

Physical Map of the Bacteriophage P22 Genome

JOAN E. RUTILA AND ETHEL NOLAND JACKSON¹

Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan 48109

Received April 3, 1981; accepted May 29, 1981

P22 mutations have been located relative to restriction endonuclease cleavage sites on the P22 chromosome by rescue of genetic markers from cloned DNA fragments. These data, in combination with prior genetic and physical mapping data, have allowed the construction of a detailed composite physical and genetic map.

Many cleavage sites for restriction endonucleases have now been mapped on the chromosome of bacteriophage P22 (1-5). This physical map of cleavage sites facilitates generation of defined genome segments for a variety of applications. Many such applications require information about the location of genes relative to restriction sites. In previous work, we have oriented the genetic map of P22 with the restriction endonuclease cleavage site map and located some mutations relative to certain restriction targets in the late region of the P22 genome (4). Comparison of the resulting physical gene map with the preexisting genetic map shows that the recombination frequency map is distorted in the late region and gives a poor approximation of physical locations of genes on the chromosome (4, 6). In the present studies, we have mapped more mutations relative to additional cleavage sites to give a higher resolution map of the late region. We have also correlated physical and genetic sites in portions of the early region not included in the previous study. The combination of these results defines a more detailed physical gene map of the P22 genome.

In order to integrate the genetic and physical maps, P22 wild type DNA was cleaved with restriction endonucleases and the resulting fragments were inserted into

the plasmid vector pBR322 (Table 1). The resulting plasmids were introduced into *Salmonella typhimurium*. Genetic procedures were then applied to define what P22 genes were present on each cloned fragment. *S. typhimurium* strains containing no amber suppressor and carrying plasmids having known P22 DNA restriction fragment inserts were used as lawns for plating various P22 amber mutant strains. Since the infection is carried out under nonpermissive conditions only those phage that have been able to recombine with wild type cloned P22 sequences or that have been complemented by plasmid-encoded P22 proteins will form plaques. Therefore an increase in plating efficiency of a P22 amber mutant strain on an Su⁻ host carrying a cloned P22 DNA fragment indicates the fragment carries the wild type allele of the amber mutation. Representative data for this type of experiment have been reported previously (4). The results of such plating tests for many mutants on strains containing cloned fragments (Table 1) are summarized in Fig. 1. The physical map coordinates for the restriction sites that define these cloned P22 fragments (Table 1) are listed in Table 2.

Markers in the nonessential *ant* (7, 8) gene were mapped by infecting permissive cells carrying a plasmid with P22 *ant*⁻ mutant strains and allowing one cycle of lytic growth before harvesting the lysate. The fraction of wild type recombinant phage in the lysate was then determined

¹To whom requests for reprints should be addressed.

