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R.*Sma*I cleavage map of the transfer region of the *E. coli* K12 sex factor F

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SUMMARY

A map of the seventeen R.*Sma*I sites within the 33·4 kb transfer region of plasmid F has been obtained. The number of R.*Sma*I sites contrasts sharply with the few sites for other restriction endonucleases with hexanucleotide specificity. Location of the R.*Sma*I sites in *λtra* transducing phages allowed physical location of lambda insertions into the *traB*, *traF* and *traH* genes.

1. INTRODUCTION

The transfer region of the F sex factor is a 33·4 kilobase (kb) length of DNA encoding the twenty or more genes required for conjugation, and lying between the kilobase coordinates 65·3–98·7 on the revised physical map of F (D. Johnson & N. S. Willetts, manuscript in preparation). Curiously, this region contains relatively few cleavage sites for the common restriction endonucleases with hexanucleotide recognition sequences. There are no sites for R.*Hin*dIII or R.*Bam*I (Ohtsubo & Ohtsubo, 1977; Skurray, Nagaishi & Clark, 1978), and only two sites for R.*Sal*I (Thompson & Achtman, 1978; D. Johnson & N. S. Willetts, manuscript in preparation), two sites for R.*Bgl*II (Thompson & Achtman, 1978; N. S. Willetts, unpublished data), and five sites for R.*Eco*RI (Ohtsubo & Ohtsubo, 1977).

Restriction sites are useful for the cloning of small fragments of particular interest (Achtman *et al.* 1978; Thompson & Achtman, 1978) and for the physical analysis of fragments cloned by *in vivo* methods (Willetts & McIntire, 1978; D. Johnson & N. S. Willetts, manuscript in preparation), and aid correlation of the genetic and physical maps of F. It was observed that the restriction endonuclease R.*Sma*I had a much larger number of cleavage sites within the transfer region than the other enzymes with hexanucleotide specificity listed above, and it was therefore decided to map these accurately. Although this enzyme, which recognizes the sequence CCCGGG, generates fragments with flush termini, the isoschizomeric enzyme R.*Xma*I generates fragments with four-base single strand extensions, allowing cloning into vectors such as pBR324 (Bolivar, 1978). In order to facilitate the analysis, advantage was taken of previous cloning of large segments of the transfer region onto pSC101 using R.*Eco*RI (Clark *et al.* 1976), or on to lambda phages by *in vivo* methods (Willetts & McIntire, 1978).

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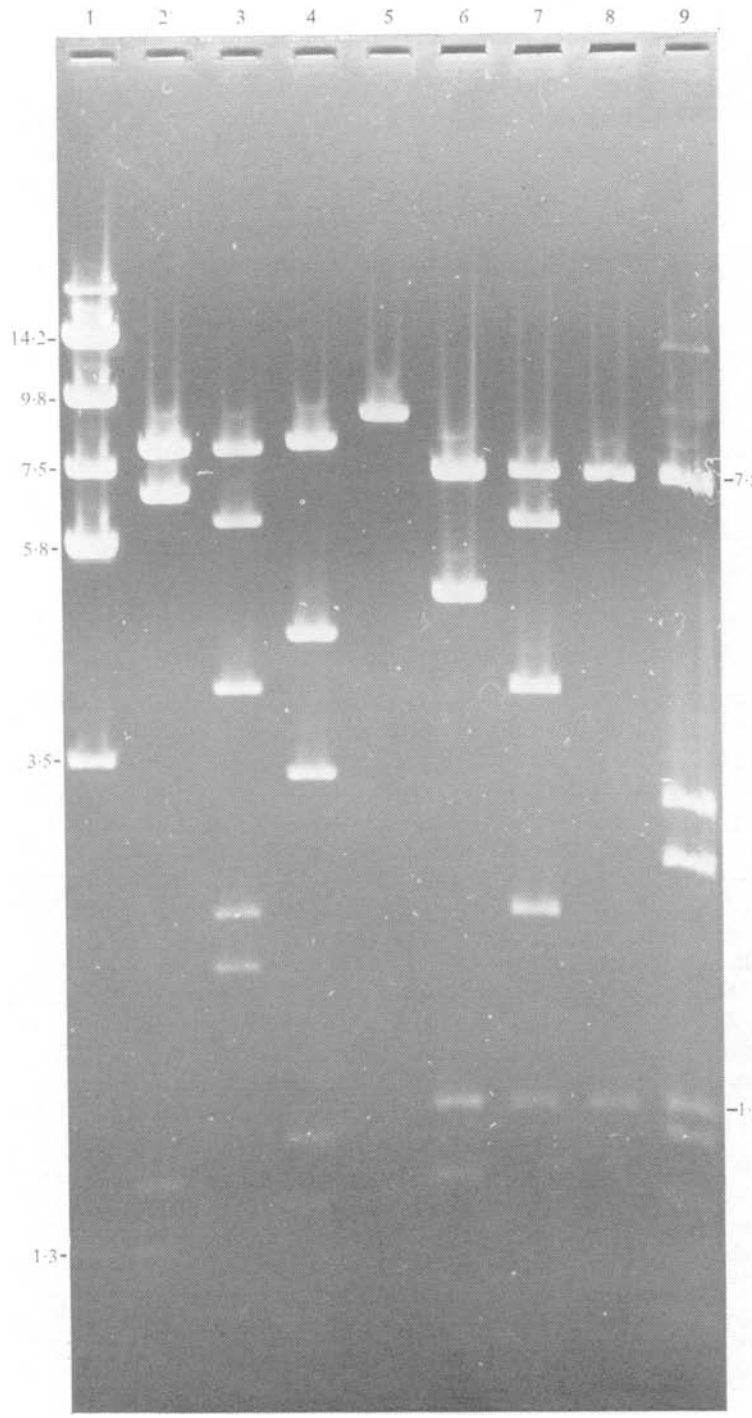

