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Restriction Maps for Twenty-One Charon Vector Phages†

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The mapping of the sites of cleavage of nine restriction endonucleases (*Eco*RI, *Hin*dIII, *Bam*HI, *Sal*I, *Kpn*I, *Sst*I, *Bgl*II, *Xho*I, and *Xba*I) on 21 Charon phage vectors is described. Maps of individual subsections were obtained and then combined to assemble the complete vector maps. Calculations of maximum and minimum sizes of inserts which may be carried by the vectors using different restriction endonucleases or pairs of restriction endonucleases are presented. The regions mapped include several parts of φ80 that had not been mapped previously.

The 21 Charon phages which were derived from bacteriophage λ make available to experimenters a wide variety of vectors for use in recombinant DNA experiments. The construction, characterization, and preliminary restriction maps of the first 20 in the series have been described in previous work (3, 13, 15). The latest EK2 certified vector, CH21A, is described elsewhere (2).

To make best use of these vectors, it is desirable to have precise maps showing sites for as many restriction enzymes as possible. Those enzymes that cut only in the replaceable region are especially important, since these enzymes are useful for cloning. Restriction sites in other regions of the vector are useful in identification and characterization of inserts. In this paper we present maps of nine restriction endonucleases in Charon phages 1 to 21.

MATERIALS AND METHODS

Restriction endonucleases. *Eco*RI was prepared as described previously (14). All other restriction endonucleases were purchased from New England Biolabs. Digestion conditions were generally those recommended by New England Biolabs. For simultaneous digestion with several enzymes, the conditions were determined empirically and generally were those appropriate for the enzyme having the more stringent requirements. In a few cases, multiple digestion was done sequentially.

DNA. DNA was prepared from phages as described previously (14). φX174 replicative-form DNA was from New England Biolabs. Plasmid DNA was prepared as described previously (7). For redigestion experiments, DNA fragments were extracted from slices of 0.5% agarose gels (0.7 cm thick) by using the elution solution and technique described by Maxam and Gilbert for acrylamide gels (8). The fragment was concentrated by ethanol precipitation and was resuspended in the buffer appropriate for the redigestion.

Gel electrophoresis. Sizes of fragments having less than 800 base pairs (bp) were determined on acrylamide gels. For larger fragments we employed agarose gels. Electrophoresis through 7.5% acrylamide-0.3% bisacrylamide gels (20 cm long by 1.5 mm thick) containing 25% glycerol was as described by Moore et al. (9). The gels were run by using voltage gradients of less than 5 V/cm.

Electrophoresis through agarose gels (10 cm long by 1.7 mm thick) was as described by Shinnick et al. (12). Thicker preparative gels were formed in shallow trays to provide edge support. Voltage gradients were less than 1 V/cm. Depending on the sizes of fragments to be resolved, agarose percentages of 0.5, 0.8, 1, or 1.2% were employed.

DNA size markers were run adjacent to the experimental digests. A standard curve was made for each gel by plotting the mobilities of the marker fragments versus the logarithms of their sizes. The sizes of DNA fragments from the experimental digests were then determined from the standard plot. The size markers for the acrylamide and for the agarose gels were as described previously (5).

Data management. Two computer programs were used. The first (11) performed least-squares analysis to provide best-fit maps from fragment sizes. The second program (Schroeder, unpublished) provided a comprehensive file management system which could be used to store, edit, and splice data files containing restriction maps of subregions of λ and the Charon phages and to construct and update maps of the 21 complete Charon phages.

RESULTS AND DISCUSSION

The complete maps of *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Kpn*I, *Sal*I, *Sst*I, *Xba*I, and *Xho*I digests of the 21 Charon phages and of phage λ are presented in Fig. 1.

The Charon phages were constructed by combining various point, deletion, insertion, duplication, and substitution mutations of λ and other

