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Novel bacteriophage λ cloning vector

(genome coverage/spi phenotype/*in vitro* packaging/*unc*-54 gene)

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ABSTRACT **A simple method for generating phage collections representing eukaryotic genomes has been developed by using a novel bacteriophage λ vector, λ1059. The phage is a *Bam*HI substitution vector that accommodates DNA fragments 6–24 kilobases long. Production of recombinants in λ1059 requires deletion of the λ *red* and *gamma* genes. The recombinants are therefore *spi*⁻ and may be separated from the *spi*⁺ vector phages by plating on strains lysogenic for bacteriophage P2. Random fragments suitable for insertion into λ1059 are obtained by partial digestion of high molecular weight eukaryotic DNA with *Sau*3a. This restriction enzyme cleaves at the sequence G-A-T-C and leaves a 5′-tetranucleotide "sticky end." Because G-A-T-C extensions are also produced by *Bam*HI cleavage, these fragments may be annealed directly to *Bam*HI-cleaved λ1059. By using these methods, a set of clones covering the entire *Caenorhabditis elegans* genome was constructed. DNA segments which include the *unc*-54 myosin heavy chain gene have been isolated from this collection.**

Fractionation of genomes was an intractable problem until the introduction of recombinant DNA techniques. These methods eliminate the necessity for physical separations of DNA segments and permit the isolation of structural genes from collections of randomly cloned DNA fragments (1–10).

Given a suitable probe, any eukaryotic gene may be isolated from a pool of cloned fragments, provided that it is large enough to give sequence representation of an entire genome. The simple multicellular eukaryote *Caenorhabditis elegans* has a haploid DNA content of approximately 8×10^7 base pairs (bp) (11). If random DNA cleavage and uniform cloning efficiency are assumed, a collection of 8×10^4 clones with an average length of 10^4 bp will be sufficient to include any genomic sequence with >99% probability. Similarly, the human genome with 2×10^9 bp will be covered by 10^6 clones 10^4 bp long.

We have developed a novel bacteriophage λ cloning vector, λ1059, with properties that simplify the construction of recombinant phage collections representing genomes. The strategy is shown in Fig. 1. The vector DNA is cleaved with *Bam*HI and the vector arms are hybridized to fragments [15–20 kilobase (kb)] of genomic DNA. Nearly random fragments of genomic DNA may be obtained by partial digestion with *Sau*3a, an enzyme which has a four-bp recognition sequence. Viable phage particles are then recovered by *in vitro* packaging (7, 12, 13), and a stock of recombinant phages is obtained by amplification of the phages harboring inserts on a strain that restricts the growth of the original vector.

MATERIALS AND METHODS

Growth of Bacteriophage DNA. Phage were grown as liquid lysates on Q358 (r^-_k, m^+_k, su^+_{II}, 80^R) bacteria in CY medium supplemented with 25 mM Tris·HCl (pH 7.4) and 10 mM $MgCl_2$. CY medium contains (per liter) 10 g of Difco Casamino acids, 5 g of Difco Bacto yeast extract, 3 g of NaCl, and 2 g of KCl and is adjusted to pH 7.0. The phages were precipitated by addition of 70 g of polyethylene glycol 6000 per liter and purified by two cycles of CsCl density gradient centrifugation (14). Phage DNA was prepared by phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol), extraction of concentrated phage suspensions and stored at 1.0 μg/ml in 10 mM Tris·HCl, pH 7.4/10 mM NaCl/0.1 mM EDTA.

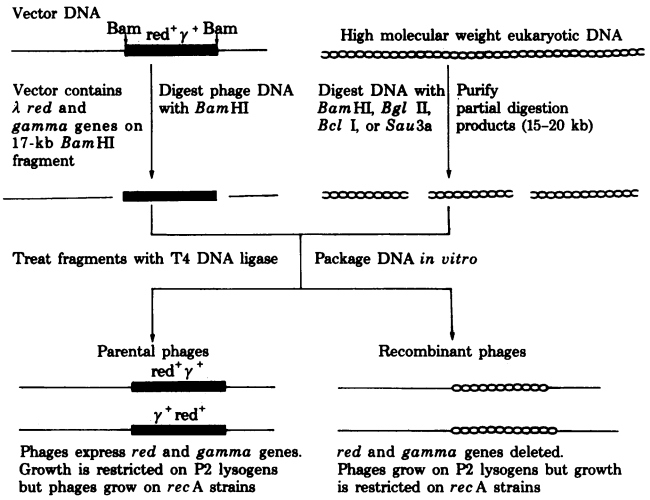

FIG. 1. Construction of recombinants by using the λ1059 vector.

