MICROBIOLOGICAL REVIEWS, Dec. 1992, p. 577–591 Vol. 56, No. 4
0146-0749/92/040577-15$02.00/0
Copyright © 1992, American Society for Microbiology

Bacteriophage Lambda as a Cloning Vector†

VIJAY M. CHAUTHAIWALE,[1]‡ A. THERWATH,[2] AND VASANTI V. DESHPANDE[1]*

Division of Biochemical Sciences, National Chemical Laboratory, Pune 411 008, India,[1] and Unit de Parasitologie Moleculaire, Faculté de Médecine, 15, Rue de L'Ecole de Médecine, 75270 Paris Cedex 06, France[2]

INTRODUCTION 578
Plasmid Vectors 578
General-purpose cloning vectors 578
Expression vectors 578
Promoter probe and terminator probe vectors 578
Cosmids 578
Phagemids 578
Bacteriophage Vectors 578
Filamentous phages 578
Double-stranded phage vectors 579
Scope of Present Review 579
LIFE CYCLE AND GENETICS OF LAMBDA 579
Development of Lambda: Two Alternative Modes 579
Lytic Cycle 579
Three stages of lytic cycle 579
DNA replication and in vivo packaging 579
Lysogeny 580
Lysogeny or Lytic Cycle 580
PHAGE LAMBDA AS A VECTOR 580
Manipulation of Restriction Sites 580
Size Limitation for Packaging 580
Transfection of Recombinant Molecules 581
Two-strain packaging 581
Single-strain packaging 581
Biological Containment 581
PHAGE VECTORS 581
Replacement Vectors 581
Insertion Vectors 581
Charon Phages 582
λ1059 and Derivatives 584
λEMBL 585
λgt11 to λgt23 585
λgt10 585
λZAP 585
Novel Lambda Vectors 585
Vectors for directional cloning 585
Vectors with controlled lytic and lysogenic events 586
Vectors for in vitro transcription of cloned inserts 586
Choice of Hosts for Lambda Vectors 586
Storage of Lambda Stocks 587
DETECTION OF RECOMBINANTS 587
Screening a Phage Library 587
Immunological screening 587
Nucleic acid probes 587
Differential and Subtractive Screening Methods 587
Differential screening 588
Subtractive screening 588

* Corresponding author. Electronic mail address: vasanthi@ncl.ernet.in.
† NCL communication no. 5228.
‡ Present address: Department of Biochemistry, University of Tennessee, Knoxville, TN 37996-0840.

CONCLUDING REMARKS 588
ACKNOWLEDGMENT 588
REFERENCES 588

INTRODUCTION

Recombinant DNA techniques have allowed the isolation and propagation of specific DNA fragments which can be easily sequenced and/or used as highly specific probes. In vitro site-directed modifications of these fragments and their reintroduction into the genome result in a modified genetic makeup of an organism. In addition, it is now possible to induce overproduction of commercially important proteins by genetically tailored microorganisms.

Several cloning strategies have been developed to meet various specific requirements. Cloning protocols have been designed for a variety of host systems. However, *Escherichia coli* still remains the most popular host of choice since its genetics, physiology, and molecular biology have been studied in great detail and a wealth of information is readily available. Many cloning vectors have also been constructed for use with *E. coli* as a host. Although this review focuses on the basic and applied aspects of bacteriophage lambda vectors, an overview of other vectors is included for comparison. In general, cloning vectors can be broadly classified as plasmid and phage vectors.

Plasmid Vectors

Plasmid vectors are convenient for cloning of small DNA fragments for restriction mapping and for studying regulatory regions. However, these vectors have a relatively small insert capacity. Therefore, a large number of clones are required for screening of a single-copy DNA fragment of higher eukaryotes. Second, the handling and storage of these clones is time-consuming and difficult. The repeated subcultures of recombinants may result in deletions in the inserts.

The plasmid vectors can be of three main types: general-purpose cloning vectors, expression vectors, and promoter probe or terminator probe vectors.

General-purpose cloning vectors. Cloning of foreign DNA fragments in general-purpose cloning vectors (e.g., pBR322 [11]) selectively inactivates one of the markers (insertional inactivation) or derepresses a silent marker (positive selection) so as to differentiate the recombinants from the native phenotype of the vector.

Expression vectors. In expression vectors (e.g., pUC18 [123]), DNA to be cloned and expressed is inserted downstream of a strong promoter present in the vector. The expression of the foreign gene is regulated by the vector promoter irrespective of the recognition of its own regulatory sequence.

Promoter probe and terminator probe vectors. Promoter probe (e.g., pJAC4 [51]) and terminator probe vectors are useful for the isolation of regulatory sequences such as promoters or terminators and for studying their recognition by a specific host. They possess a structural gene devoid of the promoter or the terminator sequence (18).

Cosmids

Cosmids (e.g., pWE15 and pWE16 [118]) are plasmids containing the lambda *cos* ends (15). They are 4 to 6 kb in size and are specifically designed for cloning of large DNA fragments (40 to 50 kb). They have (i) a drug resistance marker, (ii) a plasmid origin of replication, (iii) a fragment carrying the ligated cohesive ends (*cos*) of phage lambda, and (iv) one or more unique restriction sites for cloning. The recombinant molecules can be conveniently packaged in vitro inside a phage coat by the cleavage of two *cos* sites flanking an insert DNA (15). The resultant phages are then infected into a suitable *E. coli* host. (A cosmid molecule alone cannot be packaged because it falls short of the minimum size required for packaging.) Inside a cell, two *cos* ends are ligated by the host ligase, resulting in a circular molecule which can be propagated as a plasmid, and a drug resistance marker is expressed.

Although cloning in cosmids is preferred for certain specialized purposes, their use is associated with a few problems (recombination and deletion of the insert, low yields of recombinant DNA, etc.).

Phagemids

Phagemids (e.g., pUC118 and pUC119 [116] and pBS vectors [97]) combine desirable properties of both plasmids and filamentous phages. They carry (i) the ColE1 origin of replication, (ii) a selectable marker such as antibiotic resistance, and (iii) the major intergenic region of a filamentous phage (20–22). The segments of foreign DNA cloned in these vectors can be propagated as plasmids. When cells harboring these plasmids are infected with a suitable helper bacteriophage, the mode of replication of the plasmid changes under the influence of the gene II product of the incoming virus. Interaction of the intergenic region of the plasmid with the gene II protein initiates the rolling-circle replication to generate copies of one strand of the plasmid DNA, which are packaged into progeny bacteriophage particles (68, 129). The single-stranded DNA purified from these particles is used as a template to determine the nucleotide sequence of one strand of the foreign DNA segment, for site-directed mutagenesis or as a strand-specific probe. Phagemids provide high yields of double-stranded DNA and render unnecessary the time-consuming process of subcloning DNA fragments from plasmids to filamentous bacteriophages.

Bacteriophage Vectors

Both single-stranded (filamentous) and double-stranded *E. coli* phages have been exploited as cloning vectors.

Filamentous phages. Filamentous phages are not lytic. They coexist with the infected cells for several generations and are convenient for cloning genes which produce toxic products. Among the filamentous phages, fd, f1, and M13 have been well characterized and their genomes have been sequenced (4, 5, 115). Their gene functions and molecular mode of propagation are very similar. They infect cells via F pili, and the first mature phage appears within 15 min (66, 67).

Phage M13 is widely used in nucleotide sequencing and site-directed mutagenesis since its genome can exist either in a single-stranded form inside a phage coat or as a double-stranded replicative form within the infected cell. During replication, only the plus strand of the replicative form is selectively packaged by the phage proteins (71). The replicative form is a covalently closed circular molecule and hence can be used as a plasmid vector and transformed into

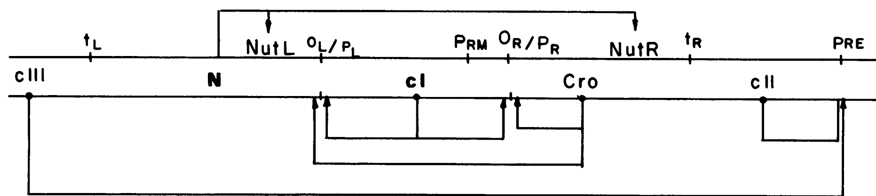


FIG. 1. Regulatory region of the lambda genome. Binding sites of specific gene products are indicated by the arrowheads.

the host by the usual transformation procedures. The vectors derived from M13, e.g., M13mp18 and M13mp19, have the same polylinker as that of pUC18 and pUC19, respectively (123). The DNA fragments having noncomplementary ends can be directionally cloned in this pair of vectors, and the two strands of DNA can be sequenced independently.

Double-stranded phage vectors. Of the double-stranded phages, bacteriophage lambda-derived vectors are the most popular tools for several reasons: (i) acceptance by the phage of large foreign DNA fragments, thereby increasing the chances of screening a single clone carrying a DNA sequence corresponding to a complete gene; (ii) development and availability of refined techniques aimed at minimizing the problems of background due to nonrecombinants; (iii) the possibility of screening several thousand clones at a time from a single petri plate; and, finally, (iv) the ease with which the phage library can be stored as a clear lysate at 4°C for months without significant loss in plaque-forming activity (75, 76).

Recently, a bacteriophage P1 cloning system has been developed which permits cloning of DNA fragments as large as 100 kbp with an efficiency that is intermediate between cosmids and yeast artificial chromosomes (105).

Scope of Present Review

The extensive knowledge of the basic biology of lambda has permitted modifications of its genome to suit the given experimental conditions. In the present review we describe how the utility of lambda as a cloning vector rests essentially in its intrinsic molecular organization. The following sections give an account of various problems encountered in constructing lambda vectors and the strategies that have been adopted to overcome them. A few commonly used vectors are described in detail, taking into account their special values and limitations. The different methods for screening and storage of genomic and cDNA libraries in lambda vectors are also discussed.

LIFE CYCLE AND GENETICS OF LAMBDA

An understanding of the basic biology of lambda, its mode of propagation, and the genetic and molecular mechanisms that control its life cycle is needed before its applications for genetic manipulations are discussed. This section deals with the basic biology of lambda (for more details, see references 12, 25, 30, 41, 62, and 119).

The lambda virus particle contains a linear DNA of 48,502 bp (95) with a single-stranded 5′ extension of 12 bases at both ends; these extensions are complementary to each other. These ends are called cohesive ends or *cos*. During infection, the right 5′ extension (*cosR*), followed by the entire genome, enters the host cell. Both the *cos* ends are ligated by *E. coli* DNA ligase (10, 23, 125), forming a covalently closed circular DNA which is acted upon by the host DNA gyrase, resulting in a supercoiled structure.

Development of Lambda: Two Alternative Modes

After infecting the host, the lambda genome may start its replication; this results in the formation of multiple copies of the genome. The protein components necessary for the assembly of mature phage particles are synthesized by the coordinated expression of phage genes. Phage DNA is packaged inside a coat, and the mature phages are released into the environment after cell lysis. This mode of propagation is called the lytic cycle.

