

BEHAVIOR OF TRANSPOSON Tn1 IN *Proteus mirabilis*

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ABSTRACT

The Tn1 transposition frequency from plasmid pTH10 to the host chromosome was higher when the host was *Proteus mirabilis* than when it was *Escherichia coli*. Moreover it is possible that the values obtained for *P. mirabilis* were underestimated since the transposon donor plasmid is unstable in the *P. mirabilis* strain PG1342.

Hybridization experiments suggested that there are more than one insertion per chromosome of *P. mirabilis* but further analyses are required to localize the insertion. If Tn1 elects a hot-spot to insert into the host chromosome this target does not cause auxotrophy when disrupted by Tn1 insertion in the clones tested.

Transposition to plasmids harbored by *P. mirabilis* does not always happen at higher levels than to the chromosome as has been reported for *E. coli*. Furthermore the frequencies of Tn1 insertions are variable depending on the target DNA. The selection of an insertion site was different for *P. mirabilis* and *E. coli* suggesting that host factors play an important role on this selection. On the other hand, Tn1 showed preferential insertion into another transposon (Tn402) independent of the host. Tn1 insertion is not stable in the latter case leading to excision of the ampicillin transposon.

INTRODUCTION

Tn1 is a transposable element that confers ampicillin resistance to the host cell. Elements Tn1, Tn2 and Tn3 are considered identical and have been extensively studied in *Escherichia coli* strains. However, apart from the role of IS elements in rearranging plasmids harbored by *Proteus mirabilis* (Mollet *et al.*, 1983) there is no information concerning the behavior of Tn1 or other movable elements in this genus.

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Our laboratory has been studying the high instability of some genetic markers presented by *P. mirabilis* (Costa *et al.*, 1982a, b, c; Costa *et al.*, 1985). The emergence of auxotrophic mutants of a *P. mirabilis* strain at high frequency has been associated to some movable element, although characterization of such an element has not been possible (unpublished results from this laboratory).

Since genetic instability can be related to the presence of movable elements, we decided to follow the behavior of Tn1 in a *P. mirabilis* host, instead of an *E. coli* strain. We used a thermo-sensitive plasmid for replication, pTH10, to determine the transposition frequency to the host chromosome. This plasmid codes for kanamycin, tetracycline and ampicillin resistance, the latter given by the Tn1 transposon. Clones with insertion into the chromosome were scored for acquisition of auxotrophic markers. We also determined transposition frequencies to three plasmids belonging to different incompatibility groups. One of these plasmids, R751, presented strong preference for Tn1 insertion inactivating its trimethoprin resistance gene and was analysed further by restriction enzyme digestion.

MATERIAL AND METHODS

Bacterial strains and plasmids

The bacterial strains and plasmids are described in Table I.

Media

Routine growth was obtained in LB, Luria Broth (Miller, 1972); YT, yeast tryptone agar was used for conjugation and antibiotics were used when necessary at the following concentrations ($\mu\text{g/ml}$): kanamycin 50; carbenicillin 250; tetracycline 20; MM, minimal medium agar (Davis and Mingioli, 1950) with nicotinic acid (7.5 $\mu\text{g/ml}$) added, was used for search of auxotrophic mutants and conjugation experiments.

Matings

One tenth of a milliliter of logarithmically growing donor cells was dropped onto solid medium (YT). Upon drying these cells were overlaid with the same volume of logarithmically growing recipient cells and the plates were incubated at 37°C for five hours. The growing cells were removed by adding 3 ml of 0.5 M sodium chloride, diluted and plated onto selective media (MM with nutritional requirements and antibiotics to check resistance markers).

Table I - Bacteria and plasmids.

Strains and plasmids	MW (kb)	Relevant genotype or phenotype <i>a</i>	Inc group	Reference <i>b</i>
Bacteria				
<i>P. mirabilis</i> PG1342	-	<i>arg met thr recA tet</i>	-	1
<i>E. coli</i> HB101	-	<i>leu pro thi recA str</i>	-	2
J53	-	<i>met pro nal</i>	-	3
Plasmids <i>c</i>				
pMR5	54	<i>Ap</i> (Tn801) <i>Tc Km</i>	P	4
pTH10	54	<i>Ap</i> (Tn1) <i>Tc Km</i>	P	5
R751	51.4	<i>Tp</i> (Tn402)	P	6
N3	50	<i>Su Sp Tc</i>	N	7
RP4	54	<i>Ap</i> (Tn1) <i>Tc Km</i>	P	8
R387	-	<i>Sm Cm</i>	K	9
pBR322	4.4	<i>Ap Tc</i>	-	10

a. recA, mutant on recombination; auxotrophic markers; *arg*, arginine; *leu*, leucine; *met*, methionine; *pro*, proline; *thi*, thiamine; *thr*, threonine; resistance markers: *Ap*, ampicillin; *Tc* and *tet*, tetracycline, *Km*, kanamycin; *Tp*, trimethoprin; *Su*, sulfanilamide; *Sp*, spectinomycin; *Sm* and *str*, streptomycin; *nal*, nalidixic acid; *Cm*, chloranphenicol.

