plate to allow the plaques corresponding to positive signals to be picked. The most reliable system we have used for this alignment involves labeling the filters and plates during adsorption by puncturing the filter at three points around its circumference with a hypodermic needle and injecting 0.1 to 0.2 μl of India ink into the agar at each point. The ink diffuses very little during the 2 days normally required for hybridization and autoradiography, and it allows absolute orientation of the filters, autoradiograms, and plates. From a plate with more than 1000 plaques, we normally pick about 20 plaques from the area of each positive signal for rescreening and plaque purification. Once pure clones are obtained, the sizes and restriction spectra of the inserted DNA's can be rapidly determined by the method of Cameron *et al.* (*16*).

The large capacity and speed of this method make practical, we believe, the isolation of eukaryotic unique genes by screening all the recombinants produced through shotgun cloning the entire genome. This should make possible isolation of a large number of genes for which physical or genetic purification schemes are not available.

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References and Notes

1. M. Thomas, R. L. White, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2294 (1976); B. E. Noyes and G. R. Stark, *Cell* **5**, 301 (1975); S. G. Clarkson, H. O. Smith, W. Schaffer, K. W. Gross, M. L. Birnstiel, *Nucleic Acids Res.* **3**, 2617 (1976); G. N. Yenikolopov, A. P. Ryskov, T. Nitta, G. P. Georgiev, *ibid.*, p. 2645; S. C. Hardies and R. D. Wells, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3117 (1976); R. Higuchi, G. V. Paddock, R. Wall, W. Salser, *ibid.*, p. 3146.
2. A shotgun experiment is defined (NIH guidelines) as one which involves the production of recombinant DNA's between the vector and total or partially purified DNA from a cellular source.
3. K. Struhl, J. R. Cameron, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1471 (1976).
4. L. Clarke and J. Carbon, *Cell* **9**, 91 (1976).
5. L. Kedes, A. Chang, D. Houseman, S. Cohen, *Nature (London)* **255**, 533 (1975); M. Grunstein and D. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
6. K. Jones and K. Murray, *J. Mol. Biol.* **96**, 455 (1975); Yu. V. Ilyin, N. A. Tchurikov, G. P. Georgiev, *Nucleic Acids Res.* **3**, 2115 (1976); B. Sanzey, O. Mercereau, T. Temynck, P. Kourilsky, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3394 (1976).
7. R. A. Kramer, J. R. Cameron, R. W. Davis, *Cell* **8**, 227 (1976).
8. B. M. Olivera and F. Bonhoeffer, *Nature (London)* **250**, 513 (1974).
9. This calculation is based on the worst case in which only 2000 plaques can be grown on each of 50 plates, the screening of which would require about five working hours over a 3-day period.
10. The usual host was *E. coli* C600 rk^- mk^+. Cells and phage are described by J. R. Cameron, S. M. Panasenko, I. R. Lehman, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3416 (1975).
11. The T plates contained 1 percent Bacto-tryptone, 0.5 percent NaCl, 1 percent agar, made to *p*H 7.5 with NaOH. The L plates contained 1 percent Bacto-tryptone, 0.5 percent Bacto yeast extract, 0.5 percent NaCl, 1.25 percent agar, made to *p*H 7.5 with NaOH. Seven percent agar was used for the T-soft and the L-soft agar.
12. Standard saline citrate phosphate buffer (SSCP) is (1×) 120 m*M* NaCl, 15 m*M* sodium citrate, 13 m*M* KH_2PO_4, 1 m*M* EDTA, titrated to *p*H 7.2 with NaOH.
13. In the model experiments described, the probe was total yeast ribosomal RNA (labeled with ^{32}P) prepared by the method of G. M. Rubin, *Methods Cell Biol.* **12**, 45 (1975).
14. λgt-Sc1109 (the gift of R. Kramer) is a recombinant λgt phage which carries two Eco RI fragments of *Saccharomyces cerevisiae* DNA, one of which carries the 5 · 8*S* and 26*S* rRNA genes. Approximately 5 percent of this phage's DNA is complementary to rRNA.
15. The λgt2 [λgti in Struhl *et al.* (*3*)] and λgt-λB were the gifts of M. Thomas.
16. J. R. Cameron, P. Philippsen, R. W. Davis, *Nucleic Acids Res.*, in press.
17. We thank T. St. John for helpful discussions and L. Horn for help in manuscript preparation. This work was supported by NSF grant PCM 76-02600 and NIH grant GM 021819-03. One of us (W.D.B.) was supported by NIH training grant 5T01-GM 01156 and by a fellowship from the Bush Foundation, St. Paul, Minn.
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Use of Phage Immunity in Molecular Cloning Experiments