Alternatively, the phage genome may enter a dormant stage (prophage) by integrating itself into a bacterial genome by site-specific recombination; during this stage it is propagated along with the host in the subsequent progeny. This stage is termed lysogeny. Changes in environmental and physiological conditions may activate the prophage stage and trigger lytic events.

Lytic Cycle

Three stages of lytic cycle. The genetic events in the lytic cycle can be divided into three stages: (i) immediate early, (ii) delayed early, and (iii) late.

Two immediate-early genes, *N* and *Cro*, are transcribed divergently and independently from the two promoters p_L and p_R, respectively (Fig. 1). The transcription terminates at t_L and t_R, respectively, after *N* and *Cro* have been transcribed. The *N* gene product binds to *nutL* and *nutR* (left and right *N* utilization sites, respectively) and acts as an antiterminator by suppressing transcription termination at t_L, t_R, and other sites with the help of host proteins synthesized by *nusA*, *nusB*, and *nusE* loci of *E. coli*. Antitermination by the *N* gene product results in the expression of delayed-early (regulatory) genes (*cII* and *cIII*), replication genes (*O* and *P*), seven recombination genes (*int*, *xis*, and others) and gene *Q*. The product of *Q* acts as an antiterminator for the transcription of late genes which comprise 10 head protein genes, 11 tail protein genes, and 2 lysis genes.

DNA replication and in vivo packaging. In lytic events, the circular phage DNA is replicated bidirectionally by a theta mode, which later switches to a rolling-circle mode resulting in the synthesis of a linear concatemeric DNA joined by *cos* ends (54). The linear molecule is then packaged into a head coat after cleavage at each *cos* end by a phage terminase (a product of the *A* gene) (6, 45). The *red* and *gam* genes of the phage and the *recA* and *recBC* genes of the host are involved in the generalized recombination events, which play an important role in the in vivo packaging of the lambda genome. The linear concatemeric DNA is a substrate for RecBC, which determines ATP-dependent exonuclease activity (3). The phage protein Gam (molecular mass, 33 kDa)

specifically inhibits all RecBC-associated activities (113) and inhibits the degradation of linear concatemeric phage DNA.

However, in *red gam* mutants, the only molecules available for packaging are exonuclease-resistant covalently closed circular molecules and their subsequent recombinations. On $recA^+$ hosts, these mutants grow poorly because the lambda DNA is a poor substrate for RecA-mediated recombination. The recombination efficiency and plaque size can be considerably improved by introduction of short DNA sequences called *chi* (crossover hot-spot instigator) in the lambda genome by site-specific mutations (100–104). The generalized recombination catalyzed by the *recA recBC* system is stimulated by *chi* sequences. Various models have been proposed for recombination stimulation by *chi* (60, 90, 98, 124).

The *red* and *gam* gene products are responsible for the Spi^+ (sensitive to P2 lysogeny) phenotype of wild-type lambda, and consequently lambda is unable to grow on P2 lysogens (63, 93, 130). Therefore *red* and *gam* mutants can be propagated on P2 lysogens (Spi^-).

Lysogeny

Transcription originating from o_L/p_L and o_R/p_R, after antitermination by N protein, continues through the *cIII* and *cII* genes, respectively. At an adequate concentration of cII protein, the two promoters p_{RE} and p_{int} are activated, resulting in the synthesis of cI repressor and integrase, respectively.

The expression of *cI* gene through p_{RE} (promoter for repressor establishment) is regulated positively by cII, in association with cIII. This promoter is recognized by RNA polymerase only in the presence of cII. The repressor cI, when synthesized from p_{RE}, binds to both p_L and p_R, repressing transcription of all other genes including *cII* and *cIII*, and lysogeny is established. The synthesis of cI from p_{RE} is also inhibited in the absence of cII and cIII. Sufficient levels of cI during lysogeny are maintained by p_{RM} (promoter for repressor maintenance) situated adjacent to o_R at the right end of the *cI* gene. RNA polymerase cannot initiate transcription at p_{RM} unless the repressor is bound to o_R. Thus it behaves as a positive regulatory protein for its own synthesis. The *cI* transcript from p_{RM} does not have any conventional ribosome-binding site and produces only a low level of repressor protein. Although a large amount of cI repressor synthesized from a strong promoter, p_{RE}, is essential for the establishment of lysogeny, even a low level of the repressor synthesized by p_{RM} is sufficient for its maintenance.

Lysogeny or Lytic Cycle

o_L and o_R each have three repressor-binding sites, i.e., o_L1, o_L2, o_L3, and o_R1, o_R2, o_R3, respectively (site 1 is the nearest to the transcription start point). In lysogeny, the binding of repressor dimers at o_R1 and o_R2 blocks the expression from p_R but promotes the expression of *cI* from p_{RM}. When the concentration of cI is in excess, it binds to o_R3 and stops its own expression from p_{RM}.

Cro protein, also termed as antirepressor, can also bind to o_L and o_R. However, its highest affinity is for o_R3, followed by those for o_R2 and o_R1. The presence of Cro at o_R3 inhibits RNA polymerase from binding at p_{RM}, thereby breaking the lysogeny maintenance circuit. It also inhibits the expression of early genes from p_L and p_R. The delayed genes are expressed from p_Q to form viable phages and cause lysis.

PHAGE LAMBDA AS A VECTOR

The large genome size and complex genetic organization of lambda had posed initial problems with its use as a vector. The problems, however, were surmounted through the sustained efforts of researchers, and lambda has been developed into an efficient vector.

The broad objectives in constructing various phage vectors are (i) the presence of cloning sites only in the dispensable fragments, (ii) the capacity to accommodate foreign DNA fragments of various sizes, (iii) the presence of multiple cloning sites, (iv) an indication of incorporation of DNA fragments by a change in the plaque type, (v) the ability to control transcription of a cloned fragment from promoters on the vector, (vi) the possibility of growing vectors and clones to high yield, (vii) easy and ready recovery of cloned DNA, and (viii) introduction of features contributing to better biological containment.

There are several difficulties in the use of lambda as a vector. Some of the problems and the general strategies adopted to overcome them are discussed in this section.

Manipulation of Restriction Sites

The major obstacle to the use of phage lambda as a cloning vector was essentially the presence of multiple recognition sites for a number of restriction enzymes in its genome. Initially, all attempts were directed toward minimizing the number of *Eco*RI sites. Murray and Murray in 1974 were able to construct derivatives of lambda with only one or two *Eco*RI sites (77). Similarly, Rambach and Toillais constructed lambda derivatives with *Eco*RI sites only in the nonessential region of the genome by repeated transfer on restrictive and nonrestrictive hosts (86). After several cycles of digestion, packaging, and growth, phage derivatives with desirable restriction sites and full retention of infectivity were obtained. All but one *Hin*dIII sites were removed by recombination of known deletion mutants or substitutions (74). Recently, oligonucleotides with specific sequences have been synthesized and introduced into the bacteriophage lambda genome. This has provided a variety of cloning sites in the genome (33, 58).

Size Limitation for Packaging

The second problem was the requirement of a minimum and maximum genome length (38 and 53 kbp, respectively) for the efficient packaging and for the production of viable phage particles (8, 111). The viability of the bacteriophage decreases when its genome length is greater than 105% or less than 78% of that of wild-type lambda. Genetic studies of specialized transducing bacteriophages showed, however, that the central one-third of the genome, i.e., the region between the *J* and *N* genes, is not essential for lytic growth. The presence of a nonessential middle fragment of the phage genome was also revealed during construction of viable deletion mutants (16, 17). These mutants lack most of the two central *Eco*RI B fragments which are not essential for lytic growth. However, too much DNA cannot be deleted because there is a minimum 38-kbp requirement essential for efficient packaging. The de novo insertion of DNA (even if heterogeneous) is essential for the formation of viable phages. This constitutes a positive selection for recombinant phages carrying insertions. This approach was successfully exploited in constructing recombinant phages carrying *E. coli* and *Drosophila melanogaster* DNA (28, 111, 112).

Transfection of Recombinant Molecules

The problem of transfection of recombinant molecules constructed in vitro was overcome by the successful in vitro assembly of viable and infectious phage particles (55, 120, 121). Two types of in vitro packaging systems have been developed so far, i.e., two-strain packaging and single-strain packaging.

Two-strain packaging. The basis of the two-strain in vitro packaging system is the complementation of two amber mutations. Two lambda lysogens, each carrying a single amber mutation in a distinctly different gene, are induced and grown separately so that they can synthesize the necessary proteins. Neither of the lysogens alone is capable of packaging the phage DNA.

The role of various phage products in DNA packaging has been studied in detail (7, 14, 29, 35, 46, 73). The E protein is the major component of the bacteriophage head, and in its absence all the viral capsid components accumulate. The D protein is involved in the coupled process of insertion of bacteriophage DNA into the prehead precursor and the subsequent maturation of the head. The A protein is required for the cleavage of the concatenated precursor DNA at the *cos* sites. Two phage lysogens carrying *A* and *E* or *D* and *E* mutations in the phage genome are induced separately, and cell extracts are prepared. Neither of the extracts can produce infectious phage particles. However, when the extracts are mixed, mature phage particles are produced by complementation (43, 46).

The major drawback of the two-strain system is the competition of native phage DNA with recombinant molecules. In both the cell extracts, native phage DNA is also present and can be packaged with an efficiency equal to that of the chimeric DNA. This reduces the proportion of recombinants obtained in a library. The problem of regeneration of endogenous phages obtained in the library was partially overcome by the use of b_2-deleted prophages, which poorly excise out of the host chromosome (27, 106) or by UV irradiation of packaging extracts (44).

Single-strain packaging. Rosenberg et al. (89, 91) have successfully developed a single-strain packaging system by introducing deletion in the *cos* region of prophage, rendering the prophage DNA unpackagable because *cos* is the packaging origin. Induction of the lysogen results in the intracellular accumulation of all protein components needed for packaging. However, packaging of phage DNA is prevented by the lack of *cos* sites on the prophage DNA. On the other hand, exogenous DNA with *cos* sites is packaged efficiently to produce an infectious bacteriophage particle. The single-strain system is superior to two-strain system in having a lower background of parental phages. In addition, it uses *E. coli* C, which lacks the *Eco*K restriction system (91), as the host for the lysogen.

Biological Containment

The biological containment of recombinant phages is an important aspect from the point of view of ethics and eventual biohazards. It is desirable that cloning vectors and recombinants have poor survival in the natural environment and require special laboratory conditions for their replication and survival. According to Blattner et al. (9), the lytic phages offer a natural advantage in this respect since the phage and the sensitive bacteria coexist only briefly. A newly inserted segment may not be compatible with *E. coli* metabolism for extended periods. To make the phage vectors more safe, three amber mutations (*S*am100, *B*am100, and *W*am403) were introduced in its genome. The new vector λgt WES λC is safer because an amber suppressor host strain is a very rare occurrence in the natural environment. Many vectors carry one of the amber mutations on the genome so that they can be propagated only on an appropriate suppressor host.