Preparation of Size-Fractionated Nematode DNA. Nematode DNA (N2 DNA) was prepared from frozen animals; bacteria and bacterial debris were removed by flotation on sucrose (11). The worms were pulverized by grinding in a mortar chilled with liquid nitrogen. DNA was prepared by CsCl density gradient centrifugation after deproteinization by phenol extraction (15). Analysis of this material on neutral and alkaline agarose gels showed the DNA to be longer than 100 kb. DNA aliquots (20 μg) were digested in 100 μl of 10 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$/10 mM 2-mercaptoethanol/50 mM NaCl (Hin buffer) containing 0.1, 0.2, 0.5, 1.0, and 2.0 units of *Sau*3a or 1, 2, 5, 10, and 20 units of *Bam*HI for 1 hr at 37°C. The digests were pooled and an aliquot of each was labeled by nick translation (16). Aliquots (50 μg) of digested DNA and of radioactively labeled DNA were fractionated by electrophoresis on 1.5 × 20 cm columns of 0.5% low-melting-temperature agarose (Bethesda Research Laboratories, Rockville, MD) in 40 mM Tris acetate, pH 8.3/20 mM Na acetate/2 mM EDTA containing 2 μg of ethidium bromide per ml (TAE buffer). Electrophoresis was for approximately 18 hr at 150 V, by which time the xylene cyanol dye had moved approximately 15 cm. DNA 15–20 kb long was recovered from slices of the gel after the agarose was melted at 70°C for 5 min. The melted gel was diluted with 10 vol of H_2O and applied to 300-μl columns of phenol neutral red-polyacrylamide affinity adsorbent (17) (Boehringer Mannheim) equilibrated with TAE buffer. DNA was eluted with 2 M $NaClO_4$ in TAE and concentrated by ethanol precipitation.

Abbreviations: bp, base pair(s); kb, kilobase(s); pfu, plaque-forming units.

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Insertion of Nematode DNA in λ1059 Arms. λ1059 DNA was digested with a 3-fold excess of *Bam*HI for 60 min at 37°C in Hin buffer. The reaction was terminated by incubation at 70°C for 5 min. Aliquots (2.0 μg) of *Bam*HI cleaved λ1059 DNA were ligated in the presence of 0–1.0 μg of 15 to 20-kb nematode DNA in 20-μl reaction mixtures containing 0.1 Weiss unit of T4 DNA ligase, 10 mM Tris·HCl (pH 7.4), 10 mM $MgCl_2$, 50 mM NaCl, and 0.1 mM ATP. After incubation at 4°C for 18 hr, phages were recovered by *in vitro* packaging. Packaging extracts were prepared by lysing 10 g of induced NS 428 (13) in 50 ml of 50 mM Tris·HCl, pH 8.0/3 mM $MgCl_2$/10 mM 2-mercaptoethanol/1 mM EDTA in a French pressure cell operated at 1000 psi (6.9 MPa). After centrifugation of the extract for 30 min at 35,000 rpm in a Ti 60 rotor, aliquots were stored at −70°C. Extracts prepared in this manner are active in *in vitro* packaging when supplemented with partially purified protein A prepared as described by Blattner *et al.* (7). Packaging was performed in 150-μl reaction mixtures containing 50 μl of extract, 10 μl of protein A, 20 mM Tris·HCl (pH 8.0), 3 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 1 mM EDTA, 6 mM spermidine, 6 mM putrescine, 1.5 mM ATP, and 2.0 μg of cleaved and religated λ1059 DNA. After incubation for 60 min at 20°C the extracts were diluted with 1 ml of sterile 10 mM Tris·HCl, pH 7.4/5 mM $MgSO_4$/0.2 M NaCl/0.1% gelatin (λ dil) and titered on Q358 and Q359 (r^-_k, m^+_k, su^+_{II}, 80^R, P2).

