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Transposon-Mediated Mutagenesis and Recombination in *Vibrio cholerae*

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An efficient method for introducing transposons into the *Vibrio cholerae* chromosome or the P plasmid was developed by using $F'_{ts}lac^+$ plasmids from *Escherichia coli* as suicide delivery vehicles. Hybrid P::Tn*1*,Tn*5* and P::Tn*1*,Tn*10* plasmids containing Tn*5* or Tn*10* in either of the two possible orientations were constructed. During conjugation, these hybrid plasmids mediated oriented transfer of markers from the sites of insertion of homologous transposons in the donor chromosome. The collection of donor strains described here permits oriented mobilization of all parts of the *V. cholerae* chromosome.

Conjugal gene transfer in *Vibrio cholerae* is mediated by the P factor, a naturally occurring 48-megadalton conjugative plasmid (5, 6, 14). Although the P factor can mobilize chromosomal genes, recombinants in P plasmid-mediated matings are produced at low frequencies, and nonselected markers are poorly linked to selected markers (17, 18). Unlike the F plasmid of *Escherichia coli*, the P plasmid of *V. cholerae* does not integrate in a stable manner into the bacterial chromosome (10, 21, 22).

S. R. Johnson and W. R. Romig developed a transposon-facilitated recombination (Tfr) system in *V. cholerae* that increased the efficiency of chromosomal gene transfer (14, 15). Their Tfr donor strains contained copies of the ampicillin resistance (Amp^r) transposon Tn*1* (Tn*801*) inserted into both the P plasmid and the bacterial chromosome (14, 21). The regions of homology between the chromosome and the P factor, provided by the transposon, are presumed to be responsible for the increase in efficiency of gene transfer during transposon-facilitated recombination (4, 7, 9).

Unfortunately, there were several liabilities inherent to the system developed by Johnson and Romig. Construction of strains containing chromosomally inserted copies of Tn*1* required an extensive series of plasmid manipulations, and these steps had to be repeated during the isolation of each independent strain containing a chromosomal insertion of Tn*1*. Additionally, Tn*1* chromosomal insertions were rare, and enrichment techniques were necessary to obtain auxotrophs with Tn*1* insertions in biosynthetic genes. Briefly, the hybrid P plasmid pSJ26, containing Tn*1*, Tn*9* (chloramphenicol resistance, Cam^r) and *hts* (a marker lethal for the host bacterium at high temperature) was transferred by conjugation to a recipient strain of *V. cholerae*. Transconjugants were incubated at 42°C to isolate strains with deletion mutants of plasmid pSJ26 that contained Tn*1* but were transfer deficient and lacked Tn*9* and *hts*. Superinfection with the incompatible, hybrid P plasmid pSJ25 (Tn*9*, *hts*) and selection for Cam^r were used to eliminate the Tn*1* vector. Isolates that remained ampicillin resistant were then cured of pSJ25 by selecting for survival at elevated temperatures. Reported uses of this approach were limited to derivatives of eltor strain RV79 and classical strain RV88, neither of which were recent clinical isolates. We attempted to extend the application of this Tn*1* system to several recent clinical isolates of *V. cholerae* but were unable to generate Tn*1* chromosomal insertions.

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The goals of the present study were to develop a generally applicable technique for introducing and utilizing transposons during *V. cholerae* genetic studies and to produce an expanded set of insertional auxotrophic Tfr donors for use in such studies. We achieved these goals by using temperature-sensitive F′ factors (7, 13) as suicide delivery vehicles to introduce the kanamycin resistance (Kan^r) transposon Tn*5* or the tetracycline resistance (Tet^r) transposon Tn*10* into the chromosome and P factor of *V. cholerae*. Tn*5* and Tn*10* insertional auxotrophs were recovered and converted to new Tfr donors capable of directed chromosomal gene transfer by introduction of P::Tn*1*,Tn*5* or P::Tn*1*,Tn*10* hybrid sex factors. The set of donor strains described here is capable of mobilizing any region of the *V. cholerae* eltor chromosome. A selected subset of these strains has recently been used to map the chromosomal locations of several eltor biotype markers (12).