PHAGE VECTORS

Many phage vectors have been constructed in the recent past, each with its own special features. There is no universal lambda vector which can fulfill all the desired objectives of the cloning experiments. The choice of a vector depends mainly on (i) the size of a DNA fragment to be inserted, (ii) the restriction enzymes to be used, (iii) the necessity for expression of the cloned fragment, and (iv) the method of screening to be used to select the desired clones.

Bacteriophage lambda vectors can be broadly classified into two types: (i) replacement vectors and (ii) insertion vectors.

Replacement Vectors

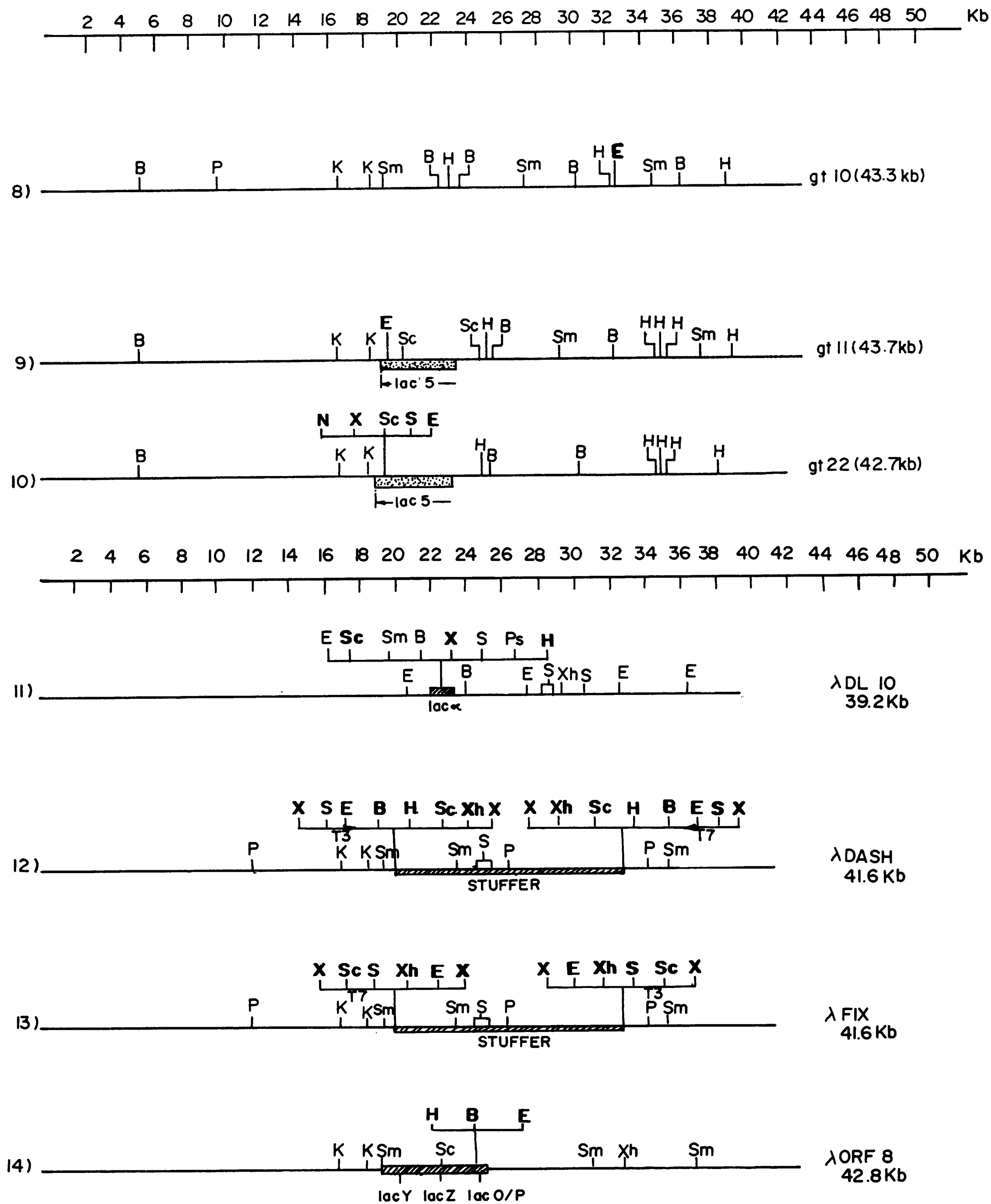
Taking advantage of the maximum and minimum genome size essential for efficient packaging and the presence of the nonessential central fragment, it is possible to remove the stuffer fragment and replace it with a foreign DNA fragment in the desired size range. This forms the basis of lambda-derived replacement vectors. Cloning of a foreign DNA in these vectors involves (i) preparation of left and right arms by physical elimination of the nonessential region, (ii) ligation of the foreign DNA fragment between the arms, and (iii) in vitro packaging and infection.

The replacement vectors contain a pair of restriction sites to excise the central stuffer fragments, which can be replaced by a desired DNA sequence with compatible ends. The presence of identical sites within the stuffer fragment but not in the arms facilitates the separation of the arms and the stuffer on density gradient centrifugation. In many vectors, sets of such sites are provided on attached polylinkers so that an insert can be easily excised. Two purified arms cannot be packaged despite their being ligated to each other, because they fall short of the minimum length required for packaging. This provides positive selection of recombinants. The replacement vectors are convenient for cloning of large (in some cases up to 24 kbp) DNA fragments and are useful in the construction of genomic libraries of higher eukaryotes. Charon and EMBL are among the popular replacement vectors.

Insertion Vectors

Because the maximum packagable size of lambda genome is 53 kb, small DNA fragments can be introduced without removal of the nonessential (stuffer) fragment. These vectors are therefore called insertion vectors. Cloning of foreign DNA in these vectors exploits the insertional inactivation of the biological function, which differentiates recombinants from nonrecombinants. Insertion vectors are particularly useful in cloning of small DNA fragments such as cDNA. λgt10 and λgt11 are examples of this type of vector.

In recent years a multitude of lambda vectors have been constructed. Many innovative approaches have been used to introduce desired properties into the vectors. The following section deals with the strategies adopted for the construction of some of the commonly used vectors (e.g., Charon, EMBL, λgt11) and their salient features, utilities, and limitations. The restriction maps of some of the vectors are shown in Fig. 2.



Charon Phages

The Charon series of phages represents a classic example of how the genome of phage lambda was exploited to construct a wide range of vectors from Charon 1 to Charon 40.

Initially (9, 87), the main focus was on (i) the removal of restriction sites from the essential region and (ii) altering the distribution of restriction sites. Various point mutations, substitutions, and deletions were introduced into wild-type lambda for this purpose. The mutations were introduced by standard genetic crosses, which were then confirmed by various techniques such as heteroduplex analysis, plating on suitable indicator bacteria, and agarose gel electrophoresis. The *Eco*RI site in the ϕ80 immunity region was removed by deletion, and the *Hin*dIII site near gene *Q* was eliminated by a point mutation. Introduction of *nin5* deletion enables transcription to be continued independent of the *N* gene product. Various amber mutations (e.g., in Charon 4A) were introduced to increase its biological containment. The presence of *lac-5* allows formation of blue plaques when 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) is included in the plating medium. Charon 1, 4, 8, 9, 10, and 14 are essentially replacement vectors which require a

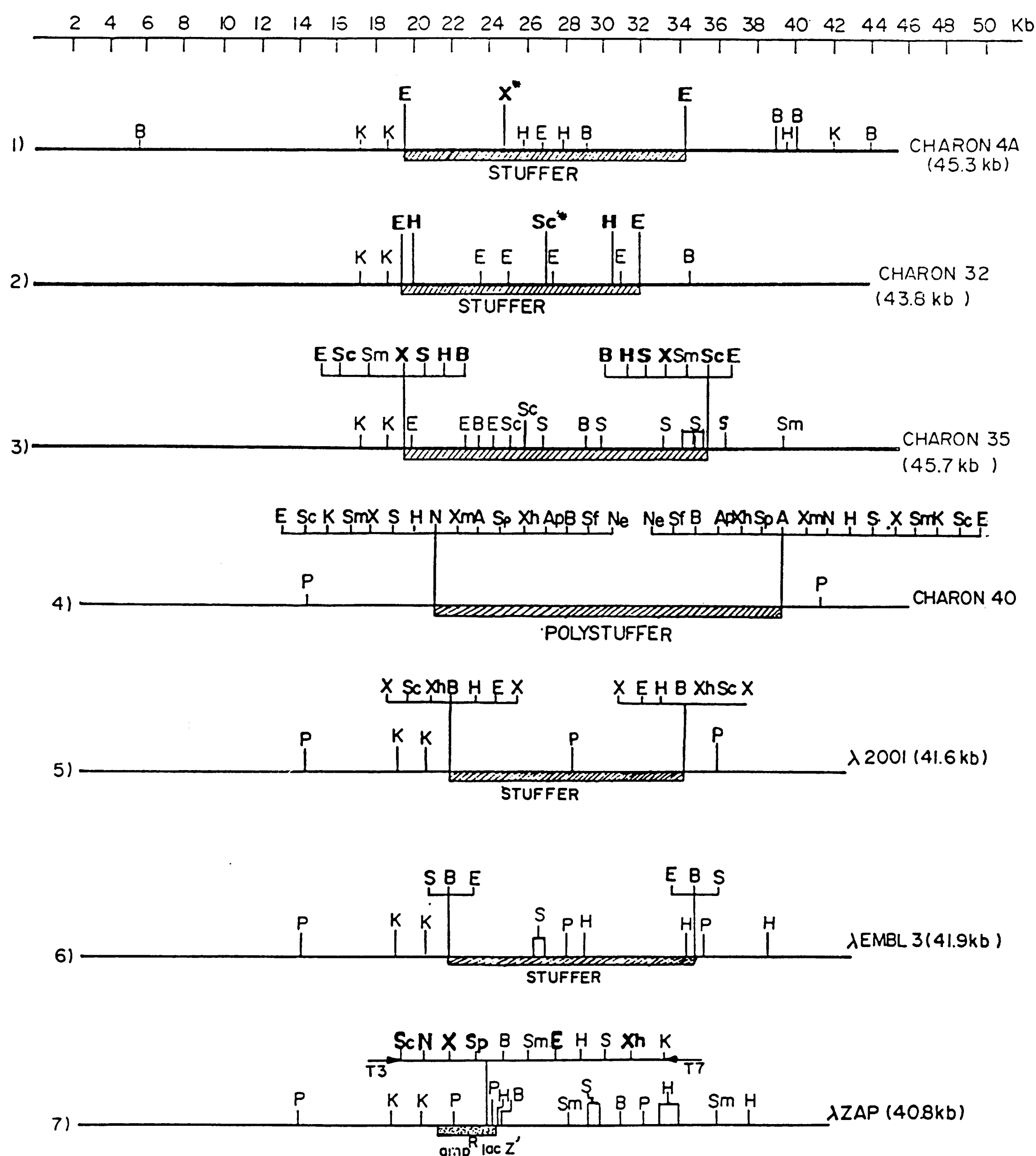


FIG. 2. Restriction maps of lambda vectors. The important restriction sites in the vectors are shown on the vector maps. The useful cloning sites are shown in boldface. Charon 4A and 32 can be used as insertion vectors by using *Xba*I and *Sac*I sites, respectively, which are shown by asterisks. Abbreviations: A, *Ava*II; Ap, *Apa*I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, *Not*I; Ne, *Nae*I; P, *Pvu*I; S, *Sal*I; Sc, *Sac*I; Sf, *Sfi*I; Sm, *Sma*I; Sp, *Spe*I; X, *Xba*I; Xh, *Xho*I; Xm, *Xma*III. 1, Charon 4A; 2, Charon 32; 3, Charon 35; 4, Charon 40; 5, λ2001; 6, λEMBL3; 7, λZAP; 8, λgt10; 9, λgt11; 10, λgt22; 11, λDL10; 12, λDASH; 13, λFIX; 14, λORF8.

certain minimum length of insert to be ligated to produce mature phages. Charon 6 and 7 form clear plaques on insertion of foreign DNA owing to the inactivation of the *c*I repressor gene. The cloning sites of many of the Charon phages are downstream from p_L (Charon 4 and 10 through 15) or the *lac* promoter (Charon 16) to enable controlled transcription of the cloned gene.