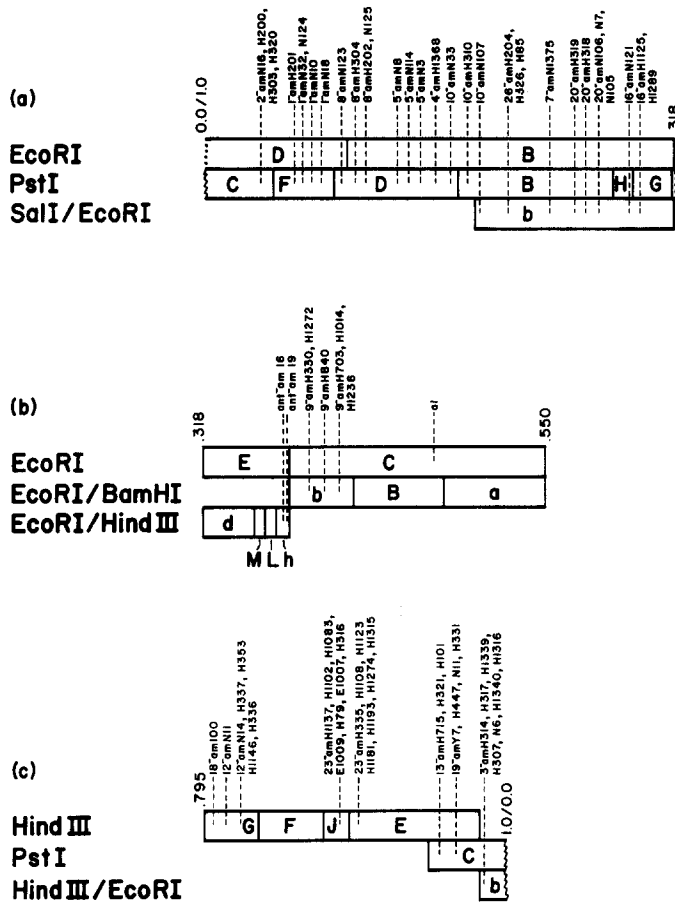


FIG. 1. P22 genes were located relative to restriction endonuclease cleavage sites by rescue of the wild type marker from restriction fragments of P22 DNA cloned in the plasmid vector pBR322. Markers in essential genes were located by plating P22 amber mutant strains on *Su*⁻ *S. typhimurium* NK1345 carrying either pBR322 or a plasmid containing cloned P22 sequences (the hybrid plasmids used are listed in Table 1). Plating efficiencies of all the P22 *am* strains on NK1345 carrying pBR322 range from 10^{-5} to 10^{-7} , relative to the *Su*⁺ host. An increase in plating efficiency of 10^2 - 10^6 on NK1345 containing one of the hybrid plasmids (Table 1) indicates the wild type allele of the amber mutation is present on the cloned P22 DNA fragment (see reference (4) for representative plating efficiency data). Mutations in nonessential genes were located by infecting liquid cultures of permissive cells carrying cloned P22 sequences with the phage mutant to be tested. The lysate produced after a single round of infection was titered on both permissive and nonpermissive hosts to determine the relative number of wild type recombinant progeny (see Table 3). Mutant phage strains used were from the collection of D. Botstein unless otherwise indicated. Results of the marker rescue experiments are summarized in the figure. The locations of P22 amber markers are shown relative to restriction endonuclease sites on P22 DNA. Dashed lines connect a mutation with all lettered P22 fragments from which the wild type allele was rescued. The position of mutations within a restriction fragment is not to scale. Each of several alleles associated with a single dashed line has been rescued from the indicated DNA fragments, but the order of alleles within the group is unknown. Physical site coordinates of restriction endonuclease cleavage sites are given in Table 2. (a) Physical gene map of P22 DNA between coordinates 0 and 0.318. Fragment D is formed by *EcoRI* cleavage at 0.096 and DNA maturation at *pac* (20). The order of the mutations in gene 1 (9), gene 8 (F. Winston and D. Botstein, unpublished experiments), gene 5 (9), and gene 20 (P. B. Berget, G. Weinstock, and D. Botstein, manuscript in preparation) has been determined previously. Amber alleles in genes 10 and 16 were ordered by these experiments. (b) Physical gene map of P22

TABLE 1
P22 DNA FRAGMENTS CLONED IN pBR322^a

Plasmid	Cloned P22 fragment	Plasmid	Cloned P22 fragment
pP22-314	<i>Hind</i> III G	pP22-451	<i>Sal</i> I/ <i>Eco</i> RI a
pP22-318	<i>Hind</i> III F	pP22-201	<i>Eco</i> RI/ <i>Bam</i> HI b
pP22-322	<i>Hind</i> III J	pP22-250	<i>Bam</i> HI B
pP22-312	<i>Hind</i> III E	pP22-512	<i>Pst</i> I C
pP22-384	<i>Eco</i> RI/ <i>Hind</i> III b	pP22-513	<i>Pst</i> I F
pP22-3	<i>Eco</i> RI D	pP22-508	<i>Pst</i> I D
pP22-4	<i>Eco</i> RI B	pP22-515	<i>Pst</i> I B
pP22-6	<i>Eco</i> RI E	pP22-502	<i>Pst</i> I H
pP22-10	<i>Eco</i> RI C	pP22-514	<i>Pst</i> I G

