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Genetics

λYES: A multifunctional cDNA expression vector for the isolation of genes by complementation of yeast and *Escherichia coli* mutations

(automatic subcloning/*cre–lox* recombination/human genes/plant genes)

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ABSTRACT **This work describes a multifunctional phage λ expression vector system, λYES, designed to facilitate gene isolation from eukaryotes by complementation of *Escherichia coli* and *Saccharomyces cerevisiae* mutations. λYES vectors have a selection for cDNA inserts using an oligo adaptor strategy and are capable of expressing genes in both *E. coli* and *S. cerevisiae*. They also allow conversion from phage λ to plasmid clones by using the *cre–lox* site-specific recombination system, referred to here as automatic subcloning. A simple method has been developed for the conversion of any plasmid into a phage λ cDNA cloning vector with automatic subcloning capability. cDNA libraries constructed in these vectors were used to isolate genes from humans and *Arabidopsis thaliana* by complementation of yeast and bacterial mutations, respectively.**

Cloning of genes by complementation is a powerful method for gene isolation in prokaryotic and lower eukaryotic systems. This technique has not been used extensively in higher eukaryotes because of the difficulty of introducing large numbers of clones into a mutant organism—e.g., humans, mice, fruit flies, and plants—and the general lack of mutations in the systems where complementation is possible—e.g., mammalian tissue culture. One approach which circumvents some of these problems is expression of genes from higher eukaryotes in organisms more amenable to complementation, using selections and screens to identify the genes of interest. Interspecies complementation has been used previously to isolate genes (1–3). The first gene from yeast was isolated by complementation of an amino acid biosynthetic mutation in *Escherichia coli* (4). The *Drosophila* phosphoribosylglycinamide formyltransferase (GART) gene was isolated by complementation of the yeast *ade8* mutation (5). A human gene complementing the *cdc2* mutation of *Schizosaccharomyces pombe* (6), a cAMP phosphodiesterase from rat brains (7), and the human GART gene (8) were isolated by using heterologous cDNA libraries in yeast expression vectors. Although interspecies complementation cannot isolate every gene of interest from higher organisms, a very large number of genes are potentially accessible using this approach, including many of the general biosynthetic genes, genes involved in conserved cellular processes such as protein sorting and transport, and cell cycle regulatory genes. A fertile ground for gene isolation using heterologous cDNA expression libraries lies in the cloning of sequence specific DNA-binding proteins and transcription factors. The basic mechanism of transcriptional activation and repression seems to be conserved in yeast and other eukaryotes, suggesting that simple artificial systems could be built to select for genes encoding proteins that activate or repress transcription in a sequence-specific fashion. We have previously devised a genetic selection for genes encoding sequence-specific DNA-binding proteins that function in *E. coli* (9, 10).

This paper describes the construction and successful testing of a multifunctional cDNA expression vector, λYES (yeast–*E. coli* shuttle), designed to facilitate the isolation of genes by complementation in heterologous organisms.

MATERIALS AND METHODS

Bacterial, Yeast, and Plasmid Strains. *E. coli* JM107 [*endA1, gyr96, thi, hsdR17, supE44, relA1,* Δ(*lac–proAB*), (F′, *traD36, proAB*$^+$, *lacI*^{q}ZΔM15)] (11) was the transformation recipient for all plasmid constructions. pMC9 (*lacI*) was a gift from M. Calos (Stanford University). *Saccharomyces cerevisiae* CMY478 (*cdc28-4, ura3-52, tyr1, ile1, trp1-1*) was a gift of C. Mann (University of Washington, Seattle). The genotype of *E. coli* AB1157 is *thr-1, leuB6, proA2, his-6, thi-1, argE3, lacY1, galK2, ara-14, xyl-5, mtl-1, tsx-33, rpsL31, supE44*. The *trpD* mutant *E. coli* strain was JMB9 Δ*trp*ld102 and its genotype is *leu, thi-1, supE44, galK2, xyl-5, lac*$^-$, *xyl*$^-$, *ara*$^-$, Δ*trp*ld102, *hsdR17* (12).