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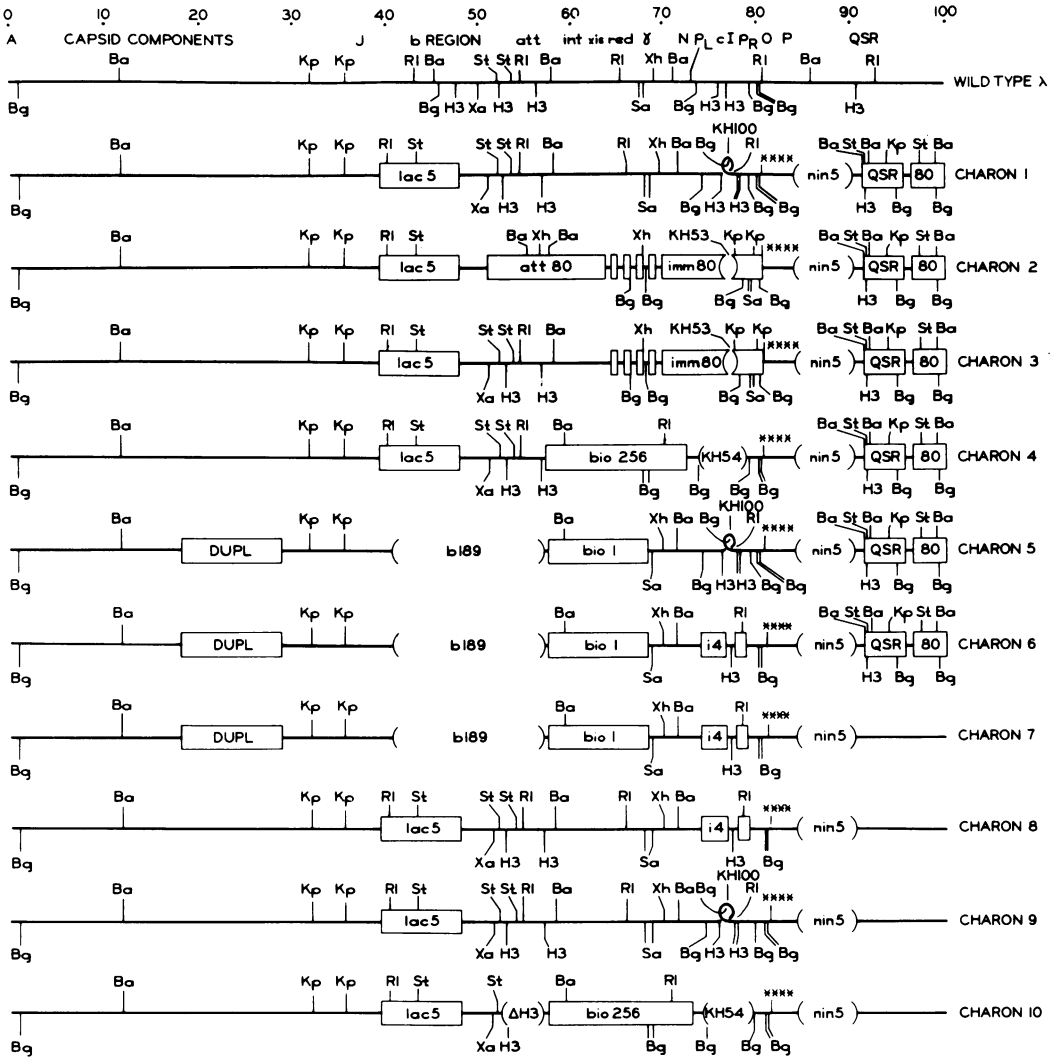

FIG. 1. *Restriction maps of λ and Charon phages 1 to 21. The phage λ map is shown on the top line with some genes identified. In the vector maps lines represent regions homologous to λ, and boxes represent deletions. Lengths of the substitution boxes represent the amount of λ DNA removed and are not proportional to the amount of inserted DNA. **** represents the locations of the EcoRI site in the O gene of wild-type lambda which is removed in the Charon phages. The abbreviations for the restriction endonucleases are as follows: Ba, BamHI; Bg, BglII; Kp, KphI; H3, HindIII; RI, EcoRI; Sa, SalI; St, SstI; Xa, XbaI; and Xh, XhoI. Base pair coordinates of cleavage sites for these nine restriction endonucleases are shown on the facing page. Abbreviations: Lend, left end; Rend, right end.*

lambdoid phages by homologous recombination (Fig. 1) (2, 3, 13, 15).

Thus, the analysis could be simplified by mapping subsections. It was possible to divide each Charon phage except Charon 2 into subsections with endpoints at restriction sites in λ DNA (i.e., within the straight-line portions of Fig. 1). In the case of Charon 2, one site in φ80 DNA was needed. The eight subsections listed in Table 1 were mapped. Complete vector maps were then assembled by splicing individual subsections together with λ, taking into account deletions and duplications.

Most subsections were mapped rather simply. Our mapping of the immunity region of φ80 was more complex, and data are presented only for that case. Figure 2 shows a map of the *imm*80 subsection, which was defined to begin with a *Bam*HI site (position 28,713 on the λ map [5]) and to end with the *****Eco*RI site in gene *O*

CHARON 1	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25737
SstI	26477
EcoRI	26693
HindIII	28023
BamHI	28623
EcoRI	32374
SalI	33422
SalI	33934
XhoI	34175
BamHI	35179
BglII	36403
HindIII	37616
BglII	38277
EcoRI	39132
HindIII	39377
HindIII	39502
BglII	40021
BglII	40672
BglII	40732
****	41086
BamHI	43133
SstI	43283
HindIII	43325
BamHI	43639
KpnI	45525
BglII	45589
SstI	47145
BamHI	47567
BglII	47744
Rend	49058

CHARON 2	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
BamHI	26923
XhoI	27228
BamHI	29998
BglII	31993
XhoI	32749
BglII	32875
KpnI	35620
BglII	36351
SalI	36642
SalI	36795
KpnI	37430
BglII	38379
****	38394
BamHI	40441
SstI	40591
HindIII	40633
BamHI	40947
KpnI	42833
BglII	42897
SstI	44453
BamHI	44875
BglII	45052
Rend	46366

CHARON 3	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25737
SstI	26477
EcoRI	26693
HindIII	28023
BamHI	28623
BglII	33920
XhoI	34676
BglII	34802
KpnI	37547
BglII	38278
SalI	38569
SalI	38722
KpnI	39357
BglII	40306
****	40321
BamHI	42368
SstI	42518
HindIII	42560
BamHI	42874
KpnI	44760
BglII	44824
SstI	46380
BamHI	46802
BglII	46979
Rend	48293

CHARON 4	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25737
SstI	26477
EcoRI	26693
HindIII	28023
BamHI	29026
BglII	32613
BglII	32747
EcoRI	34524
BglII	36071
BglII	36373
BglII	37024
BglII	37084
****	37438
BamHI	39485
SstI	39635
HindIII	39677
BamHI	39991
KpnI	41877
BglII	41941
SstI	43497
BamHI	43919
BglII	44096
Rend	45410