^a The restriction endonuclease fragments of P22 c1-7 DNA listed were inserted into the plasmid vector pBR322 and the hybrid plasmids were used in marker rescue studies to yield the maps given in Fig. 1. Construction of the hybrid plasmids containing *Eco*RI, *Eco*RI/*Sal*I, *Eco*RI/*Bam*HI (4), *Hind*III and *Hind*III/*Eco*RI (5) fragments has been described previously. The *Pst*I cleavage site map of P22 DNA was generated and *Pst*I fragments inserted into pBR322 by A. Siegel, R. Deans, and E. Jackson (unpublished experiments). All plasmids were initially introduced into *Escherichia coli*. Plasmid DNA was prepared from the *E. coli* clones and used to transform *S. typhimurium* LT2 r⁻m⁻ *his*C527*am cys*A1348*am* (DB5289, obtained from D. Botstein) or *S. typhimurium* LT2 r⁻m⁺ *gal*⁺ *rif*^R *nal*^R *cys*⁻ (NK1345, obtained from N. Kleckner) as described previously (4).

as described in Table 3. The P22 nonessential antigen conversion gene *a1* was mapped relative to restriction sites by a cell agglutination assay (see Fig. 1c).

By these approaches, various P22 genes have been mapped relative to physical markers on the chromosome. These results

are summarized in Fig. 1. Many of the mutations used in this study have been ordered previously by genetic mapping procedures (3, 7-14) and the order of markers determined by our experiments agrees with the earlier results. However, amber mutations in gene 10 have not been

DNA between coordinates 0.318 and 0.550. Rescue of alleles of gene *ant* which is not essential for lytic growth is described in Table 3. The *ant*⁻ *am* alleles used have been ordered, the *ant*⁻ *am*19 allele being the most carboxyl-terminal *ant*⁻ amber known (8, 13). The 9⁻ *am* alleles were ordered (P. B. Berget, G. Weinstock, and D. Botstein, manuscript in preparation; 15) by using a series of prophage deletion mutants. The 9⁻ *am*H330 and H1272 alleles are in the most amino-terminal group while the 9⁻ *am*H703, H1014, and H1236 alleles are in the most carboxyl-terminal group of 9⁻ amber mutations. The region (*a1*) which codes for a prophage-directed modification of the host O antigen was mapped to the *Eco*RI C fragment by an agglutination test (10, 25). NK1345 cells containing the *Eco*RI C insert in pBR322 were found to be agglutinated by antisera (O antisera factor 1, Difco) directed against the modified O antigen (factor 1). Similar tests done with cells containing cloned *Bam*HI B or *Eco*RI/*Bam*HI fragment a were negative for factor 1. (c) Physical gene map of P22 DNA between coordinates 0.795 to 1. The most amino-terminal mutation known in gene 12 is 12⁻ *am*N11, and the 12⁻ alleles N14, H336, H337, H353, H1146 make up the most carboxyl-terminal of eight groups of 12⁻ alleles mapped by marker rescue from P22 prophage deletion strains (3). Each of the two groups of 23⁻ *am* alleles (from the collections of T. Potete and D. Botstein) defined by our experiments probably include independent isolations of mutations at the same site (M. Susskind, unpublished experiments). Since the *Pst*I C and *Hind*III/*Eco*RI b fragments span the *pac* site (at coordinate 0) only a portion of each of these fragments is shown. Gene 3⁻ *am* alleles are shown on the opposite side of the *pac* site from gene 2 since these 3⁻ *am* alleles were rescued from the *Eco*RI/*Hind*III b fragment and not from the smaller subfragment, *Eco*RI D, which has an end at *pac* (1). However, we cannot exclude the possibility that gene 3 markers lie within *Eco*RI D too close to the end of the insert to yield detectable numbers of recombinants.