Media, Enzymes, Assays, and Genetic Methods. For drug selections, LB plates (27) were supplemented with either kanamycin (40 μg/ml) or ampicillin (50 μg/ml). When necessary, isopropyl β-D-thiogalactoside (IPTG) was added to a final concentration of 1 mM. Restriction endonucleases, *E. coli* DNA polymerase I large (Klenow) fragment, T4 polynucleotide kinase, T4 DNA polymerase, and T4 DNA ligase were purchased from New England Biolabs. Deoxyribonucleotides and ATP were purchased from P-L Biochemicals. Drugs were purchased from Sigma.

Construction of λKC and BNN132. pNN402, the plasmid used to recombine the *cre* gene into λ, was created in two steps. First, the *Bam*HI–*Kpn* I *lacZ*-containing fragment from λgt11 was subcloned in *Bgl* II–*Kpn* I-cut pSE345 to make pNN401. pNN401 contains the *lacZ* gene surrounded by λ DNA. pNN402 was derived from pNN401 by replacing the *lacZ* gene with the *neo* gene and a polylinker sequence. This places *neo* between stretches of DNA with λ homology, which allows it and any gene linked to it to be crossed into λ (13). pNN402 was made by a three-way ligation between *Eco*RI–*Xba* I-cut pNN401, a *Hin*dIII–*Xba* I *neo*-containing fragment from pSE251 (a *Hin*dIII–*Sma* I subclone of *neo* from Tn*5* in pUC19), and a 56-base-pair (bp) *Eco*RI–*Hin*dIII fragment from pUC19. To cross the *cre* gene into λ, the *Xho* I–*Sal* I *cre*-containing fragment from pBS39 (14) was inserted into *Sal* I-cut pNN402. Wild type λ was then grown as a plate

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Abbreviation: IPTG, isopropyl β-D-thiogalactoside.

lysate on a strain containing this recombinant plasmid (13). Resulting phage were used to infect JM107, and kanamycin-resistant lysogens were selected and tested for λ immunity and their ability to catalyze automatic excision upon infection by λYES-P. A strain satisfying these requirements was chosen for further study and named BNN132, and the phage resulting from this cross was named λKC (*kan–cre*).

Construction of λYES. pSE936 was the precursor plasmid to the expression vectors in this work. Rather than giving the precise details of the many steps in the circuitous construction of pSE936, we will describe the origin of each fragment in the plasmid and each joint. The cloning sites *Eco*RI*Xho*I-*Eco*RI (GAATTCCTCGAGGAATTC, $n = 18$) are at position 0 on the map. The 30-bp translational initiation sequence ATAATTTTTTCCTCCAGATCCTCTAGAGTC follows. CAT, nucleotides 18–20 of the junction between these sequences, forms the initiation codon for the translation of fusion proteins driven from the *lac* promoter, which transcribes in the opposite direction. Adjacent to this is the 159-bp *Alu* I fragment containing the wild-type *lac* promoter. Adjacent is the 19-bp *Hin*cII–*Hin*dIII fragment from the pUC19 polylinker. At nucleotide 217 is a 12-bp fragment of sequence GCGGCCGGCCGC. Adjacent to this is the 365-bp *Hin*dIII–*Xho* I fragment from *HIS3* (15) containing the transcriptional terminator of *HIS3*. Both *Hin*dIII sites on either side of the 12-bp fragment were destroyed. Fused to the *Xho* I site at nucleotide 594 is the 2682-bp *Sal* I–*Pvu* II fragment of YIp5 (16) containing *URA3*. The *Pvu* II site at nucleotide 3276 is fused to the 852-bp *Pvu* II–*Hpa* I *CEN4* fragment (17). The *Hpa* I site at nucleotide 4028 is fused to an approximately 200-bp *Sal* I–*Xho* I fragment from pNN454 containing directly repeated *lox* sites flanking a *Not* I site. This destroys the *Hpa* I site but retains the *Sal* I site. The *Xho* I site at nucleotide 4228 is fused to the 454-bp *Nae* I–*Hin*dIII *ARS1* fragment (18), destroying the *Xho* I site. The *Hin*dIII site is fused to the 2295-bp *Pvu* II–*Eco*RI fragment of pBR322 containing *ori* and *bla* (19), destroying the *Hin*dIII site. To the *Eco*RI site at nucleotide 6979 an 822-bp *GAL10-1* promoter fragment is fused (20), destroying the *Eco*RI site at 6979 but not 7800. This fragment is a deletion derivative of the *GAL10-1* promoter where an *Eco*RI linker was placed at a breakpoint that was 4 bp before the translational start of the *GAL1*-encoded protein (20).