MATERIALS AND METHODS

Bacterial strains and plasmids. Tables 1 and 2 list the bacterial strains and plasmids used. The strains were maintained frozen at −70°C in brain heart infusion (BHI; Difco Laboratories, Detroit, Mich.) with 15% glycerol.

Media. Meat extract agar (MEA) adjusted to pH 7.0 and BHI broth were used as nutrient media (18). Solid synthetic medium was minimal medium V (MinV) which contained (per liter): Difco agar, 15.0 g; K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $(NH_4)_2SO_4$, 1.0 g; NaCl, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; and either glucose, 0.2%, or lactose, 0.3%. Agar and salts were autoclaved separately. $MgSO_4 \cdot 7H_2O$ and carbon sources were added afterwards. MinV was supplemented with 50 μg of adenine per ml to satisfy an uncharacterized purine requirement in RV and RJ strains and as required with amino acids at 20 μg/ml. Antibiotics (Sigma Chemical Co., St. Louis, Mo.) for selection and counterselection were used at the following concentrations: kanamycin (Kan), 50 μg/ml; streptomycin (Str), 75 μg/ml; spectinomycin (Spc), 75 μg/ml; rifampin (Rif), 30 μg/ml in MEA or BHI, 20 μg/ml in synthetic medium; ampicillin (Amp), 300 μg/ml; tetracycline (Tet), 10 μg/ml.

Mating conditions. All matings were performed as plate matings. Samples containing 100 μl from overnight cultures of donor and recipient strains grown at 30°C in BHI broth were inoculated onto MEA plates and incubated at 30°C overnight.

Mutant isolation. Auxotrophic mutants derived from *V. cholerae* GN6300 (Table 1) were obtained either by treating

TABLE 1. Bacterial strains

Strain	Relevant characteristics[a]	Source (reference)
V. cholerae		
U1	Prototroph, eltor biotype, Ogawa serotype, 1980 clinical isolate from Dacca, Bangladesh, ancestor for all GN strains	A. Huq
GN6300	Spontaneous *spc-201* mutant of U1, ancestor for all other GN strains	This study
GN9003	*pro-201*::Tn*5*, *spc-201*, Kan^r	This study
GN9005	*pro-202*::Tn*5*, *spc-201*, Kan^r	This study
GN9006	*pyrA-201*::Tn*5*, *spc-201*, Kan^r	This study
GN9008	*cys-201*::Tn*5*, *spc-201*, Kan^r	This study
GN9010	*lys-201*::Tn*5*, *spc-201*, Kan^r	This study
GN9012	*pur-201*::Tn*5*, *spc-201*, Kan^r	This study
GN9015	*met-201*::Tn*5*, *spc-201*, Kan^r	This study
GN8008	*aro-202*::Tn*10*, *spc-201*, Tet^r	This study
GN6436	*aro-201*, *ile-201*, *val-201*, *spc-201*, *rif-201*	This study
GN6437	*aro-201*, *ile-201*, *ura-201*, *spc-201*, *rif-201*	This study
GN6438	*aro-201*, *ile-201*, *pro-203*, *spc-201*, *rif-201*	This study
GN7007	GN6300::Tn*1*, prototroph	This study
RV79	Prototroph, eltor biotype, Ogawa serotype, ancestor for all RJ strains, growth stimulated by purines	(15)
RJ3	*arg-2*, Str^r	(15)
RJ40	*arg-7*, *ilv-5*, *lys-1*, Rif^r, Str^r	(15)
RJ44	*arg-7*, *ilv-5*, *his-2*, Rif^r, Str^r	(15)
RJ47	*arg-7*, *ilv-5*, *trp-2*, Rif^r, Str^r	(15)
RJ57	*arg-7*, *ilv-5*, *met-4*, *thy-3*, Rif^r, Str^r	(15)
RJ234	RV79::Tn*1*-24, prototroph	(15)
RV88	*pur-1*, *leu-1*, *pro-1 str-2* (derived from classical strain 162)	(17)
E. coli		
HU735	C600 *trpΔE5*, *recA56*	R. Hull
UB1636	*lac*, Str^r, multiply auxotrophic	(20)
C600	*lac*, multiply auxotrophic	(2)