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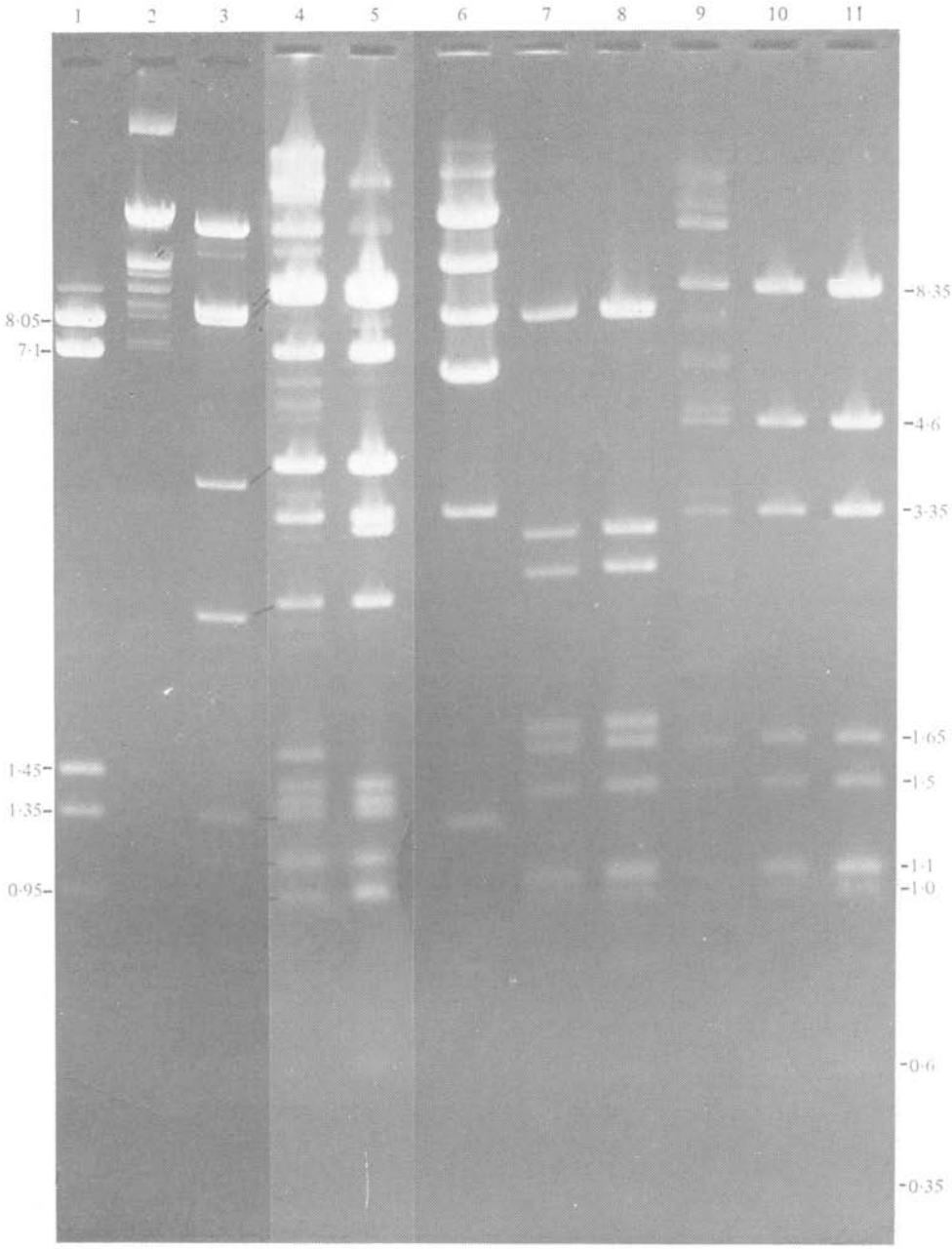