pSE936 was linearized with *Not* I and ligated into λgt6 to which *Not* I linkers had been added to the *Eco*RI sites. λgt6 is identical to λgt7 (21) except that the deletion in *c*I is replaced with the intact *c*I857 gene, making it 130 bp longer. This ligation mix was packaged and used to infect JM107 at 30°C, and ampicillin-resistant colonies were selected at 30°C. A clone containing pSE936 was partially digested with *Xho* I and filled in with the Klenow fragment of DNA polymerase I and dNTPs. A clone in which the *Xho* I site on the phage was destroyed but the site in the plasmid sequences remained was isolated and named λYES-R.

pSE937 was constructed by replacing the 822-bp *GAL1* promoter on pSE936 with a 907-bp *GAL1* fragment that had an *Eco*RI site placed into the *Ava* I site at nucleotide 88 of the coding region of the *GAL1* protein. This plasmid was linearized with *Not* I and ligated into *Not* I-cleaved gel-purified λYES-R arms. After restriction analysis, a phage was chosen that had the plasmid inserted in the same orientation as λYES-R and was named λYES-P. The sequence of the coding region of the *GAL1* protein up to the *Xho* I site is 5′-ATGACTAAATCTCATTCAGAAGAAGTGATTGTACC-TGAGTTCAATTCTAGCGCAAAGGAATTACCAAGAC-CATTGGCCGAAAAGTGCGGAATTCCTCGAG-3′. The CGA of the *Xho* I site is an in-frame codon.

DNA Sequencing. Plasmid DNAs were sequenced by the method of Sanger *et al.* (22). Single-stranded plasmid DNAs were prepared by the method of Zagursky and Berman (23), using R408 as a helper phage (24). Oligonucleotides used for double-stranded DNA sequencing directly from these cDNA clones are 5′-ACTTTAACGTCAAGGAG-3′ for reading from *GAL1* towards the *Xho* I cloning site and 5′-TGTG-GAATTGTGAGCGG-3′ for reading from the *lac* promoter towards the *Xho* I site.

cDNA Library Construction in λYES. cDNA was made by standard methods, using the procedure of Gubler and Hoffman (25) and employing avian myeloblastosis virus reverse transcriptase. After the second strand reaction, the cDNA in 400 μl was precipitated by addition of 22 μl of 100 mM spermine, incubated on ice for 30 min, pelleted for 15 min at 12,000 × *g*, washed three times for 30 min each on ice with 1 ml of spermine wash buffer [70% (vol/vol) EtOH/10 mM $Mg(OAc)_2$/0.3 M NaOAc, pH 7] and once with 1 ml of 70% EtOH (26). cDNA was resuspended in 50 μl of 10 mM Tris·HCl, pH 8.0/1 mM EDTA (TE) and the ends were made flush by treatment with T4 DNA polymerase under the conditions suggested by the supplier, followed by addition of 5 μl of 0.5 M EDTA, extractions with phenol/chloroform, and precipitation with ethanol. cDNA, approximately 4 μg, was resuspended in 7 μl of TE and was ligated to 2 μg of an equal mixture of two different adaptors in a total volume of 10 μl at 4°C overnight. The sequences of the kinase-treated adaptors were as follows: adaptor 1, "top strand" 5′-CGAGATTTACC-3′ and "bottom strand" 5′-GGTAAATC-3′; adaptor 2, "top strand" 5′-CGAGAGTTCAC-3′ and "bottom strand" 5′-GTGAACTC-3′. After ligation, 170 μl of TE, 20 μl of 1 M KCl, and 10 μl of 100 mM spermine were added, the mixture was incubated on ice for 30 min, and the cDNA was precipitated and washed as above. KCl inhibits precipitation of unligated adaptors (26). Adapted cDNA was resuspended in 20 μl of TE and electrophoresed on a 1% low-melting-point agarose gel. cDNA 600 bp and longer was gel purified for ligation into λYES-R arms; 0.1 μg of cDNA was ligated to 2 μg of λYES-R plasmid DNA in a volume of 4 μl at 4°C overnight and packaged using one Gigapack Gold packaging extract (Stratagene). Phage libraries were amplified on LE392 containing pMC9, a clone of *lacI* in pBR322, to reduce expression of the *lac* promoter.

