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Construction and Characterization of the Hybrid Bacteriophage Lambda Charon Vectors for DNA Cloning†

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Twenty hybrid lambda phages especially designed for molecular cloning have been constructed and named Charon phages. These phages differ in the ranges of sizes of DNA fragments that may be inserted, by the selections and screens which may be used to isolate and detect the incorporation of cloned fragments, by the way transcription of the cloned fragment may be controlled, by the different restriction enzymes that can be used for cloning, by the phage immunities that may be employed for controlling replication and transcription, and by the biological safety features that they contain. The crosses used to produce the vectors are described, and their genealogy is discussed. The structure of each vector has been verified by genetic tests, by DNA length determinations, by electron micrographic analysis of DNA heteroduplexes, and by gel electrophoresis of restriction enzyme digests. In the course of these constructions, a new *Eco*RI site was found in a derivative of λ *Aam32Bam1* which maps very near the left cohesive end of λ.

The genome of bacteriophage λ is organized so that the central one third (the replaceable region, Fig. 1) contains genes which are entirely dispensable for lytic growth. As a consequence of this, specialized transducing phages of λ had been used for many years before the development of in vitro DNA joining techniques (39) to propagate foreign DNA segments of *Escherichia coli*. Hence, a variety of techniques for studying the organization and expression of DNA inserted into λ is available. With the emergence of recombinant DNA technology, it was a natural extension to adapt λ so that DNA from any source could be introduced into the replaceable region of its genome.

Several groups undertook construction of λ vectors (12, 13, 19, 20, 45, 47, 57, 59). The series that we have constructed, called the Charon phages, now includes 20 vectors. The first 16 in the series have been generally described in a previous publication (12) along with the results of experiments relating to biological containment (63). In this communication, we describe the construction steps used to produce the 20 vectors now available, the rationale behind their construction, and the determination of their structures.

MATERIALS AND METHODS

Media and plates. The NZ broth used for propagation of all strains in liquid culture contains 1.0 g of $MgCl_2$, 5 g of NaCl, and 10 g of NZ-amine A (Humko-Sheffield Division of Kraft Co. Corp., Lyndhurst, N.J.) per liter. NZC broth contained in addition 1 g of Casamino Acids per liter, whereas NZY contained 5 g of yeast extract per liter. For plating media, NZ broth was supplemented with 1.2% (bottom) or 0.7% (top) agar (Difco), respectively. Indicator plates for the β-galactosidase assay in addition contained 40 mg of the dye 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG, Bachem Inc., Marina Del Ray, Calif.) per liter. The dye was first dissolved in 2 ml of dimethylformamide and added to freshly autoclaved bottom agar immediately before pouring plates (40).

Phage and bacterial strains. Table 1 lists the phage strains used in this study with relevant genotypes, sources, and references. All bacterial strains used in this study are listed in Table 2 and were derived from *E. coli* K-12.

Phage propagation. Phage stocks were propagated in liquid lysates by the preadsorb-dilute-shake method (12). After at least two cycles of purification by single-plaque isolation, well-isolated plaques were picked with a Pasteur pipette, and the agar plug was resuspended in 1 ml of plaque storage buffer (0.1 M NaCl-0.05 M Tris-hydrochloride [pH 7.9]-0.01 M $MgCl_2$-0.01% gelatin, saturated with chloroform). The plaques were either allowed to soak for 15 min or gently agitated on a Vortex mixer until the top agar dislodged from the agar plug before further handling.

Primary cultures were grown by mixing 0.1 ml of stationary-phase bacterial cells, 0.1 ml of MgCa (0.01 M $MgCl_2$-0.01 M $CaCl_2$), and 0.1 ml of the resuspended plaque in a 125-ml Erlenmeyer flask and shaking for 10 min at 37°C (the preadsorption step). A 50-ml amount of NZ, NZC, or NZY broth was added, and the culture was shaken at 37°C. The broth used depended on nutritional requirements of the host bac-

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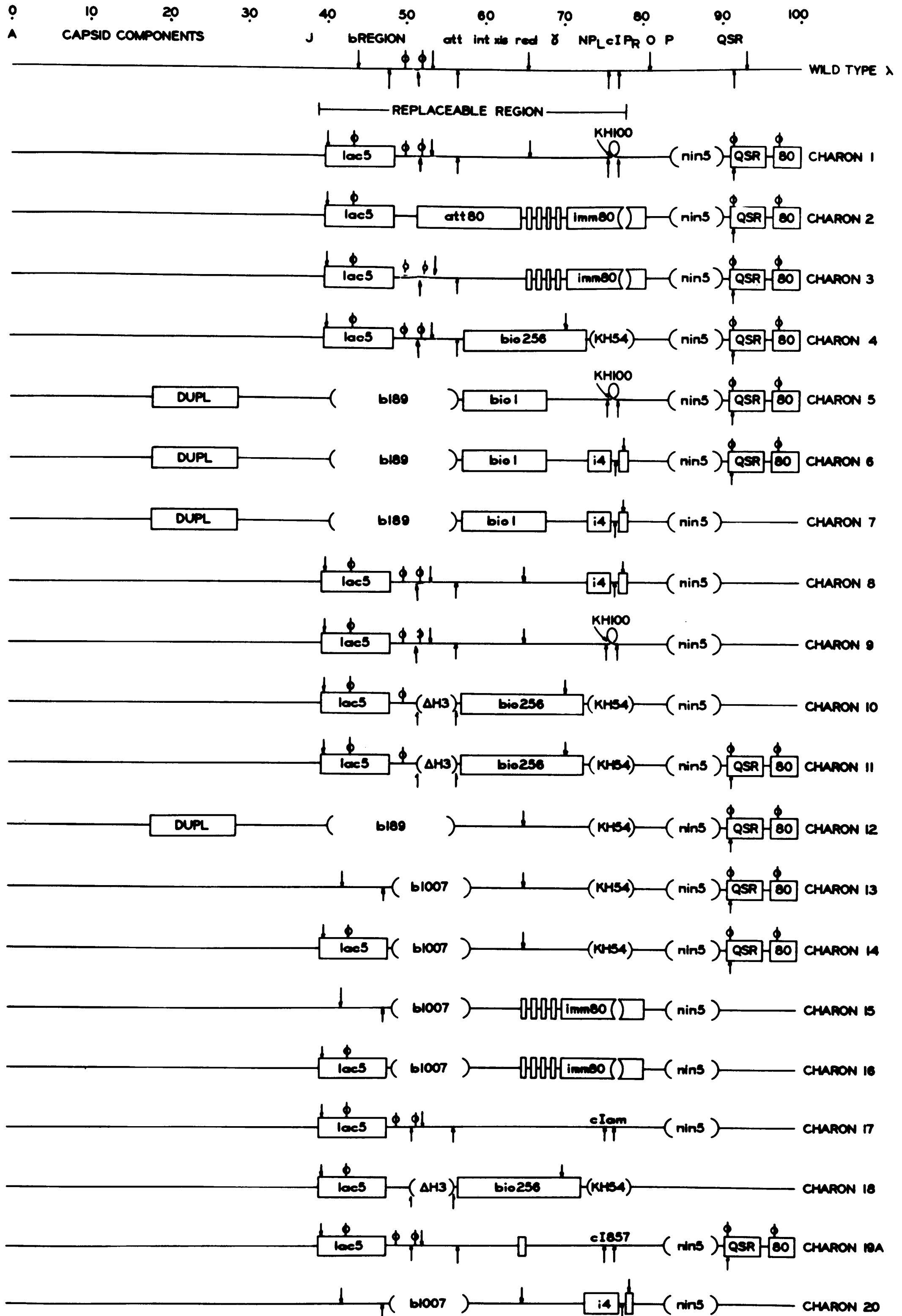

0 10 20 30 40 50 60 70 80 90 100
A CAPSID COMPONENTS J bREGION att int xis red γ N P_L cI P_R O P QSR
WILD TYPE λ
REPLACEABLE REGION
lac5 KH100 nin5 QSR 80 CHARON 1
lac5 att80 imm80 nin5 QSR 80 CHARON 2
lac5 imm80 nin5 QSR 80 CHARON 3
lac5 bio256 (KH54) nin5 QSR 80 CHARON 4
DUPL b189 bio1 KH100 nin5 QSR 80 CHARON 5
DUPL b189 bio1 i4 nin5 QSR 80 CHARON 6
DUPL b189 bio1 i4 nin5 CHARON 7
lac5 i4 nin5 CHARON 8
lac5 KH100 nin5 CHARON 9
lac5 ΔH3 bio256 (KH54) nin5 CHARON 10
lac5 ΔH3 bio256 (KH54) nin5 QSR 80 CHARON 11
DUPL b189 (KH54) nin5 QSR 80 CHARON 12
b1007 (KH54) nin5 QSR 80 CHARON 13
lac5 b1007 (KH54) nin5 QSR 80 CHARON 14
b1007 imm80 nin5 CHARON 15
lac5 b1007 imm80 nin5 CHARON 16
lac5 cIam nin5 CHARON 17
lac5 ΔH3 bio256 (KH54) CHARON 18
lac5 cI857 nin5 QSR 80 CHARON 19A
b1007 i4 nin5 CHARON 20

teria. For example, NZC was used with K802 to supply its methionine requirement. Phages making turbid plaques were visually monitored and harvested after the appearance of lysis debris, whereas clear-plaque phage were generally grown overnight. Lysates were harvested by adding chloroform, swirling, and centrifuging to remove bacterial debris (5 min at 8,000 rpm in a Sorvall GSA). The supernatant phage stock was stored at 4°C with additional chloroform.

Large-scale secondary stocks were prepared by preadsorbing 0.3 ml of cells and 0.3 ml of MgCa with 6×10^5 phage (10^{-3} multiplicity of infection [MOI]). This was then added to 1 liter of broth and shaken overnight at 37°C. We found that lysis tended to occur sooner when a higher MOI was used, but the final titer was largely insensitive to an MOI above 10^{-3} for most lambda phages tested. For phages of $\phi80$ host range, an MOI of 1.0 gave the best results with the preadsorb-dilute-shake method.

DNA preparation. Phage were grown in 12-liter batches, concentrated by evaporation, and purified by differential centrifugation, polyethylene glycol precipitation, and two steps of isopycnic centrifugation in CsCl. DNA was prepared from the phage by three phenol extractions as described previously (61).

Phage crosses. Phage crosses were done by cross-streaking parental phage stocks at titers of about 10^9 phage per ml on lawns of bacteria on plates as described elsewhere (11). The use of UV light to stimulate recombination was avoided to minimize the possibility of introducing silent mutations in the recombinant, which might complicate later genetic or DNA sequencing studies on these phages. The progeny phages were plated on the appropriate bacterial hosts for selection or screening as described below. A few candidates for each recombinant were purified by two cycles of single-plaque isolation on a host strain permissive for both parents. Single plaques were picked from each purified candidate, and primary stocks were grown in liquid. These were given isolation numbers and tested for the desired properties.

Plating tests. The various plating phenotypes used either as the basis for selections or tests in strain construction or in characterizing the recombinants included: Sus^+, the ability to plate on a strain lacking amber suppressors; Spi^-, the ability to plate on P2 lysogens (67); Fec^-, the inability to plate on *recA* strains (30, 66); h^λ, the inability to plate on λ-resistant strains; h^{80}, the inability to plate on $\phi80$-resistant strains; immunity, the inability to plate on a lysogen carrying a prophage of like immunity (34); N independence, the ability to plate on *groN* strains (26, 27). In addition to the above phenotypes, which provide the basis for selections, the following tests were also used. Clear- versus turbid-plaque morphologies were usually determined on Ymel or CSH18. K802 was not used because turbidity did not develop well on that strain. Bio-transducing phages were detected on lawns of W602DC (*bioA*, carried by both *bio*1 and *bio*256) or B583 (which is deleted for the entire Bio operon and is complemented by *bio*256 but not *bio*1) on minimal plates limiting for biotin (35). *att* site specificity was identified by measuring recombination frequencies between phage markers flanking the attachment region in test crosses with phages carrying *att*80 or *att*λ. *lac*5 was detected by the formation on dark blue plaques with blue halos on XG plates on lawns of $LacZ^+$ or $LacZ^-$ cells. Phages giving light blue plaques on $LacZ^+$ cells but colorless plaques on $LacZ^-$ cells had the Lac operator and an interrupted *lacZ* gene (12). Complementation tests were done for A^- and B^- mutations by using paper strips to cross-streak the phage to be tested against tester phages carrying either *Aam*32 or *Bam*1. A lawn of Su^o host cells, usually W3350, was used. The test was scored for lysis in the zone of intersection of the streaks. Positive controls were included in all tests where the absence of lysis indicated the phenotype.