b. 1, Eitner *et al.* (1982); 2, Maniatis *et al.* (1982); 3, Clowes and Hayes (1968); 4, Robinson *et al.* (1980); 5, Harayama *et al.* (1981); 6, Jobanputra and Datta (1974); 7, Datta and Hedges (1971); 8, Datta *et al.* (1971); 9, Hedges and Datta (1971); 10, Bolivar *et al.* (1977).

c. All plasmids are conjugative except pBR322.

Plasmidial DNA extractions

An alkaline lysis method (Birboim and Doly, 1979) and a rapid boiling method (Holmes and Quigley, 1981) were used. The plasmidial DNA purification was made according to Maniatis *et al.* (1982).

Chromosomal DNA extraction

Chromosomal DNA was prepared by a modification of Marmur (1961). The pellet of a 50 ml overnight culture was resuspended in 5 ml TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Lysozyme was added (10 mg) and after 10 minutes 0.12 ml NaCl

5 M, 0.9 ml SDS 10% and 5 mg of protease were added, and the preparation incubated for 30 minutes at 37°C. One extraction with phenol followed by one of chloroform/phenol was carried out and the DNA precipitated with ethanol. The pellet was resuspended in TE (pH 8.0) and submitted to a cesium chloride gradient (Maniatis *et al.*, 1982).

Restriction analysis

Standard methods were used as described in Sharp *et al.* (1973) and Maniatis *et al.* (1982). Treatment with restriction endonucleases was performed as recommended by suppliers.

Agarose gel electrophoresis

Electrophoresis of cleaved DNA was performed in 0.8% (wt/vol) agarose type II (Sigma), in TBE buffer (0.1 M Tris-HCl; 0.1 M boric acid and 2 mM EDTA). The gel was submitted to a tension of 80 V (about 90 A) and after the run was stained by immersion in ethidium bromide (1 µg/ml) for 20 minutes. The gel was photographed through long wave UV light.

DNA hybridization

Restriction fragments were transferred to nitrocellulose paper by the Southern blot transfer method (Southern, 1975). Hybridization was carried out following Maniatis *et al.* (1982). The filter was incubated with hybridization solution (6X SSC; 0.1% SDS; 20 µg/ml salmon sperm DNA, 2 mM sodium pyrophosphate; 50% formamide) for 2 hours at 42°C (0.2 ml/cm). Radioactive probe labeled by nick translation (Rigby *et al.*, 1977) was added to the hybridization solution (50 µl/cm) and incubated at 42°C for 12 hours. After one wash at 42°C and two at 65°C (30 minutes each) in hybridization solution lacking formamide and salmon sperm DNA, the filter was air dried and exposed to X-ray film for 24 hours at -70°C with an intensifier screen.

Frequency of transposon insertion into bacterial chromosome

Strains harboring plasmid pMR5 or pTH10 were grown in LB at 28°C to a stationary phase. Suspensions were diluted 100 times in LB and grown overnight at 42°C. The same dilutions and growth conditions were repeated twice. Samples were spread on plates containing ampicillin and incubated overnight at 42°C. Ampicillin resistant clones were scored for kanamycin resistance by tooth-picking. Insertion of Tn1 into the chromosome was associated to ampicillin resistant clones which have lost the kanamycin resistant phenotype at 42°C. The frequency of transposon inser-

tion on the bacterial chromosome was calculated following Kretschmer and Cohen, 1979): $T = (A_p - K_m)/L$; where, T = frequency of transposition; A_p = titre of ampicillin resistance; K_m = titre of kanamycin resistance; L = total titre of cells. The transposition frequency was expressed as events per cell per generation.