Some of the more recent vectors of the Charon series (Charon 32 to 40) (24, 64) have many desirable properties which make them versatile cloning vectors; e.g., the stuffer fragment of Charon 31 is replaced by a mouse stuffer lacking the sites for *Bam*HI and *Sal*I. The new vector, Charon 32, has a single site for *Bam*HI and two sites for *Sal*I in the right arm. By deleting the 1.3-kb fragment between *gam* and the *N* gene, a single *Bam*HI site and one of the two *Sal*I sites were removed to give Charon 33. This was further improved as Charon 34 by introduction of a polylinker with a *Bam*HI site at one end and *Eco*RI at the other end. Charon 34 has cloning sites for *Eco*RI, *Xba*I, *Sst*I, *Hin*dIII, *Bam*HI, and *Sal*I. It has an *E. coli* stuffer with no internal *Bam*HI site. Charon 35 has the same properties as Charon 34, except that the *E. coli* stuffer fragment contains a single *Bam*HI site. One of the

TABLE 1. Positions of restriction sites and lengths of arms and stuffers in replacement vectors

Vector and enzyme	Position (kb)	Arm length (kb)		Stuffer length (kb)
		Left	Right	
Charon 4A				
*Eco*RI	19.6, 34.3, 26.4	19.6	11.0	6.8, 7.9
Charon 32				
*Eco*RI	19.4, 31.9, 23.6, 24.8, 27.2, 30.9	19.4	11.9	1.0, 1.2, 2.4, 3.7, 4.2
*Hin*dIII	19.9, 30.5	19.9	13.3	10.6
*Sac*I	26.9	27.0	16.8	
Charon 35				
*Eco*RI	19.5, 19.8, 22.6, 24.0, 35.1	19.5	10.6	0.1, 1.4, 3.0, 11.1
*Sac*I	19.5, 25.0, 25.7, 35.1	19.5	10.6	0.7, 5.5, 10.6
*Sal*I	19.4, 26.4, 29.8, 33.1, 34.0, 34.6, 34.8, 35.1	19.6	10.5	0.2, 0.3, 0.6, 0.9, 3.3, 3.4, 6.9
*Xba*I-*Hin*dIII	19.5, 35.1	19.6	10.5	15.6
*Bam*HI	19.5, 23.1, 28.8, 35.1	19.6	10.5	3.6, 5.7, 6.3
Charon 40				
*Eco*RI, *Apa*I, *Xba*I, *Sfi*I, *Sac*I, *Avr*II, *Sal*I, *Spe*I, *Hin*dIII, *Xho*I, *Bam*HI, *Nae*I, *Kpn*I, *Not*I, *Sma*I, *Xma*I	19.2, 19.8	19.2	9.6	235-bp repeat
λEMBL				
*Eco*RI, *Bam*HI	19.9, 33.1	19.9	9.6	13.5
*Sal*I	19.5, 24.8, 25.3, 33.1	19.9	9.6	0.5, 5.3, 7.8
λ2001				
*Xba*I, *Sac*I, *Xho*I, *Bam*HI, *Hin*dIII, *Eco*RI	20.0, 32.7	20.0	9.0	12.7

two adjacent *Kpn*I sites present in the essential *J* gene of Charon 35 was removed by the insertion of a synthetic oligonucleotide duplex with *Kpn*I compatible ends but an inability to generate a *Kpn*I site. The other site was removed by repeated cycling and isolation of point mutants. The *Kpn*I site was introduced in the polylinker to form a new vector, Charon 36.

The *Sma*I site in the *P* gene of Charon 33 was abolished by the cycling method. The *Sal*I site between the *beta* and *gam* genes was eliminated by removing nonessential *beta* and *exo* genes to produce a phage with a shorter right arm. The *Eco*RI site in the polylinker and the *Sal*I site in the right arm were joined by an adapter plasmid DNA, in such a way that the *Eco*RI site was regenerated and the *Sal*I site was eliminated. This vector is called Charon 37. Charon 38 was derived by the removal of the *Apa*I site in gene *G* of Charon 37. Charon 39 and 40 were constructed by introduction into Charon 38 of a new polylinker consisting of 16 cloning sites. The two arms of the vectors have polylinkers in the opposite orientation, which facilitates the excision of the insert. Table 1 summarizes the positions of the cloning sites and the lengths of the arms and stuffers in some of the Charon vectors. (For positions of various other restriction sites, see reference 82.)

The removal of restriction sites from the essential region and their reintroduction as polylinkers was not the sole objective in the construction of these vectors. Usually, removal of the stuffer fragment also resulted in elimination of the *gam* gene. However, in Charon 32 to 40, the *gam* gene is retained on the right arm, which allows the propagation of recombinants on *recA* hosts, thereby overcoming the potential problem of recombination of duplicate segments in the target DNA. This is particularly important for cloning of higher eukaryotic DNA, which contains a large amount of repetitive DNA. In addition, an insert of up to 24 kb can be accommodated in Charon 38 to 40.

In Charon 39 and 40, a novel polystuffer approach is used for the easy removal of the stuffer fragment. A polystuffer consists of many small restriction fragments, ligated to each other to form long tandem repeats. The polystuffer can be digested with the same enzyme to make small pieces, which can be easily separated from the arms by polyethylene glycol precipitation. In addition, each unit fragment of polystuffer contains a *lac* operator region. Therefore, even a small fragment of the polystuffer which becomes ligated within the phage genome can induce β-galactosidase by means of repressor titration and is chromogenically detectable.

λ1059 and Derivatives

The property of inhibition of growth of lambda on P2 lysogen is effectively used in the construction of λ1059 (56, 57). Since this is a replacement vector, *red* and *gam* genes of the phage are removed in a stuffer, conferring the Spi$^-$ phenotype to the recombinants. Two *Bam*HI sites are suitable for the separation of the two arms from the stuffer. The ligation of only two arms (19.6 and 9.4 kb) falls short of the minimum size requirement for packaging, thus giving selective advantage to recombinants. The parental phage fails to grow on P2 lysogen but can grow on the *recA* strain, whereas the recombinants can grow on P2 lysogen but not on the *recA* strain.

Lambda 1059 also carries a pacI plasmid containing multiple *att* sites and the ColE1 origin of replication. It can therefore be grown either lytically as a phage or nonlytically as a plasmid in the presence of a repressor.

New polylinkers containing *Xba*I, *Sac*I, *Xho*I, *Bam*HI, *Hin*dIII, and *Eco*RI sites have been introduced into λ1059. The new vector lambda 2001 (58) also has a *chi* mutation that facilitates growth on *recA*$^+$ hosts. The *Bam*HI site is at the center of the polylinker, so although it cannot be regenerated (e.g., by ligation with *Mbo*I or *Sau*3AI fragments), the insert can be excised by using flanking sites.

λEMBL

The λEMBL series of phages is derived from λ1059 (32–34) by replacing the region of the *Col*E1 plasmid by a fragment carrying the *trpE* gene of *E. coli*. The removal of the ColE1 plasmid region facilitates screening of recombinants by using DNA probes cloned in ColE1-derived plasmid vectors. This vector is named λEMBL1. *Eco*RI sites are removed by repeated cycling on restricting and nonmodifying hosts to derive λEMBL2. A new polylinker is introduced into λEMBL2 to construct λEMBL3, which has *Sal*I, *Bam*HI, and *Eco*RI sites. λEMBL4 has the same sites as λEMBL3 in the reverse orientation, and λEMBL3A has two amber mutations, allowing selection of DNA sequences linked to *supF*.

The polylinker of λEMBL3 is modified to form λEMBL12, which has *Eco*RI, *Bam*HI, *Sst*I, *Xba*I, and *Sal*I sites (78). Introduction of *Not*I and *Sfi*I sites (octanucleotide-recognizing enzymes) in λEMBL3 has led to the construction of EMBL301 (61). λPJ4A, a derivative of λEMBL4A, has been constructed for cloning *Eco*RI fragments (53). A library in PJ4A can be screened by the method of Seed (96).

λgt11 to λgt23

λgt11 to λgt23 are insertion vectors which carry a part of the *E. coli* β-galactosidase gene. λgt11 is constructed by crossing λgt7–*lac-5* (containing the β-galactosidase gene) with λgt4 (having *c*I857, S100, and *nin5* mutations) (126). The *c*I857 mutation enables the phage to grow as a lysogen at 32 to 34°C or lytically at 42°C. The S100 suppressor mutation helps to achieve better biological containment. A unique *Eco*RI site at the 3′ terminus of the β-galactosidase gene is useful for cloning DNA up to 9.2 kb long. Insertional inactivation of the β-galactosidase gene enables blue-white screening on X-Gal plates. Since the foreign gene is expressed as a β-galactosidase fusion product, it can be screened by using the antibody probes (99).

λgt18 and λgt19 are derivatives of λgt11 in which two unwanted *Sal*I sites are eliminated by insertion of an oligomer within the *Sal*I sites. A polylinker containing *Sal*I, *Sac*I, *Xba*I, and *Eco*RI is then introduced. λgt18 and λgt19 have a polylinker in the opposite orientation (40).

λgt20 and λgt21 are derived from λgt18 and λgt19, respectively, by introduction of a *chi* site and elimination of *Sac*I and *Xba*I sites. These vectors have a maximum insert capacity of 8.2 kb. On the other hand, λgt22 and λgt23 (39) have *Not*I, *Xba*I, *Sac*I, *Sal*I, and *Eco*RI sites in the opposite orientation. In these vectors, a full-length cDNA can be inserted in the defined orientation at the *Sal*I and *Not*I sites because these sites are infrequent in eukaryotic DNA.