Genetic selections and recognition procedures. The selections referred to in Table 3 were made where possible by plating phages from crosses on a host chosen so that only the desired recombinants formed plaques. Where this was not possible, selection was used against only one parent, and the recombinant was identified on the basis of recognizable characteristic such as blue- or turbid-plaque morphology. In some cases screening was done in two steps. For example, blue or white plaques on one selective indicator could be transferred by toothpicks to other plates containing permissive and nonpermissive indicators. The desired recombinant could then be recognized by its plating phenotype on the latter strains. The particular stratagem employed for each cross is listed in Table 3.

Removing *Eco*RI restriction sites by cycling. The method of Arber and Kühnlein (5) was used. Cycles of growth on an *Eco*RI modifying and restricting host (R. N. Yoshimori, Ph.D. thesis, University of California-San Francisco Medical Center, San Francisco, Calif., 1971) were alternated with growth on a host lacking modification and restriction. Phage strains were constructed with only the one or two sites to be removed. Liquid cultures of the phages were grown first on pBRY-13/1100.5 carrying the *Eco*RI restriction and modification system to select for mutations resulting in the loss of the *Eco*RI site. Aliquots

FIG. 1. *Genetic and physical map of λ and the Charon phages. The phage map is drawn to scale with the replaceable region indicated relative to essential genes for lytic growth. Beneath it are the Charon phage vectors with portions from λ (indicated by a single line) aligned with the λ map. Parentheses indicate deletions. Substitutions are indicated by boxes whose end points define the λ DNA deleted and do not correspond in length to the amount of DNA substituted. The total DNA length of each vector is given in Table 5. The downward and upward arrows indicate EcoRI and HindIII sites, respectively. The symbol ϕ represents Sst I sites. DUPL is a duplicated piece of phage λ. lac5, bio1, and bio256 are substitutions from lac and bio regions of E. coli. The boxes labeled att80, imm80, and QSR80 are portions of phage $\phi80$. The region shown by four small boxes around 70%λ in some phages is from $\phi80$; it is partially homologous to λ. imm434 is a substitution for imm from $\phi434$; KH100 is an insertion in λcI.*

TABLE 1. *Phage strains used in this study*[a]

Name	Genotype	Source or reference
Hy42	λ *att*80 *imm*80 *nin*5 *QSR*λ	(53)
WB1	λ *lac*5 *att*80 *imm*21 *cIts nin*5 *Sam*7	Wayne Barnes, unpublished but see (7)
HY42 S7	λ *att*80 *imm*80 *nin*5 *Sam*7	This study ⑦ BW*24
GA1	λ *lac*5 *att*80 *imm*80 *nin*5 *Sam*7	This study ⑧
KH341	λ *b*519 *b*515 *bio*Δ(*att-N*) *imm*λ *nin*5 *QSR*λ	Karen Hass, unpublished
BW3-3	λ *b*519 *att*80 *imm*80 *nin*5 *Sam*7	This study ⑨
φ80 *imm*λ	φ80 *att*80 *imm*λ *QSR*80	(50)
Fbn°	λ *b*519 *att*80 *imm*80 *nin*5 *QSR*80	This study ⑩ no. 1414
Fbn^{15}	λ *b*519 *att*80 *imm*80 ΔKH53 BW2 *nin*5 *QSR*80	This study ⑪ no. 1415
biol	λ *bio*l	(38)
cI857 S7	λ *c*I857 *Sam*7	This laboratory; see (28)
biol S7	λ *bio*l *Sam*7	This study ①
BC4	λ *bio*l *nin*5 *QSR*80	This study ② BW9-2a
BC5	λ *lac*5 *bio*l *nin*5 *QSR*80	This study ③ BW13-3
plac5 KH100	λ *lac*5 KH100 *Pam*80	This laboratory; see (11)
Rb189	λ *b*189 *imm*80 *QSR*80	Eric Rosenvald, unpublished
Bbn°	λ *dup*L *b*189 *bio*l *nin*5 *QSR*80	This study ④ BW15-2a no. 1416
Bbn^{10}	λ *dup*L *b*189 *bio*l BW1 *nin*5 *QSR*80	This study ⑤ Bbn^{10}15-lt no. 1417
KH100 *Pam*80	λ *insertion in* cI: KH100 *Pam*80	(11)
Charon 1	λ *lac*5 KH100 BW1 *nin*5 *QSR*80	This study ⑥ BW20$_1$-2N no. 1127
Charon 5	λ *dup*L *b*189 *bio*l KH100 BW1 *nin*5 *QSR*80	This study ⑮ no. 1149
Charon 2	λ *lac*5 *att*80 *imm*80 KH53 BW2 *nin*5 *QSR*80	This study ⑫ BW22-1 no. 1153
Charon 3	λ *lac*5 *att*λ BW3 *imm*80 KH53 BW2 *nin*5 *QSR*80	This study ⑫ BW23-1 no. 1128
plac5 S7	λ *lac*5 *Sam*7	This laboratory; see (32)
FS	λ *lac*5 *bio*256 KH54 *Pam*80	This study ⑬
JS2	λ *bio*256 KH54 *Pam*80	John Salstrom
λi4 029	λ *imm*434 *Oam*29	John Salstrom
D13	λ *bio*256 KH54 BW1 *nin*5 *QSR*80	This study ⑯ no. 1064
Charon 4	λ *lac*5 *bio*256 KH54 BW1 *nin*5 *QSR*80	This study ⑭ no. 1100
ΔH3	λ *lac*5 ΔH3 (s*Hin*dIII 2-3) *c*I857	Dietmar Kamp, unpublished
509	λ *b*538 sRI3° *c*I*am*509 sRI4° s*Hin*dIII6° sRI5^{+}	Noreen Murray; (44)
509-5W	λ *b*538 sRI3° *c*I*am*509 sRI4° s*Hin*dIII6° DK1	This study ⑱ no. 1152
Charon 11	λ *lac*5 ΔH3 *bio*256 KH54 BW1 *nin*5 *QSR*80	This study ⑰ BW33-2 no. 1316
Charon 18	λ *lac*5 ΔH3 *bio*256 KH54 sRI4° s*Hin*dIII6° DK1	This study ⑲ DK19-6 no. 1373
7W	λ *imm*434 sRI4° *nin*5 s*Hin*dIII6° DK1	This study ㉒ DK7W no. 1208
Charon 10	λ *lac*5 ΔH3 *bio*256 KH54 sRI4° *nin*5 s*Hin*dIII6° DK1	This study ⑳ DK22-1 no. 1385
CB3	λ *lac*5 KH100 sRI4° s*Hin*dIII6° DK1	This study ㉑ DK12, CB3 no. 1309
Charon 17	λ *lac*5 sRI3° *c*I*am* sRI4° *nin*5 s*Hin*dIII6° DK1	This study ㉑ DK5, NRE2 no. 1189
Charon 6	λ *dup*L *b*189 *bio*l *imm*434 sRI4° *nin*5 *QSR*80	This study ㉓ L.A.F. 2b of + no. 1282
Charon 7	λ*dup*L *b*189 *bio*l *imm*434 sRI4° *nin*5 s*Hin*dIII6° DK1	This study ㉓ L.A.F. Ch6C6 no. 1321
NRE-4W	λ *dup*L *b*189 *imm*434 sRI4° *nin*5 s*Hin*dIII6° DK1	This study ㉓
*b*1007	λ *b*1007	(30)
Charon 20	λ *b*1007 *imm*434 sRI4° *nin*5 s*Hin*dIII6° DK1	This study ㉔ DK24-4 no. 1420
Charon 8	λ *lac*5 *imm*434 sRI4° *nin*5 s*Hin*dIII6° DK1	This study ㉒ DK7B
Charon 15	λ *b*1007 BW3 *imm*80 KH53 BW2 *nin*5 s*Hin*dIII6° DK1	This study ㉕ DK26-3
Charon 13	λ *b*1007 KH54 BW1 *nin*5 *QSR*80	This study ㉖ DK27-2-1
Charon 14	λ *lac*5 *b*1007 KH54 BW1 *nin*5 *QSR*80	This study ㉖ DK27-1
Charon 12	λ *dup*L *b*189 KH54 BW1 *nin*5 *QSR*80	This study ㉗ L.A.F. A131/1
Charon 19A	λ *Aam*32*Bam*1 *lac*5 BW3 *c*I857 sRI4° *nin*5 *QSR*80	This study ㊲ BWB1 no. 1747
Charon 16	λ *lac*5 *b*1007 BW3 *imm*80 KH53 BW2 *nin*5 s*Hin*dIII6° DK1	This study ㉘ DK32-6
Charon 9	λ *lac*5 KH100 sRI4° *nin*5 s*Hin*dIII6° DK1	This study ㉙ DK13-1
λA32B1	λ *Aam*32*Bam*1	William Dove; (14)
Ch3A	λ *Aam*32*Bam*1 *lac*5 BW3 *imm*80 KH53 BW2 *nin*5 *QSR*80	This study ㉚ BW26-1
Ch4A	λ *Aam*32*Bam*1 *lac*5 *bio*256 KH54 BW1 *nin*5 *QSR*80	This study ㉛ BW27-2 no. 1186

TABLE 1—*Continued*

Name	Genotype	Source or reference
Ch3AΔ*lac*	λ *Aam32Bam*1 Δ(sRI*lac*5-sRIλ2) BW3 *imm*80 KH53 BW2 *nin*5 *QSR*80	David Grunwald ㉜ no. 1366
Ch14A	λ *Aam32Bam*1 *lac*5 *b*1007 KH54 BW1 *nin*5 *QSR*80	This study ㉝ DK31-1 no. 1437
Ch13A	λ *Aam32Bam*1 *b*1007 KH54 BW1 *nin*5 *QSR*80	This study ㉞ DK29-1 no. 1433
CH15A	λ *Aam32Bam*1 *b*1007 BW3 *imm*80 KH53 BW2 *nin*5 s*Hin*dIII6° DK1	This study ㉟ DK30-1 no. 1434
Ch16A	λ *Aam32Bam*1 *lac*5 *b*1007 BW3 *imm*80 KH53 BW2 *nin*5 s*Hin*dIII6° DK1	This study ㊱ DK43-1 no. 1674
λgtWES·λ$\overleftarrow{B}$	λ *Wam*403*Eam*100 λ$\overleftarrow{B}$ ΔλC *c*I857 sRI4° *nin*5 sRI5° *Sam*100	P. Leder; (59)
BW27-1	λBW4 *Aam32Bam*1 *lac*5 *bio*256 KH54 BW1 *nin*5 *QSR*80	This study ㉛ BW27-1 no. 1273

[a] Where the source is "this study," the circled number indicates the cross in Fig. 2 and Table 3 in which the phage was constructed, followed by the originator's designation of the strain and in some cases the stock number in this laboratory. The mutations BW1, BW2, BW3, DK1, and KH53 remove *Eco*RI cleavage sites (12). *dup*L is a spontaneous duplication in the left arm of λ (12). BW3 is a hybrid which removes the *Eco*RI site in the λ *exo* gene by the substitution of an as yet undetermined amount of DNA from the φ80 Red-*gam* region where the phages share intermittent partial homology as seen in heteroduplexes between them (23). BW4 is a new *Eco*RI cut mapping 0.3%λ to the right of the left cohesive end.