Abstract. *Immunity to phage superinfection is a useful selective marker in molecular cloning experiments. Plasmids which have unique sites for several different restriction endonucleases and which specify immunity to bacteriophage* λ *are described.*

Antibiotic resistance genes carried on plasmids are useful as selective markers in molecular cloning experiments (*1*). We show here that phage λ immunity may be used as an alternative selective marker on plasmids that are carried in *Escherichia coli*. Plasmids bearing the *c*I (repressor) gene of bacteriophage λ direct the synthesis of λ repressor and render carrier cells immune to superinfection by λ (*2*).

In a model experiment, we have compared selection for immunity or antibiotic resistance, using a plasmid (pKB158) (*2*) that carries both the λ *c*I gene and a gene specifying tetracycline resistance (*tet*). Cells were transformed (*3*) with either no DNA, 1 μg of pKB158 DNA, 1 μg of pKB158 DNA digested with Eco RI, or 1 μg of pKB158 DNA digested with Eco RI and subsequently treated with T4 polynucleotide ligase. Transformants were selected on either tryptone plates plus 20 μg of tetracycline per milliliter or tryptone plates seeded with 10^9 λKH54h_λ and 10^9 λKH54h_{80} (*2*, *4*). The results are given in Table 1. Selection for λ immunity yielded about half as many transformants as did selection for tetracycline resistance. A small background of mucoid bacteria survived the selection for immunity even when no DNA was present in the transformation; however, all (36 of 36 tested) of the nonmucoid λ immune clones carried pKB158, as judged by the fact that they were tetracycline resistant. In summary, clones carrying plasmids which bear the λ *c*I gene are easily selected and distinguishable from the small background of mucoid bacteria which survive the selection procedure. A possible disadvantage of the use of phage immunity in molecular cloning experiments is that selection for immunity is not possible with phage-resistant strains (such as χ1776) and is not easily maintained during growth in liquid media with standard *E. coli* strains.

We briefly describe two well-charac-

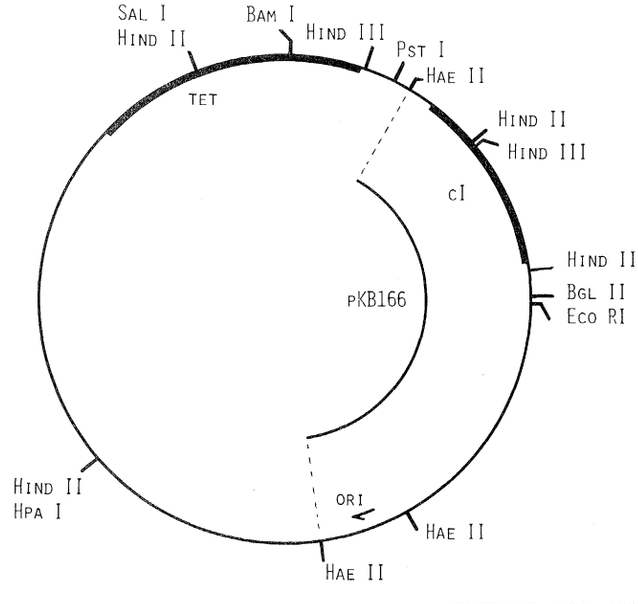

Fig. 1. Structure of pKB158. Map showing the locations of various restriction endonuclease cleavage sites on pKB158. Also shown are genes *c*I and *tet* (as heavy lines) and the Col E1 origin of replication *ori* (by an arrow). Only three of the more than ten Hae II sites are indicated. The material contained in pKB166 is indicated by an arc inside the map of pKB158. pKB158 has four Hind II sites, one of which is also recognized by Sal I and another of which is also recognized by Hpa I.

