Integration of eukaryotic genes for 5S RNA and histone proteins into a phage lambda receptor

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ABSTRACT

Highly purified HindIII restriction fragments of Xenopus laevis 5S DNA and of Psammechinus miliaris histone DNA have been covalently inserted into a derivative of phage λ. This phage, genetically constructed by Murray et al. (1), contains only a single target for HindIII in the CI gene. Viable hybrid molecules were detected as clear plaque-forming phage after transfection of E.coli, the vast majority of which were shown by hybridization to be recombinants of the desired type. The λSam7 mutation has been introduced into one hybrid phage containing histone DNA, thereby substantially increasing the yield of recombinant DNA.

INTRODUCTION

It is widely accepted that the isolation and amplification, by molecular cloning, of defined DNA fragments will rapidly enhance our understanding of chromosome structure and gene organization. This technique (reviewed in ref.1,2) is essential for the biochemical investigation of unique genes, but even for those repeated genes which can be isolated by classical centrifugation techniques (3-5), it provides individual repeat units in the amounts required for extensive analysis.

In this paper we describe the integration of highly purified 6 kilobase pair (kb) repeat units of the histone gene cluster of Psammechinus miliaris (6), and of the more variable sized repeat units of a 5S DNA fraction from Xenopus laevis, into a derivative of phage λ. In a later paper the cloning of initiator methionyl-tRNA genes from the same frog will be reported (7). For these experiments we have employed the λ598 phage vector genetically constructed by Murray et al. (1). This phage (λAb538 redB13 imm434) contains in the CI gene of the substituted imm434 region a single target for HindIII, a restriction enzyme.
known to cut both 5S DNA and histone gene clusters once per repeat unit (8, 6). The location of this HindIII site makes λ598 an appealing vector because viable recombinant DNA molecules yield easily identified clear plaques after ligation and transfection of E.coli, whereas merely rejoined vector molecules, which are still capable of producing limm repressor, give rise to turbid plaques (1). Moreover, this vector is capable of accepting DNA fragments with high efficiency, particularly if high ratios of donor to vector DNA are used in the ligase reactions. A minor disadvantage, that the DNA yield is only moderate, has been overcome by the introduction of the λSam7 mutation (9) into one of the hybrid phages carrying a histone DNA repeat unit.

MATERIALS AND METHODS

Phage and Bacterial Strains. The λ598 vector phage was the gift of K. & N.E. Murray, who also supplied E.coli 803 r− m− supII supIII Met and 803 r− m+ rec A− supII Met. E.coli C600 r− m− rec B C− Thi−Thr−Leu was obtained from Ph.Kourilsky, and E.coli lop−8 (λc1857 Sam7) from G. Weinstock.

Enzymes. Phage T4 polynucleotide ligase prepared by the method of Weiss et al. (10) was the gift of F. Rougeon. Restriction endonuclease HindIII was prepared by an adaptation of the procedure of DeFilippes (11) to be described elsewhere (7). One μl of enzyme digested 5 μg of vector DNA in 30 min at 37°C.

DNAs. PM2 DNA was the gift of T. Bickle. DNA was isolated from CsCl-purified λphage by phenol extraction (12).

Xenopus laevis 5S DNA was purified from erythrocyte DNA by three cycles of equilibrium centrifugation (Figure 1). Conditions for the first actinomycin-CsCl gradient were the same as those used for the isolation of tDNAmet1 (13). For the second gradient, pooled fractions shown by brackets in Figure 1a, together with similar fractions from 18 identical gradients, were mixed with an additional 0.7 mg of actinomycin C1. CsCl was added to N câ 1.3905 at 3°C in a total volume of 20ml, and the gradients
were centrifuged for 4 days at 35,000 rpm at 3°C in a Spinco Ti60 rotor. Fractions enriched for 5S DNA were located by hybridization and the actinomycin and CsCl removed (13). The DNA, in 10mM sodium tetraborate, was mixed with AgNO₃ to an Ag⁺: DNA-P₀₄ molar ratio of 0.27, and with Cs₂SO₄ to N_d = 1.3720 at 25°C in a total volume of 6 ml. Centrifugation was for 2 days at 42,000 rpm and 25°C in a Spinco Ti50 rotor. Ag⁺ and Cs₂SO₄ were removed (13) and the 5S DNA was concentrated by ethanol precipitation.