TABLE 2. *Bacterial strains*

Bacteria	Characteristics used in these constructions	Source and reference
Ymel	*su*3	This lab; (56, 65)
W3350	*su*°	This lab; (14)
TC600	*su*2, φ80ʳ, λˢ	This lab; derived from C600 (4)
N100	*su*°, *recA*	This lab; (29)
GroN	*su*°, *groN*	J. Salstrom; (26)
IK11	*su*°, *recA*, Δ(*lac-pro*) XIII	I. Kuhn; (48)
pBRY-13/1100.5	*su*°, contains *Eco*RI restriction-modification system	J. Davies, unpublished
QR48	*su*², *recA*	This lab; (51)
K802	*su*2, hsr$_K^-$ hsm$_K^+$, Met⁻, Gal⁻	E. Kort; (64)
B583	Δ(*gal-uvr-bio*)	S. R. Jaskunas
W602 DC	*bioA*	D. Court, through G. Kayajanian
Ymel(λ)	*su*3, λ*imm*λ	This lab
Ymel(φ80)	*su*3, φ80*imm*80	J. Salstrom
Ymel(λi21)	*su*3, λ*imm*21	This lab, see (37)
Ymel(λi434)	*su*3, λ*imm*434	This lab
Ymel(P2)	*su*3, P2 Old⁺	This lab; (52)
Ymel/φ80	φ80ʳ, *su*3	This study
Ymel(WB1)	*su*3, λp*lac*5 *att*80 *imm*21 *c*I*ts nin*5 *Sam*7	This lab
C600 (P2)	*su*2, P2 Old⁺, φ80ʳ, λˢ	This lab; (52)
W3350 (P2)	*su*°, P2 Old⁺	This lab; (52)
W3101(φ80)	*su*°, φ80*imm*80	This lab; (6)
GroP(P2)	*su*2, P2 Old⁺, *groP*	J. Salstrom; (27)
QR48(λi434)	*su*2, *recA*, λ*imm*434	This lab; (51)
SA443(λi21)	*su*°, λ*imm*21	This lab; (1)
SA439(88)	*su*°, λ*bio*24-5Δ *int-rex O-R-A-J*. (λ *c*I⁺)	This lab; (1)

were treated with chloroform, titrated, and then grown on Ymel to dilute out the modified *Eco*RI sites from the survivors, and the cycle was repeated. The restriction ratio, defined as the ratio of the titer on pBRY-13/1100.5 to the titer on Ymel, was determined after each cycle. When the restriction ratio of the cycled lysate reached 0.5, individual candidate plaques were picked, purified by plaque isolation, and grown into primary culture, and the restriction ratio was again determined. DNA from promising candidates was then subjected to gel electrophoresis after digestion with *Eco*RI and *Eco*RI/*Hin*dIII mixtures.

Gel electrophoresis of restriction digests. Gel electrophoresis was done in horizontal agarose gels as described by Shinnick et al. (49). When it was important to determine the size of both large and small fragments from a given phage, two gels were run. Large fragments were best displayed on 0.8% agarose

TABLE 3. *Crosses and construction steps for the Charon phages*[a]

No.	Cross or step	Selection or recognition procedure	Derivative names	Parental names
1	λ *bio*1 λ cI857 *Sam*7	Screen for (−) on TC600 among plaques on Ymel (P_2)	*bio*1 *S7*	*bio*1 cI857 *S7*
2	λ *bio*1 *Sam*7 λ *b*519 *att*80 *imm*80 *nin*5 *QSR*80	Selected on W3101 (φ80)	BC4	*bio*1 *S7* Fbn[0]
3	λ *bio*1 *nin*5 *QSR*80 λ *plac*5 *Sam*7	Screen for blues on C600 (P_2) XG	BC5	BC4 λ*lac*5 *S7*
4	λ *plac*5 *bio*1 *nin*5 *QSR*80 λ *b*189 *imm*80 *QSR*80	Screen for whites on Ymel (φ80) XG	Bbn[0]	BC5 Rb189
5	λ DUPL *b*189 *bio*1 sRI4⁺ *nin*5 *QSR*80 10 cycles of RI selection λ DUPL *b*189 *bio*1 BW1 *nin*5 *QSR*80	Grow alternately on Ymel and then pBRY-13/1100.5	Bbn[10]	Bbn[0]
6	λ DUPL *b*189 *bio*1 BW1 *nin*5 *QSR*80 λ *plac*5 KH100 sRI4⁺ *Pam*80	Select clear blues on IK11 XG	Charon 1	Bbn[10] *lac*5 KHlCO
7	λ *att*80 *imm*80 *nin*5 or λ *plac*5 *att*80 *imm*21 *nin*5 *Sam*7	Screened for (−) on SA443 (λ*imm*21) from plaques on Ymel (WB1)	Hy42 *S7*	Hy42 WB1
8	λ *att*80 *imm*80 *nin*5 *Sam*7 λ *plac*5 *att*80 *imm*21 *nin*5 *Sam*7	Screen for blue on Ymel (λ*imm*21)	GA1	Hy42 *S7* WB1
9	λ *plac*5 *att*80 *imm*80 *nin*5 *Sam*7 λ *b*519 *b*515 *bio-att-N* *nin*5	Screen for white on Ymel (λ)	BW3-3	GA1 KH341
10	λ *b*519 *att*80 *imm*80 *nin*5 *Sam*7 φ80 *imm*λ *QSR*80	Select on SA439 (88)	Fbn[0]	BW3-3 φ80 *imm*λ
11	λ *b*519 *att*80 *imm*80 *nin*5 *QSR*80 ↑ ↑ 15 cycles of RI selection λ *b*519 *att*80 *imm*80 KH53* BW2* *nin*5 *QSR*80	Grow alternately on Ymel and then pBRY-13/1100.5	Fbn[15]	Fbn[0]

TABLE 3—*Continued*

No.	Cross or step	Selection or recognition procedure	Derivative names	Parental names
12	λ *b519* *att80* *imm80* KH53* BW2* *nin5* *QSR80*	Screen blues on Ymel (λ)XG	Charon 2	Fbn[15]
	Ch2 Ch3		Charon 3	
	λ *plac5* *att*λ KH100 *Pam80*			*plac5* KH100
13	λ *plac5* KH100 BW1* *nin5* *QSR80*	Screen blues on GroP (P_2)	FS	Charon 1
	λ *bio256* KH54 P80			JS2
14	λ *plac5* *bio256* KH54 P80	Screen clear blues on W3350	Charon 4	FS
	λ *dupL* *b189* *bio1* BW1* *nin5* *QSR80*			Bbn[10]
15	λ *dupL* *b189* *bio1* BW1* *nin5* *QSR80*	Screen clear whites on Ymel (P_2)	Charon 5	Bbn[10]
	λ *plac5* KH100 BW1* *nin5* *QSR80*			Charon 1
16	λ *plac5* *bio256* KH54 BW1* *nin5* *QSR80*	Screen whites on W3350 (P_2)	D13	Charon 4
	λ *imm434* *Oam29*			λi4 029
17	λ *plac5* ΔH3 cI857	Select blues on Ymel (P_2)	Charon 11	ΔH3
	λ *bio256* KH54 BW1* *nin5* *QSR80*			D13
18	λ *b538* *s*RI3° c*Iam* sRI4° *sHind*III6° *s*RI5⁺	Alternate growth on W3350 and then on pBRY-13/1100.5	509-5W	509
	3 cycles of RI selection			
	λ *b538* *s*RI3° c*Iam* sRI4° *sHind*III6° DK1*			
19	λ *b538* *s*RI3° c*Iam* sRI4° *sHind*III6° DK1*	Screen for (−) on GroN from clear blues on Ymel (P_2)	Charon 18	509-5W
	λ *plac5* ΔH3 *bio256* KH54 BW1* *nin5* *QSR80*			Charon 11
20	λ *plac5* ΔH3 *bio256* KH54 sRI4° *sHind*III6° DK1*	Screen for blue clears on Ymel (*imm434*) and Ymel (P_2) from GroN	Charon 10	Charon 18
	λ *imm434* sRI4° *nin5* *sHind*III6° DK1*			7W
21	λ *plac5* KH100 BW1* *nin5* *QSR80*	Screen on GroN among blues on Ymel XG	Charon 17	Charon 1
	CB3→ CH17		CB3	
	λ *b538* *s*RI3° c*Iam* sRI4° *sHind*III6° DK1*			509-5W
22	λ *imm434* *Oam29*	Screen (+) on GronN among SA88 (+)	Charon 8	λi4 029
	7W Ch8		7W	
	λ *plac5* cIam sRI4° *nin5* *sHind*III6° DK1*			Charon 17

TABLE 3—*Continued*

No.	Cross or step	Selection or recognition procedure	Derivative names	Parental names
23	λ *plac5* *imm434* sRI4" *nin5* s*Hin*dIII6" DK1* NRE4W→ Ch7→ Ch6↘ λ *dupL* *b189* *bio1* KH100 BW1* *nin5* *QSR80*	From whites on SA88, screen on Ymel (P_2): (+) for CH6, CH7, (−) for NRE-4W	Charon 6, Charon 7, NRE-4W	Charon 8 Charon 5
24	λ *plac5* *imm434* sRI4° *nin5* s*Hin*dIII6" DK1* λ *b1007*	Screen whites on on SA88	Charon 20	Charon 8 λb1007
25	λ *b1007* *imm434* sRI4" *nin5* s*Hin*dIII6" DK1* λ *plac5* BW3 *imm80* KH53 BW2* *nin5* *QSR80*	Screen for whites on Ymel (P_2)	Charon 15	Charon 20 Charon 3
26	λ *b1007* *imm434* sRI4" *nin5* s*Hin*dIII6" DK1* Ch13 Ch14 λ *plac5* *bio256* KH54 BW1* *nin5* *QSR80*	Screen for (−) on Ymel (λ*imm434*) among (+) N100	Charon 13, Charon 14	Charon 20 Charon 4
27	λ *dupL* *b189* *imm434* sRI4"·*nin5* s*Hin*dIII6" DK1* λ *plac5* *bio256* KH54 BW1* *nin5* *QSR80*	Screen for whites among (+) on QR48 (λ*imm434*)	Charon 12	NRE-4W Charon 4
28	λ *b1007* BW3 *imm80* KH53 BW2* *nin5* s*Hin*dIII6" DK1* λ *plac5* *b1007* KH54 BW1* *nin5* *QSR80*	Screen for (+) on SA88 among blues on Ymel (P_2) XG	Charon 16	Charon 15 Charon 14
29	λ *imm434* sRI4" *nin5* s*Hin*dIII6" DK1* λ *plac5* KH100 sRI4" s*Hin*dIII6" DK1*	Screen clear (+) on Ymel (λ*imm434*) among blues on GroN	Charon 9	7W CB3
30	λ *Aam32Bam1* λ *plac5* BW3 *imm80* KH53 BW2* *nin5* *QSR80*	Screen (−) on W3350 among blues on Ymel (P_2)	Charon 3A	$\lambda A_{32}B_1$ Charon 3
31	λ *Aam32 Bam1* λ *plac5* *bio256* KH54 BW1* *nin5* *QSR80*	Screen (−) on W3350 among blues on Ymel (P_2)	Charon 4A	$\lambda A_{32}B_1$ Charon 4
32	λ *Aam32Bam1* *plac5* *imm80* KH53 BW2* *nin5* *QSR80* ↑ ↑*Eco*RI, baycovin, T4 DNA ligase λ *Aam32 Bam1* ↑Δ*lac* ↑ *imm80* KH53 BW2* *nin5* *QSR80*	Pick whites on KH802	Charon 3A Δ*lac*	Charon 3A

TABLE 3—*Continued*

No.		Cross or step	Selection or recognition procedure	Derivative names	Parental names
33	λ	*Aam32 Bam1 Δlac imm80* KH53 BW2* *nin5 QSR80*	Screen (−) on N100 among blues on QR48	Charon 14A	Charon 3A Δ*lac*
	λ	*plac5 b1007* KH54 BW1* *nin5 QSR80*			Charon 14
34	λ	*Aam32 Bam1 plac5 bio256* KH54 BW1* *nin5 QSR80*	Screen (−) on N100 among whites on QR48	Charon 13A	Charon 4A
	λ	*b1007* KH54 BW1* *nin5 QSR80*			Charon 13
35	λ	*Aam32 Bam1 plac5 bio256* KH54 BW1* *nin5 QSR80*	Screen (−) on N100 among whites on QR48	Charon 15A	Charon 4A
	λ	*b1007 imm80* KH53 BW2* *nin5* s*Hin*dIII6° DK1*			Charon 15
36	λ	*Aam32 Bam1 b1007 imm80* KH53 BW2* *nin5* s*Hin*dIII6° DK1*	Screen (−) on W3350 among blues on TC600	Charon 16A	Charon 15A
	λ	*plac5 b1007 imm80* KH53 BW2* *nin5* s*Hin*dIII6° DK1*			Charon 16
37	λ	*Aam32 Bam1 plac5 attλ* BW3 *imm80* KH53 BW2 *nin5 QSR80*	Select blues on Ymel (φ80)	Charon 19A	Charon 3A
	λ	*Wam403 Eam100* $\lambda\overleftarrow{B}$ Δ*λC* cI857 sRI4° *nin5* sRI5° *Sam100*			λgtWES·$\lambda\overleftarrow{B}$

[a] The number of each step is keyed to the genealogy of Fig. 2. The relevant markers of each parent are shown above and below the line which indicates the probable recombination event which produced the desired recombinant or derivative. The names of the derivatives and parents are keyed to Table 1 which enumerates their complete genotype. "Screen for (−)" indicates that individual plaques were tested for the inability to plate on the specified indicator strain. "Screen for blue on Ymel XG" indicates the use of strain Ymel on XG indicator plates. "Cycles of RI selection" are described in the text.

with 1 V/cm and loading less than 0.2 μg/10-μl slot. Thinner gels required less sample and increase resolution. The *Eco*RI enzyme used in the digestion of DNA was prepared as described (61). *Hin*dIII was purchased from New England Biolabs.