terized plasmids which carry the λ *c*I gene and which may be useful as cloning vectors. Plasmid pKB158, used in the experiments described above, contains unique cleavage sites for the restriction endonucleases Eco RI, Bgl II, Pst I, Bam I, Sal I, and Hpa I (see Fig. 1). Hpa I cleavage of pKB158 produces a flush-ended linear molecule, which may be used to clone flush-ended DNA fragments generated by a variety of restriction endonucleases (*5*). Joining of flush-ended DNA molecules to each other is mediated by high concentrations of T4 polynucleotide ligase (*2*).

We have prepared a smaller derivative of pKB158 which lacks the *tet* gene, in the following manner: pKB158 was digested with Bam I and Hpa I. The larger fragment produced (see Fig. 1) contained the λ *c*I gene and the Col E1 origin of replication. This fragment was partially digested with Hae II, diluted, and treated with T4 polynucleotide ligase. Cells were transformed with this DNA and λ immune transformants were selected as described above. About 5000 immune colonies were washed from their plates with tryptone broth, and a drop of this pool was used as an inoculum for a culture from which plasmid DNA was prepared. The plasmid DNA was separated by polyacrylamide gel electrophoresis and the smallest plasmid present on the gel was eluted and used to transform cells; this plasmid was named pKB166 (see Fig. 1).

Plasmid pKB166 contains only two Hae II fragments (2000 and 350 base pairs long), including the one reported to contain the Col E1 origin of replication (*6*). It is one of the smallest plasmids described to date, about 2400 base pairs long (1.6×10^6 daltons). Of those 2400 base pairs, 1100 are derived from the λ immunity region (*c*I and adjacent sequences) and 1300 are derived from Col E1. Since *E. coli* strains stably maintain pKB166 and are immune to colicin E1 when they harbor pKB166, the 1300 base pairs of pKB166 which are derived from Col E1 [corresponding to the region from 0.79 to 1.00 on the map in (*6*)] contain sufficient genetic information to direct replication and to express immunity to colicin E1.

pKB166 has unique recognition sites for Eco RI, Bgl II, and Hind III, and has only two recognition sites each for Hae II and Hind II. DNA fragments generated by Bam I or Mbo I as well as Bgl II may be cloned in the Bgl II site in pKB166, because all three endonucleases produce identical sticky ends. DNA fragments generated by Hae II may be cloned in partially digested pKB166. DNA fragments cloned in the Hind III or Hind II sites in pKB166 will destroy *c*I or its promoter and eliminate the expression of λ immunity. pKB166 is comparable with the so-called mini Col E1 plasmids (*7*) in that it is extremely small, can be amplified by treatment with chloramphenicol, determines two selectable phenotypes (immunity to phage λ and to colicin E1), and has several restriction endonuclease cleavage sites in which DNA fragments may be cloned.

Table 1. Selection of pKB158-bearing clones by λ immunity or by tetracycline resistance. Cells (5 to 6 × 10^7) from a transformed culture were spread on selective plates (described in the text) and incubated overnight at 37°C. The number of colonies which grew are tabulated.