CHARON 5	
Lend	0
BglII	482
BamHI	5526
KpnI	23815
KpnI	25337
BamHI	27338
SalI	30105
XhoI	30346
BamHI	31350
BglII	32574
HindIII	33787
BglII	34448
EcoRI	35303
HindIII	35548
BglII	36192
BglII	36843
BglII	36903
****	37257
BamHI	39304
SstI	39454
HindIII	39496
BamHI	39810
KpnI	41696
BglII	41760
SstI	43316
BamHI	43738
BglII	43915
Rend	45229

CHARON 6	
Lend	0
BglII	482
BamHI	5526
KpnI	23815
KpnI	25337
BamHI	27338
SalI	30105
XhoI	30346
BamHI	31350
HindIII	33360
EcoRI	33596
BglII	34499
BglII	34559
****	34913
BamHI	36960
SstI	37110
HindIII	37152
BamHI	37466
KpnI	39352
BglII	39416
SstI	40972
BamHI	41394
BglII	41571
Rend	42885

CHARON 7	
Lend	0
BglII	482
BamHI	5526
KpnI	23815
KpnI	25337
BamHI	27338
SalI	30105
XhoI	30346
BamHI	31350
HindIII	33360
EcoRI	33596
BglII	34499
BglII	34559
****	34913
Rend	41550

CHARON 8	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25737
SstI	26477
EcoRI	26693
HindIII	28023
BamHI	28623
EcoRI	32374
SalI	33422
SalI	33934
XhoI	34175
BamHI	35179
HindIII	37189
EcoRI	37425
BglII	38328
BglII	38388
****	38742
Rend	45379

CHARON 9	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25737
SstI	26477
EcoRI	26693
HindIII	28023
BamHI	28623
EcoRI	32374
SalI	33422
SalI	33934
XhoI	34175
BamHI	35179
BglII	36403
HindIII	37616
BglII	38277
EcoRI	39132
HindIII	39377
HindIII	39502
BglII	40021
BglII	40672
BglII	40732
****	41086
Rend	47723

CHARON 10	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25739
BamHI	26742
BglII	30329
BglII	30463
EcoRI	32240
BglII	33787
BglII	34089
BglII	34740
BglII	34800
****	35154
Rend	41791

FIG. 1, part 2

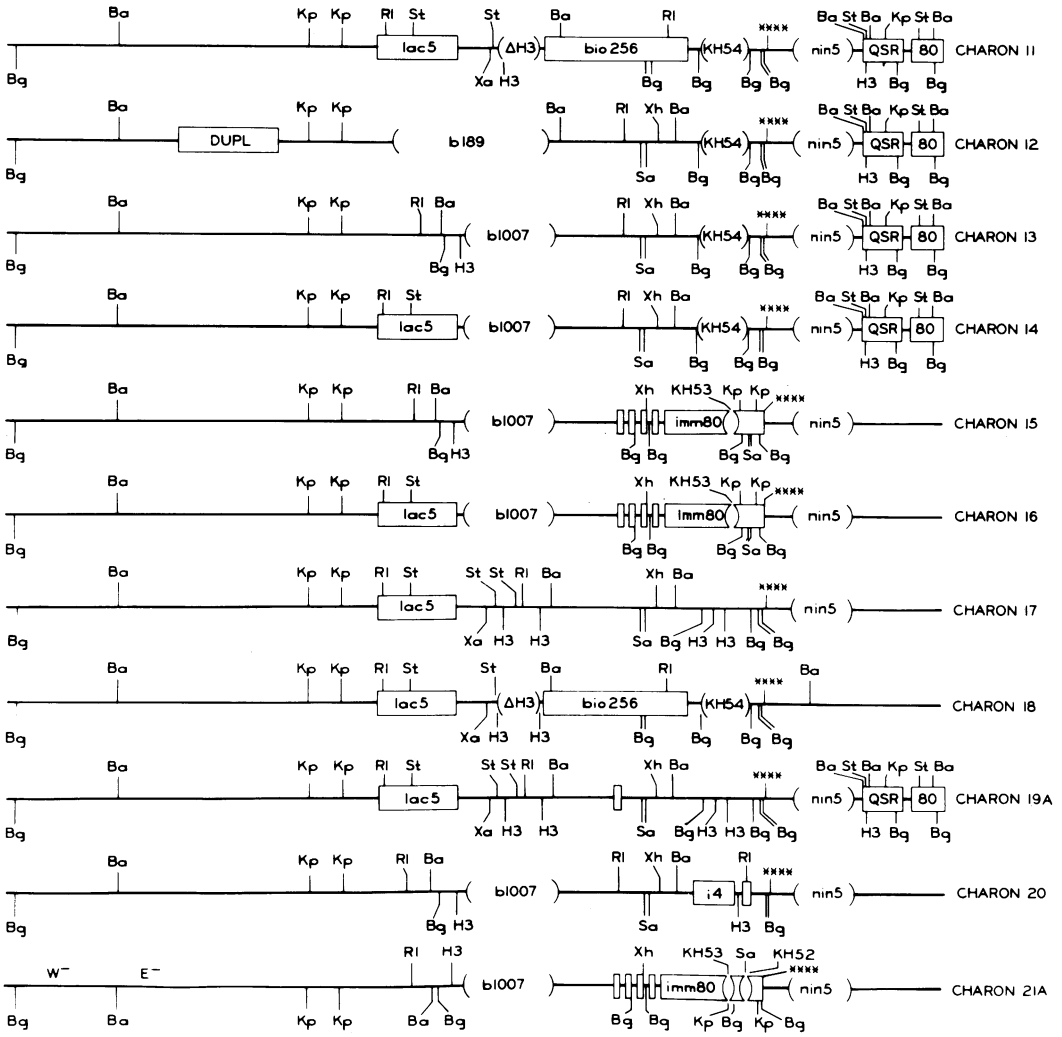

FIG. 1, part 3

(position 40,000 on the λ map). This subsection, which includes the KH53 deletion, is used in five of the Charon phages (Charon phages 2, 3, 15, 16, and 21).