The sizes of restriction fragments were obtained by comparison of mobility to a reference mixture of *Eco*RI-digested λ phages whose fragment lengths were calibrated in reference 58 and by our electron micrographic measurements. These reference size standards have been adequate to confirm the phage structures in this study independent of their absolute sizes. However, a detailed comparison of λ restriction fragment sizes with restriction fragments whose absolute lengths are known from their complete DNA sequence is being done at this time, showing some deviations greater than 5% between our reference and absolute fragment lengths. For this reason, the sizes of DNA fragments quoted in this work should be interpreted as the names of those fragments whose absolute lengths will be presented in a later study. Where DNA sizes are quoted in kilobase pairs (kbp), the calibration of reference 12 is used (100%λ = 49.4 kbp). Size estimates of fragments larger than 30%λ length were usually either taken from electron microscopic analysis of heteroduplexes or deduced by subtracting the summed lengths of the smaller fragments from the total DNA length of the phage because the gels did not permit accurate measurement of very large fragments.

Heteroduplex analysis. Heteroduplexes were prepared from intact phage by NaOH denaturation, formamide reannealing, spreading, staining with uranylacetate, and shadow casting with platinum as described (16, 17, 60). Measurements were made on projected slides. Circular DNA of double-stranded phage PM2 (20.44%λ; M. Fiandt, personal communication) and in some cases single-stranded φX174 (10.88%λ, D. Daniels, footnote 34 of reference 12) were used as references (61).

Total DNA size determination. Intact phage were centrifuged to equilibrium in a Beckman model E analytical ultracentrifuge, and the buoyant density was determined with λ^+, λ*cb*2 (88%λ length), or both as reference standards. The total DNA length was then determined as described by Szybalski and Szybalski (55).

Restriction site nomenclature. Restriction sites are variously referred to by their map position, the genes or segments in which they cut, or the standard nomenclature (31) in which the sites are numbered from the left end of the vegetative λ map. None of these is adequate to systematically accommodate hybrid lambdoid phages and their deletion, insertion, and substitution derivatives.

RESULTS AND DISCUSSION

Construction of *Eco*RI no-cut hybrid phages. Native lambda has two *Eco*RI sites on the right arm as shown in Fig. 1 (3, 58), and thus it is not suitable as a DNA cloning vector with *Eco*RI. Because the family of lambdoid phages contains blocks of nonhomologous DNA containing genes grouped for similar function and which can in many cases be substituted for the corresponding λ blocks, a survey of the distribution of *Eco*RI restriction sites was undertaken of various lambdoid phages and hybrids to determine which might be used to eliminate the unwanted *Eco*RI sites. Gels were run of *Eco*RI restriction enzyme digests of DNA from the various phages. The gel analyses showed that the *QSR* region of φ80 eliminates the *Eco*RI site near gene *S*, but the *Eco*RI site within gene *O* was present in all phages tested, including 21, 434, φ80, φ82, *nin*5 deleted λ, and several immunity hybrids of these. Because no deletion or substitution was found which removed the *Eco*RI site in gene *O*, it was necessary to remove it by mutation.

To efficiently select for removal of a restriction site, it is necessary to provide a parent which has only one or a few sites. Our survey revealed that restriction sites in the replaceable region could be eliminated by the *b*519 deletion, which eliminates the 44.5% site; the *b*189 deletion, which eliminates both the 44.5 and 54.8% cuts; the *att*80 region substitution, which eliminates the 54.8% cut without introducing any cuts; and the *bio*1 substitution, which removes the 65.6% cut without introducing a site (12). Many of these observations have also been made by others (45, 57).

Two hybrids combining these various elements were constructed as parents for selection of viable mutants lacking this site. Bbn° (Table 3, no. 4) is the product of the first four crosses shown in Fig. 2 and is a λ immunity hybrid phage containing only the *Eco*RI site in gene *O*. Fbn° (Table 3, no. 10) is a φ80 immunity hybrid phage designed to provide the basis for vectors with replication and immunity specificities differing from λ. This is the product of crosses 7 through 10 in Fig. 2. It contains two *Eco*RI sites, one in the φ80 immunity region and one in gene *O*. These two phages were cycled to remove their *Eco*RI cuts as described above. Bbn^{10} and Fbn^{15} had restriction ratios of 1 (see above). The precursors had restriction ratios of 8×10^{-2} (Bbn° one cut) and 9×10^{-3} (Fbn° two cut) compared with 3×10^{-5} for λ*c*72 (five cut). The loss of the *Eco*RI sites was confirmed by agarose gel electrophoresis of the phage DNA incubated with *Eco*RI followed by heating (15) to dissociate the left and right ends of the DNA (data not shown). The mutation of Bbn^{10} was named BW1 and does not display any structural irregularity when heteroduplexed with λ*c*72. The mutation in gene *O* of Fbn^{15}, BW2, also shows complete homology in heteroduplexes with λ*c*72. However, heteroduplexes with the φ80 immunity hybrid phage *hy*42, combined with buoyant den-

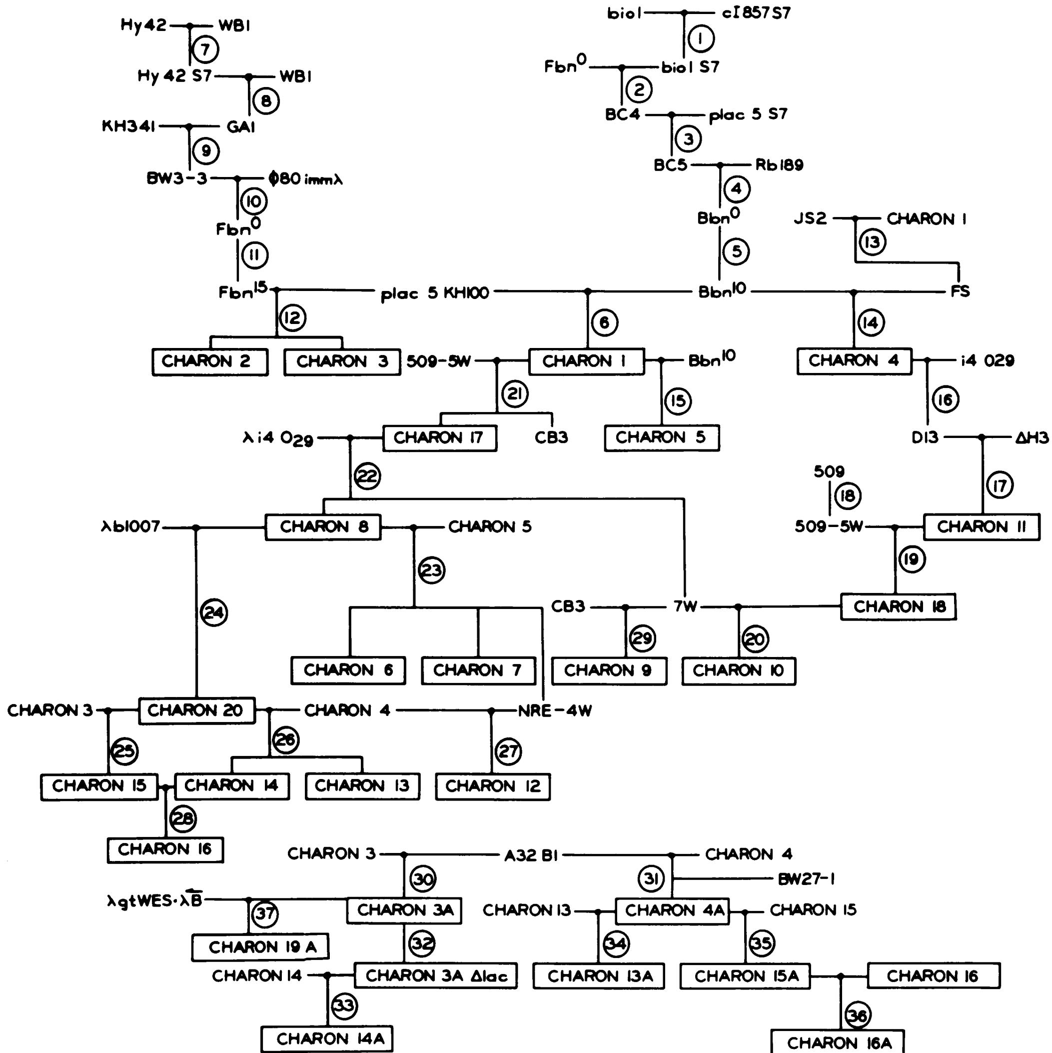

FIG. 2. *Genealogy of the Charon phages. The pedigree of the Charon phages lists the abbreviated names of each phage used in this study, which are keyed to their complete genotypes in Table 1. The crosses in which they participate are indicated by the dots and are keyed to genetic crosses in Table 3 by the circled numbers. Boxes are placed around the names of phages in the crosses where they were produced.*

sity analysis (12), showed that the other mutation of Fbn^{15}, called KH53, is a deletion of about 1.1%λ length. Because the plaque morphology of Fbn^{15} had changed from turbid to clear after cycling, it is most likely that KH53 is a deletion either in the repressor of φ80 or some other component of the φ80 lysogenization mechanism.

Because BW1 and BW2 are independent mutations which result in the same phenotype as similar mutations also isolated by Murray (in strain 509 used herein) (44) and by Davis (in λgt) (57), we have indicated wherever possible in the crosses of Table 3 which allele was present on each parent. However, some recombinants could have received their mutant allele from either. Only specific reference to the genealogy of Fig. 2 and the crosses of Table 3 can determine whether or not the particular allele can be identified in any given Charon phage.

Construction of cloning vectors. The Fbn^{15} and Bbn^{10} phages, both of which lack any *Eco*RI restriction sites, were recombined with replaceable regions from other well-characterized phages to produce vectors for DNA cloning with many different features. Our survey of restriction sites in lambdoid phages and other published work suggested a number of deletion,

substitution, and insertion mutations which might be used to eliminate or insert *Eco*RI sites in more favorable positions in the dispensable region.

The resulting 20 Charon vectors are shown diagrammatically in Fig. 1. They were constructed by the 37 steps detailed in Table 3, according to the genealogy in Fig. 2. The rationale which directed the development of these vectors will be presented in the following sections.

Size constraints and vectors design. The rationale for vector construction must first be considered within the framework of the size constraints placed on cloning by the phage DNA size limits, which require a minimum of 38 and a maximum of 53 kbp of DNA between the cohesive ends (*cos* sites) of the phage genome to produce a viable phage particle (9, 10). The fact that there are both upper and lower limits on the amount of DNA which can be packaged into viable phage means that there must be different vector configurations for the cloning of the largest and the smallest restriction fragments possible. A vector designed to accommodate the largest possible cloned restriction fragment would confine the essential genes of the vector to the smallest possible DNA segment. For plaque-forming λ the minimum would be about 27 kbp. This would allow a maximum insert size of about 26 kbp. Such a vector would also have a minimum requirement for inserted DNA at least 11 kbp in length. To avoid a minimum size requirement as would be needed in an optimum vector for cloning small restriction fragments, down to size 0, at least 38 kb of DNA must remain on the left and right vector ends after appropriate enzyme digestion. Such a vector would have a cloning capacity 0 to 15 kbp. Clearly, different vectors are needed for cloning the shortest and longest restriction fragments. As one considers the use of multiple enzymes, the consideration of size capacity must be made for each enzyme and combination thereof.