With the increasing popularity of λgt11 and its derivatives as expression vectors, various improved methods have been developed for cloning (52, 114), screening (31, 117), insert amplification (19), and hyperproduction of recombinant protein (107).

TABLE 2. Cloning sites of insertion vectors

Vector	Enzyme(s)	Arm length (kb)	
		Left	Right
λgt10	*Eco*RI	32.7	10.6
λgt11	*Eco*RI	19.5	24.2
λgt18	*Sal*I, *Eco*RI	19.5	23.7
λgt20	*Sal*I, *Xba*I, *Eco*RI	19.5	23.2
λgt22	*Not*I, *Xba*I, *Sac*I, *Sal*I, *Eco*RI	19.5	23.2
λZAP	*Sac*I, *Not*I, *Xba*I, *Spe*I, *Eco*RI, *Xho*I	21.8	18.1

λgt10

Lambda gt10 is a versatile insertion vector that is ideal for screening with nucleic acids probes (50). It has a unique *Eco*RI site in the *c*I repressor gene. Insertional inactivation of the *c*I gene gives clear plaques, compared with the turbid plaques of *c*I$^+$ nonrecombinants. Moreover, recombinants can be directly selected on a high-frequency lysogeny (*hfl*$^+$) strain because *c*I$^+$ parental phages lysogenize efficiently and recombinants (*c*I mutants) replicate well, resulting in a reduced background of nonrecombinants (49).

λZAP

Lambda ZAP is very useful for in vivo excision of the insert. It is an insertion vector with Bluescript SK(−) phagemid sequences which enable excision of the phagemid, including an insert, with the help of a helper M13 phage. The entire DNA insert can be recovered within the phagemid polylinker, which has 21 restriction sites, 6 of them (*Sac*I, *Not*I, *Xba*I, *Spe*I, *Eco*RI, and *Xho*I) being unique in the vector. The maximum insert capacity of λZAP is 10 kb (97).

Table 2 describes the cloning sites and arm lengths of various insertion vectors.

Novel Lambda Vectors

Besides the vectors described above, several others have been constructed in the recent past for certain specialized purposes. They are derivatives of commonly used lambda vectors and have been engineered to fulfill defined objectives.

Vectors for directional cloning. Directional cloning facilitates insertion of a DNA fragment in a known orientation with respect to the regulatory sequences of the vector and is particularly desirable for cloning of cDNA fragments. Because both the arms of the vector have noncomplementary ends, the dephosphorylation step can be bypassed. This approach also gives a low background of nonrecombinants.

λORF8 is an insertion vector designed for directional cloning and for the construction of a cDNA expression library (69). This vector has a polylinker (*Hin*dIII-*Bam*HI-*Eco*RI) situated within the *lacZ* gene which enables blue-white screening. cDNAs in which oligo(dT) priming is used for first-strand synthesis can be directionally cloned as an *Eco*RI-*Bam*HI, *Eco*RI-*Hin*dIII, or *Bam*HI-*Hin*dIII fragment. A specially designed linker containing a *Bam*HI site creates a *Hin*dIII site when ligated to the 3′ end (i.e., near

the $\frac{AAA}{TTT}$ end) of the cDNA fragment which can be directionally cloned in ORF8.

Recently Charon BS (+/−) vectors have been designed for high-efficiency directional cDNA cloning (109). The library constructed in this vector can be transferred to plasmids in one step for further analysis. The vector consists of Bluescript KS M13 (+) or KS M13 (−) plasmid DNA digested with *Not*I and ligated to a mixture of *Not*I adapters with *Hin*dIII and *Eco*RI ends, followed by cloning between Charon 15 lambda arms generated by *Hin*dIII and *Eco*RI digestion. The presence of an F1 origin and T3/T7 RNA polymerase promoters is useful for preparing single-stranded circular DNA or strand-specific RNA for cDNA cloning.

Vectors with controlled lytic and lysogenic events. It is possible to propagate a recombinant as a phage by lytic events or as an autonomously replicating plasmid or lysogen. The temperature-sensitive mutation of *c*I repressor (*c*I857) is widely exploited for controlled lytic or lysogenic events (83). When such a mutation is present on the phage genome, the phage can be propagated as a lysogen at 32°C. The viable phage particles can be obtained simply by shifting the growth temperature to 42°C. The repressor is inactivated at 42°C, and lytic events are initiated. In the case of λgt11 (126), although the library can be propagated and screened as a phage, stable lysogens can be maintained for the large-scale production of the desired protein. λSV2 can be maintained in an integrated state at 32°C or as an autonomous plasmid at 42°C (47, 48). Similarly, λNM:pBR322 can grow lytically or as a plasmid under different sets of conditions (70).

λSE4 is a derivative of λ1059 with a very low copy number replication system and a spectinomycin resistance gene. In a nonimmune host (i.e., strains lacking the *c*I gene), the phage grows lytically and the recombinants are *spi*, as is λ1059. On the other hand, in an immune host, phage-specific functions are repressed. The plasmid can replicate at low copy number and confers spectinomycin resistance. DNA fragments in the range of 2 to 19 kb can be cloned into the *Bam*HI site, and the genes from prokaryotic organisms can be isolated by complementation of *E. coli* mutants (26).

λDL10 and λDL11 contain an α-complementing fragment of *lacZ* and a polylinker of M13mp10 and M13mp11, respectively. The unique *Sac*I, *Xba*I, and *Hin*dIII sites can be used for cloning of DNA fragments up to 12 kb. α-complementing activity is expressed under the control of *lacP* of the vector and $lacI^q$ repressor of the host. Therefore, induction by isopropyl-β-D-thiogalactopyranoside (IPTG) is essential for expression of the *lacZ* α-fragment. In addition, stable lysogens can be generated as a result of the presence of *int* and *att* loci on the vector. These two properties make this pair of vectors attractive for cloning of genes that might be lethal when cloned in a high-copy plasmid (122).

Vectors for in vitro transcription of cloned inserts. Large amounts of single-stranded RNA can be synthesized in vitro from a cloned insert by initiating transcription from a bacteriophage promoter adjacent to the cloned insert. The strong promoters derived from *E. coli* bacteriophages T7 and T3 (108, 110) or *Salmonella typhimurium* phage SP6 (37) are used for this purpose. There are several advantages of using RNA probes synthesized in vitro. These promoters are transcribed specifically by RNA polymerases encoded by the respective phages. On the other hand, the specific RNA polymerases do not recognize bacterial and eukaryotic promoters of the insert or other vector promoters (94). Thus, highly specific probes complementary to the sequence downstream of these promoters can be synthesized in vitro by using a specific RNA polymerase and the four nucleotide triphosphates. The newly synthesized RNA sequences can be used as strand-specific probes. Since the template DNA can be transcribed several times in vitro, several copies of the probe can be generated. Because of high stability of DNA-RNA hybrids (13), RNA probes generally give strong signals.

In λZAP, the presence of T7 and T3 promoters, one on either side of the polylinker, permits in vitro transcription of the insert (97). Similarly, in λT7-T3/E-H, *Eco*RI and *Hin*dIII sites are present between the promoters, embedded in the *lacZ* gene (38), and in replacement vectors λDASH and λFIX, these promoters are adjacent to the polylinker (94). The λSWAJ series of vectors is derived from λgt10 by insertion of pGEM2 (81). SWAJ1, in which *Xba*I and *Sac*I sites are positioned between the SP6 and T7 promoters, can be used for the directional cloning of cDNA fragments. A 535-bp fragment of pGEM1 or pGEM2 containing both the promoters and cloning sites is present in SWAJ2 and SWAJ3, respectively, whereas in λSWAJ1, λSWAJ4, and λSWAJ5, the entire pGEM sequence is present. In λSWAJ2 to λSWAJ5, an *Eco*RI site is present along with *Xba*I and *Sac*I. Since the polylinker in λSWAJ4 and λSWAJ5 is flanked by *Spe*I sites, the library in these vectors can be efficiently converted into a plasmid library by digestion with *Spe*I, ligation, and transformation of a suitable host with the ligated DNA.

The presence of promoters on either side of an insert permits in vitro transcription of both sense and antisense strands of insert. If the cDNA is directionally cloned, the strand-specific probes can be easily generated by transcribing the appropriate strand of an insert DNA. This approach is very useful for the differential and subtractive hybridization techniques (94) and for cloning of the rare tissue-specific genes. A few other vectors such as λpt13 are useful in the study of transcriptional control in *E. coli* (42), and λNMT can be used for efficient transduction of mammalian cells with cDNA clones (79).

Table 3 summarizes the salient features of some of the novel vectors.

Choice of Hosts for Lambda Vectors

It is critical to use particular strains of *E. coli* as hosts on the basis of the genotypes of different vectors (94). The hosts which are widely used are derivative strains of *E. coli* K-12, which codes for the restriction enzyme *Eco*K. The vectors can be protected by being grown in a modifying strain. However, to protect the foreign DNA, the recombinants are propagated in a restriction-deficient (r_k^-) strain. *hsdR* strains are defective in restriction but can modify *Eco*K sites (r_k^-, m_k^+), whereas *hsdS* strains are deficient in both restriction and modification (r_k^-, m_k^-). Amber suppressor hosts such as *supE* and *supF* are essential for the propagation of vectors in which specific amber mutations are introduced, so as to attain biological containment. The problem of recombination and rearrangement between homologous DNA sequences is overcome by propagating the phages in a host that is defective in *recA recBC* pathways.

The restriction systems McrA and McrBC, encoded by *mcrA* and *mcrBC* genes, respectively, of wild-type *E. coli* (85), are methylcytosine-requiring systems that attack DNA only when it is methylated at specific cystosines, whereas Mrr, encoded by *mrr* (84), is a methyladenine-requiring restriction system that attacks DNA only when it is methylated at specific adenines. The restriction of the cloned DNA by these methylation-specific systems results in a substan-

TABLE 3. Novel lambda vectors

Vector	Size (kb)	Cloning sites		Reference
		Enzyme(s)	Position(s) (kb)	
λSWAJ4	46.2	*Eco*RI, *Xba*I, *Sac*I	32.9	81
λT7-T3/E-H	41.1	*Hin*dIII-*Eco*RI	28.6	38
λSE4	48.7	*Bam*HI	24.1, 40.1	26
λORF8	42.8	*Eco*RI, *Bam*HI, *Hin*dIII	24.7	69
λDASH	41.6	*Xba*I, *Sal*I, *Eco*RI, *Bam*HI, *Hin*dIII, *Sac*I, *Xho*I	20.0, 33.8	94
λFIX	41.6	*Xba*I, *Sac*I, *Sal*I, *Xho*I, *Eco*RI	20.0, 33.8	94

tially reduced recovery of the sequences cloned in lambda vectors. The problem can be avoided by the use of strains in which these mechanisms are rendered nonfunctional by mutation. A strain completely disabled for restriction will be defective at the *hsd*, *mcrA* and *mcrBC*, and *mcrR* loci and will be a more permissive host for propagation of lambda clones.