[a] Allele numbers are arbitrary and follow the suggested guidelines of Parker et al. (17). Gene symbols are those of Bachmann (3).

cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Sigma) (1) or by insertion of transposon Tn*5* or Tn*10* (see below). Auxotrophic mutants were detected and characterized by previously described replica plating procedures (8, 11). Spontaneous antibiotic-resistant mutants were selected by plating ca. 10^9 bacteria on MEA containing appropriate antibiotics.

Auxotrophic mutants of GN6300 containing Tn*5* or Tn*10* insertions were isolated in the following manner. GN6300 was mated with *E. coli* HU735(F$'_{ts}$*lac*$^+$ *trp*$^+$ Tn*5*) or *E. coli* C600(F$'_{ts}$*lac*$^+$ Tn*10*). GN6300 transconjugants were isolated by replica plating onto lactose MinV containing kanamycin or tetracycline and incubating the plates at 30°C for 24 to 48 h. Colonies isolated in this way were subcultured in BHI with kanamycin or tetracycline at 30°C overnight. The cultures were diluted 1:100 into fresh BHI without antibiotics and incubated at 40°C for 6 to 8 h. GN6300 Kan^r or Tet^r strains were isolated on MEA containing kanamycin or tetracycline and screened for auxotrophy by replica plating onto glucose MinV. Strains that were auxotrophic, Kan^r or Tet^r, and also lactose negative were presumed to be the result of Tn*5* or Tn*10* chromosomal insertions. These strains were called GN6300::Tn auxotrophs (see Table 1).

Strain GN7007, a prototrophic derivative of GN6300 containing a Tn*1* chromosomal insertion, was isolated by the following procedure. *V. cholerae* GN6300 was mated with *E. coli* UB1636(pMR5). A GN6300(pMR5) transconjugant was isolated by selection on MinV containing tetracycline and was repeatedly subcultured at 30°C in BHI containing tetracycline to permit accumulation of Tn*1* transpositions. Elimi-

TABLE 2. Bacterial plasmids

Plasmid	Relevant characteristics[a]	Source (reference)
P	*V. cholerae* fertility plasmid	(14)
pSJ5	P::Tn*1*, Amp^r (Tn*1*, plus orientation)	(15)
pSJ13	P::Tn*1*, Amp^r (Tn*1*, minus orientation)	(15)
pJN2	pSJ5::Tn*5*, Amp^r, Kan^r (Tn*5*, minus orientation)	This study
pJN8	pSJ5::Tn*5*, Amp^r, Kan^r (Tn*5*, plus orientation)	This study
pJN15	pSJ13::Tn*5*, Amp^r, Kan^r	This study
pJN5	pSJ5::Tn*10*, Amp^r, Tet^r (Tn*10*, minus orientation)	This study
pJN7	pSJ5::Tn*10*, Amp^r, Tet^r (Tn*10*, plus orientation)	This study
F$'_{ts}$ *lac*$^+$*trp*$^+$ Tn*5*	Tn*5* Kan^r, Lac$^+$, temperature sensitive for maintenance	R. Hull
F$'_{ts114}$*lac*$^+$ Tn*10*	Tn*10* Tet^r, Lac$^+$, temperature sensitive for maintenance	(7)
pMR5	Kan^r, Amp^r (Tn*1* or Tn*801*), Tet^r, temperature sensitive for maintenance	(20)

[a] See footnote *a*, Table 1.

nation of pMR5 was accomplished by growth at 42 to 44°C without antibiotic selection. Tn*1* insertion strain GN7007 was identified by screening for its Amp^r, Kan^s, Tet^s phenotype and by demonstrating its ability to function as a Tfr donor after introducing P::Tn*1*,Tn*5* hybrid plasmids.