Amplification and Screening of Recombinant Phage Collections. Recombinant phage collections were prepared from plate stocks including at least 10^5 infective centers on Q359 bacteria. These were stored over chloroform at 4°C in λ dil and had titers of approximately 10^9 recombinants per ml. The plaque hybridization technique of Benton and Davis (18) was used to identify phages harboring *unc*-54 sequences. The probe was a 1.1-kb *Hin*dIII fragment cloned in M13 mp2 (ref. 19; unpublished data). Because the M13 mp2 vector contains bacterial *lac* sequences which hybridize to chromosomal DNA released in phage plaques, bacterial hosts with chromosomal *lac* deletions were used for plating recombinant phages [D 91 (r^+_k m^+_k Δ lac-pro) or Q364 (r^-_k m^+_k, su^+_{III}, Δ lac-pro, P2)].

Enzymes. T4 ligase was prepared from a λ T4 gene 30 recombinant supplied by N. Murray (20). Restriction endonucleases were prepared by published procedures (21–27).

Safety. These experiments were performed under C1 and C2 physical containment in accordance with the recommendations of the British Genetic Manipulation Advisory Group.

RESULTS

Principle of the Method. λ1059 is one of a family of "phasmid" phage vectors. These vectors carry pacl plasmids (ColEl plasmids with cloned λ *att* sites) inserted into phage arms by site-specific recombination events (unpublished data). The resulting phasmid phages carry multiple λ *att* sites and ColEl origins of replication. They may be grown lytically as phages or nonlytically as plasmids in the presence of λ repressor. The plasmids may be "released" from the phage arms by infecting a λ integrase constitutive strain. In our nomenclature the plasmid inserts are identified by placement between < and >. Details of the construction of λ1059 will be presented elsewhere. The first step involved cloning the *Bgl* II fragment from the λ immunity region (738–780) in the *Bam*HI site of pacl 29. Plasmids specifying λ immunity were inserted into an immunity 434 genome to yield a phasmid with the structure *h*λ Δ{sR1 plac-sR1 λ2} < *c*I857 pacl 29 > Δ{*int*-*c*III} 434 *c*II *chi* 153 *P*am 902. One "phasmid" was found in which the cloned immunity region was in the same orientation as in the phage genome and this gave recombinants with an *h*80 Δ{*att*80 − λ*c*III} phage (28) yielding *h*80Δ{*att* 80 − λ*c*III} *c*I857 pacl 29 P.P′ Δ {*int* − *c*III} 434 *c*II *chi* 153 *P*am 902. The right arm was substituted to give *h*80 Δ{*att* 80 − *c*III} *c*I 857 pacl P.P′ *b*1319 81 *c*Its *chi* 3. This was then crossed with λ sBam 1° *b*189 *int* 29 *nin* L44 *c*I 857 to give λ sBam 1° *b*189 < *int* 29 *nin* L44 *c*I857 pacl 29 > *b*1319 81 *c*Its *chi* 3. λ1059 was obtained by crossing with another phasmid λ sBam 1° *b*189 < pacl 29 > Δ{*int* − *c*III} KH54 *nin* 5 *chi* 3.

The structure of λ1059 is shown in Fig. 2. The phage is a *Bam*HI substitution vector composed of three *Bam*HI fragments: a 19.6-kb left arm carrying the genes for the λ head and tail proteins, a 17-kb central fragment, and a 9.4-kb right arm carrying the λ replication and lysis genes. The two arms of the vector contain all the essential functions required for λ replication and maturation in a DNA sequence 58.2% of the wild-type length. Viable phages are produced when these arms are hybridized with internal DNA fragments 12.8% to 49.8% the length of wild-type λ (6.3–24.4 kb). The two arms alone do not produce viable phages, because lambdoid phages require genome sizes between 70% and 108% of the wild-type DNA to fill the phage heads properly (37, 38).