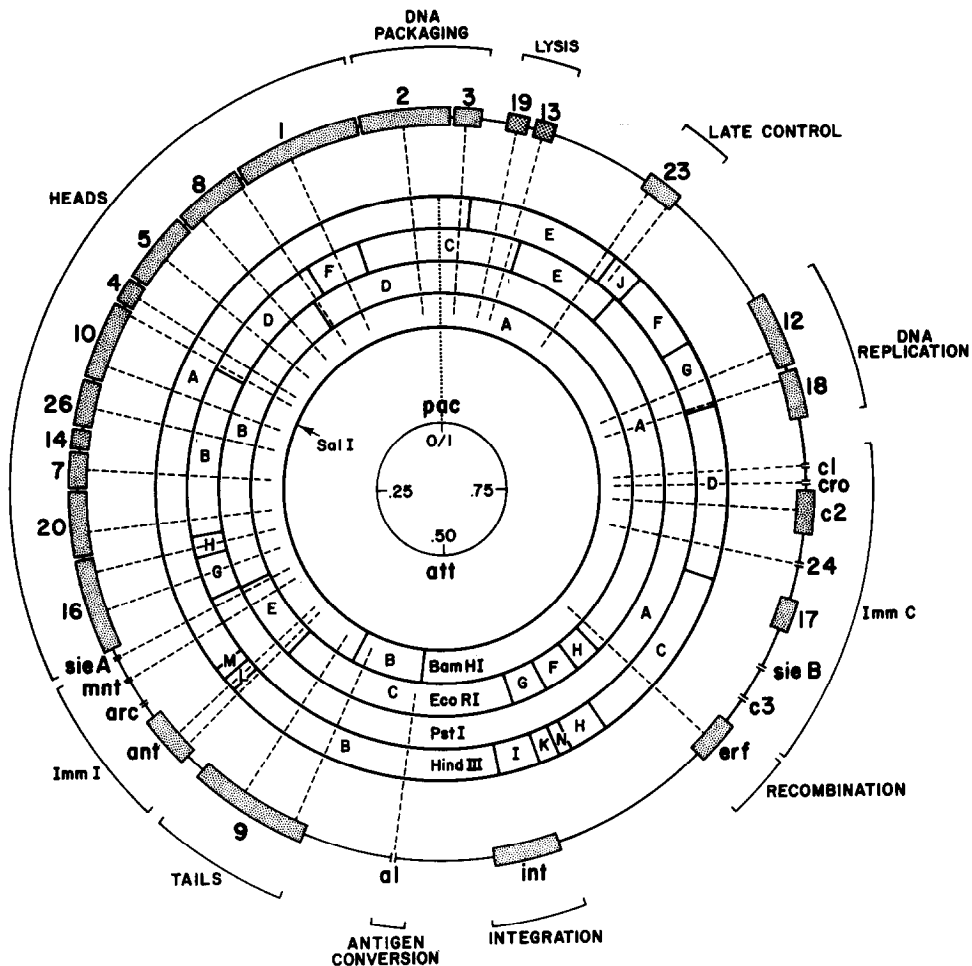


FIG. 2. The physical map of the P22 genome shows the approximate location of genes relative to restriction enzyme cleavage sites. Because mature linear P22 DNA molecules are circularly permuted, the cleavage map is circular (1). Physical map coordinates, indicated on the inner circle, are fractions of the length of one complete set of genes (41.6 kb, 1). *Pac* is the site at which headful packaging of P22 DNA initiates and *att* is the prophage attachment site (16, 17, 20, 26). The fragment maps shown as four concentric rings are produced by digestion with *Bam*HI (3, 4); *Eco*RI (1, 4, 20; fragment D is a segment of *Eco*RI A generated by DNA maturation at *pac*); *Pst*I (5, A. Siegel, R. Deans, and E. Jackson, unpublished experiments); and *Hind*III (2, 23). The position of the single cleavage site for *Sal*I (3, 4) is indicated by an arrow. The approximate physical map locations of P22 genes are shown on the outer ring as shaded bars of length proportional to the size of the protein encoded (27), or as short parallel lines where the protein size is unknown. Dashed lines from a gene intersect those restriction fragments known to include at least a portion of the gene. Gene order and gene functions have recently been reviewed (14). Three additional genes, 14 (27), *arc* (13), and *cro* (28) are shown here. Genes from the DNA replication region counterclockwise through the antigen conversion region have been assigned to restriction fragments by marker rescue from cloned fragments (Fig. 1; Table 3; 4), except for *sieA* and *mnt* which were located by assaying expression of the genes from cloned fragments (J. Rutila and E. Jackson, manuscript in preparation). Insertions in *ant* were shown to lie in *Hind*III L (2), so *Hind*III site 3 lies in gene *ant* (see Fig. 1). Weinstock and Botstein (6) have mapped Tn1 insertions in genes 20, 16, *ant*, 9, and the *a1* region relative to *Eco*RI and *Bam*HI sites, and their conclusions are in agreement with this map. Missense mutations (P. B. Berget, G. Weinstock, and D. Botstein, manuscript in preparation) and insertions within gene 9 (6) have been mapped in the *Bam*HI B fragment, indicating that gene