Fig. 1. Photographs of agarose gels of R. *Sma*I fragments obtained from the chimeric plasmids pRS27, 29 and 31, and from the *λtra* phages EDλ90, 96 and 97.

(*a*) Track 1, EDλ97 + *Eco*RI; 2, pRS27 + *Sma*I; 3, pRS29 + *Sma*I; 4, pRS31 + *Sma*I; 5, pSC101 + *Sma*I; 6, pRS27 + *Sma*I + *Eco*RI; 7, pRS29 + *Sma*I + *Eco*RI; 8, pSC101 + *Sma*I + *Eco*RI; 9, pRS31 + *Sma*I + *Eco*RI. Bands of < 1 kb cannot be seen on this gel, but fragments down to about 0·4 kb were identified on other gels.

(*b*) Track 1, pRS27 + *Sma*I; 2, pRS27 + *Sma*I partial digest; 3, EDλ90 + *Sma*I; 4, EDλ96 + *Sma*I; 5, EDλ97 + *Sma*I; 6, EDλ97 + *Eco*RI; 7, pRS31 + *Sma*I + *Eco*RI; 8, as 7 but twice as much DNA; 9, pRS31 + *Sma*I partial digest; 10, pRS31 + *Sma*I; 11, as 10, but twice as much DNA. Tracks 4–11 are rearranged from one gel, and tracks 1–3 are from a second gel: band correspondence between the two gels is indicated.

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2. MATERIALS AND METHODS

(i) *Plasmids and bacteriophages*

pRS27, pRS29 and pRS31 were described by Clark *et al.* 1976; their DNA (the gift of R. Everett) was prepared by a method based upon that of Humphreys, Willshaw & Anderson (1975). The *λtra* phages EDλ90, EDλ92, EDλ96 and EDλ97 were isolated from an abnormal insertion of λ into *traB* (Willetts & McIntire, 1978), and their DNA was obtained as described by Willetts (1977).

(ii) *Endonuclease digestion and electrophoresis techniques*

R.*Eco*RI was prepared in this laboratory, and R.*Sma*I was purchased from Boehringer Mannheim GmbH. Digestions with R.*Sma*I were carried out for 120 min at 30° in a buffer containing 30 mM Tris-HCl pH 9·0, 3 mM $MgCl_2$, 15 mM KCl and 0·1 % gelatine. Partial digests were for 5 min. When required, R.*Sma*I digestions were followed by R.*Eco*RI digestions after adjustment of the buffer, and these and the electrophoretic separation and analysis of the fragments obtained were as described by Willetts & McIntire (1978). Standards were usually obtained by digestion of EDλ97 with R.*Eco*RI (ibid.), to give fragments with sizes 14·2 kb (f1), 9·8 kb, 7·5 kb (λB), 5·8 kb (λC), 3·5 kb (λF), 1·3 kb (f15), and 0·8 kb (f17): f19 (0·2 kb) was not usually seen. The revised sizes of the R.*Eco*RI F fragments (f1, 14·2 kb; f2, 13·2 kb; f6, 8·3 kb; f15, 1·3 kb; f17, 0·8 kb) were derived by D. Johnson and N. S. Willetts (manuscript in preparation). The R.*Sma*I map of λ was given by McParland, Brown & Pearson, (1976).