DNA	Selection for	
	λ Immunity	Tetracycline resistance
None	6	0
pKB158	248	451
pKB158 + Eco RI	9	1
pKB158 + Eco RI + T4 ligase	28	51

The polypeptides encoded by pKB166 are few and with the exception of λ repressor are small. This we infer from experiments involving a plasmid (pKB266) which is nearly identical to pKB166, differing principally in that pKB266 has *lac* promoter fragments which transcribe *c*I [compare pKB158 and pKB252 in (*2*)]. A minicell-producing strain, χ1274 (*8*), was transformed with pKB266 and minicells were purified as described by Inselberg (*9*). The minicells were labeled with [^{35}S]methionine and extracts of the labeled minicells were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The principal (> 95 percent) ^{35}S-labeled polypeptide greater than 8000 daltons present in such extracts was λ repressor (molecular weight, 26,000).

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References and Notes

1. S. N. Cohen, A. C. Y. Chang, H. W. Boyer, R. Helling, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3240 (1973); C. Covey, D. Richardson, J. Carbon, *Mol. Gen. Genet.* **145**, 155 (1976); R. L. Rodriguez, F. Bolivar, H. Goodman, H. W. Boyer, M. Betlach, in *Molecular Mechanisms in Control of Gene Expression*, D. Nierlich, W. S. Rutter, C. F. Fox, Eds. (Academic Press, New York, 1976), p. 471; M. So, R. Gill, S. Falkow, *Mol. Gen. Genet.* **142**, 239 (1975).
2. K. Backman, M. Ptashne, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4174 (1976).
3. S. N. Cohen, A. C. Y. Chang, L. Hsu, *ibid.* **69**, 2110 (1972).
4. Phage λKH54 [F. Blattner, M. Fiandt, K. K. Hass, P. A. Twose, W. Szybalski, *Virology* **62**, 458 (1974)] has a deletion in its *c*I gene. A deletion mutant is used to select λ immune clones because point mutants revert to *c*I$^+$ at a low frequency and produce lysogens which spuriously survive the selection. The use of two host ranges (h_λ and h_{80}) reduces the background by eliminating clones which are resistant to one of the host ranges employed.
5. K. Backman, unpublished results.
6. A. Oka and M. Takanami, *Nature (London)* **264**, 193 (1976).
7. V. Hershfield, H. W. Boyer, L. Chow, D. R. Helinski, *J. Bacteriol.* **126**, 447 (1976); H. Avni, P. E. Berg, A. Markovitz, *ibid.* **129**, 358 (1977).
8. H. I. Adler, W. D. Fisher, A. Cohen, A. A. Hardigree, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 321 (1967).
9. J. Inselberg, *J. Bacteriol.* **102**, 642 (1970).
10. We wish to thank M. Ptashne, in whose laboratory these experiments were performed, for support and helpful discussions.

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Hybridization in situ of SV40 Plaques: Detection of Recombinant SV40 Virus Carrying Specific Sequences of Nonviral DNA

Abstract. *The detection and recovery of SV40 genomes containing foreign DNA sequences can be facilitated, and the risk of accidental dispersal reduced, by in situ hybridization and radioautography.*

Specific nucleic acid sequences contained in the cells of bacterial colonies can be detected without prior isolation of the cell's DNA by in situ hybridization of radioactive nucleic acid probes (RNA or DNA) to imprints of the bacterial colonies immobilized on nitrocellulose disks (*1*). Similarly, λ phage plaques, formed on lawns of sensitive bacteria, can be screened for the presence of recombinant phages carrying specific DNA segments (*2*). Beside facilitating detection and isolation of bacterial or phage clones carrying recombinant DNA molecules, such methods decrease the risk of dispersion of these organisms by reducing the scale and simplifying the number of mechanical and chemical manipulations generally used to identify organisms carrying specific recombinant DNA molecules.

SV40 DNA has been used as a vector for cloning and propagating foreign DNA segments in animal cells (*3–5*). Where the desired recombinants do not possess a distinguishable phenotype, the recombinant virus genomes are detected by hybridization of the viral DNA isolated from cells infected with each of the putative recombinant virus stocks (*3–5*). But this procedure is cumbersome and time-consuming; furthermore, because the number of plaques that can be screened is limited, the ability to detect