Construction of P::Tn*1*,Tn*5* hybrid plasmids. *V. cholerae* GN6300(pSJ5) and GN6300 (pSJ13) were individually mated with *E. coli* HU735(F$'_{ts}$*lac*$^+$ *trp*$^+$ Tn*5*). GN6300 Spc^r Amp^r Kan^r transconjugants were isolated from each mating by replica plating on glucose MinV containing kanamycin and confirming the phenotypes of the selected strains. After growth in BHI at 40°C, GN6300 Spc^r Amp^r Kan^r isolates were tested for their ability to transfer both Amp^r and Kan^r determinants to RJ3 recipients. Selected RJ3 Amp^r Kan^r transconjugants were mated with GN6300 recipients to identify strains that transferred the Kan^r and Amp^r determinants with 100% coinheritance. The presence of plasmids with the expected molecular mass of the P::Tn*1*,Tn*5* hybrid was confirmed by agarose gel electrophoresis. P::Tn*1*,Tn*5* plasmids were transferred by conjugation from RJ3 donor strains into selected GN6300::Tn*5* auxotrophs, and the newly constructed strains were tested as Tfr donors. Two P::Tn*1*,Tn*5* plasmids derived from pSJ5 were identified that mediated transposon-facilitated transfer of chromosomal markers in opposite directions from the site of the Tn*5* chromosomal insertion in the donor strain. These two plasmids were designated pJN2 and PJN8. A third P::Tn*1*,Tn*5* plasmid derived from pSJ13 was isolated and designated pJN15.

Construction of P::Tn*1*,Tn*10* hybrid plasmids. *V. cholerae* GN6300(pSJ5) was mated with *E. coli* C600 (F$'_{ts}$*lac*$^+$ Tn*10*), and two P::Tn*1*,Tn*10* plasmids were obtained by using methods analogous to those used to construct pJN2 and pJN8. These two plasmids were designated pJN5 and pJN7.

Construction and characterization of Tfr donors. Appropriate P::Tn*1*,Tn*5* or P::Tn*1*,Tn*10* mobilizing plasmids were introduced by conjugation into GN6300::Tn strains to create new Tfr donors. Each new Tfr donor was tested in plate matings with a variety of *V. cholerae* auxotrophic recipient strains of eltor and classical biotypes. Bacteria from these matings were replicated to appropriately supplemented glucose MinV synthetic medium, and the donor strains were counterselected with rifampin (or streptomycin for matings with RV88 as the recipient strain) and omission of required growth factors. Colony counts were performed after incubating the plates at 30°C for 48 to 72 h.

RESULTS AND DISCUSSION

By using the conditions described, a number of auxotrophic mutations resulting from Tn insertions into biosynthetic genes of *V. cholerae* eltor strain GN6300 were isolated (see Table 1). More than one type of auxotroph was usually recovered from a culture, but only one isolate of each phenotype was analyzed. This was done to insure that each Tn insertion strain was the result of an independent transposition event. We isolated a collection of eight auxotrophic Tn strains with seven different requirements (*met*, *lys*, *cys*, *pro*, *aro*, *pyrA*, and *pur*) (Table 1). These eight Tn strains were converted to Tfr donors by introducing the P hybrid sex factors pJN2, pJN8, pJN5, or pJN7 (Table 2). Conjugal transfer of these plasmids was detected by positive selection

TABLE 3. Patterns of inheritance of selected donor markers in transposon-facilitated recombination in *V. cholerae*[a]