Preparation of "T-Filled" λYES-R Vector Arms. Fifty micrograms of λYES-R plasmid DNA was cleaved with *Xho* I, precipitated with ethanol to remove Mg^{2+}, and resuspended in 190 μl of TE, and 10 μl of 10 mM spermine was added and the mixture was rapidly mixed by inversion. λ DNA precipitates were pelleted for 2 sec in an Eppendorf centrifuge, then washed as described above. DNA was resuspended in 90 μl of TE and 10 μl of 10× *Taq* DNA polymerase buffer (1× = 50 mM KCl/10 mM Tris·HCl, pH 8.3/2 mM $MgCl_2$/0.01% gelatin/25 μM dTTP) was added. The mixture was incubated at 72°C for 5 min, then 1 unit of *Taq* DNA polymerase (Cetus) was added and incubated at 72°C for 2 min. This was then diluted to 190 μl with water and 10 μl of 10 mM spermine was added; the mixture was rapidly mixed by inversion, and DNA was pelleted and washed as above. DNA was resuspended in 50 μl of TE and used for ligation. A 10- to 100-fold increase in plaque-forming units was observed upon addition of properly adapted insert DNA. The largest libraries were obtained when the vector was T-filled for the shortest amount of time.

Automatic Subcloning: Conversion of a Library in λYES-R into a Plasmid Library. Phage (10^8) were incubated with 1 ml of a fresh overnight culture of JM107(λKC) cells, BNN132, in 10 mM $MgCl_2$ for 30 min at 30°C without shaking. Two milliliters of LB was added and cells were incubated with shaking for 1 hr at 30°C. Cells were then plated on 150-mm LB plates with ampicillin at 50 μg/ml and 0.1% glucose and incubated at 37°C overnight. Ampicillin-resistant cells were scraped from these plates, added to 3 liters of "terrific broth" (ref. 27, p. A.2) with ampicillin, and grown to stationary

phase. This culture yielded 2 mg of plasmid DNA after CsCl purification. This DNA was used to transform CMY478 by the method of Schiestl and Gietz (28), using total yeast RNA as carrier.

Complementation of *E. coli* Mutants. *E. coli* strains were infected with λKC, and lysogens were selected by plating on LB plates with kanamycin at 40 μg/ml. The lysogens were grown overnight in LB maltose with 10 mM IPTG and resuspended in 10 mM $MgSO_4$. Then 6×10^6 phage from the cDNA library were incubated with 10^9 cells for 30 min at 30°C without shaking. One milliliter of M9 minimal medium with 0.2% mannitol, 1 mM IPTG, and all of the amino acid supplements required by the strain was added and the culture was incubated for 2 hr at 30°C with shaking. The cells were washed with water and resuspended in 1 ml of 10 mM $MgSO_4$, and 1 ml was plated at 30°C on selective plates containing ampicillin at 50 μg/ml, 0.2% mannitol as a carbon source, and the required amino acid supplements lacking the selection amino acid as required.