Restriction mapping information from each of these phages was supplemented with data from the KH53$^+$ precursor phage Fbn (13) and the plasmid pFbn3 (1). This plasmid was made by splicing the *imm80* *Eco*RI fragment from Fbn into the mini ColE1 plasmid vector pVH51.

The difference between these substrates had to be taken into account in analyzing the mapping data. For example, phage Fbn has two *Eco*RI sites not present in the cloning vectors. One of these is in the middle of the immunity region (removed by deletion KH53), and the other is within gene *O* (removed by point mutation BW2). The KH53-BW2 combination appears in Charons 2, 3, 15, and 16, whereas Charon 21 also includes the KH52 deletion which was isolated by in vitro excision of the *Sal*I subfragment of *imm80*. The plasmid pFbn3 contains both *Eco*RI sites and neither of the deletions.

The first step of analysis of *imm80* was to determine which of the nine restriction enzymes cut in the *imm80* subsection. Since a comprehensive map of λ was available, the easiest way to do this was to digest Charon 15 DNA with each enzyme and to compare the results with λ (Fig. 1). This experiment showed that *Xho*I cut once, *Kpn*I and *Sal*I cut twice, and *Bgl*II cut four times in the *imm80* subsection.

The second step was the determination of the order of restriction fragments in the region.

CHARON 11	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25739
BamHI	26742
BglII	30329
BglII	30463
EcoRI	32240
BglII	33787
BglII	34089
BglII	34740
BglII	34800
****	35154
BamHI	37201
SstI	37351
HindIII	37393
BamHI	37707
KpnI	39593
BglII	39657
SstI	41213
BamHI	41635
BglII	41812
Rend	43126

CHARON 12	
Lend	0
BglII	482
BamHI	5526
KpnI	23815
KpnI	25337
BamHI	26819
EcoRI	30570
SalI	31618
SalI	32130
XhoI	32371
BamHI	33375
BglII	34599
BglII	34901
BglII	35552
BglII	35612
****	35966
BamHI	38013
SstI	38163
HindIII	38205
BamHI	38519
KpnI	40405
BglII	40469
SstI	42025
BamHI	42447
BglII	42624
Rend	43938

CHARON 13	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	21569
BamHI	22703
BglII	22784
HindIII	23513
EcoRI	27354
SalI	28402
SalI	28914
XhoI	29155
BamHI	30159
BglII	31383
BglII	31685
BglII	32336
BglII	32396
****	32750
BamHI	34797
SstI	34947
HindIII	34989
BamHI	35303
KpnI	37189
BglII	37253
SstI	38809
BamHI	39231
BglII	39408
Rend	40722

CHARON 14	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
EcoRI	27642
SalI	28690
SalI	29202
XhoI	29443
BamHI	30447
BglII	31671
BglII	31973
BglII	32624
BglII	32684
****	33038
BamHI	35085
SstI	35235
HindIII	35277
BamHI	35591
KpnI	37477
BglII	37541
SstI	39097
BamHI	39519
BglII	39696
Rend	41010

CHARON 15	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	21569
BamHI	22703
BglII	22784
HindIII	23513
BglII	28898
XhoI	29654
BglII	29780
KpnI	32525
BglII	33256
SalI	33547
SalI	33700
KpnI	34335
BglII	35284
****	35299
Rend	41936

CHARON 16	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
BglII	29188
XhoI	29944
BglII	30070
KpnI	32815
BglII	33546
SalI	33837
SalI	33990
KpnI	34624
BglII	35574
****	35589
Rend	42226

CHARON 17	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25737
SstI	26477
EcoRI	26693
HindIII	28023
BamHI	28623
SalI	33422
SalI	33934
XhoI	34175
BamHI	35179
BglII	36403
HindIII	37616
HindIII	38201
BglII	38845
BglII	39496
BglII	39556
****	39910
Rend	46547

CHARON 18	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25739
BamHI	26742
BglII	30329
BglII	30463
EcoRI	32240
BglII	33787
BglII	34089
BglII	34740
BglII	34800
****	35154
BamHI	37769
Rend	44656

CHARON 19	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	19801
SstI	20905
XbaI	25072
SstI	25336
HindIII	25737
SstI	26477
EcoRI	26693
HindIII	28023
BamHI	28623
SalI	33422
SalI	33934
XhoI	34175
BamHI	35179
BglII	36403
HindIII	37616
HindIII	38201
BglII	38845
BglII	39496
BglII	39556
****	39910
BamHI	41957
SstI	42107
HindIII	42149
BamHI	42463
KpnI	44349
BglII	44413
SstI	45969
BamHI	46391
BglII	46568
Rend	47882

CHARON 20	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	21569
BamHI	22703
BglII	22784
HindIII	23513
EcoRI	27354
SalI	28402
SalI	28914
XhoI	29155
BamHI	30159
HindIII	32169
EcoRI	32405
BglII	33308
BglII	33368
****	33722
Rend	40359

CHARON 21	
Lend	0
BglII	482
BamHI	5526
KpnI	17290
KpnI	18812
EcoRI	21569
BamHI	22703
BglII	22784
HindIII	23513
BglII	28898
XhoI	29654
BglII	29780
KpnI	32525
BglII	33256
SalI	33547
KpnI	34182
BglII	35131
****	35146
Rend	41783