Donor strain and genotype	Recipient strain and genotype[b]												
	RJ57 *met*	RJ44 *ilv*	GN6436 *val*	RJ40 *lys*	RJ44 *arg*	GN6437 *ile*	GN6438 *pro*	RV88 *leu*	GN6437 *ura*	GN6437 *aro*	RJ44 *his*	RJ47 *trp*	RJ57 *met*
GN9015 *met-201*::Tn*5*(pJN8)	*	+	ND	+	+/−	ND	ND	ND	−	ND	−	−	*
GN9015 *met-201*::Tn*5*(pJN2)	*	+/−	ND	+	+	ND	ND	ND	+	ND	+	+	*
GN9012 *pur-201*::Tn*5*(pJN8)	+	−	+/−	* +	+	+	ND	ND	+/−	+	+	+	+
GN9012 *pur-201*::Tn*5*(pJN2)	−	+	+	* +/−	−	−	ND	ND	−	−	−	−	−
GN9010 *lys-201*::Tn*5*(pJN8)	+/−	−	−	*	+	+	+	ND	+	+	+	+	+/−
GN9010 *lys-201*::Tn*5*(pJN2)	+/−	+	+	*	−	−	−	ND	−	+/−	+/−	+/−	+/−
GN9008 *cys-202*::Tn*5*(pJN8)	+/−	−	−	−	− *	+	+	ND	+	ND	+	+	+/−
GN9008 *cys-202*::Tn*5*(pJN2)	−	+	+	+	+ *	−	−	ND	−	ND	−	−	−
GN9003 *pro-201*::Tn*5*(pJN8)	+/−	+	+	+	+	+	*	+/−	+/−	−	+/−	+/−	+/−
GN9003 *pro-201*::Tn*5*(pJN2)	+/−	+/−	−	−	−	−	*	+	+	+	+	+	+/−
GN9005 *pro-202*::Tn*5*(pJN8)	+/−	−	+/−	−	−	−	*	+/−	+	+	+	−	+/−
GN9005 *pro-202*::Tn*5*(pJN2)	−	+	+	+	+	+	*	+	−	−	−	−	−
GN9006 *pryA-201*::Tn*5*(pJN8)	+/−	+/−	−	−	−	−	− *	+	+	+	+	+	+/−
GN9006 *pryA-201*::Tn*5*(pJN2)	+	+	+	+	+/−	+	+ *	−	−	−	−	−	+
GN8008 *aro-202*::Tn*10* (pJN5)	−	ND	ND	ND	+	ND	+	+	+	*	−	−	−
GN8008 *aro-202*::Tn*10*(pJN7)	+	ND	ND	ND	−	ND	−	−	−	*	+	+	+
GN7007::Tn*1*(pJN8)	−	−	ND	−	−	ND	ND	ND	−	ND	+	+ *	−
GN7007::Tn*1*(pJN15)	+	−	ND	−	−	ND	ND	ND	−	ND	−	− *	+
RJ234::Tn*1*-24(pSJ5)	−	−	ND	− *	+	ND	ND	ND	+	ND	−	+/−	−
RJ234::Tn*1*-24(pSJ13)	+	+	ND	+ *	−	ND	ND	ND	−	ND	−	+/−	+
GN6300(pJN2) or (pJN8)	−	−	−	−	−	−	−	−	−	−	−	−	−
GN6300(pJN5) or (pJN7)	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] For each mating, the selected recombinants expressed the Rif^r or Str^r phenotype of the recipient strain and the wild-type donor allele corresponding to the specified auxotrophic marker of the recipient. The relevant auxotrophic markers of the recipient strains are listed in the sequence that corresponds to their order on the genetic map of *V. cholerae* (see Fig. 1). The circular genetic map was broken at the *met* locus to give the linear order presented here. For each pair of donor strains, matings with all of the recipient strains tested were performed at the same time.