RESULTS

Construction of λYES. λYES was designed for the construction of large cDNA libraries with a high percentage of inserts, regulated expression in yeast or *E. coli*, and simple conversion from a phage to a plasmid to facilitate both recovery of inserts and introduction of libraries into yeast. A plasmid vector, pSE936 (Fig. 1), was constructed containing the ColE1 origin of replication, *lac* promoter, and *bla* gene for replication, expression, and selection in *E. coli* and the *URA3* (29), *ARS1* (18, 30), *CEN4* (17, 31), and *GAL1* (20) promoter sequences for replication, expression, and selection in *S. cerevisiae*. The *GAL* and *lac* promoters were placed in a convergent orientation on opposite sides of the *Xho* I cloning site. Since the cDNA cloning strategy is nondirectional, half of the inserts are in the proper orientation for expression in yeast and half for expression in *E. coli*. The *lac* promoter contains a ribosome-binding sequence optimally spaced from an AUG start codon and is capable of making protein fusions. The *GAL1* promoter used lacks a translational start. Adjacent to the *lac* promoter is a termination sequence derived from *HIS3* (15) in the same orientation as *GAL1* initiated transcripts. pSE936 also contains two direct repeats of a *lox* site flanking a *Not* I restriction site. This 7.8-kb plasmid was linearized with *Not* I and inserted into a modified λgt6 vector (21) in which the *Eco*RI sites were converted into *Not* I sites by using linkers and the *Xho* I site was removed to make λYES-R (RNA), a 42.6-kb phage with an 8.4-kb insert capacity. A second vector, λYES-P (protein), contains the *GAL1* promoter and DNA encoding the first 29 amino acids of the *GAL1* protein adjacent to the cloning site and is capable of making protein fusions in yeast.

The *GAL* promoter on λYES-R was tested by insertion of a promoterless *lacZ* cassette. Galactose-grown cells showed 500-fold more β-galactosidase activity than glucose-grown cells, and RNA blot analysis confirmed this (data not shown). Since some message was detected in glucose-grown cells, complementation may not always be reversed by growth on glucose. The *lac* promoter on λYES-R was also tested by using a specially constructed *lacZ* cassette fused in frame to the *Eco*RI site and shown to produce high levels of β-galactosidase activity in the presence of 1 mM IPTG (data not shown).

Automatic Subcloning Using the *cre–lox* System in λYES. Site-specific recombination is used in the life cycle of bacteriophage P1 to circularize the linear phage upon infection of *E. coli* and to resolve the dimeric products of P1 replication. P1-encoded cre protein catalyzes this efficient site-specific recombination between 24-bp sequences called *lox* sites (32). The plasmid portion of λYES is flanked by direct repeats of *lox* sites, and cre-mediated site-specific recombination can be used to convert the phage form of the vector into a plasmid. The *cre* gene linked to *neo* from Tn*5* was crossed into wild-type λ to create λKC and lysogenized in JM107, creating the strain BNN132. Infection of BNN132 with λYES at 37°C resulted in the production of ampicillin-resistant colonies with an efficiency of 50% relative to plaque-forming units. Each colony tested had undergone a precise excision of the DNA between the *lox* sites, leaving a plasmid with only one *lox* site (Fig. 2). The presence of the λ repressor produced by