One way to clone a wider range of fragment sizes is provided by vectors which contain duplications such as *dup*L, Fig. 1. This 13.5%λ duplication arose spontaneously during the attempt to isolate a recombinant predicted to measure only 76.5%λ (12). CsCl gradients of phages containing *dup*L generally exhibit three phage bands of different buoyant density, which correspond to phages containing one, two, or three copies of the duplicated sequence. By mixing vector DNA prepared from different CsCl bands, one can clone a wide range of DNA molecules.

Selections and recognition procedures for cloning. The ideal cloning system would allow the experimenter to work with a mixture of vector and target DNA, and obtain plaques only from phages with cloned DNA fragments. Short of this, various recognition procedures can be used to distinguish phages containing cloned DNA from those containing only vector DNA. The design of these selections and recognition procedures is very different for vectors intended for cloning large fragments and those which can clone very small fragments.

With vectors designed for cloning small fragments, successful insertion of foreign DNA may be recognized by the inactivation of a gene on the vector whose expression is disrupted by the insertion. Useful *Eco*RI sites within genes include those in *lac*5, where insertion produces a white plaque on an XG plate with a Lac^- host (12); *exo,* where clones fail to plate on a *polA* host (58); and *imm*434, where insertion produces clear plaques (43).

The term "size selection" (57) has been used with vectors designed for cloning large fragments. This characterizes the requirement for insertion of DNA into vectors whose right- and left-end restriction fragments together are not long enough to produce a viable phage. However, to be propagated such phages must contain replaceable DNA stuffer fragments, which make their own genome long enough to be propagated. Because the size selection does not select against reincorporation of vector stuffer fragments, the power of size selection to identify plaques containing foreign DNA is limited unless one can select or screen against reincorporation of the stuffer fragments or remove them physically. Unless the stuffer fragments are physically removed, more than half the recombinant phages can represent reincorporation of stuffer fragments.

Among the useful options for genes on stuffer fragments are the following: a fragment carrying the λ Red and *gam* genes and their promotor would be strongly selected against by the Spi (sensitive to *p*2 inhibition) selection (67); a Lac-containing fragment may be easily scored visually as a blue plaque among colorless plaques (12) on XG plates. A Bio fragment may be detected by picking individual plaques and regrowing them on Bio^- bacteria and biotin-limiting plates (35). A fragment containing the genes *int* and *xis* can be assayed similarly by complementation with the red-plaque test (21). Because a different selection or recognition procedure is needed for each different stuffer fragment, vectors should contain the minimum number of different fragments, optimally one, with a good selection or recognition procedure available.

Charons 1, 2, and 3. Charon 1 was constructed to accommodate large *Eco*RI DNA

fragments. The size of the left and right ends was reduced nearly to a minimum by the use of *lac*5 and KH100 *Eco*RI cuts and inclusion of the *nin*5 deletion. Charon 2 was designed for small *Eco*RI fragments and provides the convenient Lac color test to identify clones. Charon 3 offers increased maximum cloning capacity over Charon 2 (from 4.4 to 9.5 kbp), but the utility of the Lac color test is reduced because the Lac operator is removed during cloning. The increased capacity has made Charon 3 very useful for cloning a broad size range of fragments out of diverse mixtures (61). Furthermore, the ϕ80 replication and immunity specificity has been exploited in the cloning of the λ origin of replication (42) and its subsequent genetic (25) and physical (18) characterization. Charon 3A has also been used for cloning complementary DNA by polydeoxyadenylic acid:polydeoxythymidylic acid tailing (H. Faber and O. Smithies, personal communication). Because the stuffer fragment is unnecessary, Charon 3 can be improved by eliminating it (called Charon 3Δ*lac*).

Charons 4, 10, 11, and 18. An improved large capacity vector was constructed by using the *Eco*RI site in the *bio*256 substitution to define a new right end. Although this right end is 1 kb longer than Charon 1, its advantages include: (i) the phage promoter p_L can be used to promote transcription across a cloned fragment under experimental control (33); (ii) the presence of gene *N* on the vector enhances growth and overcomes many blocks to transcription that might occur on cloned fragments (2, 24, 41); and (iii) the deletion KH54 increases cloning capacity as well as deleting the *c*I repressor which is needed to maintain lysogeny. Charon 4 has two rather than three stuffer fragments. These contain *lac*5 and *bio*256, respectively, and one can screen for the presence of each.

However, when it became available, the deletion ΔH3 was added to eliminate the *Eco*RI site between the *lac*5 and *bio*256 fragments (crosses 16 and 17, Fig. 2). The resulting vector, Charon 11, has a single stuffer fragment with a visual recognition procedure. With Charon 11, inserts of foreign DNA can be identified at a glance as white plaques on Lac$^+$ bacteria on XG plates. To produce Charon 10, a no-cut right end (NRE) version of Charon 11 (see below), we first constructed Charon 18 (cross 19, Fig. 2). Charon 10 was then constructed from this precursor (cross 20, Fig. 2). Charon 10 has a slightly greater capacity than Charon 11, and it can also be used to clone *Hin*dIII fragments.

NRE: an improved right arm for multienzyme cloning. When the enzyme *Hin*dIII became available, it was attractive as an enzyme for cloning. Although the left arm of λ contains no *Hin*dIII sites, there is one site in the essential region of λ near the *Q* gene.

Although the *QSR*80 region eliminates the *Eco*RI site at the right end of the λ genome, it still has a *Hin*dIII site, and thus *QSR*80 vectors as well as *QSR*λ vectors are unsuitable for cloning with *Hin*dIII. *QSR*80 also contains *Bam*HI, *Sst* I, *Sal* I, *Bgl* II and *Sma* I sites, all of which are lacking in *QSR*λ (J. deWet, personal communication). In a cross between λ and ϕ80, N. Murray constructed a strain (509) lacking the *Hin*dIII site but which had the *Eco*RI site (44). Our electron micrographic analysis of this strain revealed apparently perfect homology between the right end of strain 509 and λ (12). To provide a right end that could be used for vectors to clone with either *Eco*RI or *Hin*dIII, we selected a second mutation in strain 509, removing the *Eco*RI site near gene *S*, to produce an NRE. We found that NRE, like *QSR*λ, has no *Bam*HI, *Sst* I, *Bgl* II, or *Sma* I sites.

The ability to clone with more than one enzyme in individual vectors also presents the possibility of using combinations of enzymes, provided the restriction sites in the replaceable region of the vector are arranged so that digestion of the vector produces heterologously terminated left and right ends. Besides the advantages of increased flexibility from the use of the different enzymes to define different target fragments and vector configurations, short target fragments which are terminated with different cuts will not be subject to monomolecular self-closure reactions which compete with bimolecular target-vector reactions during ligation.

Charons 5, 6, 7, and 12. As pointed out by N. Murray, the *Eco*RI site in the *c*I gene of *imm*434 offers a good recognition procedure for clones. One can easily detect a clear plaque among 10^4 turbid ones, possibly among many more. Therefore, Charon 6 was designed as an *Eco*RI insertion vector for small fragments by using the single cut in the *imm*434 substitution (cross 23, Fig. 2 and Table 3). Charon 7 is an NRE adaptation of Charon 6, which derived from the same cross, and includes *Eco*RI, *Hin*dIII, and *Hin*dIII/*Eco*RI cloning capability. Charon 12 was designed as an insertion vector by using the *Eco*RI cut in *exo* to insert fragments under the transcriptional control of p_L. It is anticipated that a *gam* deletion of Charon 12 might provide a vector which could be used to select for inserted fragments on a P2 lysogen. Charons 5, 6, 7, and 12 all contain the 13.5%λ left-arm duplication, *dup*L. Thus, each of them will accommodate a wide range of insert sizes (see Table 7).

Charons 8, 9, and 20. Charons 8 and 9 both approach the maximum capacity that our pres-

ent knowledge makes possible for *Eco*RI cloning. Charon 20 is a maximum capacity vector for *Hin*dIII cloning. Moreover, Charon 20 contains only one replaceable fragment when *Hin*dIII is used, against which there is the strong Spi selection. All three vectors can be used with either *Sst* I, *Hin*dIII, or *Eco*RI, and many combinations can also be used.

Charons 17 and 19A. Charon 17 is the first vector in the Charon series with the ability to form lysogens containing cloned DNA. The *cIam* mutation limits lysogen formation to Su$^+$ hosts. Although the vector can be used with *Hin*dIII, *Sst* I and *Eco*RI, the use of *Hin*dIII destroys the ability of the vector to lysogenize. Charon 19A includes the temperature-sensitive repressor cI857 and is fully competent for lysogenization of *Eco*RI clones at 30°C. Ch19A was originally constructed with amber mutations in genes *A* and *B* for possible safety testing.

Biological containment. Biological containment is the use of safeguards built into the biology of a host-vector system to prevent replication and survival of a cloned fragment in case of accidental infection or inadvertant release into the environment (see references 8, 12, and 46 for extended discussions). Our principal strategy was to block the formation of stable lysogens, because free phage themselves were shown to be inactivated in the "wild" of the sewer (63) and of the gastrointestinal tract (12). Moreover, λ-sensitive hosts are notably lacking in these environments. Charons 3 and 4 were chosen for adaptation and testing for EK-2 certification (National Institutes of Health Guidelines [46]), because they had proven their utility and they carried deletions in their immunity regions to block the formation of lysogens. Especially in conjunction with the *nin*5 deletion, these immunity deletions insure that these phage will destroy any sensitive cell that they encounter by productive lytic infection. To further reduce the host range, amber mutations in gene *A* for DNA maturation and *B* for an essential capsid protein (36) were crossed first to Charons 3 and 4 to produce the first of the "A" amber series of derivatives 3A and 4A.

Charons 13, 14, 15, and 16. Charons 13 through 16 were designed with additional blocks to the already low lysogenization frequencies observed for Charons 3A and 4A (12, 63) by eliminating most or all homology with *E. coli*. This should minimize any possible integration into the host by general recombination through homologous segments. The deletion *b*1007 was included to delete both the phage attachment site and part of the gene *int* which are necessary for site-specific integration (22). Charon 13 completely eliminates bacterial homology and has a reasonably high capacity with a single replaceable fragment. Transcriptional control of the cloned fragment is possible from p_L. Charon 14 has a larger capacity than 13, and the Lac color test can be used to detect reincorporation of the stuffer fragment. Fewer than 200 bp of bacterial DNA remain in clones identified as Lac$^-$. Charons 15 and 16 are vectors for cloning small *Eco*RI fragments by insertion, and they contain the NRE right end. Charon 15 was designed with the capability to clone with *Eco*RI, *Hin*dIII, or the combination *Eco*RI/*Hin*dIII. Charon 16 is an *Eco*RI insertion vector which would retain the entire *lac*5 homology after incorporation of a cloned fragment. Charon 16 also can be used to clone with *Sst* I and the *Sst* I/*Eco*RI combination, but it contains no *Hin*dIII sites. Charons 13 through 16 were crossed to *Aam32Bam*1 to produce Charons 13A through 16A for increased safety. (Each of the vectors Charons 3A, 4A,

TABLE 4. *Diagnostic plating phenotypes of the charon vectors*[a]

