	-	-	14.5
4	1287	-	+	75.8
21	1400	-	-	15.8
34	1541	-	+	152.7
30	1560	-	-	11.0

a. Cells were grown and transaminase C activity was assayed as described elsewhere (11). Specific activity is expressed as nanomoles of pyruvate produced per minute per milligram of protein.
b. Positions of insertions based on coordinates in Fig. 2. Approximate position for #44 was determined by restriction analysis (10).

avtA expression (Fig. 2, Table 1). From these considerations, it appears that *avtA* encodes a 226 amino acid polypeptide. In addition, there are two short open reading frames in the sequenced region (from positions 34 to 204 and from 1404 to 1704), which are probably not biologically significant. The differential effects of two insertions (#35 and 15) located upstream of the *avtA* coding region, and a computer analysis of the upstream sequence led to the identification of a potential promoter whose -10 region lies at position 325-330. The strength of this promoter, as predicted by the homology index, is comparable to lambda pRE, a weak promoter whose optimal function relies on a trans-activator protein (17).

Unusual effects of nondisruptive insertions

Position effects associated with the insertion of transposable elements outside of a structural gene have been reported previously (22-25). We have found that a group of insertions distributed within a 435 bp region downstream of *avtA* (Fig. 1) affected the level of transaminase C activity (10, Table 2). These insertions fall into two phenotypic classes due to different levels of transaminase activity. One class completely lacked transaminase activity, while the other had partial activity. The data presented in Fig. 1 might indicate a correlation between phenotype and the orientation of insertion. However, the phenotypes of other insertions located in this region (10), suggest that the down-regulation of *avtA* is independent of the orientation of gamma-delta. The position effect of gamma-delta on adjacent gene activity might be explained by a differential

stability of mRNA. An alternative explanation, which we favor, is that the rate of transcription initiation is altered by changes in the DNA topology caused by the presence of gamma-delta insertions. This preference is based on the following findings: Val+ Ile+ revertants of strains carrying null activity insertions (#21 and 30) are found to arise without loss of the insertion sequence. Ten out of 10 such revertants contain a dimeric form of the parental plasmid. The mutant phenotype of these dimers is regained upon monomerization, indicating that a change in the plasmid size reversed the position effect. The topological changes due to gamma-delta insertion must be subtle since insertions as little as 19 bp apart (# 30 and #34, Fig. 2) can have dramatically different effects upon the expression of the adjacent gene (Table 1). Furthermore, since not all genes are subject to topological regulation (26,27), the ability of gamma-delta to exert a position effect on a given gene would depend upon whether or not its transcription is sensitive to changes in DNA topology.

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REFERENCES

1. Sanger, F., Nicklen, S. & Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA **74**, 5463-5467.
2. Korneluk, R.G., Quan, F. & Gravel, R.A. (1985) Gene **40**, 317-323.
3. Barnes, W.M., Bevan, M. & Son, P.H. (1983) Methods in Enzymol. **101**, 98-122.
4. Messing, J. (1983) Methods in Enzymol. **101**, 20-79.
5. Henikoff, S. (1984) Gene **28**, 351-359.
6. Guyer, M.S. (1983) Methods in Enzymol. **101**, 362-369.
7. Sancar, A. & Rupp, W.D. (1979) Biochem. and Biophys. Res. Commun. **90**, 123-129.
8. Guyer, M.S. (1978) J. Mol. Biol. **126**, 347-365.
9. Whalen, W.A., Wang, M.-D. & Berg, C.M. (1985) J. Bacteriol. **164**, 3350-3352.
10. Wang, M.-D., Liu, L., Wang, B.-M. & Berg, C.M. (1987) J. Bacteriol. **169**, 4228-4234.
11. Whalen, W.A. & Berg, C.M. (1982) J. Bacteriol. **150**, 739-746.
12. Berg, C.M., Whalen, W.A. & Archambault, L.B. (1983) J. Bacteriol. **155**, 1009-1014.
13. Wang, B.M., Liu, L., Groisman, E.A., Casadaban, M.J. & Berg, C.M. (1987) Genetics. **116**, 201-206.
14. Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
15. Shine, J. & Dalgarno, L. (1974) Nature **254**, 34-38.
16. Reed, R.R., Young, R.A., Steitz, J.A., Grindley, N.D.F. & Guyer, M.S. (1979) Proc. Natl. Acad. Sci. USA **76**, 4882-4886.

17. Harley, C.B. & Reynolds, R.P. (1987). Nucl. Acids Res. **15**, 2343-2361.
18. Berg, C.M. & Berg, D.E. (1987) in *Escherichia coli* and *Salmonella typhimurium:* Cellular and Molecular Biology, eds. Neidhardt, C., Ingraham, J., Low, K.B., Magasanik, B., Schaechter, M. & Umbarger, H.E. (American Society for Microbiology, Washington, DC).
19. Berg, D.E. & Berg, C.M. (1983) Biotechnology **1**, 417-435.
20. Ahmed, A. (1985) Gene **39**, 305-310.
21. Adachi, T., Mizuuchi, M., Robinson, E.A., Appella, E., O'Dea, M.H., Gellert, M. & Mizuuchi, K. (1987) Nucl. Acids Res. **15**, 771-784.
22. DiNardo, S., Voelkel, K.A., Sternglanz, R., Reynolds, A.E. & Wright, A. (1982) Cell **31**, 43-51.
23. Pfeifer, F., Betlach, M., Martienssen, R., Friedman, J. & Boyer, H.W. (1983) Mol. Gen. Genet. **191**, 182-188.
24. Stokes, H.W. & Hall, B.G. (1984) Proc. Natl. Acad. Sci. USA **81**, 6115-6119.
25. Heller, K., Mann, B. J. & Kadner, R. J. (1985) J. Bacteriol. **161**, 896-903.
26. Wang, J.C. (1983) in Genetic Rearrangement, eds. Chater, K.F., Cullis, C.A., Hopwood, D.A., Johnston, A.W.B. & Woolhouse, H.W. (Sinaur, Amherst, MA), pp. 1-26.
27. Drlica, K. (1984) Microbiol. Rev. **48**, 273-289.