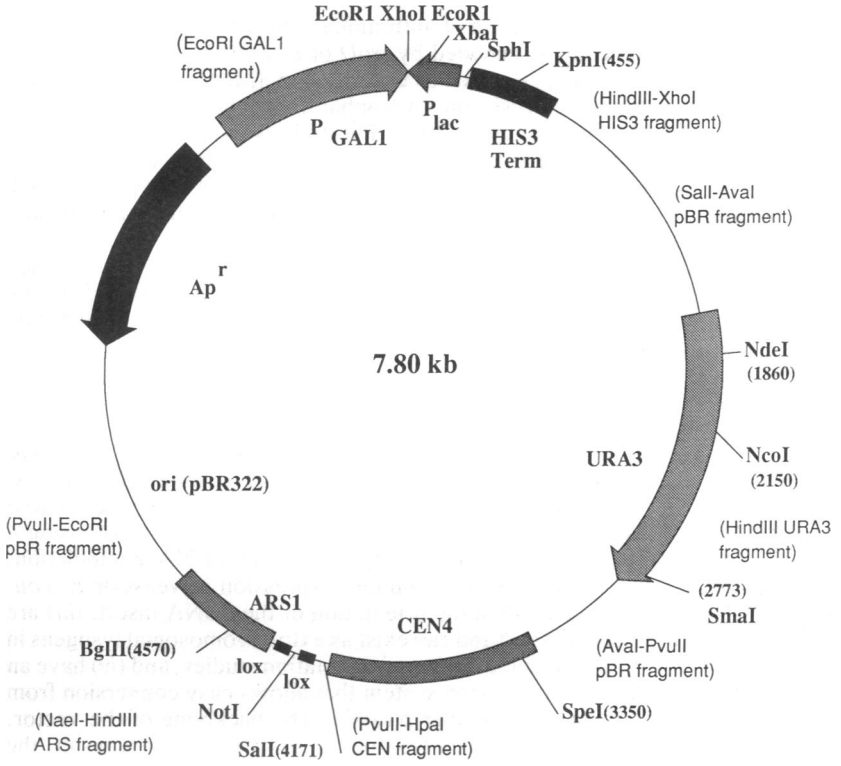

FIG. 1. Map of pSE936, the plasmid precursor of λYES-R. Nucleotide positions (in parentheses) of unique restriction enzyme cleavage sites are shown. The origins of many of the fragments used to construct this plasmid are shown in parentheses outside the circle. Functional sequences are depicted as filled boxes and are labeled inside of the circle. Boxes with arrows are genes transcribed in the direction of the arrow. The *Xba* I site lies between the *lac* promoter and the translational start. kb, Kilobases.

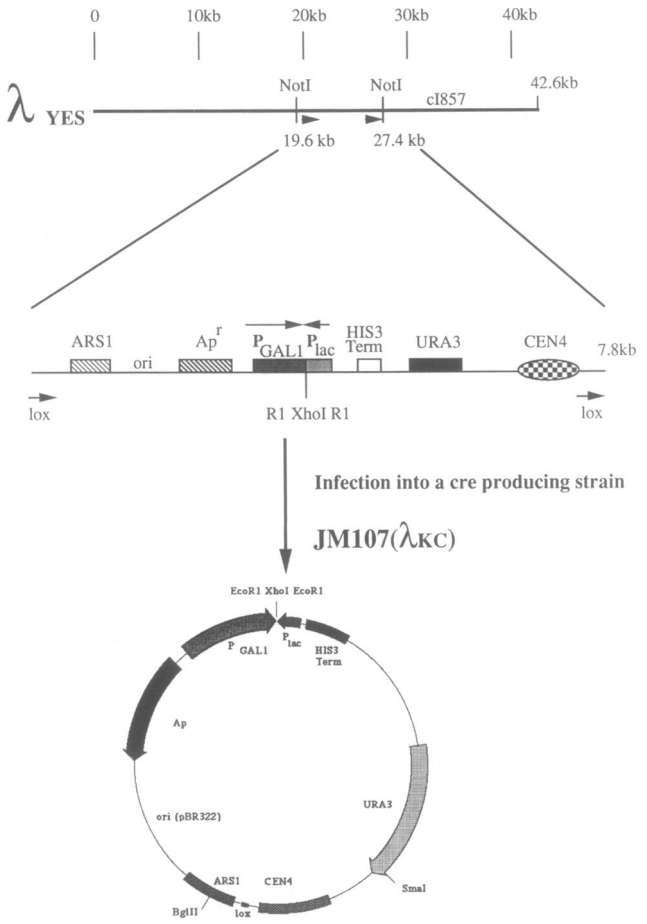

FIG. 2. Schematic representation of *cre–lox*-mediated automatic subcloning. λYES is illustrated as a line. The λ arms are derived from λgt6, which is a *c*I857 phage constructed with three major deletions to allow it to accommodate inserts. It has a deletion between 19.6 kb (the λ*plac* left arm) and 26.107 kb (*Eco*RI site), the *b*522 deletion between 27.66 kb and 31.042 kb, and the *nin*5 deletion between 40.501 kb and 43.307 kb on the wild-type λ sequence. The *Eco*RI sites of λgt6 were converted into *Not* I sites, and *Not* I linearized pSE936 inserted at this site. The *Xho* I site originally at 33.498 kb in the wild-type λ sequence was destroyed to make the plasmid-derived *Xho* I site unique. The arrows indicate the position and orientation of the *lox* sites. Infection of JM107(λKC) cells by these phage allows the cre recombinase to catalyze a site-specific recombination between the two *lox* sites to produce a plasmid with only one *lox* site shown below.