TABLE 2

PHYSICAL MAP COORDINATES OF RESTRICTION ENDONUCLEASE CLEAVAGE SITES ON P22 DNA^a

Restriction endonuclease cleavage site	Map coordinate	Restriction endonuclease cleavage site	Map coordinate
<i>EcoRI</i>		<i>HindIII</i>	
1	0.096	1	0.353
2	0.318	2	0.360
3	0.376	3	0.368
4	0.550	4	0.530
5	0.576	5	0.554
6	0.603	6	0.566
7	0.623	7	0.573
		8	0.596
<i>PstI</i>		9	0.699
1	0.045	10	0.795
2	0.087	11	0.832
3	0.172	12	0.876
4	0.277	13	0.894
5	0.290	14	0.982
6	0.317		
7	0.879		
8	0.948		
		<i>BamHI</i>	
<i>SaII</i>		1	0.420
1	0.183	2	0.481

^a The map coordinate for each restriction endonuclease site is given as a fraction of the length of the P22 genome, 41.6 kb (1), where the origin of the coordinate system is at *pac* (4). The *EcoRI*, *HindIII*, *SaII*, and *BamHI* cleavage sites are numbered in order of map location. Site locations were reported previously (4). The *PstI* sites were mapped by A. Siegel, R. Deans, and E. Jackson (unpublished experiments). The coordinate for the *PstI* site 1 was adjusted to agree with unpublished data of S. Casjens.

ordered previously. The plating efficiencies of three different 10^{-am} alleles on Su⁻ hosts carrying various cloned P22 fragments show that there is both a *PstI* and a *SaII* cleavage site in gene 10. The map order of the three 10^{-am} mutations shown in Fig. 1 was inferred from the known map order of these restriction targets. The same type of analysis was used in ordering gene 16 alleles as shown in Fig. 1.

This map generated from the marker rescue experiments (Fig. 1) extends our previous results (4) and is the most complete and detailed description now available of the physical location of P22 genes

in the regions 0 to 0.555 and 0.795 to 1.0. Several physical gene maps of segments of these regions have been made previously by other approaches. Chan and Botstein (9) constructed a physical map of a portion of the genome near the attachment site from electron microscopic studies of insertions, deletions, and substitutions. Weinstock and Botstein (6) correlated genetic and physical maps in the interval between genes 20 and 9 by measuring the distances between Tn1 insertions and sites of cleavage by *EcoRI* or *BamHI* in the region. Conditional lethal mutations in gene 9 have been mapped

9 overlaps *BamHI* site 1 (see Fig. 1). Early region genes between *a1* and 18 were placed on the map by restriction endonuclease digestion of phages carrying insertions, deletions, or substitutions as follows: *int* and *erf*, Youderian and Susskind (23); 24, *c2*, *c1*, Jackson *et al.* (1), Deans and Jackson (2), Poteete *et al.* (29). Genes *c3*, *steB*, and 17 are located between genes 24 and *erf* by recombination frequency data (14). The order of these three genes is uncertain.