FIG. 1, part 4

TABLE 1. *Subsection maps*[a]

Subsection	Enzyme	Coordinate
lac5	*Kpn*I	0 (19,181)
	Left end of *lac5*	
	*Eco*RI	989
	*Sst*I	2,093
	Right end of *lac5*	
	*Xba*I	6,259(25,161)
lac5-att80	*Kpn*I	0 (19,181)
	Left end of *lac5*	
	*Eco*RI	989
	*Sst*I	2,093
	*Bam*HI	8,111
	*Xho*I	8,416
	*Bam*HI	11,186
	Right end of *att80*	
	*Bgl*II	13,180 (5,297, *imm80*)
bio256	*Hin*dIII	0 (28,113)
	Left end of *bio256*	
	*Bam*HI	1,003
	*Bgl*II	4,590
	*Bgl*II	4,724
	*Eco*RI	6,401
	Right end of *bio256*	
	*Bgl*II	8,047 (36,494)
b189-bio1	*Kpn*I	0 (19,181)
	b189 and left end of *bio1*	
	*Bam*HI	2,001
	Right end of *bio1*	
	*Sal*I	4,767 (34,024)
imm80	*Bam*HI	0 (28,713)
	Left end of *imm80*	
	*Bgl*II	5,297
	*Xho*I	6,053
	*Bgl*II	6,179
	*Kpn*I	8,924
	*Bgl*II	9,655
	*Sal*I	9,942
	*Sal*I	10,095
	*Kpn*I	10,734
	*Bgl*II	11,683
	*Eco*RI	11,697 (40,000)
imm434	*Bam*HI	0 (35,269)
	Right end of *imm434*	
	*Hin*dIII	2,010
	*Eco*RI	2,246
	Right end of *imm434*	
	*Bgl*II	3,148 (39,586)
KH100	*Hin*dIII	0 (37,700)
	Left end of KH100	
	*Bgl*II	661
	*Eco*RI	1,516
	Right end of KH100	
	*Hin*dIII	1,760 (38,291)
QSR80	*Eco*RI	0 (40,000)
nin5	*nin5*	
	Left end of *QSR80*	
	*Bam*HI	2,047
	*Sst*I	2,197
	*Hin*dIII	2,239
	*Bam*HI	2,553
	*Kpn*I	4,439
	*Bgl*II	4,503
	*Sst*I	6,059
	*Bam*HI	6,481
	*Bgl*II	6,658
	Right end of ϕ80	7,971 (49,502)

[a] Coordinates are listed for restriction sites within subsections, with the left end of each subsection defined as zero. Deletion and substitution boundaries are inserted in their corresponding positions, but coordinates are not given since these were not measured. Coordinates in parentheses are the λ restriction map (5) coordinates of sites which occur in λ. All subsection map endpoints are in λ except for the *Bgl*II site at 13,180 of the *lac5-att80* subsection map, which corresponds to a *Bgl*II site in the *imm80* subsection map. The *Eco*RI site at 11,697 in the *imm80* subsection does not occur in the vectors, as it has been removed by mutation. This *Eco*RI site has been shown by sequence to be the endpoint of the *imm80* substitution (10).

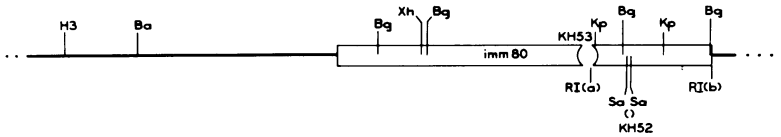

FIG. 2. *Restriction map of the imm80 region of the λ-φ80 hybrid Fbn. The straight line represents λ DNA, and the box represents φ80 DNA. The parentheses indicate the deletion KH53, which eliminates the EcoRI site, RI(a). In the Charon phages the EcoRI site, RI(b) has been removed by the point mutation BW2. The fragment bounded by EcoRI sites RI(a) and RI(b) was inserted into pVH51 to construct the plasmid pFbn3. For abbreviations, see legend to Fig. 1.*

Digestion of pFbn3 showed that all *Kpn*I and *Sal*I sites, as well as two of the *Bgl*II sites, were present in the plasmid and thus are located to the right of ΔKH53, leaving two closely spaced *Bgl*II sites and the *Xho*I site to the left of ΔKH53. The relative arrangement of these sites was established by single and multiple digestions of Charon 3 DNA. We observed that a 882-bp *Bgl*II fragment was cut into 756- and 126-bp subfragments by *Xho*I. Since no other small fragments were observed, we conclude that *Xho*I cuts once in between the *Bgl*II sites. To determine the order of the 756- and 126-bp fragments, the nearby *Kpn*I site was used as a reference. The *Kpn*I-*Bgl*II subfragment from this area measured 2,745 bp, whereas the *Kpn*I-*Xho*I frag-

ment measured 126 bp larger. Thus, the smaller of the *Xho*I-*Bgl*II subfragments was assigned to the right of the *Xho*I site. In a similar way, a series of single and double digests of pFbn3 and Charon 3 was analyzed, leading to the assignments shown in Fig. 2.

Mapping experiments with pFbn3 were done to analyze the *Bgl*II, *Kpn*I, and *Sal*I sites to the right of KH53 in *imm*80, as the restriction patterns from the plasmid were much simpler than those from phage DNA. The pair of *Eco*RI sites in the plasmid, neither of which is present in the Charon phages, also provided convenient reference points from which to measure the locations of the restriction sites situated between them.