λKC ensures that λYES switches into the lysogenic rather than the lytic mode upon infection. A 200-bp *Sal* I–*Xho* I fragment containing *lox* sites in direct repeats flanking a *Not* I site has been constructed on plasmid pNN934 and can be used to convert any plasmid into an automatic subc. ning phage.

A Biochemical Selection for Inserts and cDNA Library Construction in λYES. A partial fill-in strategy using *Xho* I sites and adaptors was employed to select for inserts (33). In this strategy the vector can ligate only to the cDNA and vice versa. Adaptors eliminate the need to methylate the cDNA or treat it with restriction enzymes. This protocol routinely results in libraries with 95–99% inserts. Very large libraries were an unanticipated consequence of this procedure. Using 2 μg of vector, 0.1 μg of cDNA, and a single packaging extract (Gigapack Gold, Stratagene), we produced libraries with an average of 3×10^7 recombinants (averaged over 16 libraries, with a range of 6×10^6 to 2×10^8 recombinants).

The sequence around the *Xho* I site is palindromic, GAATTCCTCGAGGAATTC. The use of a single adaptor (8 bp of double-stranded DNA with 3-base overhang) to provide cloning ends to the cDNA will result in an inverted repeat of 19 bp at the beginning and end of the cDNAs when cloned in the vector. Secondary structure is known to influence translation and stability of messages. Although this was not demonstrated to be a problem here, this situation was nevertheless avoided by the synthesis of two adaptors of differing sequence. They were mixed in equimolar amounts prior to ligation to the cDNA to produce a mixture of cDNAs with heterologous ends.

Cloning the Human *CDC28* Homolog by Complementation of a *cdc28* Mutation in *S. cerevisiae*. A human cDNA library in λYES-R, the construction of which will be described later, was used to infect BNN132 and plasmid DNA was prepared as described in *Materials and Methods*. This library DNA was used to transform CMY478 (*cdc28-4*). Uracil prototophs were selected and grown to stationary phase; 2×10^6 independent transformants were obtained. Approximately 1×10^6 Ura^+ cells spread onto yeast extract/peptone/galactose plates were incubated at the nonpermissive temperature of 34°C for 5 days. Twenty colonies were retested at 34°C on media containing galactose and on media containing glucose. Three colonies that failed to grow on the glucose plates were chosen for further study. Plasmids recovered from these clones by transformation of *E. coli* were shown to contain the same 1.6-kb insert by restriction endonuclease mapping and hybridization analysis. The insert was subcloned in pICEM-19R+ in both orientations and sequenced from each end. Sequence analysis revealed it was identical to the human homolog of the *Schizosaccharomyces pombe CDC2* gene previously isolated by Lee and Nurse (6). Our cDNA was smaller than that obtained by Lee and Nurse (2 kb) and lacked a poly(A) tail, indicating that it was likely to have resulted from an internal priming event. This result demonstrates that the vector can function to isolate human genes by complementation of yeast mutations.

Cloning the *Arabidopsis thaliana* Homologs of the *trpD* and *leuB* Genes by Complementation of *E. coli* Mutants. A λYES-R cDNA library prepared with mRNA from *Arabidopsis* plants was used to complement a deletion of the *trpD* gene in *E. coli*. *trpD* was chosen because an auxotrophic mutant of *Arabidopsis thaliana* has been identified and shown to be defective in the production of anthranilate phosphoribosyltransferase, the enzyme encoded by *trpD* of *E. coli* (34). Complementation was performed as described in *Materials and Methods*. Ampicillin-resistant tryptophan-independent colonies arose at a frequency of 10^{-5}. Plasmids isolated from 10 clones allowed growth on anthranilic acid in place of tryptophan upon retransformation. All cDNA inserts cross-hybridized and were single copy in genomic Southern analysis (data not shown).