The central *Bam*HI fragment of the vector balancing the phage arms carried the λ *red* (*exo* and β genes) and *gamma* functions under the control of the leftward promoter (pL) and λ repressor (*c*I 857). These genes confer *spi*$^+$ phenotype on the vector, which is therefore able to grow on *rec*A strains but is unable to grow on strains lysogenic for phage P2 (39, 40). When the vector DNA is cleaved with *Bam*HI, the vector fragments are ligated, and the DNA is packaged *in vitro*, parental phages with the central fragment cloned in both orientations are produced. Both of these phages are *spi*$^+$ because transcription is initiated at pL on the central fragment and therefore does not depend on the orientation of this segment.

When the arms of the vector, cleaved with *Bam*HI, are ligated in the presence of foreign DNA, both parental and recombinant phage genomes are produced. The phages harboring inserts in place of the central fragment have a *spi*$^-$ phenotype and are able to grow on P2 lysogens but not on *rec*A strains.

Genomic DNA suitable for insertion into λ1059 may be prepared with various restriction enzymes. *Bam*HI cleaves at the sequence G ↓ G-A-T-C-C and is representative of a family of restriction enzymes that leave G-A-T-C as a 5′-tetranucleotide "sticky" end (21–23). Other members of this family include *Bgl* II (A ↓ G-A-T-C-T) (24, 25), *Bcl* 1 (T ↓ G-A-T-C-A) (26), and *Sau*3a (↓ G-A-T-C) (27). Cleavage of genomic DNA by *Sau*3a is an effective technique for generating a nearly random population of high molecular weight DNA fragments because the recognition sequence for *Sau*3a, G-A-T-C, should occur once every 256 bp in DNA with 50% G + C, and only 1/80th of these sites need to be cleaved to produce DNA fragments 20 kb long. The frequency of *Sau*3a sites will not vary appreciably with changes of base composition. In DNA with 67% G+C (or A+T), sites should occur once every 324 bp.

Preparation of Nematode DNA Fragments. Rigorous size fractionation of the DNA to be cloned is essential to avoid spurious linkage produced by multiple ligation events. If fragments longer than 14 kb are ligated to the λ1059 vector arms, any dimers or multimers formed during the ligation reactions will exceed the 24-kb cloning capacity of the phage and will not appear in the recombinant phage population. Fragments shorter than 12 kb are frequently cloned as multiples.

In preliminary experiments using *Bam*HI-digested DNA fractionated by sucrose gradient centrifugation, we found a high proportion of clones containing unrelated sequences of nematode DNA. These were produced by the inadvertant ligation of two or more short *Bam*HI fragments contaminating the higher molecular weight DNA fraction. Purification of