The third and final step of analysis of the *imm*80 subsection was the least-squares calculation of map coordinates for the 12 restriction sites. To obtain the best estimates of fragment sizes, all available measurements for each of 22 fragments resulting from various combinations of enzymes were averaged. In addition, the size of the *Bgl*II-*Eco*RI fragment at the right of the subregion map was shown to be 14 bp by DNA sequence studies (10). These measurements served as input for the least-squares program of Schroeder and Blattner (11). As Fig. 3 shows, the 12 restriction sites were positioned so that the maximum error between predicted and measured fragment sizes was 2.6%.

Mapping of other subgroups proceeded along similar but generally simpler lines. Most of the restriction sites for the nine enzymes which were mapped could be determined on the basis of the sizes of DNA restriction fragments or upon the results of digestion of a phage DNA with a pair of enzymes.

The ordering of the *Bam*HI, *Sst*I, and *Hin*dIII restriction sites in the leftmost region of the *QSR*80 substitution proved difficult because of their close spacing and the lack of reference sites outside the region (Table 1). Digestion of *QSR*80 with *Bam*HI produced a fragment of 506 bp which was cut by *Hin*dIII into fragments of 192 and 314 bp. *Sst*I cut the 506-bp fragment into 356- and 150-bp pieces. It was also shown by acrylamide gel electrophoresis that the *Sst*I site was 42 bp from the *Hin*dIII site and that *Sst*I cut the smaller of the two *Bam*HI-*Hin*dIII fragments. However, it was not possible to determine the order of the two *Bam*HI-*Hin*dIII fragments, and consequently it was difficult to tell whether the *Sst*I site was to the left or to the right of the *Hin*dIII site. This problem was solved by isolating the *Hin*dIII fragment from the right end of Charon 3, using a preparative agarose gel. This DNA was redigested with *Bam*HI and analyzed on a 7.5% acrylamide gel. The larger, 315-bp *Bam*HI-*Hin*dIII fragment was found to be present, and the smaller one was absent. This showed that the closer of the two *Bam*HI sites was located to the left of the *Hin*dIII site. Since the *Bam*HI-*Hin*dIII fragment contains the *Sst*I site, this site had to be located ~45 bp to the left of the *Hin*dIII site. Once the order of these sites had been established, final map positions could be assigned by the least-squares program.

In order to complete the Charon maps, it was necessary to determine the length of each deletion and the duplication *dup*L, as well as to establish which restriction map interval is occupied by each of these mutations. In the case of ΔH3 and ΔKH52, which were generated in vitro by restriction enzyme cleavage, this was not a problem. These deletions remove 2,286 and 153 bp, respectively. Determination of the total shortening of fragments allowed the lengths of the in vivo-generated deletions to be

SITE NUMBER	MAP COORDINATE	INTERVAL
1	-602.4	.0
2	.0	602.4
3	5297.3	5297.3
4	6053.5	756.2
5	6179.0	125.5
6	8923.6	2744.6
7	9654.9	731.3
8	9942.1	287.2
9	10095.4	153.3
10	10734.3	638.9
11	11683.5	949.2
12	11697.5	14.0

FRAGMENT	SIZE ADJUSTMENTS: ORIGINAL	COMPUTED	CHANGE
1 2	602.0	602.4	.1%
1 4	6700.0	6655.8	.7%
2 3	5270.0	5297.3	.5%
3 4	740.0	756.2	2.1%
3 5	907.0	881.6	2.6%
4 5	125.0	125.5	.4%
4 6	2890.0	2870.1	.7%
5 6	2740.0	2744.6	.2%
5 7	3445.0	3475.9	.9%
5 11	5030.0	5004.5	.5%
6 7	730.0	731.3	.2%
6 10	1825.0	1810.7	.8%
7 8	285.0	287.2	.8%
7 10	1100.0	1079.4	1.9%
7 11	2050.0	2028.6	1.1%
8 9	153.0	153.3	.2%
8 12	1710.0	1755.8	2.6%
9 10	640.0	638.9	.2%
9 11	1590.0	1588.1	.1%
9 12	1600.0	1602.1	.1%
10 11	950.0	949.2	.1%
10 12	964.0	963.1	.1%
11 12	14.0	14.0	.0%

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FIG. 3. *Assignment of coordinates to restriction sites in the imm80 subsection by least-squares analysis. The site numbers at the top correspond to the restriction sites shown in Fig. 2. The map coordinate column gives the computed map positions of the restriction sites, with the BamHI site in λ defined as zero. Under fragment size adjustments are listed the sizes of fragments as measured (original) and as assigned by the program (computed) and the percent difference between these values (change). The fragments are identified in the left column by the numbers of the restriction sites which bound the fragment. A full description of the computational method appears in reference 12.*

TABLE 2. *Cloning capacity of Charon phages 1 through 21 calculated by using single restriction enzymes and pairs of enzymes*[a]