The choice of lytic or lysogenic growth of recombinants can be controlled by selective use of different hosts. For λgt11, use of Y1090 as the host facilitates lytic growth and subsequent screening with antibody or nucleic acid probes, whereas Y1089 is useful for the construction of recombinant lysogens when a large amount of recombinant protein is required.

Storage of Lambda Stocks

Most of the lambda strains are stable for several years when stored at 4°C in SM buffer containing 0.3% freshly distilled chloroform (94). The master stocks of bacteriophage lambda are kept in 0.7% (vol/vol) dimethyl sulfoxide at −70°C for long-term storage (94). Klinman and Cohen (59) have developed a method for storage of a phage library at −70°C by using top agar containing 30% glycerol.

DETECTION OF RECOMBINANTS

The efficiency of a cloning vector rests mainly on the availability of an easy and convenient method for differentiating recombinants from nonrecombinants. Ideally, when the replacement vectors are used for cloning, there should be no background of nonrecombinants. However, an inefficient removal of stuffer and/or the presence of packagable phage DNA in packaging extracts may give some background. In Charon 40, religation of the stuffer with the arms can be easily detected on X-Gal plates because each unit fragment in the polystuffer contains a *lac* operator region, which gives blue plaques as a result of repressor titration. The inability of λ1059 to grow on P2 lysogen, in contrast to the ability of recombinants to grow on such a host, provides a direct selection for recombinants.

The other method commonly used for selection of recombinants is the insertional inactivation of genetic markers present on the vector. The use of high-frequency lysogenic (*hfl*) hosts (such as NM514), combined with the insertional inactivation of the *c*I gene in lambda gt10, provides a condition in which only the recombinants (*c*I mutants) can form plaques (49). The use of a unique *Eco*RI site for cloning inactivates the β-galactosidase gene in λgt11, differentiating blue nonrecombinant plaques from colorless recombinant plaques on X-Gal plates. Thus, by a careful choice of host and vector, it is possible to detect recombinants conveniently.

Screening a Phage Library

The phage library can be screened for the presence of a desired gene by using either specific immunological or nucleic acid probes.

Immunological screening. Immunological selection can be used for the vectors in which the desired gene is expressed as a protein (antigen), even at a very low level. The recombinants which express a desired gene can be picked up by their selective reaction with the specific antibodies. The expression of a cloned gene in λgt11 as a fusion protein of β-galactosidase facilitates screening with specific immunological probes. This approach has been especially successful in isolation of genes for which no probes other than specific antisera are available (80, 126, 127).

Nucleic acid probes. Screening with nucleic acid probes is convenient both when the cloned gene is not expressed and when an easy method for detection of gene product is not available. The method is based on the assumption that the same genes from diverse sources have at least a partial homology in their nucleotide sequences.

Oligonucleotide probes are synthesized either on the basis of the known protein sequence or by studying highly conserved sequences in other, related proteins. Alternatively, a cloned gene fragment from the heterologous sources can be used as a probe for screening. The success of screening with nucleic acid probes depends on (i) the extent of homology between the desired gene and the probe and (ii) the stringency of the hybridization conditions.

Double-stranded oligonucleotide sequences can be used as a probe to identify clones that express specific DNA-binding proteins (94). The cDNA expression library is screened with radiolabeled double-stranded probe containing a specific DNA-binding site. Generally, such a probe is self-ligated to form concatemers before being used for hybridization. Such a concatenated probe with multiple binding sites gives much stronger signals than a probe with a single binding site.

Seed (96) has developed a method for in vivo selection of recombinants in which probe sequences are inserted into a very small plasmid vector and introduced into a suitable host (79). The phage library is then propagated on these transformed cells. Phages bearing sequences homologous to the probe acquire an integrated copy of the plasmid by reciprocal recombination, and these phages can be detected by a suitable marker on the plasmid.

Differential and Subtractive Screening Methods

A few methods are available for screening of the gene of interest that is expressed in type of the cells but not in the other (94). In these methods, the recombinants expressing genes common to both the cell types can be either differentiated or eliminated so that the set of recombinants which are specific to only one particular cell type may be obtained.

This approach is very useful for screening tissue-specific or differentially regulated genes.

Differential screening. In the differential screening method, total poly(A)$^+$ RNA is extracted from the two types of cells (one expressing the gene of interest and the other not expressing it) and used as templates to generate radioactive probes by using reverse transcriptase. A cDNA library of cells expressing the desired gene is independently screened with the two types of probes. The clones that hybridize to both probes represent common sequences in the two cell types, whereas clones that hybridize with only one type of probe are specific to that cell type.

Subtractive screening. The sensitivity of differential screening can be enhanced by subtractive hybridization. As in case of differential screening, poly(A)$^+$ RNA is extracted from the two types of cells. However, radioactive probes are generated only from the mRNA population extracted from the cells that express the gene of interest. This cDNA probe is hybridized to an excess of mRNA that does not represent the desired sequence (i.e., mRNA derived from the cells which do not express the gene of interest). The DNA-RNA hybrids are removed on a hydroxyapatite column, and nonhybridized radioactive single-stranded cDNA is used as a probe to screen a library constructed from the cells that express the desired gene. Alternatively, nonhybridized single-stranded cDNA can be used as templates to generate the second strand and can subsequently be cloned in a suitable vector to construct a subtracted library.

CONCLUDING REMARKS

The introduction of coliphage lambda as a cloning vector in the late 1970s has led to the construction of many useful derivatives of lambda. Initially, these attempts were directed toward removing restriction sites from the essential region of the genome and reintroducing them in suitable polylinkers. The entire Charon series is an example of such developments. Manipulations of restriction sites, biological containment, increase in the insert capacity, introduction of polylinkers, easy removal of stuffer fragments, and easy detection of recombinants are some of the important criteria applied in development of the new vectors. The Spi$^+$ phenotype of wild-type lambda is exploited in λ1059. Introduction of *chi* sequences is useful in packaging in *recA*$^+$ strains. To overcome the problem of unwanted recombination of repetitive sequences cloned in lambda vectors, Charon 32 to 40 phages have been developed, because they can be propaged on *recA* hosts.

The failure of expression of eukaryotic genes is mainly due to inability to recognize eukaryotic promoters and the absence of RNA-processing machinery in *E. coli*. These problems have been partially overcome by the use of cDNA cloning in the expression vectors such as λgt11. Lambda T7-T3/EH or SWAJ4 has the additional advantage of transcription of both strands of the insert. Directional cDNA cloning is a desirable strategy for which many lambda vectors have been developed. Introduction of *Not*I and *Sfi*I sites into vectors facilitates excision of intact inserts. Increasing the insert capacity is yet another important goal. Charon 38 to 40 have the maximum insert capacity (24 kbp) produced so far among the lambda vectors. Controlled lytic or lysogenic events can be achieved by using vectors such as λgt11 or λSV2 in which the temperature-sensitive *c*I repressor mutation (*c*I857) is introduced.

In addition to the development of cloning vectors, many other techniques have been developed simultaneously to facilitate DNA manipulations with lambda vectors. Partial filling of *Sal*I-*Eco*RI termini of vector arms and *Sau*3AI-cut inserts produces complementary ends which can be ligated easily (128). Efficiency of in vitro packaging is an important factor for successful cloning in lambda vectors. Many commercial packaging extracts that yield an efficiency of 10^8 PFU/μg of DNA are available. A recent study suggests that Stratagene Gigapack Gold packaging extracts give maximum efficiency compared with other commercial packaging extracts (65). A wide variety of protocols are now available for construction of libraries in lambda vectors (1, 32, 50, 94) and for rapid isolation and purification of lambda DNA (2, 36). Roditi et al. have developed a rapid method for determination of the orientation of inserts cloned in lambda vectors (88).

Libraries in lambda vectors can be amplified for easy screening of rare sequences. However, repeated amplifications may result in the underrepresentation or loss of poorly growing clones. Amplification is not advisable in λgt11 because there is always a danger that healthy nonrecombinants will overgrow recombinants.

The development of the polymerase chain reaction technique has allowed rare sequences to be amplified several thousand-fold and has therefore facilitated the detection of rare genes (72, 92). Collectively, these contributions have made molecular cloning and gene manipulations in lambda vectors accessible to any student of biology who has access to relatively simple laboratory setups.

ACKNOWLEDGMENT

We are grateful to C. Sivaraman for his valuable suggestions during the preparation of the final text.

REFERENCES

1. **Aguan, K., T. Kusano, N. Suzuki, and Y. Kitagawa.** 1990. An improved method for construction of high efficiency cDNA library in plasmid and lambda vector. Nucleic Acids Res. **18:**1071.
2. **Bansal, O. B., and R. H. Das.** 1989. A simple and rapid method for the isolation of plasmid and lambda phage DNAs. Nucleic Acids Res. **17:**10129.
3. **Barbour, S. D., and A. J. Clark.** 1970. Biochemical and genetical studies of recombination proficiency in Escherichia coli. I. Enzymatic activity associated with recB$^+$ and recC$^+$ genes. Proc. Natl. Acad. Sci. USA **65:**955–961.
4. **Beck, E., R. Sommer, E. A. Austrswald, C. Kurz, B. Zink, G. Osterberg, H. Schaller, K. Sugimoto, H. Sugisaki, T. Okamoto, and M. Takanami.** 1978. Nucleotide sequence of bacteriophage fd DNA. Nucleic Acids Res. **5:**4495–4503.
5. **Beck, E., and B. Zink.** 1981. Nucleotide sequence and genome organization of filamentous bacteriophage f1 and fd. Gene **16:**35–58.
6. **Becker, A., and H. Murialdo.** 1990. Bacteriophage lambda DNA: the beginning of the end. J. Bacteriol. **172:**2819–2824.
7. **Becker, A., H. Murialdo, H. Lucko, and J. Morell.** 1988. Bacteriophage lambda DNA packaging: the product of F1 gene promotes the incorporation of the prohead to the DNA terminase complex. J. Mol. Biol. **199:**597–607.
8. **Bellet, A. J. D., H. G. Busse, and R. L. Baldwin.** 1971. Tandem genetic duplication in a derivative of phage lambda, p. 501–513. *In* A. D. Hershey (ed.), Bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Blattner, F. R., B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L. E. Furlong, D. J. Grunwald, D. O. Keifer, D. D. Moore, J. W. Schumm, and D. Smithies.** 1977. Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. Science **196:**161–169.
10. **Bode, V. C., and A. D. Kaiser.** 1965. Changes in the structure and activity of lambda DNA in superinfected immune bacte-

rium. J. Mol. Biol. **14**:399–417.