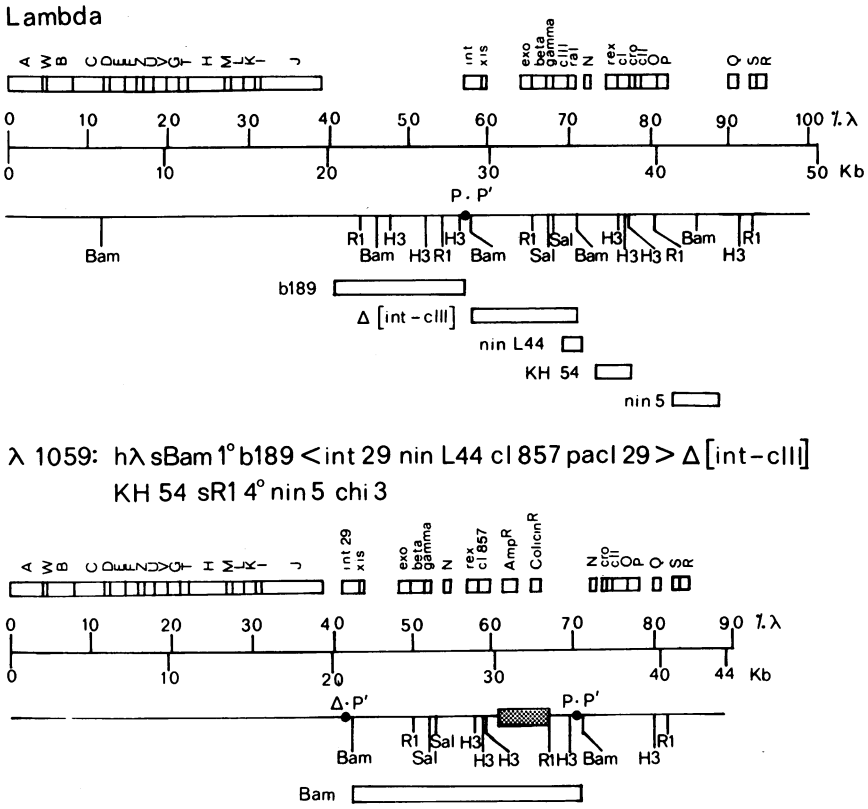

FIG. 2. Structure of λ1059. Above is shown the *Bam*HI, *Eco*RI, *Hin*dIII, and *Sal*I, restriction maps of λ and the positions of many of the known λ genes. The bars underneath the λ map indicate the map positions of the deletions used in the construction of λ1059. A restriction map of λ1059 is shown below. The phage is a *Bam*HI substitution vector composed of three regions separable by cleavage by *Bam*HI or by recombination at the duplicated λ *att* sites (Δ.P′ and P.P′). The left arm of the phage carries the λ structural genes A–J. The sBam 1° (29) mutation and the *b*189 deletion (30) remove the *Bam*HI sites from this arm. The central fragment carries the λ sequence from the first *att* site (Δ.P′) to the *Bgl* II site at coordinate 745 in the *cro* gene (coordinates indicate the map position with reference to the wild-type sequence in units of 0.1% λ). At this juncture, sequences from the mini ColEl plasmid pacl 29 (stippled region) are introduced (unpublished data). This plasmid introduces the β lactamase gene (*Amp*^{R}) and colicin immunity gene (*Colicin*^{R}).

The central fragment terminates in a duplicated λ *att* site (P.P′). This sequence is present in wild-type λ from the *Eco*RI site at 543 to the *Bam*HI site at 578. Because the *red* (*exo* and *beta* genes) and *gamma* genes are present on the central fragment, the vector is *spi*$^+$. These genes are transcribed from pL which is regulated by repressor (cI 857). Because pL is present on the central fragment, the *red* and *gamma* genes are expressed when this fragment is inverted. The *Bam*HI site at 714 has been removed from the central fragment by the *nin* L44 deletion (31). The right arm carries a deletion (32) Δ{*int-c*III} originally made *in vitro* by removing DNA from between the two *Bam*HI sites at 580 and 714 by M. Gottesman, the KH54 deletion (33) removing the *rex* and cI genes, and the *nin* 5 deletion (34). The bar underneath the restriction map of λ1059 indicates the region of the phage removed in genetic manipulation experiments. Substitution of the central fragment produces a *spi*$^-$ phage with a *b*189 arm, a single λ *att* site, a 10- to 20-kb insert cloned between the *Bam*HI sites at 580 and 714, and an immunity arm with the KH54 and *nin* 5 deletions. The growth of these phages is enhanced by the *chi* 3 mutation present on the right arm of the vector (35, 36).

molecules approximately 18 kb long by preparative agarose gel electrophoresis eliminated this problem. High molecular weight nematode DNA (>100 kb) was fragmented with either *Bam*HI or *Sau*3a. We were concerned that abnormal distributions of restriction sites could bias the distribution of fragments obtained by a single digestion condition. We therefore digested the nematode DNA in five separate reactions in which enzyme concentration was varied over a 20-fold range. The partially digested DNAs were pooled, and an aliquot was incubated with DNA polymerase I and [α-^{32}P]dATP to provide radioactive marker molecules (16). The radioactive and nonradioactive fragments were then fractionated by electrophoresis through columns of 0.5% low-melting-temperature agarose. DNA molecules of different sizes were recovered from sections of the gel by melting the agarose and adsorbing the DNA to small columns of phenol neutral red-polyacrylamide (17). Molecules of 18 ± 3 kb (mean ± SD) can be reproducibly obtained, and these were used to prepare the recombinant phage collections.