The same strategy was used to isolate cDNAs complementing the *leuB* mutation of *E. coli* strain AB1157. Complementing clones were isolated at frequency of approximately 1 in 250,000.

DISCUSSION

A series of multifunctional λ expression vectors, λYES, have been constructed with a number of features designed to facilitate the creation and use of large cDNA libraries. These vectors (*i*) have a biochemical selection for inserts using a fill-in adaptor strategy, which simplifies cDNA manipulation; (*ii*) are capable of regulated expression in yeast or *E. coli*, depending upon the orientation of the cDNA insert; (*iii*) are phasmids (13) and can exist as extrachromosomal lysogens in *E. coli*, facilitating complementation studies; and (*iv*) have an automatic excision system that allows easy conversion from a phage to a smaller plasmid. The backbone of the vector, λgt6, was designed for optimal growth and, as a result, the

λYES vectors are very healthy phage. This fact, coupled with the adaptor strategy employed for insert selection, may be responsible for the large libraries produced in this vector (10^7 to 10^8 recombinants per μg of vector and 10^8 to 10^9 recombinants per μg of cDNA). Replication of the libraries as phage facilitates standard hybridization and antibody screening (35). Although such standard physical methods as hybridization and antibody screening are practical for only a small fraction of the number of recombinants in these libraries, genetic selections are limited only by the number of clones that can be introduced into a given organism ($>10^{10}$ for λ vectors in *E. coli*; $>10^6$ for plasmid vectors in yeast (28, 36)].

The two promoters used in the expression vectors, the *E. coli lac* promoter and the yeast *GAL1* promoter, are both tightly regulated. This facilitates the identification of complementing clones in a background of revertants and may reduce the loss of "toxic" clones during propagation of the libraries. In *E. coli*, cDNAs can be expressed either as fusion proteins to the translational start site in the vector or from internal translational start sites. Translation from the vector ATG and ribosome binding site will usually allow more efficient expression of a cDNA-encoded protein, but translational fusions can reduce or abolish the activity of a protein. In one version of the vector, λYES-R, cDNAs are expressed in yeast as transcriptional fusions to the *GAL1* message. In λYES-P, the cDNAs are translationally fused to the portion of the *GAL1* gene encoding the first 29 amino acids. Translational fusions produce altered proteins, some of which may show novel or unregulated activities which can be selected.

The *cre–lox* automatic excision method is both simpler and more efficient than the coinfection of single-stranded helper phage and λ used in λZAP (37) and can be used to convert an entire library into plasmid DNA without further biasing due to packaging efficiency variation among different single-stranded phage. Most plasmids can be converted into an excisable phasmid by incorporation of the small fragment of DNA containing the *lox* sites flanking the *Not* I site and cloning in the λYES arms. These arms, 19.6 and 15.8 kb, total 35.4 kb in length. Since the maximum packaging capacity of λ is 51 kb, that leaves 15.6 kb to be divided between the plasmid and insert capacity. During the course of this work a similar excision system was developed by Palazzolo *et al.* (38). They found that, unlike pBR322, pUC plasmids that carry a *lox* site give poor yields of DNA in cre-producing strains, including BNN132 (M. J. Palazzolo and H. D. Lipshitz, personal communication).

That these vectors can be used to isolate cDNA clones from higher eukaryotes was demonstrated by complementation of mutations in yeast and *E. coli*. This approach is likely to provide a powerful method of isolating the basic genes which are common to many organisms. However, it is not limited to the isolation of genes for which homologous functions exist in *E. coli* or yeast. In principle, any gene can be isolated for which an artificial selection can be devised. For example, we have previously developed a general genetic selection method for isolation of genes encoding sequence-specific DNA-binding proteins that function in *E. coli*. We have recently developed a similar selection system that functions in yeast (S.J.E. and W. Harper, unpublished results). These methods, combined with creative selections and screens, will facilitate the isolation of many genes.

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