TABLE 3
 RESCUE OF *ant* ALLELES FROM CLONED P22 RESTRICTION FRAGMENTS^a

Phage strain	Source of lysate				
	NK1345/ pBR322	NK1345/ pP22-306	NK1345/ pP22-320	NK1345/ pP22-351	NK1345/ pP22-201
<i>ant⁻am</i> 16	1.1×10^{-7}	1.1×10^{-7}	4.0×10^{-7}	1.0×10^{-5}	1.6×10^{-7}
<i>ant⁻am</i> 19	2.7×10^{-7}	3.3×10^{-7}	4.0×10^{-7}	8.3×10^{-6}	1.9×10^{-7}
Wild type	1.2	0.8	0.7	0.6	0.7

^a Cultures of the Su⁻ strain *S. typhimurium* NK1345 (LT2 r⁻m⁺ gal⁺ rif^R nal^R cys⁻, obtained from N. Kleckner) containing the hybrid plasmids indicated were infected while growing exponentially in L broth (24) at 37° with one of the indicated P22 strains (8, 13) at a multiplicity of five phage per cell. The infected culture was incubated with aeration at 37° for 25 min, and then chloroform was added to complete lysis. The lysate was then titered on DB5057 (8) and NK1345. *Ant⁺* phage will grow on both hosts. *Ant⁻* phage grow on NK1345 but not DB5057 (8). The ratio of the titer on DB5057 to the titer on NK1345 is given for each lysate. Both *ant⁻am* alleles rescue from the hybrid plasmid which contains the P22 *EcoRI/HindIII* h fragment (NK1345/pP22-351), although the small size of this fragment (about 300 base pairs) limits the frequency of wild type recombinants obtained.

relative to deletion endpoints and the physical locations of some amber sites related to the size of amber fragments of gene 9 protein (15, P. B. Berget, G. Weinstock, and D. Botstein, manuscript in preparation). Each method of correlating physical and genetic maps has advantages for each particular application. The rescue of genetic markers from cloned restriction fragments is a convenient way to relate genetic markers in all regions of the chromosome to physical markers which are present in all P22 strains. Many different alleles of a gene can be quickly mapped once a set of cloned fragments is available, and both essential and nonessential genes can be placed relative to restriction targets. When genes are located relative to restriction enzyme targets, cleavage at those sites can be used to produce fragments of the chromosome of known genetic content.

Comparison of the P22 recombination frequency map with the physical maps available previously had shown that the late gene region is expanded on the genetic map (1, 4, 6, 11). The more detailed physical gene map of Fig. 1 further defines the magnitude and extent of the distortion. Genes 3, 2, 1, 8, and 5 occupy over 40% of the recombination frequency map (11) but less than 20% of the physical map (Fig.

1). The expansion of the genetic map in the vicinity of genes 3, 2, 1, 8, and 5 is probably a consequence of the P22 headful packaging mechanism which generates circularly permuted, terminally redundant linear DNA molecules (16-18). Only a limited set of permuted molecules are packaged, since ends of mature linears fall predominantly in the region 0.03 to 0.15 on the physical map (1, 4, 16). Because the permuted molecules are terminally redundant, genes in the region 0 to 0.15 will appear in heterozygotes in a genetic cross at high frequency. When the terminally redundant heterozygotic chromosome infects the next host cell, an essential early step in the replicative cycle is recombination between redundant ends (19), thereby providing opportunity for generating recombinant genomes. The finding that genes 3, 2, 1, 8, and 5 are expanded on the genetic map relative to the physical map is therefore further confirmation of the location of the packaging origin, the direction of sequential headful packaging, and the number of sequential headfuls inferred from other data (16, 20-22).

A composite physical map of the complete P22 genome can be generated by correlating the map of Fig. 1 with other physical and genetic mapping data for the region 0.550 to 0.795. Figure 2 depicts the

approximate physical location of all P22 genes of known function. In addition, the physical map of genes for some proteins not essential for lytic growth which are encoded in the region 0.42 to 0.63 has been reported (23). The function of most of these proteins is not yet known, and the genes encoding them are not included on the map. The relative distances between genetic markers is different on the physical map of Fig. 2 than on previous P22 maps (6, 11, 14), and is a more accurate representation of gene placement on the phage chromosome. This map will be useful since genes throughout the chromosome have been located relative to physical markers that can be used to generate defined chromosomal fragments.