[b] Results are based on average colony counts from three or more independent experiments. +, >100 recombinant colonies per plate; −, either <30 recombinant colonies per plate or <1/10 the number of recombinant colonies observed with the isogenic donor strain containing the hybrid plasmid with the opposite orientation of the mobilizing transposon; +/−, intermediate results. *, Position of the transposon insertion in the chromosome of the donor strain. ND, Not done. For examples of absolute numbers of recombinants observed and analysis of the linkage of unselected markers to selected markers in matings performed by this method, see reference 12. We did not observe transposon-facilitated recombination between the following pairs of donor and recipient alleles: *lys-201* and *lys-1*, *pro-201* and *pro-203*, or *pro-202* and *pro-203*. Numbers of *met*$^+$ recombinants were consistently above background levels by approximately two-fold in matings between GN9015 *met-201*::Tn*5*(pJN2) and RJ57 *met-4*, demonstrating that the *met-201* and *met-4* mutations are not at identical sites.

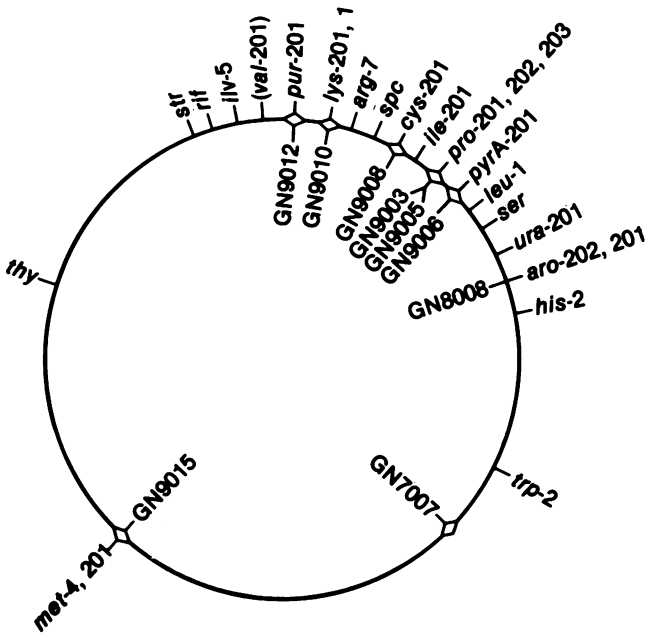

FIG. 1. Circular genetic map of *V. cholerae* eltor strain GN6300. The map is a composite based on published linkage data obtained with classical and eltor strains of *V. cholerae* (17, 21) and the data in Table 3. Relative distances between genetic markers are approximate. The sites of chromosomal insertions of transposons in our Tfr donor strains are indicated by diamonds on the map adjacent to the strain numbers. The positions of the alleles *ura-201*, *ile-201*, and *val-201* were determined relative to the sites of chromosomal insertion of the transposons in our donor strains. Our results with *aro-202* place it on the opposite side of *his-2* from the reported position of the *aro-10* locus (21). Our data indicated that the segment of the genetic map of GN6300 from *pro-201* to *ura-201* was inverted with respect to the map reported for the classical strain *V. cholerae* 162 (21). We also confirmed that the *ilv-lys* segment in *V. cholerae* eltor was inverted with respect to its orientation in the classical strain *V. cholerae* 162 (15, 21). The position of *val-201* has not been determined precisely by linkage analysis. Alleles without numerical designations were not used in the present study.

for the Amp^r phenotype controlled by Tn*1* and counterselection of the donor strain by omission of required growth factors. By this approach, we eliminated the need to use nonselective screening tests such as the lacunae assay (6) or agarose gel electrophoresis to detect acquisition of the P plasmid during construction of new donor strains.