The R.*Sma*I fragments making up the transfer region were numbered consecutively without regard to size (Sma-1, etc.). No data were obtained for cleavage sites elsewhere in the F plasmid, except that the sizes of the first (Sma-1) and last (Sma-18) fragments of the transfer region were found to be 5·8 kb and 3·4 kb respectively, by determining the cleavage maps of chimeric plasmids covering these regions (data not shown).

3. RESULTS AND DISCUSSION

pRS27, pRS29 and pRS31 contain R.*Eco*RI fragments of F cloned onto pSC101 (Clark *et al.* 1976), and taken together they cover the entire transfer region (f6, f15, f1, f17, f19 and f2). They were subjected to R.*Sma*I digestion to determine the number of cleavage sites and to R.*Sma*I and R.*Eco*RI double digestion to begin to locate these sites. pSC101 itself has a single R.*Sma*I site, located 1·8 kb from the R.*Eco*RI site, and 2·45 kb from the R.*Sal*I site. Photographs of representative gels showing the fragments formed are given in Fig. 1 (*a*) and (*b*).

pRS27 carries R.*Eco*RI F fragments f6 and f15, and there proved to be three R.*Sma*I cleavage sites in f6 and one in f15. The 0·95 and 1·45 kb fragments (Fig. 2) did not contain R.*Eco*RI sites and so could not be ordered initially. However, partial digests of pRS27 with R.*Sma*I (Fig. 1 (*b*), tracks 1 and 2) gave fragments of 2·3 kb and 2·4 kb, but not 2·8 kb, suggesting the order shown in Fig. 2. This was confirmed later by digestion of EDλ90 DNA (Fig. 1 (*b*) track 3; Fig. 3): this gave fragments of 2·55 (Sma-6), 1·15 (Sma-5), 1·35 (Sma-4) and 0·95 kb (which must therefore be Sma-3) but not 1·45 kb (which must be Sma-2). Similar but approximate locations for the R.*Sma*I sites in f6 were presented by Thompson & Achtman (1978).

Digestions of pRS29 showed that there were four R.*Sma*I cleavage sites in R.*Eco*RI F fragment f1, but did not allow the 2·55, 4·1 and 6·45 kb fragments (Fig. 2) to be ordered. However, an R.*Sal*I site is located 2·2 kb from the R.*Eco*RI site at the beginning of f1 (D. Johnson and N. S. Willetts, manuscript in preparation), and R.*Sma*I and R.*Sal*I double digests (not shown), proved that this R.*Sal*I site was in the 2·55 kb