Frequency of Tn1 transfer from host chromosome to plasmids

Plasmids from different incompatibility groups were introduced by conjugation into *P. mirabilis* and *E. coli* clones harboring Tn1 in the chromosome. Transconjugants were selected for ampicillin resistance and resistance to drugs specified by the incoming plasmid. After overnight incubation at 28°C the plasmids were transferred to *E. coli* strain J53 by conjugation. The frequency of transposition of Tn1 to plasmids was calculated as: $F = \text{efficiency of transposon transfer}/\text{efficiency of plasmid resistance marker transfer}$.

RESULTS AND DISCUSSION

Cells harboring the thermo-sensitive plasmid pTH10 were grown overnight at 28°C, an optimum temperature to elicit transposition, and then a dilution of this culture was incubated further at 42°C to prevent plasmid replication. Insertion of Tn1 into the host chromosome is related to loss of a plasmid resistance marker (kanamycin) and maintenance of ampicillin determiner (Tn1) at the restrictive temperature (42°C).

The chromosomal DNA from six clones resistant to ampicillin and sensitive to kanamycin was digested with EcoRI and hybridized with a radioactive labeled probe, pBR322, which has an ampicillin determinant originated from Tn3. All clones exhibited at least one insertion of Tn1 whereas chromosome DNA from *P. mirabilis* that had not been exposed to Tn1 did not give any signal of probe hybridization (Figure 1).

Although each sample in the gel has approximately the same amount of DNA, some signals in the Southern blot were much stronger than others (lanes 1, 10, 14 and 15 in Figure 1), suggesting that such clones have more than one insertion per chromosome. Further analysis to confirm this supposition and better localize the insertions could be obtained by digesting the chromosomal DNA with restriction enzymes that cut at more sites. Table II displays the transposition frequency found in *P. mirabilis* PG1342 strain and also in *E. coli* HB101 which was used as comparison.

A slightly higher frequency of Tn1 insertion into the chromosome was observed in *P. mirabilis* when compared to *E. coli*. This difference has been consistently observed. Additionally the plasmid pTH10 was lost at a rate of 30% during overnight cultures at 28°C from *P. mirabilis* cells, while it was maintained by *E. coli* cells under the same conditions. The rapid loss of pTH10 from *P. mirabilis* implies

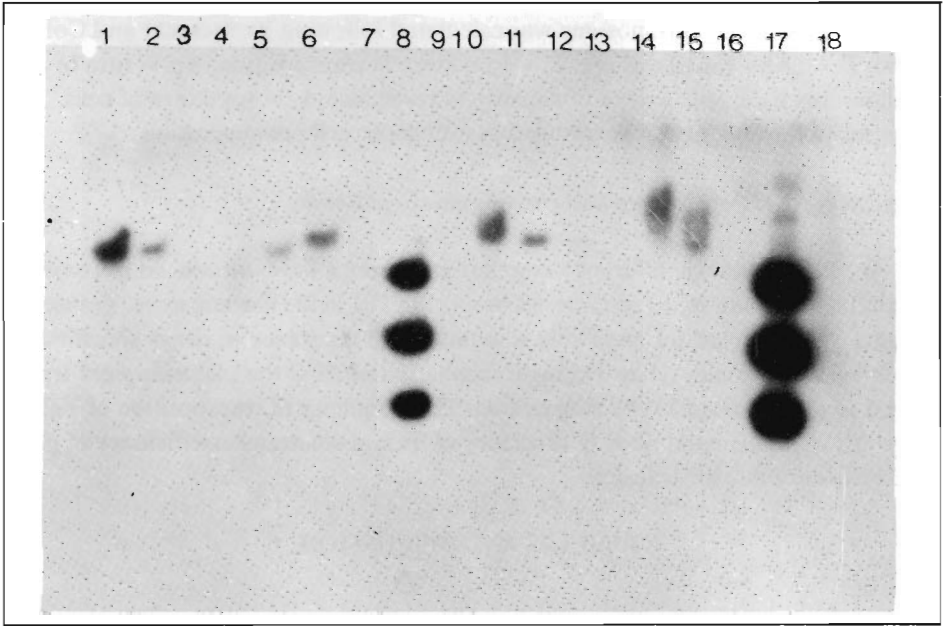


Figure 1 - Southern blot from chromosomal DNA digestion of *P. mirabilis* hybridized to pBR322. Chromosomal DNA from lanes 1 to 7 were digested with EcoRI and from lanes 10 to 16 with XhoI. Lanes: 1. PG1342:: Tn1 clone 48-2; 2. PG1342:: Tn1 clone 48-1; 3. PG1342:: Tn1 clone 4-2; 4. PG1342:: Tn1 clone 2-1; 5. PG1342:: Tn1 clone 1-2; 6. PG1342:: Tn1 clone 1-1; 7. PG1342; 8. pBR322 digested with EcoRI; 9. λ phage digested with HindIII; 10. PG1342:: Tn1 clone 48-2; 11. PG1342:: Tn1 clone 48-1; 12. PG1342:: Tn1 clone 4-2; 13. PG1342:: Tn1 clone 2-1; 14. PG1342:: Tn1 clone 1-2; 15. PG1342:: Tn1 clone 1-1; 16. PG1342; 17. pBR322 digested with EcoRI; 18. λ phage digested with HindIII.