Charon	Phenotype
1	Blue, Fec$^+$, Spi$^+$, clear, *imm*λ, Sus$^+$, Nin$^-$
2	Blue, *att*80, Fec$^+$, Spi$^-$, clear, *imm*80
3	Blue, clear, *imm*80
4	Blue, *bioA*$^+$, Bio$^+$, Fec$^-$, Spi$^-$, clear, *imm*λ, Nin$^-$
5	*bio*A$^+$, Bio$^-$, Fec$^-$, Spi$^-$, clear, immλ, Nin$^-$
6	White, Spi$^-$, *imm*434, Nin$^-$
7	White, Fec$^-$, Spi$^-$, *imm*434, Nin$^-$
8	Blue, Sus$^+$, Fec$^+$, Spi$^+$, *imm*434, Nin$^-$
9	Blue, Fec$^+$, Spi$^+$, *imm*λ, Nin$^-$, Sus$^+$
10	Blue, Spi$^-$, *imm*λ, Nin$^-$
11	Blue, Spi$^-$, Fec$^-$, clear, *imm*λ, Nin$^-$
12	White, Spi$^+$, Fec$^+$, Sus$^+$, *imm*λ, Nin$^-$
13	White, Spi$^+$, Fec$^+$, Sus$^+$, *imm*λ, Nin$^-$
14	Blue, Spi$^+$, Fec$^+$, Sus$^+$, *imm*λ, Nin$^-$
15	White, Spi$^-$, Fec$^+$, Sus$^+$, *imm*80
16	Blue, Spi$^-$, *imm*80
17	Blue, Spi$^+$, Fec$^+$, turbid, *imm*λ, Nin$^-$
18	Blue, Spi$^-$, Fec$^-$, Nin$^+$, clear, *imm*λ
19A	Blue, Sus$^-$, Spi$^+$, *imm*λ, *A*$^-$, *B*$^-$
20	White, Spi$^+$, Fec$^+$, Sus$^+$, *imm*434, turbid, Nin$^-$
3A	Blue, Spi$^-$, *imm*80, Sus$^-$, *A*$^-$, *B*$^-$
4A	Blue, Spi$^-$, *imm*λ, Sus$^-$, *A*$^-$, *B*$^-$
13A	White, Spi$^+$, Sus$^-$, *imm*λ
14A	Blue, Spi$^+$, Fec$^+$, Sus$^-$, *imm*λ
15A	White, Spi$^-$, Fec$^+$, Sus$^-$, *imm*80
16A	Blue, *imm*80, Sus$^-$, *A*$^-$, *B*$^-$

[a] The individual phenotypes and the genetic tests which determine them are described in the text. Only selected tests were applied to each individual vector to characterize critical features of the recombinants. Nin abbreviates N independence. The Fec, Spi, and Nin phenotypes are subject to interaction with different immunities: for λ, 434, or 21 immunities, Fec$^-$ and Spi$^-$ indicate the absence of Red and *gam* (which are removed by *bio*1 and *bio*256) and Nin indicates the absence of t_{R2} (which is removed by the *nin*5 deletion). φ80 and λ*imm*80 phages plate on *recA, groN*, and P2 lysogenic strains.

TABLE 5. *Comparison between predicted and observed lengths of Charon phage DNA*

Structural components	Deletion	Insertion	Net change[a]	Charon	Predicted[b] length (%λ)	Observed length (%λ)
Substitutions						
*plac*5	7.0	7.0	0% bd	1	(100.1)	100.5
*att*80	14.0	10.0	−4.0% EM (23)	2	(93.2)	95.1
*bio*1	10.9 (54)	6.6[c]	−4.4% bd (54)	3	(97.2)	98.7
*bio*256	15.3 (54)	14.9[c]	0.4% bd (54)	4	(92.3)	93.6
*imm*80	11.0	12.3	+1.3% EM (23)	5	(92.5)	93.5
*imm*434	5.8	3.5	−2.2% bd (55)	6	(87.1)	88.6
*QSR*80	9.4	11.7	+2.3% bd	7	(84.8)	86.2
Insertions				8	(92.4)	92.8
*dup*L		13.5	+13.5% bd	9	(97.8)	97.6
KH100		3.1	+3.1% bd	10	(85.9)	85.3
Deletions				11	(88.2)	89.0
*b*189	16.7		−16.7% EM	12	(89.4)	91.0
ΔH3	4.4		−4.4% EM	13	(82.7)	83.5
*b*1007	9.9		−9.9% EM	14	(82.7)	83.7
KH53	1.1		−1.1% bd	15	(85.0)	86.0
KH54	4.4		−4.4% bd (11)	16	(85.0)	86.0
*nin*5	5.3		−5.3% bd	17	(94.7)	95.0
				18	(91.5)	91.3
				19A	(97.0)	97.4
				20	(82.5)	82.2
				3A	(97.2)	98.7
				4A	(92.6)	93.7
				13A	(82.7)	83.5
				14A	(82.7)	83.7
				15A	(85.0)	86.0
				16A	(85.0)	86.0

[a] Net changes of individual components were measured either from bouyant density analysis of two phages which differed only with respect to the component in question (bd), or from electron micrographic analysis of heteroduplexes (EM). Numbers in parentheses are literature references to measurements not done in this lab.

[b] The predicted lengths were determined by applying the sum of the net changes shown here for each component of the vector.

[c] From E. H. Szybalski and W. Szybalski, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, S275, p. 325.

13A, 14A, 15A, and 16A was submitted for EK2 certification [62]. Of these Charons, 3A, 4A, and 16A were eventually certified.)

Determination of vector structures. Primary stocks of each vector were genetically tested as described above. Their plating phenotypes are shown in Table 4 and are in every case consistent with the phenotypes predicted from genotypes indicated in Fig. 1 and Table 1.

The total DNA length of each vector was calculated from the measured buoyant density of the purified phage as described above. The predicted DNA length of each vector was calculated by applying the known sizes (Table 5) of the various deletions, insertions and substitutions shown in Fig. 1 to 100%λ length. The predicted sizes are compared with the observed sizes in Table 5 and agree with the structural assignments shown in Fig. 1.

Heteroduplexes were made between the Charon phages and λ, and measurements were made to identify each insertion, deletion, and substitution by both its size and position. One can compare the structures shown in Fig. 1 with the heteroduplexes by noting that the boxes (substitutions) in Fig. 1 correspond with either two-sided substitution bubbles or split ends in the heteroduplexes of Fig. 3, whereas the deletions or insertions of Fig. 1 appear as loops emanating from the side of the continuous double-stranded heteroduplex of Fig. 3. The substitution *QSR*80, shown by two boxes in Fig. 1, does not always pair along the central 0.5%λ homology segment or at the end. The schematic chosen to represent *QSR*80 in Fig. 3 depended on the predominant species in each preparation. Although far too few molecules were measured in some cases to provide a strong quantitative basis for mapping, the technique provides verification of each structural component in each phage tested, as well as a check that no unusual deletion, insertion, substitution, or rearrangement has occurred. Charons 7, 17, 18, and 20 were not tested by heteroduplex analysis.

The deletion KH53 in the immunity region of φ80 was displayed by heteroduplexing versus λ-

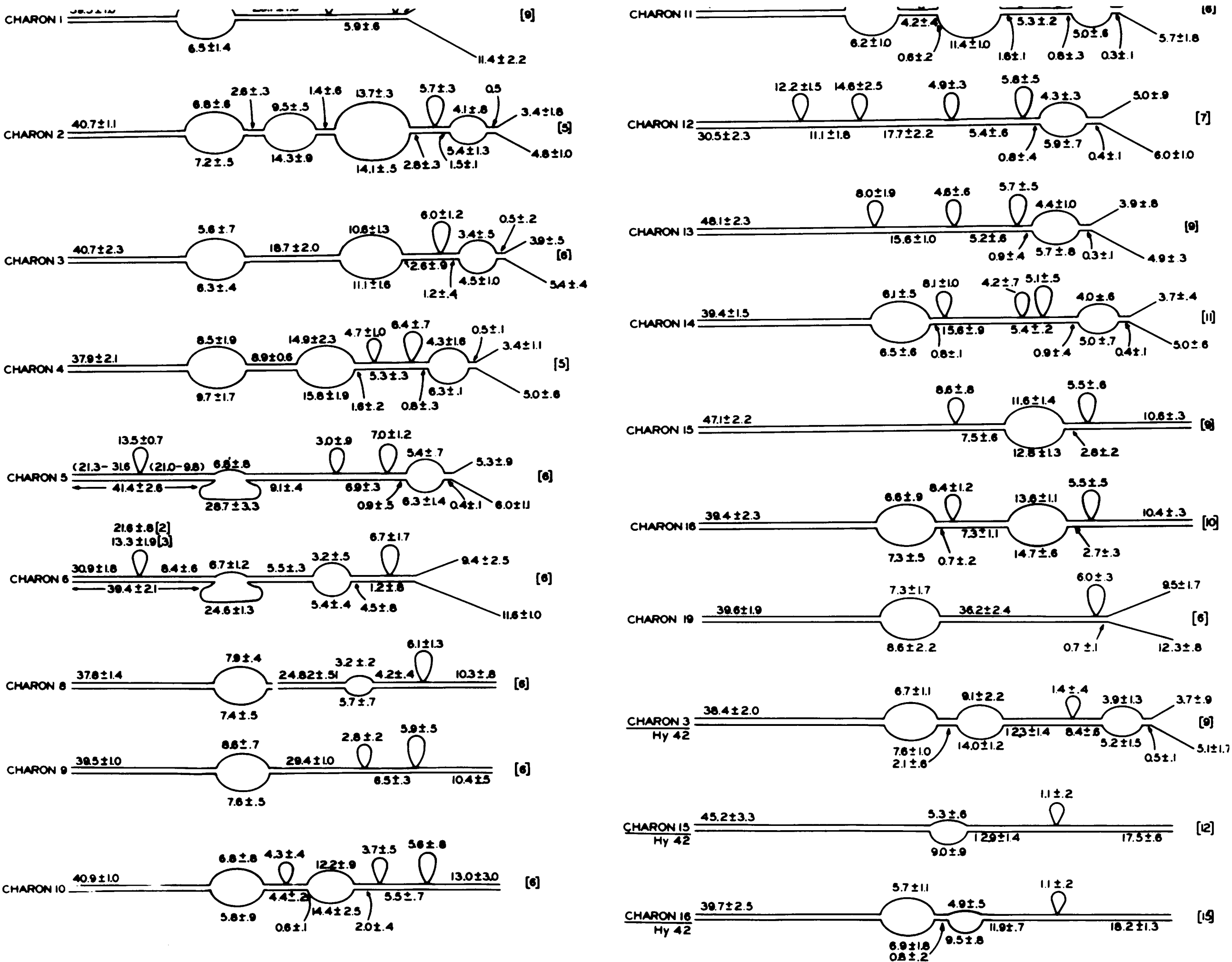

FIG. 3. *Electron micrographic analysis of heteroduplexes. The structures labeled with only Charon numbers are heteroduplexes between λc72 and the individual vector. Charons 3, 15, and 16 were paired with hy42, which carries the immunity of φ80, to display the KH53 deletion within the immunity 80 substitution. The measurements are derived from measured length comparisons with circular double-stranded DNA of coliphage PM2, and in some cases single-stranded circular φx174 DNA length standards on the same photographic field. The measurements are in %λ length ± the standard deviation (see text).*

TABLE 6. *Sizes of EcoRI and HindIII restriction fragments produced from Charon phages*[a]

Charon	*Eco*RI fragment size (%λ)	*Hin*dIII fragment size (%λ)
1	39.5, 14.6, 11.8, 14.0, 20.6	52.2, 4.4, 18.8, 3.4, 7.1, 11.6
2	39.5, 55.6	83.5, 11.6
3	39.5, 14.6, 42.6	52.2, 4.3, 27, 11.6
4	39.5, 14.6, 17.0, 22.5	52.2, 4.4, 24.5, 11.9
5	72.7, 20.6	71, 3.6, 7.3, 11.9
6	68.3, 19.9	70, 7.1, 12.0
7	68.3, 17.6	70, 17
8	39.5, 14.6, 11.8, 10.2, 17.6	48.5, 4.3, 18.0, 17.0
9	39.5, 14.6, 11.8, 14.0, 17.4	52.2, 4.3, 20.0, 3.4, 16.4
10	39.5, 27.2, 20.2	52.2, 33.1
11	39.5, 25.0, 21.3	52, 25, 11.9
12	64, 27.0	79, 12.0
13	44.5, 11.8, 27.0	48.5, 22.5, 12.0
14	39.5, 16.0, 27.0	71, 12.0
15	44.5, 41.5	48.5, 38.1
16	39.5, 47.1	86
17	39.5, 14.6, 40.9	48.5, 4.0, 19.7, 1.3, 15.5
18	NT[b]	52.2, 43.4
19A	39.5, 13.7, 38.1	52.2, 4.2, 18.5, 1.6, 7.3, 11.5
20	44.5, 12.0, 10.2, 16.5	48.5, 17.4, 16.5
3A	39.5, 14.6, 45	NT
4A	39.5, 14.6, 17.0, 22.5	52.2, 4.4, 24.5, 11.9
13A	44.5, 11.9, 27.5	48.5, 22.5, 12.0
14A	39.5, 16.0, 27.5	71, 12.0
15A	44.5, 41.5	48.5, 38.1
16A	39.5, 47.1	NT

[a] The sizes presented here are from single gels. The tabulated values are intended only to identify the presence or absence of the restriction sites indicated in Fig. 1. The fragments are arranged from left to right as shown in Fig. 1.