Insertion of Nematode DNA into λ1059 Arms. λ1059 DNA was cleaved with *Bam*HI, and 2-μg aliquots were religated with T4 DNA ligase in the presence of 0–0.6 μg of 15- to 20-kb fragments produced by *Bam*HI or *Sau*3a cleavage of nematode DNA. After *in vitro* packaging the total number of phages generated by the ligation reaction was determined by plating on Q358, a nonrestrictive strain (Q358 is r_k^- m_k^+, 80^R, su^+_{II}); the number of phages harboring nematode inserts was determined by plating on Q359, a P2 lysogen of Q358. The results of this experiment are plotted in Fig. 3.

Cleavage and religation of λ1059 in the absence of nematode DNA produced more than 1×10^6 phage particles per μg of phage DNA. These phages grew on Q358 but less than 2×10^3 plaque-forming units (pfu) were detected on Q359. This background was reduced to less than 2×10^2 pfu/μg of DNA on CQ6, a more stringent strain (CQ6 is r_c^- m_c^-, su^-). However, because recombinant phages tended to produce smaller plaques on CQ6 than on Q359, we prefer to use Q359 and derivative strains.

Cleavage and ligation of λ1059 DNA in the presence of nematode DNA fragments produced recombinant phages that were selectively detected by plating on Q359. The yield of recombinants was proportional to the amount of nematode DNA added until a saturating DNA concentration was reached. The ligation reaction tended to saturate with >2-fold molar excess

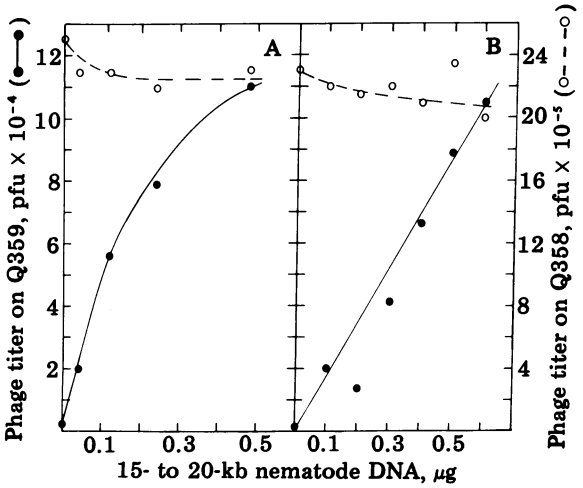

FIG. 3. Insertion of nematode DNA into λ1059 arms. Aliquots (2.0 μg) of *Bam*HI-cleaved λ1059 DNA were ligated in the presence of various amounts of 15- to 20-kb nematode DNA (N2 DNA) cleaved by *Bam*HI (*A*) or *Sau*3a (*B*). The ligated DNAs were packaged *in vitro* by using extracts of heat-induced NS 428, supplemented with partially purified protein A, and titrated on Q358 (to give total phage) and Q359 (to give recombinant phage) bacteria.

of insert DNA to vector DNA (>0.5 μg of insert DNA per 1.0 μg of vector DNA) and yielded 2.4 to 5.4 × 10^5 recombinant phages per μg of 15- to 20-kb nematode DNA. This is approximately 10 times higher than the yield reported by Maniatis *et al.* (6) who obtained 3.8 to 6.0 × 10^4 recombinants per μg of eukaryotic DNA. Because the nematode genome (8 × 10^7 bp) is represented by 4 × 10^4 clones of this size, <1 μg of nematode DNA is sufficient to produce a covering collection. Similarly, <10 μg of human DNA would be required to produce a 10^6-phage clone pool containing eight genome equivalents.