Table II - Tn1 transposition frequency from the pTH10 plasmid to the host chromosome.

Host	Transposition frequency <i>a</i>
<i>P. mirabilis</i>	
PG1342	2.8×10^{-5}
<i>E. coli</i>	
HB101	5.8×10^{-6}

a. Expressed as events per cell per generation.

that the donor replicon of Tn1 stays for a shorter period of time in the same environment as the host chromosome when compared to the *E. coli* strain. This shorter ex-

posure could account for underestimation of transposition frequency observed in *P. mirabilis*. The addition of kanamycin while the cells were growing at 28°C, in an attempt to maintain the plasmid longer, leads to insertion of the whole plasmid into the chromosome, hindering the detection of any Tn1 insertion. Although the strains used are *recA* one cannot rule out the possibility that a mobile element, other than Tn1, is responsible for insertion of the whole plasmid into the host chromosome.

Among three hundred clones of *P. mirabilis* PG1342:: Tn1 scored for auxotrophic mutations and none were found. Nor could we find new auxotrophic markers in clones of *E. coli* that harbor Tn1 insertion into the chromosome. Harayama *et al.* (1981) using the same plasmid, pTH10, reported 3% auxotrophy among clones of *E. coli* that still are resistant to ampicillin at 42°C. However such auxotrophic clones are described as being kanamycin resistant at the restrictive temperature. We therefore think that Tn1 might not be responsible for those mutations.

The transposition frequencies of Tn1 from the host chromosome to plasmids from three different incompatibility groups were obtained for both genera as described in material and methods. For the Inc-N plasmid N3 the insertion frequency observed in *P. mirabilis* was $5\text{-}4 \cdot 10^{-6}$ whereas in *E. coli* it was much higher ($5\text{-}4 \cdot 10^{-3}$).

Kretschmer and Cohen (1979) have reported that the insertion of this class of transposon occurs more often from the chromosome to plasmids than the other way around. This was not always observed in *P. mirabilis*.

No Tn1 insertion could be detected into the Inc-K plasmid R387 when it was hosted by *P. mirabilis* PG1342:: Tn1 cells, although the chloramphenicol marker of the plasmid was transferred with high efficiency. Nevertheless, when the experiment was carried out with the *E. coli* strain HB101:: Tn1 a frequency of Tn1 insertion of $2.3 \cdot 10^{-4}$ was observed. The absence of transposition to plasmid R387 in *P. mirabilis* could be explained if we assume that Tn1 insertion occurred in an abortive way or inside the *tra* operon, preventing plasmid conjugation. On the other hand, it is also possible that Tn1 has suffered no transposition at all to the R387 replicon while harbored by *P. mirabilis* PG1342:: Tn1. The data clearly indicate that the selection of an insertion site is not the same for *P. mirabilis* and *E. coli*. Results of transposition to the plasmid N3 also agree with that hypothesis. It is known that, some host factors are required for transposition (Wiater and Grindley, 1988). Our data suggest that differences in the genetic background of both genera have an important role in selecting insertion spots.

Higher frequencies of Tn1 insertions were observed into the Inc-P plasmid R751 when it was hosted by PG1342:: Tn1 ($1 \cdot 10^{-1}$) than when it was harbored by *E. coli* cells ($7.2 \cdot 10^{-3}$). Moreover, in both genera, almost 60% of Tn1 insertions inactivated the trimethoprin determinant of the plasmid which also resides on a transposon (Tn402). Plasmids that lost trimethoprin resistance by acquisition of Tn1 were isolated and the DNA was digested with EcoRI and Sall separately and with