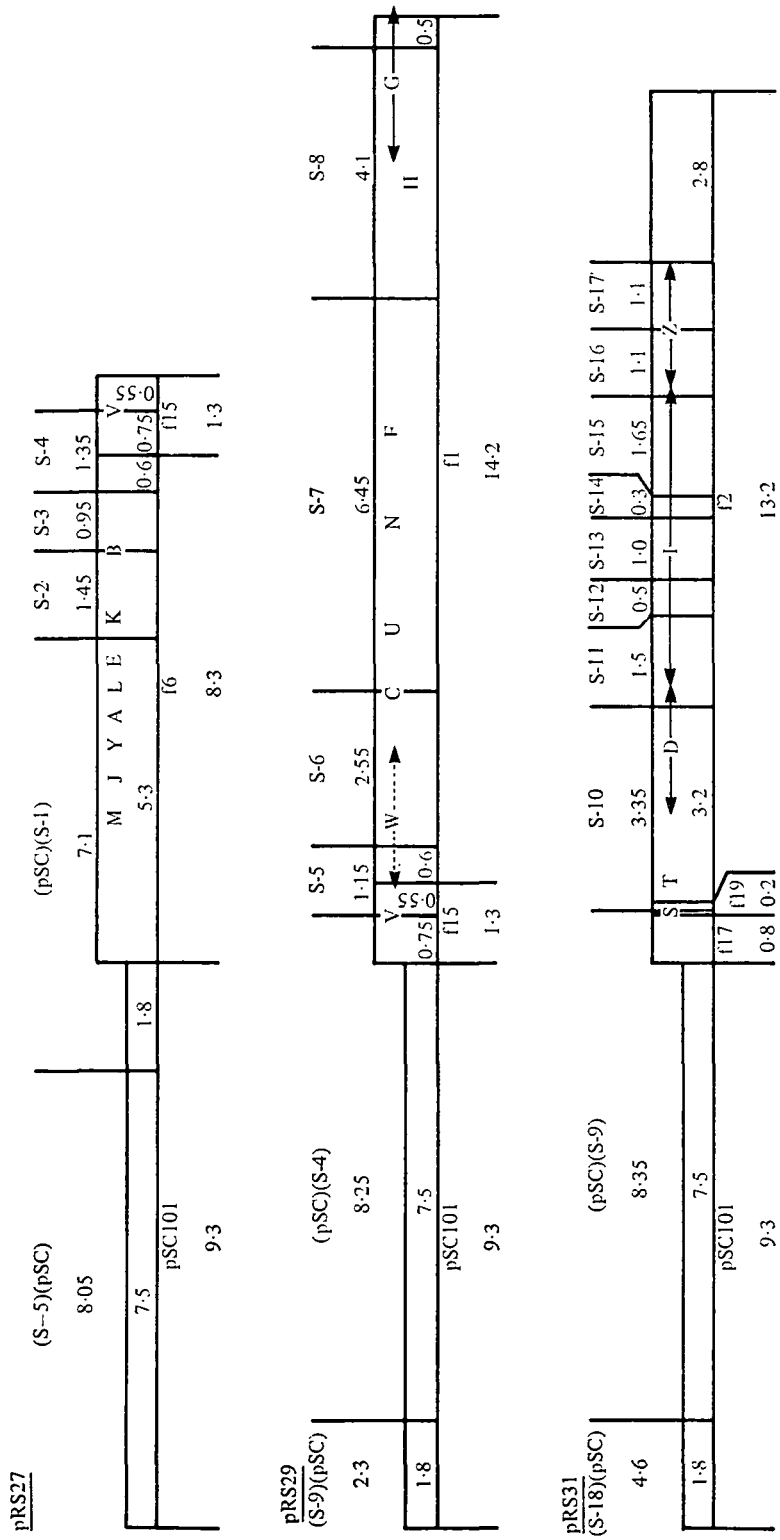

Fig. 2. R.*Sma*I and R.*Eco*RI restriction sites on the chimeric plasmids pRS27, 29 and 31. R.*Sma*I fragments are shown above the lines, R.*Eco*RI fragments below the lines, and R.*Sma*I – R.*Eco*RI double digestion fragments between the lines. Sma-n is abbreviated to S-n for reasons of space, and parentheses indicate that only a part of the *Sma*I fragment is present. The locations of the *tra* genes are shown between the lines, the lengths of long genes with one or more cleavage sites being indicated by arrows. Lengths are given in kilobases, and represent averages from 2–10 measurements of each fragment. The orientations of the *tra* regions relative to the pSC101 vector (Skurray *et al.* 1978) were A for pRS27 (which carries the *tra* promoters) and B for pRS29 and pRS31 (which express the *tra* genes from a pSC101 promoter).