[b] NT, Not tested.

φ80 hybrid 42 carrying both the *att* and *imm* regions of φ80. The duplication *dup*L in Charons 5 and 6 has the expected property that it can undergo single-strand branch migration in the formation of the duplex along the entire length of the duplicated segment, appearing to slide between different positions in different molecules (as shown in Charon 5 versus λ). It can exist in two or three copies as shown in Charon 6 versus λ. The arrangements of deletions, insertions, and substitutions and their measurements shown in Fig. 3 confirm the structures shown in Fig. 1 for each phage tested.

The distribution of *Eco*RI and *Hin*dIII restriction sites of each Charon phage was determined from horizontal agarose gel electrophoresis, and the results are summarized in Table 6 as fragment sizes from left to right on the map. The approximate fragment sizes given in Table 6 are adequate to verify which restriction cuts are present in the vectors shown, but the tabulated values represent single measurements. The detailed mapping of the Charon phages will be presented in a subsequent paper, in which more accurate sizes for all restriction fragments studied will be presented. The results shown in Table 6 are consistent in every case with the expected distribution of *Eco*RI and *Hin*dIII restriction sites from the construction steps shown in Table 3, except for BW27-1 discussed below.

New *Eco*RI site in λ. In the course of this series of crosses, a new *Eco*RI cut arose spontaneously in one of the two candidates for Charon 4A, from cross 31 of Table 3. The results from the genetic characterization of the two candidates BW27-1 and BW27-2 were identical to those shown in Table 4, including the presence in both of *Aam*32. The results of the density, heteroduplex, *Eco*RI, and *Hin*dIII gel analyses also appeared identical. One of the first workers to receive the Charon phages, T. Maniatis, soon informed us that DNA prepared from stock 1706 grown from BW27-1 was very inefficient for cloning due to an inability to anneal the cohesive ends. He suggested the possibility that an *Eco*RI cut near the left end of the DNA phage BW27-1 may be present. Our original gels did not detect this because the very small new fragment would have run off the gel, and the left end would have been shortened by an imperceptible fraction of its length.

The new cleavage site, now called BW4, was confirmed and mapped by comparing restriction patterns of DNA from Charon 4, BW27-1, and BW27-2 digested simultaneously with *Eco*RI and *Bam*HI. *Bam*HI cleaves Charon 4 into six fragments, including an 11.4%λ left end and 3.1%λ right end which contain no *Eco*RI sites. The cohesive termini on the left and right ends of λ DNA were annealed together and sealed with T4 DNA ligase before the *Bam*HI restriction enzyme digestion. A fusion fragment of the left and right ends of Charon 4 is produced whose length is sensitive to any *Eco*RI cleavage near the cohesive end. The results showed the *Bam*HI fusion fragment was clearly visible in Charon 4 and BW27-2. But BW27-1 showed two smaller bands corresponding to a 3.4% fusion piece, 0.3%λ longer than the *Bam*HI right end, and a fragment slightly shorter than the *Bam*HI left end of Charon 4. Thus, the new cleavage site maps 0.3%λ to the right of the left end of λ. Close inspection in this region of the heteroduplexes between BW27-1 and λ revealed no abnormalities, eliminating the possibility that the new cut was introduced by insertion, substitution, or deletion of more than 100 bp. This site has not been characterized further.

We therefore distributed stock 513 of Charon 4A derived from BW27-2 to all workers who had

TABLE 7. *Characteristics of Charon cloning phages 1 through 20*[a]

Vectors	Typical titers	Fragment	Left-end fragment length (%λ)	Right-end fragment length (%λ)	Cloning capacity (kbp)	Promoter control	Genetic test
*Charon 1	3×10^{10}	*Eco*RI	39.5	20.6	8.6–21.6		g
Charon 2	2×10^{10}	*Eco*RI	39.5	55.6	0–4.4	p_{Lac}	a
*Charon 3	6×10^{10}	*Eco*RI	39.5	45.2	0–9.5	p_L	f
*Charon 4	1×10^{10}	*Eco*RI	39.5	22.5	7.6–20.6	p_L	d
Charon 5	2×10^{10}	*Eco*RI	59.2[0]	20.6	0–11.9		h
		*Eco*RI	72.7[1]	20.6	0–5.3		h
		*Eco*RI	86.2[2]	20.6	0–0.1		h
Charon 6	1×10^{10}	*Eco*RI	68.3[1]	19.9	0–7.8 (14.4)[0]		c
		*Eco*RI	80.8[2]	19.9	0–1.7		c
*Charon 7	2×10^{10}	*Eco*RI	68.3[1]	17.6	0–8.9 (15.5)[0]		c
		*Eco*RI	81.8[2]	17.6	0–2.3		c
		*Hin*dIII	67.3[1]	18.6	0–8.9 (15.5)[0]		c
		*Hin*dIII	80.8[2]	18.6	0–2.3		c
		*Eco*RI/*Hin*dIII	67.3[1]	17.6	0–9.4 (16.0)[0]		c
		*Eco*RI/*Hin*dIII	80.8[2]	17.6	0–2.3		c
Charon 8	5×10^{10}	*Eco*RI	39.5	17.6	10.0–23.0		g
		*Hin*dIII	48.5	18.5	5.2–18.2		g
		Sst	42.0	37.9	0–11.9	p_L	f
		*Hin*dIII/*Sst*	42.0	18.5	8.4–21.4		g
*Charon 9	2×10^{11}	*Eco*RI	39.5	18.3	9.7–22.7		g
		*Hin*dIII	48.5	17.6	5.6–18.6		g
		*Eco*RI/*Hin*dIII	39.5	17.6	10.0–23.0		g
		Sst	42.0	43.2	0–9.3	p_L	h
		Sst/*Hin*dIII	42.0	17.6	8.8–21.2		g
*Charon 10	3×10^{10}	*Eco*RI	39.5	20.2	8.8–21.2	p_L	b
		*Hin*dIII	52.2	33.1	0–9.2	p_L	h
		Sst	42.0	34.0	0.8–13.8	p_L	b
		*Hin*dIII/*Sst*	42.0	33.1	1.2–14.2	p_L	b
*Charon 11	5×10^{10}	*Eco*RI	39.5	22.5	7.6–20.6	p_L	b
*Charon 12	1×10^{11}	*Eco*RI	64.2	26.8	0–6.4	p_L	e
Charon 13	1×10^{11}	*Eco*RI	44.5	26.8	3.1–16.1	p_L	g
Charon 14	2×10^{10}	*Eco*RI	39.5	26.8	5.5–18.5	p_L	b
Charon 15	1×10^{11}	*Eco*RI	44.5	41.5	0–8.9	p_L	h
		*Hin*dIII	48.5	37.5	0–8.9	p_L	h
		*Eco*RI/*Hin*dIII	44.5	37.5	0–10.8	p_L	h
*Charon 16	3×10^{10}	*Eco*RI	39.5	46.5	0–8.9	p_{Lac}	a
		Sst	42.0	44.0	0–8.9	p_{Lac}	a
		*Eco*RI/*Sst*	39.5	44.0	0–10.1	p_{Lac}	f
*Charon 17	2×10^{11}	*Eco*RI	39.5	39.9	0–12.1	p_L	f
		*Hin*dIII	52.2	16.9	4.1–17.1		j
		*Eco*RI/*Hin*dIII	39.5	16.9	10.4–23.4		k
		Sst	42.0	40.7	0–10.5		f
		*Hin*dIII/*Sst*	42.0	16.9	9.1–22.1		k
Charon 18	3×10^{10}	*Eco*RI	39.5	25.5	6.2–19.2	p_L	b
		*Hin*dIII	52.2	38.4	0–6.6	p_L	
		Sst	42.0	38.6	0–11.5	p_L	f
		*Hin*dIII/*Sst*	42.0	38.4	0–11.6	p_L	f
*Charon 19A	4×10^{10}	*Eco*RI	39.5	42.2	0–11.0	p_L	f
Charon 20	2×10^{10}	*Eco*RI	44.5	17.6	7.6–20.6		j
		*Hin*dIII	48.5	18.6	5.1–18.1		i

TABLE 7—*Continued*

[a] The left- and right-end fragment lengths for each useful vector-enzyme combination are given in percent lambda, as determined by agarose gel electrophoresis or electron microscopy (or both). The summed lengths determine the cloning capacity, calculated by their difference from 38 kb, the smallest phage found to grow well in this laboratory (KK1, reference 18), and 51 kb, the largest clone found to grow well (Charon 357, reference 61). The symbol p_L under the heading "Promoter control" indicates whether or not this major vector promoter would be expected to transcribe the cloned fragment. Other promoters (p'_R, p_{re}, or p_{bio}) might be useful in some cases. Suggested genetic tests to indicate successful cloning are as follows: a, colorless plaques on Lac^- cells provide definite indication of foreign DNA insertion; b, colorless plaques on Lac^+ cells provide definite indication of foreign DNA insertion without reinsertion of dispensable vector fragment; c, clear plaques provide definite indication of foreign DNA insertion; d, combination of colorless plaques in Lac^+ cells with Bio^- phenotype provides definite indication of foreign DNA substitution without reinsertion of either dispensable fragment. Bio^+ phenotype is detected by a ring of bacterial growth around plaques on Bio^- cells on biotin-deficient plates; e, lack of ability to plate on *polA*$^-$ cells provides definite indication of foreign DNA insertion; f, colorless plaques on Lac^+ cells indicate removal of dispensable fragment but does not insure that insertion of foreign DNA has taken place; g, plaque formation indicates DNA insertion but does not insure that reincorporation of dispensable fragments has not taken place; h, no particularly useful tests currently available. Not all genetic tests have been verified in all cases; i, plaques on a P2 lysogen provide positive identification of foreign DNA insertion without reinsertion of the dispensable vector fragment; j, plaques on a P2 lysogen indicate removal of a dispensable fragment, but does not insure that insertion of foreign DNA has occurred; k, colorless plaques on Lac^+, P2 lysogens strongly suggest that insertion of foreign DNA has occurred. These tests are inferred from well-established phenotypes, but have not been confirmed with each particular vector-clone combination possible. The superscripts 0, 1, and 2 correspond to vectors containing zero, one, or two copies of *dup*L. The asterisks denote vectors that have been used successfully for cloning at the University of Wisconsin.

requested Charon 4A. This has been used successfully for cloning by Maniatis and others.

The characteristics relevant to the use of the Charon phages for DNA cloning are presented in Table 7. The availability of detailed information on these various vectors should provide expanded convenience and capability to those who wish to use them, including the ability to adapt combinations of these and other vectors to their individual requirements.

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LITERATURE CITED

1. **Adhya, S., P. Cleary, and A. Campbell.** 1968. A deletion analysis of prophage λ and adjacent genetic regions. Proc. Natl. Acad. Sci. U.S.A. **61**:956–962.
2. **Adhya, S., M. Gottesman, and B. deCrombrugghe.** 1974. Release of polarity in *E. coli* by gene N of phage λ: termination and antitermination of transcription. Proc. Natl. Acad. Sci. U.S.A. **71**:2534–2538.
3. **Allet, B., P. G. N. Jeppesen, K. J. Katagiri, and H. Delius.** 1973. Mapping of the DNA fragments produced by cleavage of λ DNA with endonuclease RI. Nature (London) **241**:120–122.
4. **Appleyard, R.** 1954. Segregation of new lysogenic types during growth of a double lysogenic strain derived from *Escherichia coli* K-12. Genetics **39**:440–452.
5. **Arber, W., and U. Kühnlein.** 1967. Mutationeller Verlust B-spezifischer Restriktion des Bakteriophagen fd. Pathol. Microbiol. **30**:946–952.
6. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
7. **Barnes, W. M., R. B. Siegel, and W. S. Reznikoff.** 1974. The construction of λ transducing phages containing deletions defining regulatory elements of the *lac* and *trp* operons in *E. coli*. Mol. Gen. Genet. **129**: 201–215.
8. **Beers, R. F., and E. G. Bassett (ed.).** 1977. Recombinant molecules: impact on science and society. Raven Press, New York.
9. **Bellett, A. S. D., H. G. Busse, and R. L. Baldwin.** 1971. Tandem genetic duplications in a derivative of phage lambda, p. 501–514. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Berg, D. E.** 1971. Regulation in phage with duplications of the immunity region, p. 667–678. *In* A. D. Hershey (ed.), The bacteriohage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Blattner, F. R., M. Fiandt, K. K. Hass, P. A. Twose, and W. Szybalski.** 1974. Deletions and insertions in the immunity region of coliphage lambda: revised measurements of the promoter-startpoint distance. Virology **62**:458–471.
12. **Blattner, F. R., B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies.** 1977. Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. Science **196**:161–169.
13. **Borek, K., J. D. Beggs, W. J. Brammar, A. S. Hopkins, and N. E. Murray.** 1976. The construction *in vitro* of transducing derivatives of phage lambda. Mol. Gen. Genet. **146**:199–207.
14. **Campbell, A.** 1961. Sensitive mutants of bacteriophage λ. Virology **14**:22–32.