both BamHI and HindIII. None of the enzymes have recognition sites in the Tn1 sequence. In agarose gels we could not see any increase in molecular weight of bands that could indicate the presence of Tn1. Digestion with PstI would yield a Tn1 internal fragment of 2.8 kb. In the agarose gel presented in Figure 2 no band corresponding to a 2.8 kb fragment could be seen. Furthermore two other bands were expected corresponding to fragments of 5.7 and 1.8 kb as predicted by the restriction map of this plasmid (Meyer and Shapiro, 1980). The 1.8 kb PstI fragment is included inside the transposon Tn402. The absence of these bands suggest that some rearrangement occurred before the experiments, since the control plasmid is also changed. Neither the PstI analysis nor other digestions carried out were enough to predict what kind of recombination this plasmid has undergone. The absence of the 2.8 kb internal fragment of Tn1 is noteworthy. Although it has been transferred by this plasmid our data suggest that the transposon no longer resides on the R751 plasmid. As the host still shows an ampicillin resistance phenotype we supposed that the transposon Tn1 was excised from the plasmid but a copy is still inserted into the host chromosome.

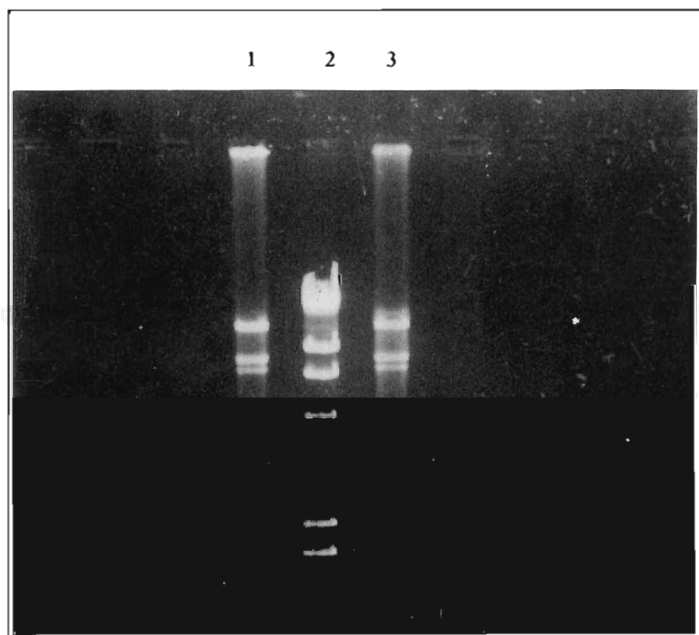


Figure 2 - Agarose gel electrophoresis of R751:: Tn1 plasmid digested with PstI. Lanes: 1. R751:: Tn1 DNA (clone 1-1); 2. lambda phage DNA digested with HindIII; 3. R751:: Tn1 DNA (clone 3-1). No bands corresponding to 2.8, 5.7 or 1.8 kb fragments, as predicted by restriction map of R751 plasmid, were observed.

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RESUMO

A frequência de transposição do Tn1 do plasmídeo pTH10 para o cromossomo da célula hospedeira foi maior em *Proteus mirabilis* que em *Escherichia coli*. Contudo pode-se supor que os valores obtidos em *P. mirabilis* foram subestimados desde que o plasmídeo doador do transposon é instável na linhagem PG1342.

Experimentos de hibridação sugerem que existem mais do que uma inserção por cromossomo de *P. mirabilis*, mas análises adicionais são necessárias para a localização dessas inserções.

Se o transposon Tn1 se insere em um ponto quente do cromossomo da célula hospedeira, esse alvo não causou auxotrofia em nenhum dos clones testados de PG1342:: Tn1.

Transposições entre plasmídios albergados por *P. mirabilis* parecem não ocorrer em níveis maiores do que para o cromossomo, conforme relatado para *E. coli*. Outrossim, as frequências de inserção do Tn1 variam de acordo com o DNA alvo. A seleção de um sítio de inserção ocorre diferentemente nos dois gêneros, sugerindo que fatores dos hospedeiros têm uma participação importante nessa seleção. Por outro lado, Tn1 mostrou inserção preferencial em outro transposon (Tn402), independentemente do hospedeiro. Neste caso a inserção do Tn1 não é estável, levando à excisão do transposon ampicilina.