11. **Bolivar, F., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crasa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
12. **Brammer, W. J., and C. Hadfield.** 1984. A programme for construction of lambda phage. J. Embryol. Exp. Morphol. **83**(Suppl.):75–88.
13. **Casey, J., and N. Davidson.** 1977. Rates of formation and thermal stabilities of RNA:DNA and DNA:DNA duplexes at high concentrations of formamide. Nucleic Acids Res. **4**:1539–1545.
14. **Casjens, S., T. Hohn, and A. D. Kaiser.** 1972. Head assembly steps controlled by genes F and W in bacteriophage lambda. J. Mol. Biol. **64**:551–563.
15. **Collins, J., and B. Hohn.** 1978. Cosmids: a type of plasmid gene-cloning vector that is packageable *in vitro* in bacteriophage lambda heads. Proc. Natl. Acad. Sci. USA **75**:4242–4246.
16. **Davidson, N., and W. Szybalski.** 1971. Physical and chemical characteristics of lambda DNA, p. 45–82. *In* A. D. Hershey (ed.), Bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Davis, R. W., and J. S. Parkinson.** 1971. Deletion mutants of lambda. III. Physical structure of attO. J. Mol. Biol. **56**:403–423.
18. **Deng, Z., and D. Hopwood.** 1987. Activity of *Streptomyces* terminator in *E. coli*. Nucleic Acids Res. **15**:2665–2675.
19. **Dorfman, D. M., L. I. Zon, and S. H. Orkin.** 1989. Rapid amplification of λgt11 library inserts from plaque using PCR. BioTechniques **7**:568–570.
20. **Dotto, G. P., K. Horinuchi, and N. D. Zinder.** 1984. The functional origin of bacteriophage f1 DNA replication: its signal and domains. J. Mol. Biol. **172**:507–521.
21. **Dotto, G. P., and N. D. Zinder.** 1983. The morphogenetic signal of bacteriophage f1. Virology **130**:252–256.
22. **Dotto, G. P., and N. D. Zinder.** 1984. Increased intracellular concentration of an initiator protein markedly reduces the minimal sequence required for initiation of DNA synthesis. Proc. Natl. Acad. Sci. USA **81**:1336–1340.
23. **Dove, W. F., and J. J. Weigle.** 1965. Intracellular state of chromosome of bacteriophage lambda. J. Mol. Biol. **12**:620–629.
24. **Dunn, I. S., and F. R. Blattner.** 1987. Charon 36 to 40: multienzyme, high capacity, recombination deficient replacement vectors with polylinkers and polystuffers. Nucleic Acids Res. **15**:2677–2698.
25. **Echols, H.** 1986. Bacteriophage lambda development: temporal switch and choice of lysis and lysogeny. Trends Genet. **2**:26–30.
26. **Elledge, S. J., and G. C. Walker.** 1985. Phasmid vectors for identification of genes by complementation of *Escherichia coli* mutants. J. Bacteriol. **162**:777–783.
27. **Enquist, L., and N. Sternberg.** 1979. *In vitro* packaging of λDAM vectors and their use in cloning DNA fragments. Methods Enzymol. **68**:281–298.
28. **Enquist, L., D. Teimeier, 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.
29. **Feiss, M.** 1986. Terminase and the recognition, cutting and packaging of lambda chromosomes. Trends Genet. **2**:100–104.
30. **Freifielder, D.** 1987. Molecular biology, p. 577–616. Jones & Bartlett Publishers, Inc., Boston.
31. **Friedman, K. D., N. L. Rosen, P. J. Newman, and R. R. Montgomery.** 1990. Screening of λgt11 libraries, p. 253–258. *In* M. A. Innis (ed.), PCR protocols, Guide to Methods and Applications. Academic Press, Inc., San Diego, Calif.
32. **Frischauf, A.** 1987. Construction and characterization of genomic library in lambda. Methods Enzymol. **152**:190–199.
33. **Frischauf, A., L. Hans, A. Poustuka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
34. **Frischauf, A., N. Murray, and H. Lehrach.** 1987. Phage vectors—EMBL series. Methods Enzymol. **153**:103–115.
35. **Georgopoulos, C., K. Tilly, and S. Casjens.** 1983. Lambda phage head assembly, p. 279–304. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Gonzalez, A., and J. Gomez-Merquez.** 1990. Purification of bacteriophage DNA by gel filtration chromatography. Genet. Anal. Tech. Appl. **7**:2–4.
37. **Green, M. R., T. Maniatis, and D. A. Melton.** 1983. Human β-globin pre-mRNA synthesized in vitro is accurately spliced in *Xenopus* oocyte nuclei. Cell **32**:681–694.
38. **Grimaldi, G., G. Manfioletti, and C. Schneider.** 1987. A lambda vector for directional cDNA cloning and *in vitro* transcription. Nucleic Acids Res. **15**:9608.
39. **Han, J. H., and W. J. Rutter.** 1987. Lambda gt22, an improved lambda vector for directional cloning of full length cDNA. Nucleic Acids Res. **15**:6304.
40. **Han, J. H., C. Stratova, and W. J. Rutter.** 1987. Isolation of full length putative rat lysophospholipase cDNA using improved methods for mRNA isolation and cDNA cloning. Biochemistry **26**:1617–1625.
41. **Herskowitz, I., and F. Banuette.** 1983. Interaction of phage, host and environmental factors in governing lambda lysis-lysogeny decision, p. 59–73. *In* V. L. Chropa (ed.), Genetics: new frontiers. Proceedings of the International Congress. Oxford IBH Publishing Co., New Delhi, India.
42. **Hirano, M., and R. W. Davis.** 1983. Construction and characterization of plasmid and lambda phage vector systems for study of transcriptional control in *Escherichia coli*. Gene **57**:89–99.
43. **Hohn, B.** 1975. DNA as a substrate for packaging into bacteriophage lambda *in vitro*. J. Mol. Biol. **98**:93–106.
44. **Hohn, B.** 1979. *In vitro* packaging of lambda and cosmid DNA. Methods Enzymol. **68**:299–309.
45. **Hohn, B., and T. Hohn.** 1974. Activity of empty, headlike particles for packaging of DNA of bacteriophage lambda *in vitro*. Proc. Natl. Acad. Sci. USA **71**:2372–2376.
46. **Hohn, B., and K. Murray.** 1977. Packaging recombinant DNA molecules in bacteriophage particles *in vitro*. Proc. Natl. Acad. Sci. USA **74**:3259–3263.
47. **Howord, B. H., and M. E. Gottesman.** 1983. SV2, a plasmid cloning vector that can be stably integrated in *E. coli*, p. 137–153. *In* M. Inouye (ed.), Experimental manipulations of gene expression. Academic Press, Inc., New York.
48. **Howord, B. H.** 1982. Vectors that can be propagated by integration into *E. coli* chromosomes, p. 211–216. *In* Y. Gluzman (ed.), Eukaryotic viral vectors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
49. **Hoyt, M. A., D. M. Knight, A. Das, H. Miller, and H. Echols.** 1982. Control of phage lambda development by stability and synthesis of cII protein: role of the viral cIII and host hflA, himA and himD genes. Cell **31**:565–573.
50. **Huynh, T. V., R. A. Young, and R. W. Davis.** 1985. Construction and screening of cDNA libraries in lambda gt10 and lambda gt11, p. 49–78. *In* D. M. Glover (ed.), DNA cloning: a practical approach. IRL Press, Oxford.
51. **Jaurin, B.** 1987. A promoter probe vector (pJAC4) that utilizes ampC β-lactamase gene of *Escherichia coli*. Nucleic Acids Res. **15**:8567.
52. **Jendrisak, J., R. A. Young, and J. D. Engel.** 1987. Cloning cDNA in λgt10 and λgt11. Methods Enzymol. **152**:359–371.
53. **Joergensen, P., and T. Mikkeelsen.** 1986. Lambda PJ4A, a lambda replacement vector carrying amber mutations for cloning EcoRI fragments. Nucleic Acids Res. **14**:9538.
54. **Kaiser, D.** 1971. Lambda DNA replication, p. 195–210. *In* A. D. Hershey (ed.), Bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
55. **Kaiser, D., and T. Masuda.** 1973. In vitro assembly of bacteriophage lambda heads. Proc. Natl. Acad. Sci. USA **70**:260–264.
56. **Karn, J., S. Brammer, and L. Bernett.** 1983. New bacteriophage lambda vector with positive selection for cloned isert.

Methods Enzymol. **101**:3–9.