Phage	Restriction enzyme(s)	Cloning capacity (kbp)
Charon 1	*Eco*RI	8.3–20.3
	*Xba*I	0–0.9
	*Sal*I	0–1.5
	*Xho*I	0–0.9
	*Xba*I-*Sal*I	0–9.8
	*Xba*I-*Xho*I	0–10.0
	*Sal*I-*Xho*I	0–1.7
Charon 2	*Eco*RI	0–3.6
	*Xho*I	0–3.6
	*Sal*I	0–3.8
	*Eco*RI-*Xho*I	4.6–16.6
Charon 3	*Eco*RI	0–8.6
	*Xba*I	0–1.7
	*Xho*I	0–1.7
	*Sal*I	0–1.9
	*Eco*RI-*Xho*I	4.6–16.6
	*Eco*RI-*Sal*I	8.6–20.6
	*Xba*I-*Xho*I	0–11.3
	*Xba*I-*Sal*I	3.4–15.4
	*Xba*I-*Sal*I	0–5.8
Charon 4	*Eco*RI	7.3–19.3
	*Xba*I	0–4.6
Charon 5	*Sal*I	0–4.8
	*Xho*I	0–4.8
	*Eco*RI	0–4.8
	*Sal*I-*Xho*I	0–5.0
	*Sal*I-*Eco*RI	0–10.0
	*Xho*I-*Eco*RI	0–9.7
Charon 6	*Sal*I	0–7.1
	*Xho*I	0–7.1
	*Eco*RI	0–7.1
	*Sal*I-*Xho*I	0–7.4
	*Sal*I-*Eco*RI	0–10.6
	*Xho*I-*Eco*RI	0–10.4
Charon 7	*Sal*I	0–8.5
	*Xho*I	0–8.5
	*Eco*RI	0–8.5
	*Sal*I-*Xho*I	0–8.7
	*Sal*I-*Eco*RI	0–12.0
	*Xho*I-*Eco*RI	0–11.7
	*Hin*dIII	0–8.5
	*Sal*I-*Hin*dIII	0–11.7
	*Xho*I-*Hin*dIII	0–11.5
	*Hin*dIII-*Eco*RI	0–8.7
Charon 8	*Eco*RI	10.2–22.2
	*Sst*I	0–10.2
	*Xba*I	0–4.6
	*Hin*dIII	4.1–16.1
	*Sal*I	0–5.1
	*Xho*I	0–4.6
	*Sst*I-*Hin*dIII	8.9–20.9
	*Sst*I-*Sal*I	5.6–17.6
	*Sst*I-*Xho*I	5.9–17.9
	*Xba*I-*Hin*dIII	4.7–16.7
	*Xba*I-*Sal*I	1.5–13.5
	*Xba*I-*Xho*I	1.7–13.7
	*Sal*I-*Xho*I	0–5.4
Charon 9	*Eco*RI	9.6–21.6
	*Sst*I	0–7.8
	*Xba*I	0–2.3
	*Hin*dIII	3.9–15.9
	*Sst*I	0–2.8
	*Xho*I	0–2.3
	*Eco*RI-*Hin*dIII	9.9–21.9
	*Sst*I-*Sal*I	3.3–15.3
	*Sst*I-*Xho*I	3.5–15.5
	*Sst*I-*Hin*dIII	8.7–20.7
	*Xba*I-*Sal*I	0–11.1
	*Xba*I-*Xho*I	0–11.4
	*Xba*I-*Hin*dIII	4.6–16.6
	*Sal*I-*Xho*I	0–3.0
Charon 10	*Eco*RI	8.6–20.6
	*Sst*I	0.6–12.6
	*Xba*I	0–8.2
	*Hin*dIII	0–8.2
	*Sst*I-*Hin*dIII	1.0–13.0
	*Xba*I-*Hin*dIII	0–8.9
Charon 11	*Eco*RI	7.3–19.3
	*Xba*I	0–6.9
Charon 12	*Eco*RI	0–6.1
	*Sal*I	0–6.6
	*Xho*I	0–6.1
	*Eco*RI-*Sal*I	0–7.6
	*Eco*RI-*Xho*I	0–7.9
	*Sal*I-*Xho*I	0–6.8
Charon 13	*Eco*RI	3.1–15.1
	*Sal*I	0–9.8
	*Xho*I	0–9.3
	*Eco*RI-*Sal*I	4.6–16.6
	*Eco*RI-*Xho*I	4.9–12.9
	*Sal*I-*Xho*I	0–10.0
Charon 14	*Eco*RI	4.8–16.8
	*Sal*I	0–9.5
	*Xho*I	0–9.0
	*Eco*RI-*Sal*I	6.5–18.4
	*Eco*RI-*Xho*I	6.6–18.6
	*Sal*I-*Xho*I	0–9.7
Charon 15	*Eco*RI	0–8.1
	*Hin*dIII	0–8.1
	*Xho*I	0–8.1
	*Sal*I	0–8.2
	*Eco*RI-*Hin*dIII	0–10.0
	*Eco*RI-*Xho*I	4.1–16.1
	*Eco*RI-*Sal*I	8.2–20.2