REFERENCES

- Birboim, H.C. and Doly, J.A. (1979). A rapid alkalyne extraction procedure for screening recombinant plasmid DNA. *Nucl. Acids Res.* 7: 1513-1519.
- Bolivar, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heynecker, H.L., Boyer, H.W., Crosa, J.H. and Falkow, S. (1977). Construction and characterization of new cloning vehicles. II-A multipurpose cloning system. *Gene* 2: 95-106.
- Chandler, M., Allet, B., Gallay, E., Boy de La Tour, E. and Caro, L. (1977). Involvement of IS1 in the dissociation of the r-determinant and RTF components of the plasmid R100. *Mol. Gen. Genet.* 153: 289-295.
- Clowes, R.C. and Hayes, W. (1968). *Experiments in microbial genetics*. Blackwell Scientific Publications, Oxford. 244 pp.
- Costa, S.O.P., Bianco, M. and Bonato, M.C.M. (1982a). Genetic instability in *Proteus mirabilis* as evidence for plasmid coded protease. *Microbial Genet. Bull.* 51: 2-7.
- Costa, S.O.P., Bonato, M.C.M. and Bianco, M. (1982b). Instability of the protease (gelatinase) trait in *Proteus mirabilis*. I - A study of newly isolated strains. *Rev. Microbiol.* 13: 35-45.
- Costa, S.O.P., Bonato, M.C.M. and Bianco, M. (1982c). Instability of the protease (gelatinase) trait in *Proteus mirabilis*. II - Behavior of protease-excreting and non-excreting cells in the presence of gelatin. *Rev. Microbiol.* 13: 46-49.
- Costa, S.O.P., Bonato, M.C.M. and Bianco, M. (1985). Influence of RP4 plasmid on extracellular protease secretion in *Proteus mirabilis*. *Braz. J. Med. Biol. Res.* 18: 397-399.
- Datta, N. and Hedges, R.W. (1971). Compatibility groups among fi-factors. *Nature* 234: 220-222.
- Datta, N., Hedges, R.W., Shaw, E.J., Sykes, R.B. and Richmond, M.A. (1971). Properties of an R factor from *Pseudomonas aeruginosa*. *J. Bacteriol.* 108: 1244-1249.
- Davis, B.D. and Mingioli, E.S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B12. *J. Bacteriol.* 60: 17-28.

- Eitner, G., Adler, B., Lanzov, V.A. and Hoffemeister, J. (1982). Interspecies RecA protein substitution in *Escherichia coli* and *Proteus mirabilis*. *Mol. Gen. Genet.* 185: 481-486.
- Harayama, S., Tsuda, M. and Iino, T. (1981). Tn1 insertion mutagenesis in *Escherichia coli* K-12 using a temperature-sensitive mutant of plasmid RP4. *Mol. Gen. Genet.* 184: 52-55.
- Hedges, R.W. and Datta, N. (1971). fi-R factors giving chloramphenicol resistance. *Nature New Biol.* 234: 220-221.
- Holmes, O.S. and Quigley, R. (1981). A rapid boiling method for the preparation of bacterial plasmid. *Anal. Biochem.* 114: 193-197.
- Jobanputra, R.S. and Datta, N. (1974). Trimethoprim resistance factors in enterobacteria from clinical specimens. *J. Med. Microbiol.* 7: 169-175.
- Kretschmer, P.J. and Cohen, S.N. (1979). Effect of temperature on translocation frequency of the Tn3 element. *J. Bacteriol.* 139: 515-519.
- Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982). *Molecular cloning - a laboratory manual*. Cold Spring Harbor Laboratory, New York, 545 pp.
- Marmur, J.A. (1961). A procedure of the isolation of desoxyribonucleic acid from microorganisms. *J. Mol. Biol.* 3: 208-218.
- Meyer, J.R. and Shapiro, A.J. (1980). Genetic organization of the broad host range Inc-P-I plasmid R751. *J. Bacteriol.* 143: 1362-1373.
- Miller, H.J. (1972). *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, New York, 466 pp.
- Mollet, B., Iida, S., Shepherd, J. and Arber, W. (1983). Nucleotide sequence of IS26, a new prokaryotic mobile genetic element. *Nucl. Acids Res.* 11: 6319-6330.
- Rigby, P.W.J., Dieckmann, M., Rhoades, R.H. and Berg, P. (1977). Labeling deoxy-ribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113: 237-243.
- Robinson, M.K., Bennett, P.M., Falkow, S. and Dodd, H.M. (1980). Isolation of a temperature-sensitive derivative of RP1. *Plasmid* 3: 343-347.
- Sharp, R.A., Sugden, B. and Sambrook, J. (1973). Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose. *Biochem.* 12: 3055-3063.
- Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98: 503-517.
- Wiater, L.A. and Grindley, N.D.F. (1988). Gama-delta transposase and integration host factor bind cooperatively at both ends of gama-delta. *EMBO J.* 7: 1907-1911.

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