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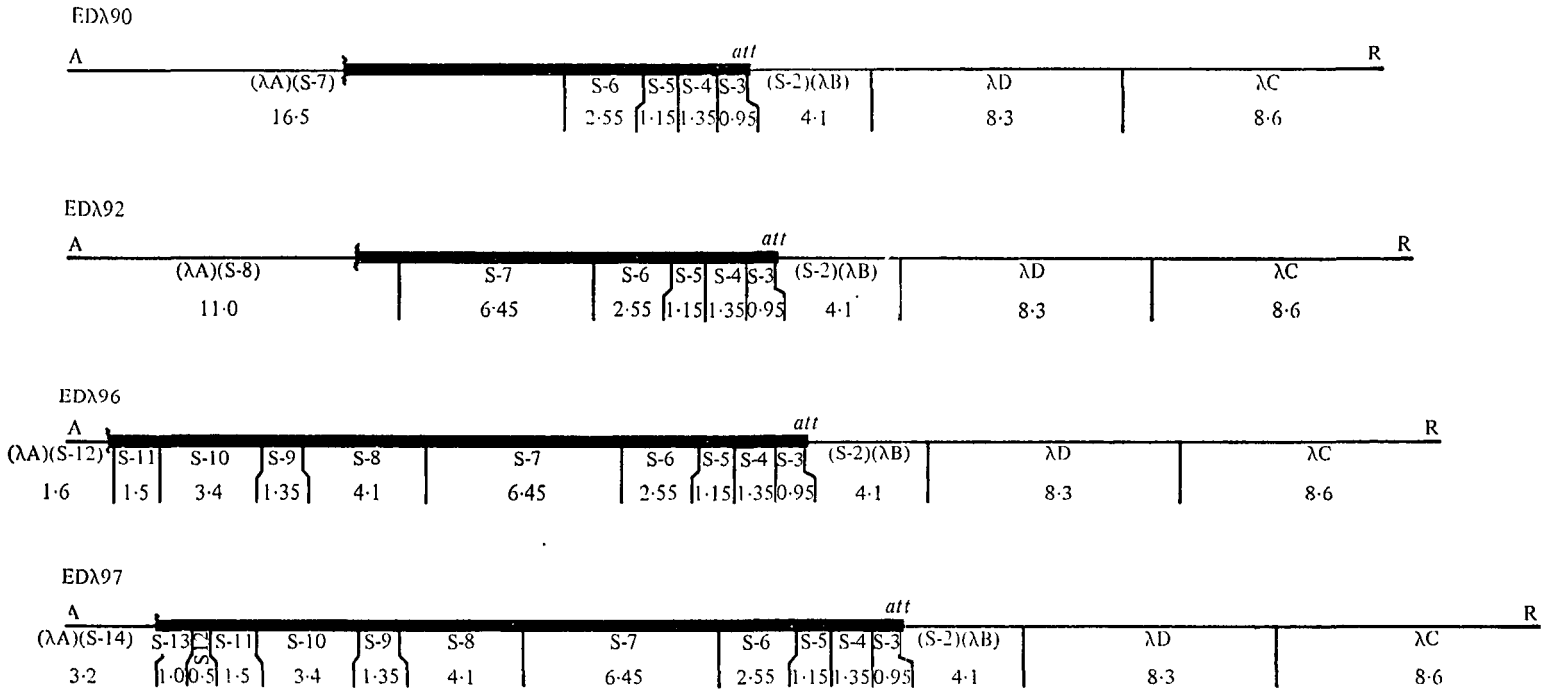

Fig. 3. R.*Sma*I sites on the phages EDλ90 (*λtra(B)VWC*), EDλ92 (*λtra(B)VWCUNF (H)*), EDλ96 and EDλ97 (both *λtra(B)-VWCUNFHGSTD*). *tra* segments are shown as thick lines; other conventions are as for Fig. 2. The sizes of the phage genomes were calculated by summing the R.*Sma*I fragments to be 43·5, 44·4, 45·4 and 48·5 kb respectively, closely similar to the values previously found (Willetts & McIntire, 1978). The lengths of the *tra* segments of EDλ96 and EDλ97 have been increased from those determined previously in the electron microscope (*ibid*), to (just) accommodate the R.*Sma*I fragments that they contain.

fragment which is therefore Sma-6 located as shown in Fig. 2. The 4·1 and 6·45 kb fragments were ordered by digestion of EDλ92 DNA; this gave the 6·45 kb but not the 4·1 kb fragment (Fig. 3), so that the former must be Sma-7 and the latter Sma-8.

pRS31 carries the R.*Eco*RI F fragments f17, f19 and f2. There were no R.*Sma*I sites in f17 but there was one in f19, and eight in f2 (Fig. 1(*b*)). Partial R.*Sma*I digestions of pRS31 were made (Fig. 1(*b*) track 9) in an attempt to order the seven fragments of f2 that did not contain R.*Eco*RI sites, and the order deduced is given in Fig. 2. A similar, though not identical, R.*Sma*I cleavage pattern for pRS31 was found by P. Manning, R. Thompson and M. Achtman (personal communication). Further information about the R.*Sma*I sites in f17, f19 and f2 was obtained by digestion of DNA from the phages EDλ96 and EDλ97 (Fig. 1(*b*) tracks 4 and 5; Fig. 3). Both give a 1·35 kb fragment that was not obtained from pRS29 or pRS31; this must be Sma-9 which spans the f1/f17/f19 R.*Eco*RI sites. EDλ96 gave fragments Sma-3, 4, 5, 6, 7, 8, 9, 10 and 11, while EDλ97 gave in addition 0·5 kb (Sma-12) and 1·0 kb (Sma-13) fragments. This is consistent with the order deduced from the partial digest of pRS31.