TABLE 2—*Continued*

Phage	Restriction enzyme(s)	Cloning capacity (kbp)
	*Hin*dIII-*Xho*I	2.2–14.2
	*Hin*dIII-*Sal*I	6.3–18.3
	*Xho*I-*Sal*I	0.1–12.1
Charon 16	*Eco*RI	0–7.8
	*Sst*I	0–7.8
	*Xho*I	0–7.8
	*Sal*I	0–7.9
	*Eco*RI-*Sst*I	0–8.9
	*Eco*RI-*Xho*I	5.9–17.9
	*Eco*RI-*Sal*I	10.0–22.0
	*Sst*I-*Xho*I	4.8–16.8
	*Sst*I-*Sal*I	8.9–20.9
	*Xho*I-*Sal*I	0–11.8
Charon 17	*Eco*RI	0–10.3
	*Sst*I	0–9.0
	*Xba*I	0–3.5
	*Hin*dIII	3.9–15.9
	*Sal*I	0–4.0
	*Xho*I	0–3.5
	*Eco*RI-*Sal*I	5.6–17.6
	*Eco*RI-*Xho*I	5.8–17.8
	*Sst*I-*Hin*dIII	8.7–20.7
	*Sst*I-*Sal*I	4.5–16.5
	*Sst*I-*Xho*I	4.7–16.7
	*Xba*I-*Hin*dIII	4.6–16.6
	*Xba*I-*Sal*I	0.3–12.3
	*Xba*I-*Xho*I	0.6–12.6
	*Sal*I-*Xho*I	0–4.2
	*Eco*RI-*Hin*dIII	9.9–21.9
Charon 18	*Eco*RI	5.8–17.8
	*Sst*I	0–9.8
	*Xba*I	0–5.3
	*Hin*dIII	0–5.3
	*Sst*I-*Hin*dIII	0–10.2
	*Xba*I-*Hin*dIII	0–6.0
Charon 19	*Eco*RI	0–9.0
	*Xba*I	0–2.1
	*Sal*I	0–2.6
	*Xho*I	0–2.1
	*Eco*RI-*Sal*I	4.3–16.3
	*Eco*RI-*Xho*I	4.5–16.5
	*Xba*I-*Sal*I	0–11.0
	*Xba*I-*Xho*I	0–11.2
	*Sal*I-*Xho*I	0–2.9
Charon 20	*Eco*RI	8.5–20.5
	*Hin*dIII	6.3–18.3
	*Sal*I	0–10.2
	*Xho*I	0–9.6
	*Sal*I-*Xho*I	0–10.4
Charon 21	*Eco*RI	0–8.2
	*Hin*dIII	0–8.2
	*Xho*I	0–8.2
	*Sal*I	0–8.2
	*Eco*RI-*Hin*dIII	0–10.2
	*Eco*RI-*Xho*I	4.3–16.3
	*Eco*RI-*Sal*I	8.2–20.2
	*Hin*dIII-*Xho*I	2.4–14.4
	*Hin*dIII-*Sal*I	6.3–18.3
	*Xho*I-*Sal*I	0.1–12.1

[a] It is assumed that a viable phage must have a total length between 38,000 and 50,000 bp. Cloning capacities are indicated in kilobase pairs. Charons 5, 6, 7, and 12 contain *dup*L. Phage which have either lost or gained a copy of the duplication due to unequal crossing-over occur in the population. A portion of the phage therefore has a cloning capacity enlarged or reduced by 6.5 kbp (the size of *dup*L).

determined. *Nin*5 was found to delete 2,865 bp, *b*1007 deleted 4,754 bp, *b*189 deleted 8,051 bp, and KH54 deleted 2,138 bp. KH53 was not measured since the *imm*80 subsection was defined to contain it. The insertion KH100 (IS5) was determined to be 1,176 bp long.

A spontaneous duplication of a region of the left arm of λ, designated *dup*L, is present in Charon phages 3, 6, and 7 (3, 13). The *Pvu*I site at 12,376 on the left arm of λ (5) is in the region of DNA which is duplicated in *dup*L, and thus *Pvu*I produces a restriction fragment which corresponds to the size of the duplication. Digestion of Charon 12 DNA with *Pvu*I yielded a band with a size of 6,525 bp. This agrees well with a value of 13.4% of the λ genome (6,580 bp at 491 bp/1% of λ) for the size of *dup*L, as determined by buoyant density measurements in an analytical ultracentrifuge (13).

The final step of mapping the Charon vectors was to splice the subregions together with appropriate sections of λ to prepare Fig. 1. This figure gives base pair coordinates for each restriction site in each vector. Preparation of the figure was aided by the use of a computer program which combined subregions (Schroeder, unpublished). All of the splice points used were restriction sites in λ DNA, with the exception of the joints connecting *att*80 to *imm*80 in Charon 2 (Table 1).

It should be emphasized that the number of significant figures in Fig. 1 does not indicate the accuracy of each coordinate. There is simply no way to round the figures off that preserves the ability to subtract coordinates to obtain the lengths of small fragments.

The accuracy of the final map is dependent in part on the accuracy of each subsection map and in part on the errors inherent in the use of subsections. For each of the subsections the sizes of fragments predicted from the map agreed with electrophoresis measurements within ±5%. The subsection approach did not allow inclusion of large fragments spanning subsection boundaries. Despite this, the total length of Charons 1 through 20 as determined by restriction mapping agrees well with the sizes that were determined by buoyant density centrifugation (13). The average difference in length for the vectors arrived at by these two methods was 0.6% of the λ genome, and the greatest difference in size was 1.6% of the total λ genome. Another uncertainty associated with the subsection approach is the possibility that one of the vectors might contain unsuspected mutations that delete or add sites. Because of the large number of experiments that would be needed, it was not possible to check every vector with every enzyme. There is precedent for such problems in that a new *Eco*RI site was found in a stock of Charon 4A (13). A similar case is presented by the *ind*⁻ mutation, which adds a *Hin*dIII site to the immunity region of λ (4). We have confirmed that this site is present in the KH100-containing phages (Charons 1 and 9), as was reported previously (1). Charon 19, which contains *c*I857, does not have the *Hin*dIII site and thus presumably is *ind*⁺. Further experience in using the phages may be the only way to learn whether other anomalies of this sort occur.

The completed maps show that many of the Charon phages possess restriction sites for the enzymes *Xho*I, *Sal*I, and *Xba*I solely within the dispensable regions, and thus these enzymes can be used for cloning. This accordingly increases the number of pairwise combinations of enzymes which may be used. Table 2 lists the possibilities that are now available and the sizes of fragments that can be cloned, assuming that λ capsids can hold between 38 and 50 kilobase pairs of DNA (3). These capacities are conservative, since λ phages having at least 52,000 bp have been isolated (13).

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