Next, the newly constructed Tn*5* and Tn*10* donor strains were characterized in conjugal matings (Table 3). Inocula from the mating mixtures were transferred by replica plating onto appropriate selective media, and the numbers of selected recombinant colonies were counted. For each pair of donor and recipient strains, we determined whether the selected donor allele was inherited at an increased frequency characteristic of transposon-facilitated recombination, an intermediate frequency, or a low frequency that was independent of Tfr. Presentation of the data in this manner facilitated identification of groups of markers, presumed to be contiguous on the genetic map of *V. cholerae*, that were inherited at higher frequency from specific Tfr donor strains. The results presented in Table 3 can be summarized as follows: (i) in each Tfr mating a specific subset of donor markers was inherited at an increased frequency; (ii) for each Tn chromosomal insertion, a different subset of donor markers was inherited at a higher frequency; (iii) reversal of the orientation of the mobilizing transposon on the P hybrid sex factor caused a change in the subset of markers that was detected in recombinants at an increased frequency; (iv) the behavior of our newly isolated Tn*5* and Tn*10* donor strains was comparable with that of the previously characterized Tn*1* donors RJ234::Tn*1*(pSJ5) and RJ234::Tn*1*(pSJ13) under the conditions of our experiments; and (v) the control strains GN6300(pJN2), GN6300(pJN8), GN6300(pJN5), and GN6300(pJN7), which contained Tn*5* or Tn*10* in the hybrid P plasmid but not in the bacterial chromosome, did not function as Tfr donor strains. The data presented in Table 3 are consistent with the order of markers shown on the genetic map of *V. cholerae* eltor in Fig. 1. For example, the *ile*, *pro*, *ura*, *his*, and *trp* markers were inherited at highest frequency from the GN9008(pJN8) donor, whereas the *arg*, *lys*, *val*, and *ilv* markers were inherited at highest frequency from the GN9008(pJN2) donor. The assumptions that the donor chromosome is mobilized in an oriented manner from the site of the chromosomal Tn*5* insertion, that chromosomal mobilization is usually incomplete, and that Tn*5* is present in opposite orientations in the pJN8 and pJN2 sex plasmids provided a rational explanation for these findings. We concluded that the chromosomal *cys-201* locus, corresponding to the site of insertion of Tn*5* in strain GN9008, was located between *arg* and *ile*. Similar reasoning permitted us to assign relative locations on the genetic map for the sites of the Tn insertions in each of the independently isolated Tn strains. Donor markers that were inherited at intermediate frequencies in these matings were usually located adjacent to the markers inherited at high frequencies, but opposite from the sites of insertion of Tn*5* on the genetic map. We concluded that the transition from high to intermediate to low frequencies of inheritance of selected markers most likely represented a decreasing probability of transfer and recombination of the corresponding markers from the Tfr donors into the recipient strains during conjugation. The circularity of the chromosome of *V. cholerae* eltor was strongly supported by the overlapping patterns for the groups of genetic determinants that were inherited at elevated frequency from the different Tfr donor strains shown in Table 3.

In conclusion, we developed generally applicable methods for introducing transposons into *V. cholerae* for use in genetic studies, produced a significantly expanded set of Tfr donor strains, and determined the locations of the chromosomal transposon insertions in these strains on a circular genetic map of *V. cholerae* eltor. In related studies we have successfully applied the general methods described here for mapping determinants of the eltor biotype of *V. cholerae* (12) and for constructing a DNase-negative strain of *V. cholerae* by recombinant DNA techniques (J. W. Newland, B. A. Green, J. Foulds, and R. K. Holmes, manuscript in preparation). It should also be possible to generalize the techniques we have developed with transposons Tn*1*, Tn*5* and Tn*10* in *V. cholerae* for use with other transposons. This will enable investigators to tailor the choice of transposons for use in future studies based on specific or unique properties of individual transposons (16). Finally, we developed the first mating system in *V. cholerae* that uses strains derived directly from a virulent clinical isolate. The strains described here should be particularly useful for genetic studies of virulence in *V. cholerae*.

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