ACKNOWLEDGMENTS

We thank David Botstein, Miriam Susskind, Peter Berget, Nancy Kleckner, and Tony Poteete for generously supplying the *S. typhimurium* and P22 strains. We also thank Miriam Susskind, Peter Berget, and Sherwood Casjens who provided access to unpublished data. This work was supported by Grant number AI-12369 from the National Institutes of Health. JER was supported by training Grant number 1-T32-GM-07544-03 awarded by the National Institutes of Health.

REFERENCES

- JACKSON, E. N., MILLER, H. I., and ADAMS, M. L., *J. Mol. Biol.* **118**, 347-363 (1978).
- DEANS, R. J., and JACKSON, E. N., *Virology* **95**, 359-372 (1979).
- WEINSTOCK, G. M., Ph.D. thesis, Massachusetts Institute of Technology (1977).
- CHISHOLM, R. L., DEANS, R. J., JACKSON, E. N., JACKSON, D. A., and RUTILA, J. E., *Virology* **102**, 172-189 (1980).
- DEANS, R. J., Ph.D. thesis, University of Michigan (1979).
- WEINSTOCK, G. M., and BOTSTEIN, D., *Virology* **106**, 92-99 (1980).
- LEVINE, M., TRUESDELL, S., RAMAKRISHNAN, T., and BRONSON, J. J., *J. Mol. Biol.* **91**, 421-438 (1975).
- BOTSTEIN, D., LEW, K. K., JARVIK, V., and SWANSON, C. A., *J. Mol. Biol.* **91**, 439-462 (1975).
- CHAN, R. K., and BOTSTEIN, D., *Virology* **49**, 257-267 (1972).
- GOUGH, M., and SCOTT, J., *Virology* **50**, 603-605 (1972).
- BOTSTEIN, D., CHAN, R. K., and WADDELL, C. H., *Virology* **49**, 268-282 (1972).
- POTEETE, A. R., and KING, J., *Virology* **76**, 725-739 (1977).
- SUSSKIND, M. M., *J. Mol. Biol.* **138**, 685-713 (1980).
- SUSSKIND, M. M., and BOTSTEIN, D., *Microbiol. Rev.* **42**, 385-413 (1978).
- SMITH, D. H., BERGET, P. B., and KING, J., *Genetics* **96**, 331-352 (1980).
- TYE, B. K., HUBERMAN, J. A., and BOTSTEIN, D., *J. Mol. Biol.* **85**, 501-537 (1974).
- RHOADES, M., MACHATTIE, L. A., and THOMAS, C. A. JR., *J. Mol. Biol.* **37**, 21-40 (1968).
- ISRAEL, V., *Virology* **106**, 100-106 (1980).
- BOTSTEIN, D., and MATZ, M. J., *J. Mol. Biol.* **54**, 417-440 (1970).
- JACKSON, E. N., JACKSON, D. A., and DEANS, R. J., *J. Mol. Biol.* **118**, 365-388 (1978).
- WEAVER, S., and LEVINE, M., *J. Mol. Biol.* **118**, 389-411 (1978).
- CHAN, R. K., Ph.D. thesis, Massachusetts Institute of Technology (1974).
- YOUDEIRIAN, P., and SUSSKIND, M. M., *Virology* **107**, 270-282 (1980).
- SMITH, H. O., and LEVINE, M., *Virology* **31**, 207-216 (1967).
- YOUNG, B. G., FUKAZAWA, Y., and HARTMAN, P. E., *Virology* **23**, 279-283 (1964).
- RAO, R. N., and SMITH, H. O., *Virology* **36**, 328-330 (1968).
- YOUDEIRIAN, P., and SUSSKIND, M. M., *Virology* **107**, 258-269 (1980).
- WINSTON, F., and BOTSTEIN, D., manuscript in preparation.
- POTEETE, A. R., PTASHNE, M., BALLIVET, M., and EISEN, H., *J. Mol. Biol.* **137**, 81-91 (1980).