57. **Karn, J., S. Brammer, L. Barnett, and G. Cesareni.** 1980. Novel bacteriophage lambda cloning vector. Proc. Natl. Acad. Sci. USA **77**:5172–5176.
58. **Karn, J., H. W. D. Matthes, M. J. Gait, and S. Brammer.** 1984. A new selective phage cloning vector lambda2001, with sites for XbaI, BamHI, HindIII, EcoRI, SstI and XhoI. Gene **32**:217–224.
59. **Klinman, D. M., and D. I. Cohen.** 1987. Preserving primary cDNA libraries. Anal. Biochem. **161**:65–88.
60. **Kobayashi, L., M. M. Stahl, and F. W. Stahl.** 1984. The mechanism of the Chi-Cos interaction in RecA-RecBC mediated recombination in phage lambda. Cold Spring Harbor Symp. Quant. Biol. **49**:497–506.
61. **Lathe, R., J. L. Vilotte, and A. J. Clark.** 1987. Plasmid and bacteriophage vectors for excision of intact inserts. Gene **57**:193–201.
62. **Lewin, B.** 1987. Genes III, p. 269–289. John Wiley & Sons, Inc., New York.
63. **Lindahl, G., G. Sironi, H. Biely, and R. Calender.** 1970. Bacteriophage lambda: abortive infection of bacteria lysogenic for phage P2. Proc. Natl. Acad. Sci. USA **66**:587–594.
64. **Loenen, W. A. M., and F. R. Blattner.** 1983. Lambda Charon vectors (Charon 32, 33, 34 and 35) adapted for DNA cloning in recombinant-deficient hosts. Gene **26**:171–179.
65. **Maloisel, V., and B. Perbal.** 1989. Efficiency of commercial *in vitro* packaging extracts. Methods Mol. Cell. Biol. **1**:41.
66. **Marvin, D. A., and B. Hohn.** 1969. Filamentous bacterial viruses. Bacteriol. Rev. **33**:172–209.
67. **Marvin, D. A., and E. J. Wachtel.** 1975. Structure and assembly of filamentous bacterial viruses. Nature (London) **253**:19–23.
68. **Mead, D. A., E. Szczesna-Skorupa, and B. Kemper.** 1985. Single stranded DNA SP6 promoter plasmids for engineering mutant RNAs and proteins: synthesis of a "stretched" prepro-parathyroid hormone. Nucleic Acids Res. **13**:1103–1118.
69. **Meissner, P. S., W. P. Sisk, and M. L. Berman.** 1987. Bacteriophage lambda cloning system for the construction of directional cDNA libraries. Proc. Natl. Acad. Sci. USA **84**:4171–4175.
70. **Mel'nikov, A. A., A. P. Chernov, and I. I. Fodor.** 1985. Lambda plasmid-phages and their properties. Mol. Biol. (Moscow) **19**:610–616.
71. **Meyer, T. F., K. Geider, C. Kurz, and H. Schaller.** 1979. Cleavage site of bacteriophage fd gene II protein in the origin of viral strand replication. Nature (London) **278**:365–367.
72. **Mullis, K. B., and F. A. Faloona.** 1987. Specific synthesis of DNA *in vitro* via a polymerase catalysed chain reaction. Methods Enzymol. **155**:335–350.
73. **Muralido, H., and A. Becker.** 1978. Head morphogenesis of complex double-stranded deoxyribonucleic acid bacteriophages. Microbiol. Rev. **42**:529–576.
74. **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.
75. **Murray, N. E.** 1983. Phage lambda and molecular cloning, p. 395–432. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
76. **Murray, N. E.** 1983. Lambda vectors, p. 677–684. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
77. **Murray, N. E., and K. Murray.** 1974. Manipulations of restriction targets in phage lambda to form receptor chromosomes for DNA fragments. Nature (London) **251**:476–481.
78. **Natt, E., and G. Scherer.** 1986. EMBL 12, a new lambda replacement vector with sites for SalI, XbaI, BamHI, SstI and EcoRI. Nucleic Acids Res. **14**:7128.
79. **Okayama, H., and P. Berg.** 1985. Bacteriophage lambda vector for transducing a cDNA clone library into mammalian cells. Mol. Biol. **5**:1136–1142.
80. **O'Reilly, M., P. W. O'Toole, and T. J. Foster.** 1988. Screening lambda libraries and detection of recombinants. FEMS Symp. **40**:187–197.
81. **Palazzolo, M. J., and E. M. Meyerowitz.** 1987. A family of lambda phage cDNA cloning vectors, lambda SWAJ, allowing the amplification of RNA sequences. Gene **52**:197–206.
82. **Perbal, B.** 1986. A practical guide to molecular cloning, p. 201–296. John Wiley & Sons, Inc., New York.
83. **Ptashne, M.** 1971. Repressor and its action, p. 221–237. *In* A. D. Hershey (ed.), Bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
84. **Raleigh, E. A., N. E. Murray, H. Revel, R. M. Blumenthal, D. Westaway, A. D. Reith, P. W. J. Rigby, J. Elhai, and D. Hanahan.** 1988. McrA and McrB restriction phenotypes of some of *E. coli* strains and implications for gene cloning. Nucleic Acids Res. **16**:1563–1575.
85. **Raleigh, E. A., and G. Wilson.** 1986. *Escherichia coli* K-12 restricts DNA containing 5-methyloytosine. Proc. Natl. Acad. Sci. USA **83**:9070.
86. **Rambach, A., and P. Toillais.** 1974. Bacteriophage lambda having EcoRI endonuclease site only in the non-essential region of the genome. Proc. Natl. Acad. Sci. USA **71**:3927–3930.
87. **Rimm, D. L., D. Horness, J. Kucera, and F. R. Blattner.** 1980. Construction of coliphage lambda Charon vectors with BamHI cloning sites. Gene **12**:301–309.
88. **Roditi, I., E. Koenig, and R. O. Williams.** 1989. A rapid method for determining the orientation of inserts in bacteriophage lambda vectors. Nucleic Acids Res. **17**:10506.
89. **Rosenberg, S. M.** 1987. Improved *in vitro* packaging of lambda DNA. Methods Enzymol. **153**:95–103.
90. **Rosenberg, S. M.** 1988. Chi stimulated patches are heteroduplex, with recombinant information on the phage lambda *r* chain. Cell **48**:855–865.
91. **Rosenberg, S. M., M. M. Stahl, J. Kobayashi, and F. W. Stahl.** 1985. Improved *in vitro* packaging of coliphage lambda DNA: a one-strain system free from endogenous phage. Gene **38**:165–175.
92. **Saiki, R., S. Scharf, F. Faloona, K. Mullis, G. Horn, H. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230**:1350–1354.
93. **Sakaki, Y., A. E. Karu, S. Linn, and H. Echols.** 1973. Purification and properties of gamma-protein specified by bacteriophage lambda: an inhibitor of the host recBC recombination enzyme. Proc. Natl. Acad. Sci. USA **70**:2215–2219.
94. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, vol. I. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
95. **Sanger, F., A. R. Coulson, G. F. Hong, D. F. Hill, and G. B. Peterson.** 1982. Nucleotide sequence of bacteriophage lambda DNA. J. Mol. Biol. **162**:729–773.
96. **Seed, B.** 1983. Purification of genomic sequences from bacteriophage libraries by recombination and selection *in vivo*. Nucleic Acids Res. **11**:2427–2445.
97. **Short, J. M., J. M. Fernandez, J. A. Orge, and W. D. Huse.** 1988. Lambda ZAP. A bacteriophage lambda expression vector with *in vivo* excision properties. Nucleic Acids Res. **16**:7583–7599.
98. **Smith, G. R., S. K. Amundsen, A. M. Chaudhari, K. C. Cheng, A. S. Ponticelli, C. M. Roberts, D. W. Schultz, and A. F. Taylor.** 1984. Role of recBC enzyme and Chi sites in homologous recombination. Cold Spring Harbor Symp. Quant. Biol. **42**:485–495.
99. **Snyder, M., S. Elledge, D. Sweetner, R. A. Young, and R. W. Davis.** 1987. Lambda gt11: gene isolation with antibody probes and other applications. Methods Enzymol. **154**:107–128.
100. **Sprague, K. U., D. H. Faulds, and G. R. Smith.** 1978. A single base-pair change creats a Chi recombinational hot spot in bacteriophage lambda. Proc. Natl. Acad. Sci. USA **75**:6182–6186.
101. **Stahl, F. W.** 1979. Special sites in generalized recombination. Annu. Rev. Genet. **13**:7–21.

102. **Stahl, F. W., J. M. Crasemann, and M. M. Stahl.** 1975. Rec mediated recombinational hot spot activity in bacteriophage lambda. III. Chi mutations are site-mutations stimulating rec-mediated recombination. J. Mol. Biol. **94:**203–212.
103. **Stahl, F. W., I. Kobayashi, and M. M. Stahl.** 1983. Chi is activated by a variety of routes. UCLA Symp. Mol. Cell. Biol. New Ser. **10:**773–783.
104. **Stahl, M. M., I. Kobayashi, F. W. Stahl, and S. K. Huntington.** 1983. Activation of Chi, a recombinator by the action of an endonuclease at a distant site. Proc. Natl. Acad. Sci. USA **80:**2310–2313.
105. **Sternberg, N.** 1990. Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs. Proc. Natl. Acad. Sci. USA **87:**103–107.
106. **Sternberg, N., D. Tiemeier, and L. Enquist.** 1977. *In vitro* packaging of λDam vector containing EcoRI DNA fragments of *Escherichia coli* and phage P1. Gene **1:**255–280.
107. **Stoker, N. G., K. A. Grant, H. M. Dockrell, C. R. Howard, N. F. Jouy, and K. P. W. J. McAdam.** 1989. High level expression of genes cloned in phage lambda gt11. Gene **78:**93–99.
108. **Studier, F. W., and A. H. Rosenberg.** 1981. Genetic and physical mapping of the late region of bacteriophage T7 DNA by use of cloned fragments of T7 DNA. J. Mol. Biol. **153:**503–525.
109. **Swaroop, A., and S. M. Weissman.** 1988. Charon BS (+) and (−), versatile lambda phage vectors constructing directional cDNA libraries and their efficient transfer to plasmids. Nucleic Acids Res. **16:**8739.
110. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
111. **Thomas, M., J. R. Cameron, and R. W. Davis.** 1974. Viable molecular hybrids of bacteriophage lambda and eukaryotic DNA. Proc. Natl. Acad. Sci. USA **71:**4579–4583.
112. **Tiemeier, D., L. Enquist, and P. Leder.** 1976. Improved derivatives of phage lambda EK2 vector for cloning recombinant DNA. Nature (London) **263:**596–598.
113. **Unger, R. C., H. Echols, and A. J. Clark.** 1972. Interaction of recombination pathways of bacteriophage lambda and host *Escherichia coli*: effect on lambda recombination. J. Mol. Biol. **70:**531–537.
114. **Van, D., J. Leen, A. Van-Belkum, and T. Koss.** 1989. Improved cDNA cloning into bacteriophage lambda gt11. Nucleic Acids Res. **17:**9496.
115. **Van Weizenbeck, P. M. G. F., T. J. M. Hulsebos, and J. G. G. Schoenmakers.** 1980. Nucleotide sequence of the filamentous bacteriophage M13 DNA genome: comparison with phage fd. Gene **11:**129–138.
116. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153:**3–11.
117. **Villarreal, R. B.** 1989. A method for screening of fusion protein expression by lambda gt11 recombinant clones without preparation of lysogens. Nucleic Acids Res. **17:**6421.
118. **Wahl, G. M., K. A. Lewis, J. C. Ruiz, B. Rothenberg, J. Zhao, and G. A. Evan.** 1987. Cosmid vectors for rapid genomic walking, restriction mapping and gene transfer. Proc. Natl. Acad. Sci. USA **84:**2160–2164.
119. **Watson, J. D., N. H. Hopkins, J. W. Roberts, J. A. Steitz, and A. M. Weiner.** 1987. Molecular biology of the gene, vol. 1, 2nd ed., p. 503–548. Benjamin/Cummings Publishing Co., Inc., Redwood City, Calif.
120. **Weigle, J.** 1966. Asembly of phage lambda *in vitro*. Proc. Natl. Acad. Sci. USA **55:**1462–1466.
121. **Weigle, J.** 1968. Studies on head-tail union in bacteriophage lambda. J. Mol. Biol. **33:**483–489.
122. **Windle, B. E.** 1986. Phage lambda and plasmid vectors with multiple cloning sites and lacZ-α-complementation. Gene **45:**95–99.
123. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
124. **Yogil, E., and S. Inna.** 1985. Rec mediated recombinational activity of two adjacent Chi elements in bacteriophage lambda. Genet. Res. **45:**1–8.
125. **Young, E. T., and R. L. Sinshemer.** 1964. Novel intracellular forms of lambda DNA. J. Mol. Biol. **10:**562–564.
126. **Young, R. A., and R. W. Davis.** 1983. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. USA **80:**1194–1198.
127. **Young, R. A., and R. W. Davis.** 1985. Immunoscreening of recombinant DNA expression libraries, p. 29–41. *In* J. K. Setlow and A. Hallaender (ed.), Genetic engineering, vol. 7. IRL Press, Oxford.
128. **Zabarovsky, E. R., and R. L. Allikmets.** 1986. An improved technique for the efficient construction of gene libraries by partial filling-in of cohesive ends. Gene **42:**119–123.
129. **Zagursky, R. J., and M. L. Berman.** 1984. Cloning vectors that yield high levels of single-stranded DNA for rapid DNA sequencing. Gene **27:**183–191.
130. **Zaissler, J., E. Singer, and F. Schaefer.** 1971. The role of recombination in growth of bacteriophage lambda. II. Inhibition of growth by prophage P2, p. 469–476. *In* A. H. Hershey (ed.), Bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.