Appproximately 10% of the total phages produced in a ligation reaction saturated with nematode DNA contained inserts. The total yield of phages decreased somewhat upon addition of nematode DNA to the ligation reaction. This may be due to the addition of trace quantities of inhibitors of the T4 ligase or the result of sequestering of vector arms by broken nematode fragments.

Isolation of Clones Containing the *unc*-54 Myosin Heavy Chain Gene. The *unc*-54 gene specifies a major myosin heavy chain (M_r, 210,000) present in the body wall muscle cells of *C. elegans* (41–43). Mutations in this gene lead to severe paralysis of the animal, but the pharynx is unaffected, allowing the animal to feed and survive. One mutant of the *unc*-54 gene, E675, produces a shortened myosin heavy chain with an internal deletion near the COOH terminus of the molecule (43). The deletion is also present in the genomic DNA sequence and may be detected in Southern gel hybridization experiments (unpublished data). Analysis of E675 demonstrates unambiguously that *unc*-54 is the structural gene for the major myosin heavy chain of the body wall musculature and that *unc*-54 sequences are unique.

The *Bam*HI and *Sau*3a clone collections were screened by plaque hybridization for clones containing *unc*-54 sequences. The probe sequence was a 1.1-kb H3 fragment cloned in M13 that includes the sequence deleted in E675 (unpublished data). Approximately 1 plaque in 12,000 should hybridize to this probe because the haploid DNA content of *C. elegans* is 8 × 10^7 and the clone collections contain inserts 18 ± 3 kb long as determined by CsCl density gradient centrifugation (data not shown). When a total of 160,000 plaques from the *Bam*HI recombinant pool and 120,000 plaques from the *Sau*3a were