In total, then, there were seventeen R.*Sma*I cleavage sites in the transfer region, many more than found for other enzymes with hexanucleotide specificity. Many of these sites fell into two clusters, covering regions of 6 to 8 kb near the beginning and at the end of the *tra* operon. The R.*Sma*I sites may prove useful for mapping purposes, and for cloning pilus-determining regions from within the *tra* operon where there are relatively few other restriction enzyme cleavage sites (see Fig. 2). However, R.*Sma*I sites will not be so useful for similar studies of transfer gene expression or of DNA metabolism since the relevant genes or sites all fall on the 5·8 kb Sma-1 fragment covering the first 3–4 kb of the transfer region, or are located in a region extensively cleaved by R.*Sma*I in the last 13 kb of the transfer region.

For the four λ*tra* phages described, the length of the fragment spanning the rightward (as drawn in Fig. 3) *tra*/λ DNA junction was 4·1 kb. This is only slightly greater than the distance from att_{λ} to the rightward λ R.*Sma*I site (4·04 kb; McParland *et al.* 1976), showing that very little of the Sma-2 fragment DNA is present. The initial insertion of EDλ4 into F*lac* giving EDFL223 (F*lac traB*::EDλ4) from which all the λ*tra* phages had been isolated, must therefore have been immediately prior to the cleavage site at the end of fragment Sma-2. This location is within 0·1 kb of that calculated from the R.*Eco*RI fragment sizes for these phages (Willetts & McIntire, 1978). Similarly, the presence of R.*Sma*I sites in *tra* DNA carried by transducing phages isolated from EDFL228 (F*lac traF*::EDλ4) and EDFL229 (F*lac traH*::EDλ4; McIntire & Willetts, 1979 and unpublished data), has allowed the physical locations of the λ insertions, and thus of points within the *traF* and *traH* genes, to be calculated as 4·55 kb from the beginning of Sma-7, and 2·1 kb from the beginning of Sma-8, respectively.

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REFERENCES

Achtman, M., Skurray, R. A., Thompson, R., Helmuth, R., Hall, S., Beutin, L. & Clark, A. J. (1978). Assignment of *tra* cistrons to *Eco*RI fragments of F sex factor DNA. *Journal of Bacteriology* **133**, 1383–1392.

Bolivar, F. (1978). Construction and characterisation of new cloning vehicles. III. Derivatives of plasmid pBR322 carrying unique *Eco*RI sites for selection of *Eco*RI generated recombinant DNA molecules. *Gene* **4**, 121–136.

Clark, A. J., Skurray, R. A., Crisona, N. J. & Nagaishi, H. (1976). Use of molecular cloning in the genetic and functional analyses of the plasmid F. In *Molecular Mechanisms in the Control of Gene Expression*, pp. 565–579. New York: Academic Press.

HUMPHREYS, G. O., WILLSHAW, G. A. & ANDERSON, E. S. (1975). A simple method for the preparation of large quantities of pure plasmid DNA. *Biochimica et biophysica acta* **383**, 457–463.

MCINTIRE, S. & WILLETTS, N. S. (1979). Transfer-deficient cointegrates of F*lac* and lambda prophage. *Molecular and General Genetics* (submitted for publication).

MCPARLAND, R. H., BROWN, L. R. & PEARSON, G. D. (1976). Cleavage of lambda DNA by a site-specific endonuclease from *Serratia marcescens*. *Journal of Virology* **19**, 1006–1011.

OHTSUBO, H. & OHTSUBO, E. (1977). Repeated DNA sequences in plasmids, phages, and bacterial chromosomes. In *DNA Insertion Elements Plasmids and Episomes*, (ed. A. I. Bukhari, J. A. Shapiro and S. Adhya), pp. 49–64. Cold Spring Harbor Laboratory, New York.

SKURRAY, R. A., NAGAISHI, H. & CLARK, A. J. (1978). Construction and *Bam*HI analysis of chimeric plasmids containing *Eco*RI DNA fragments of the F sex factor. *Plasmid* **1**, 174–186.

THOMPSON, R. & ACHTMAN, M. (1978). The control region of the F sex factor DNA transfer cistrons: restriction mapping and DNA cloning. *Molecular and General Genetics* **165**, 295–304.

WILLETTS, N. S. (1977). The transcriptional control of fertility in F-like plasmids. *Journal of Molecular Biology* **112**, 141–148.

WILLETTS, N. S. & MCINTIRE, S. (1978). Isolation and characterisation of *λtra* transducing phages from EDFL223 (F*lac traB*::ED*λ*4). *Journal of Molecular Biology*, **126**, 525–549.