15. **Davidson, N., and W. Szybalski.** 1971. Physical and chemical characteristics of lambda DNA, p. 45-82. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Davis, R. W., and J. S. Parkinson.** 1971. Deletion mutants of bacteriophage lambda. III. Physical structure of *att*$^{\phi}$. J. Mol. Biol. **56**:403-423.
17. **Davis, R. W., M. Simon, and N. Davidson.** 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. Methods Enzymol. **21**:413-428.
18. **Denniston-Thompson, K., D. D. Moore, K. E. Kruger, M. E. Furth, and F. R. Blattner.** 1977. Physical structure of the replication origin of bacteriophage lambda. Science **198**:1051-1056.
19. **Donoghue, D. J., and P. A. Sharp.** 1977. An improved bacteriophage lambda vector: construction of model recombinants coding for kanamycin resistance. Gene **1**: 209-227.
20. **Enquist, L., D. Tiemeier, P. Leder, R. Weisberg, and N. Sternberg.** 1976. Safer derivatives of bacteriophage λgt·λC for use in cloning of recombinant DNA molecules. Nature (London) **259**:596-598.
21. **Enquist, L., and R. A. Weisberg.** 1976. The red plaque test: a rapid method for identification of excision defective variants of bacteriophage lambda. Virology **72**: 147-153.
22. **Enquist, L., and R. A. Weisberg.** 1977. Genetic analysis of *att int xis* region of coliphage λ. J. Mol. Biol. **111**: 97-120.
23. **Fiandt, M., Z. Hradecna, H. Lozeron, and W. Szybalski.** 1971. Electron micrographic mapping of deletions, insertions, inversions, and homologies in the DNAs of coliphages lambda and phi 80, p. 329-354. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Franklin, N. C.** 1974. Altered reading of genetic signals fused to the N operon of bacteriophage λ: genetic evidence for modification of polymerase by the protein product of the N gene. J. Mol. Biol. **89**:33-48.
25. **Furth, M. E., F. Blattner, C. McLeester, and W. Dove.** 1977. Genetic structure of the replication origin of bacteriophage lambda. Science **198**:1046-1051.
26. **Georgopoulis, C. P.** 1971. Bacterial mutants in which the gene N function of bacteriophage lambda is blocked have an altered RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. **68**:2977-2981.
27. **Georgopoulis, C. P., and I. Herskowitz.** 1971. *Escherichia coli* mutants blocked in lambda DNA synthesis, p. 553-564. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Goldberg, A. R., and M. Howe.** 1969. New mutations in the S cistron of bacteriophage lambda affecting host cell lysis. Virology **83**:200-202.
29. **Gottesman, M. E., and M. B. Yarmolinsky.** 1968. Integration-negative mutants of bacteriophage lambda. J. Mol. Biol. **31**:487-505.
30. **Henderson, D., and J. Weil.** 1975. Nature and origin of a class of essential gene substitutions in bacteriophage lambda. Virology **67**:124-135.
31. **Hopkins, A. S., N. E. Murray, and W. J. Brammar.** 1976. Characterization of λ*trp*-transducing bacteriophages made *in vitro*. J. Mol. Biol. **107**:549-569.
32. **Ippen, K., J. A. Shapiro, and J. R. Beckwith.** 1971. Transposition of the *lac* region to the *gal* region of the *Escherichia coli* chromosome: isolation of λ*lac* transducing bacteriophages. J. Bacteriol. **108**:5-9.
33. **Jaskunas, S. R., A. M. Fallon, M. Nomura, B. G. Williams, and F. R. Blattner.** 1977. Expression of ribosomal protein genes cloned in Charon vector phages and identification of their promoters. J. Biol. Chem. **252**:7355-7364.
34. **Kaiser, A. D., and F. Jacob.** 1957. Recombination between related temperate bacteriophages and the genetic control of immunity and prophage localization. Virology **4**:509-521.
35. **Kayajanian, G.** 1968. Studies on the genetics of biotin-transducing, defective variants of bacteriophage λ. Virology **36**:30-41.
36. **Kellenberger, E., and R. S. Edgar.** 1971. Structure and assembly of phage particles, p. 271-293. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **Liedhe-Kulke, M., and A. D. Kaiser.** 1967. The c-region of coliphage 21. Virology **32**:475-481.
38. **Manly, K. F., E. R. Signer, and C. M. Radding.** 1969. Nonessential functions of bacteriophage λ. Virology **37**: 177-188.
39. **Mertz, J. E., and R. W. Davis.** 1972. Cleavage of DNA by RI restriction endonuclease generates cohesive ends. Proc. Natl. Acad. Sci. U.S.A. **69**:3370-3374.
40. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Moir, A., and W. J. Brammar.** 1976. The use of specialized transducing phages in the amplification of enzyme production. Mol. Gen. Genet. **149**:87-99.
42. **Moore, D. D., K. Denniston-Thompson, M. E. Furth, B. G. Williams, and F. R. Blattner.** 1977. Construction of chimeric phages and plasmids containing the origin of replication of bacteriophage lambda. Science **198**:1041-1046.
43. **Murray, N. E., W. J. Brammar, and K. Murray.** 1977. Lambdoid phages that simplify the recovery of *in vitro* recombinants. Mol. Gen. Genet. **150**:53-61.
44. **Murray, K., and N. E. Murray.** 1975. Phage lambda receptor chromosomes for DNA fragments made with restriction endonuclease III of *Haemophilus influenzae* and restriction endonuclease I of *Escherichia coli*. J. Mol. Biol. **98**:551-564.
45. **Murray, N. E., and K. Murray.** 1974. Manipulation of restriction targets in phage λ to form receptor chromosomes for DNA fragments. Nature (London) **251**: 476-481.
46. **National Institutes of Health.** 1976. Guidelines for recombinant DNA research. Fed. Reg. **41**(no. 131): 27902-27943.
47. **Rambach, A., and P. Tiollais.** 1974. Bacteriophage λ having *Eco*RI endonuclease sites only in the nonessential region of the genome. Proc. Natl. Acad. Sci. U.S.A. **71**:3927-3930.
48. **Schrenk, W. J., and J. H. Miller.** 1975. Specialized transducing phages carrying fusions of the *trp* and *lac* regions of the *E. coli* chromosome. Mol. Gen. Genet. **131**:9-19.
49. **Shinnick, T. M., E. Lund, O. Smithies, and F. R. Blattner.** 1975. Hybridization of labeled RNA to DNA in agarose gels. Nucleic Acids Res. **2**:1911-1929.
50. **Signer, E. R.** 1964. Recombination between coli-phages λ and φ80. Virology **22**:650-651.
51. **Signer, E. R., and J. Weil.** 1968. Recombination in bacteriophage λ. J. Mol. Biol. **34**:261-271.
52. **Sironi, G.** 1969. Mutants of *Escherichia coli* unable to be lysogenized by the temperate bacteriophage P2. Virology **37**:163-176.
53. **Szpirer, J., R. Thomas, and C. M. Radding.** 1969. Hybrids of bacteriophages λ and φ80. A study of nonvegetative functions. Virology **37**:585-596.
54. **Szybalski, E. H., and W. Szybalski.** 1974. Physical mapping of the *att-N* region of coliphage lambda: apparent oversaturation of coding capacity in the *gam-ral* segment. Biochimie **56**:1497-1503.
55. **Szybalski, W., and E. H. Szybalski.** 1971. Equilibrium density gradient centrifugation, p. 311-354. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic

acid research, vol. 2. Harper and Row, New York.

56. **Taylor, M., and C. Yanofsky.** 1964. Transformation of bacterial markers and transfer of phage markers with DNA isolated from a λ-φ80 hybrid phage carrying the tryptophan genes of *E. coli*. Biochem. Biophys. Res. Commun. **17**:798–804.
57. **Thomas, M., J. Cameron, and R. W. Davis.** 1974. Viable molecular hybrids of bacteriophage lambda and eukaryotic DNA. Proc. Natl. Acad. Sci. U.S.A. **71**: 4579–4583.
58. **Thomas, M., and R. W. Davis.** 1975. Studies on the cleavage of bacteriophage lambda DNA with *Eco*RI restriction endonuclease. J. Mol. Biol. **91**:315–328.
59. **Tiemeier, D., L. Enquist, and P. Leder.** 1976. Improved derivative of a phage λ EK2 vector for cloning recombinant DNA. Nature (London) **263**:526–527.
60. **Westmoreland, B. C., W. Szybalski, and H. Ris.** 1969. Mapping of deletions and substitutions in heteroduplex DNA molecules of bacteriophage lambda by electron microscopy. Science **163**:1343–1348.
61. **Williams, B. G., F. R. Blattner, S. R. Jaskunas, and M. Nomura.** 1977. Insertion of DNA carrying ribosomal protein genes of *Escherichia coli* into Charon vector phages. J. Biol. Chem. **252**:7344–7354.
62. **Williams, B. G., D. D. Moore, J. W. Schumm, D. J. Grunwald, A. E. Blechl, and F. R. Blattner.** Application for EK2 Certification of a Host Vector System for DNA Cloning. Original application submitted June 1, 1976.

    Supplement I. Part I. New dap bacterial host, DP50, for productive growth of Charon 3A and 4A phages. Part II. Two phages derived from Charon 3A and 4A (16A and 14A) with deleted attachment sites, and two (15A and 13A) which are in addition devoid of bacterial DNA. F. R. Blattner, A. E. Blechl, H. E. Faber, L. A. Furlong, D. J. Grunwald, D. O. Kiefer, E. L. Sheldon, and O. Smithies.

    Supplement II. Marker rescue tests. F. R. Blattner, D. O. Kiefer, and D. D. Moore.

    Supplement III. Additional marker rescue data on Charon 3A and Charon 4A. F. R. Blattner, D. O. Kiefer, and D. D. Moore.

    Supplement IV. Further tests on DP50 (χ1953) and DP50 SupF (χ2098). O. Smithies, F. R. Blattner, A. E. Blechl, H. E. Faber, E. L. Sheldon, B. G. Williams, and L. A. Furlong.

    Supplement V. Additional data requested by the phage working group. F. R. Blattner, B. G. Williams, and D. O. Kiefer.

    Supplement VI. Rearmament of Charon phages in sewage. F. R. Blattner, D. O. Kiefer, H. E. Faber, and O. Smithies.

    Supplement VII. Marker rescue tests on Charon phages having amber mutations in genes W and E instead of genes A and B. F. R. Blattner and D. O. Kiefer.
63. **Williams, B. G., D. D. Moore, S. W. Schumm, D. J. Grunwald, A. E. Blechl, and F. R. Blattner.** 1977. Construction and testing of safer phage vectors for DNA cloning, p. 261–272. *In* R. F. Beers, Jr., and E. G. Bassett (ed.), Recombinant molecules: impact on science and society. Raven Press, New York.
64. **Wood, W.** 1966. Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16**: 118–133.
65. **Yanofsky, C., and J. Ito.** 1966. Nonsense codons and polarity in the tryptophan operon. J. Mol. Biol. **21**: 313–334.
66. **Zissler, J., E. Signer, and F. Schaefer.** 1971. The role of recombination in growth of bacteriophage lambda. I. The gamma gene, p. 455–468. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
67. **Zissler, J., E. Signer, and F. Schaefer.** 1971. The role of recombination in growth of bacteriophage lambda. II. Inhibition of growth by prophage P_2, p. 469–475. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.