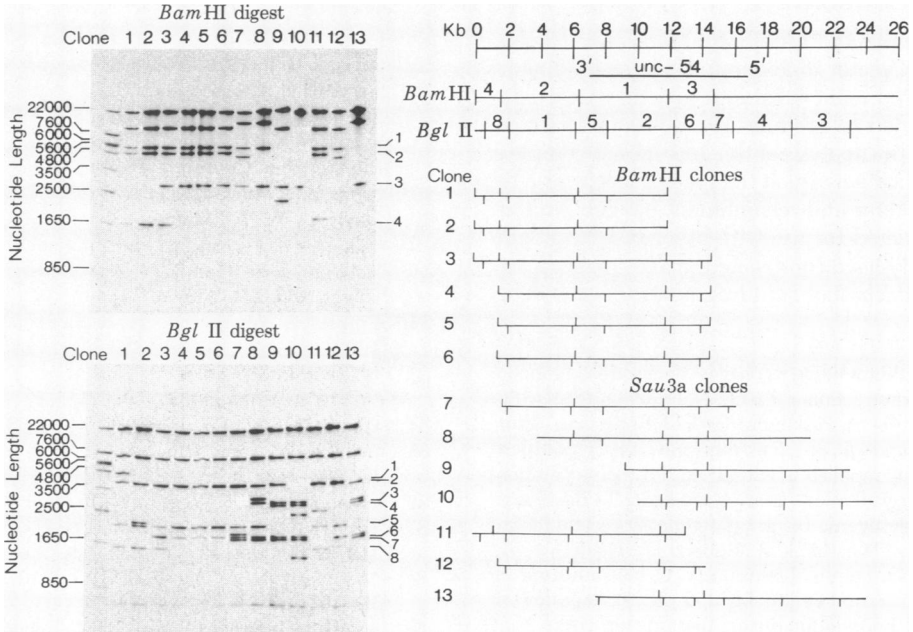

FIG. 4. Restriction endonuclease mapping of clones containing *unc*-54 myosin heavy chain gene sequences. λ DNA was digested with *Bam*HI or *Bgl* II, and nick-translated restriction fragments were separated by electrophoresis through 1% agarose gels in TAE buffer. The mobility of restriction fragments was determined from autoradiographs of the dried gel. Nick-translated *Eco*RI-cleaved λ DNA mixed with nicked-translated *Hae* III-cleaved M13 DNA were included as size markers (unnumbered track). Clones 1–6 were purified from recombinant pools prepared from *Bam*HI-cleaved nematode DNA. Clones 7–13 were isolated from collections harboring *Sau*3a-generated DNA fragments. The clones together define a 26-kb region of nematode DNA. A composite restriction map is shown at the right. Restriction maps of the clones (excluding the vector arms) are aligned beneath the composite map. In the clone maps the vertical bars above the line indicate the position of *Bam*HI cuts, and the bars below the line indicate the position of *Bgl* II cuts. Several of the *Sau*3a clones show *Bam*HI fragments not present in the nematode genome. These arise whenever a *Bam*HI site is created at the junction between the vector arm and the inserted *Sau*3a fragment. The restriction fragments present in the genome have been numbered on the composite restriction map and the positions of these fragments in the agarose gel are indicated by the scale at the right of the autoradiographs.

screened, 7 *Bam*HI-generated clones and 8 *Sau*3a-generated clones were detected which hybridized strongly to the *unc*-54 probe. Thus, *unc*-54 specific sequences are recovered at approximately the expected frequency, confirming that the recombinant pools contain nearly complete sequence representation of the *C. elegans* genome.

Thirteen of these clones were purified and their DNA was analyzed by restriction endonuclease mapping. Fig. 4 shows the restriction fragments obtained after *Bam*HI- and *Bgl* II-cleavage of the clones. Each of the *Bam*HI clones included a 5.4-kb *Bam*HI fragment (fragment 1) together with additional neighboring fragments. This fragment included the probe sequences and the 3′-terminal sequence of *unc*-54 mRNA. The distribution of *Bam*HI cuts about the 5.4-kb fragment was asymmetric. All the clones contained a 4.6-kb fragment mapping to the 3′ side of *unc*-54 gene (fragment 2) but no clone included fragments to the 5′ side of fragment 1 beyond the 2.8-kb fragment 3. This suggests that the next *Bam*HI fragment mapping to the 5′ side of fragment 3 is longer than 15 kb and therefore would be too large to be cloned together with fragments 1 and 3.

The *Sau*3a-generated clones showed a random distribution of sequences mapping in both the 3′ and 5′ sites of the *unc*-54 gene. As predicted, there were no *Bam*HI sites for at least 12 kb to the 5′ side of fragment 3. A few of the *Sau*3a-generated termini restored *Bam*HI sites at the junction between the vector arm and the nematode insert. When these clones were cleaved with *Bam*HI, novel fragments were detected.

The *Bam*HI and *Sau*3a clones contained overlapping sequences which together defined a 26-kb region of the nematode genome surrounding the *unc*-54 gene sequence. The 3′ end of the gene has been mapped within *Bam*HI fragment 1; the exact position of the 5′ end is not yet known (unpublished data).

COMMENT

We have designed simple and efficient methods for using a novel bacteriophage λ vector to generate large recombinant phage collections that represent eukaryotic genomes. Less than 10 μg of DNA is needed to produce 10^6 clones of 18 kb, a number sufficient for the analysis of the genomes of complex organisms. In our method we exploit the *spi* phenotype of λ to distinguish between vector and recombinant phages. Similar vectors can be constructed for use with other restriction enzymes, and we recently have constructed phages that can accommodate *Eco*RI and *Xho* I or *Sal* I fragments. These will be described elsewhere.

We thank the following for sending us phages containing the genetic components used in the construction of λ1059: N. Franklin, 80 Δ{*att*80 − *c*III}; K. Murray, λsBam 1°; A. Parkinson, *b*189; M. Gottesman, Δ{*int* − *c*III}; J. Weir, *b*1319 and *chi* 3; J. Salstrom, *nin*L44; F. Blattner, KH54. Rich Roberts kindly supplied us with strains for the preparation of restriction enzymes, as well as preparation of *Sau*3a. T4 ligase was prepared from a strain lysogenic for a λ-T4 gene 30 recombinant, prepared by N. Murray. We thank R. Roberts, P. Goelet, A. R. MacLeod, and J. Martin for helpful discussions. G.C. held a European Molecular Biology Organization Fellowship and J.K. was a Fellow of the Helen Hay Whitney Foundation during most of this work.

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