Psammechinus miliaris sperm DNA was 20-fold enriched for histone DNA sequences by actinomycin-CsCl gradient centrifugation (5). 12μg of this DNA was digested with HindIII and electrophoresed at 40V overnight in a 1.2% agarose slab gel (16x16x 0.5 cm). The 6 kb histone DNA band (6) was excised under long wave (366nm) light and syringed through a 26 gauge needle into 1 ml of 10mM sodium tetraborate. 1.63 gm CsCl was added and the suspension was left at room temperature for 3 hr with occasional shaking. Agarose was pelleted in a Spinco SW50.1 rotor at 45,000 rpm for 30 min at 20°C. The supernatant was adjusted to N_d = 1.4000 in a 1.5ml final volume, and the DNA banded by centrifugation for 24 hr at 45,000 rpm and 20°C in a Spinco SW50.1 rotor. 30 ng of strongly sheared ³²P-λ⁺DNA had been added to the agarose suspension, which helped the detection of the DNA band in this steep CsCl gradient. The DNA was dialyzed against 50mM Tris-HCl (pH 7.6), 0.25mM EDTA, concentrated versus Sephadex G25 to about 50μl, and dialyzed again.

RNAs. Uniformly labelled (³H-uridine) 5S RNA was isolated from X.laevis tissue culture kidney cells (14). Labelled 9S histone mRNA, and five subfractions each coding for one of the histone proteins H1, H2A, H2B, H3 and H4, were isolated from cleaving P.miliaris embryos (15).

Construction of recombinant DNAs. HindIII endonuclease reactions were done in 10 mM Tris-HCl (pH 7.6), 10mM MgCl₂, 50mM NaCl and 14mM mercaptoethanol. Before ligation the DNAs were heated at 70°C for 5 min to inactivate the endonuclease and to dissociate the λ cohesive ends. The 5S DNA ligation reactions (15μl) contained 820 ng of restricted λ vector DNA, 20-570 ng
of restricted 5S DNA, 50mM Tris-HCl (pH 7.6), 10mM MgCl$_2$, 10mM dithiothreitol, 0.07mM ATP, and 1 µl of a 1:100 dilution of T4 ligase. After 30 min at 14°C, EDTA was added to 25mM and samples were kept on ice until transfection. The histone DNA ligation reaction (20 µl) was similar except that it contained 320 ng of restricted vector DNA and an estimated 50 ng of restricted gel-purified 6 kb histone DNA.

**Calcium-dependent transfection.** Cells were prepared for transfection essentially as described by Mandel and Higa (16). E.coli C600 r$^{-}$m$^{-}$ rec B$^{-}$C$^{-}$ was grown in L broth at 37°C to 2 x 10$^8$ cells/ml, chilled, centrifuged and resuspended in 0.5 volume of 0.1M CaCl$_2$. After 15 min on ice, the cells were again centrifuged and resuspended in 0.1 volume of 0.1M CaCl$_2$ plus 0.05 volume of a solution containing 1 part SSC and 2 parts 0.1M CaCl$_2$. The cells, stored on ice, were competent for transfection for at least 90 min. The DNA samples (1 - 10 µl) were gently shaken with 0.15 ml of calcium-treated cells for 5 min at 37°C, mixed with 3 ml of soft agar containing 1mM MgSO$_4$ and overlayed on L-agar plates. Plates were incubated overnight at 37°C. These experiments, and the subsequent growth of recombinant phage, were done under "Low Risk" conditions as defined in the "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (17).

**Phage growth.** Clear plaques from transfection plates were picked into 1 ml of L-broth and replated on the C600 rec B$^{-}$C$^{-}$ host to obtain isolated plaques. The plaque-purified phages were propagated on the 803 rec A$^{-}$ strain either on plates or by growth in liquid culture. For the latter, cells were grown in L-broth at 37°C to 2 x 10$^8$ cells/ml and infected at a multiplicity of 1-3. When lysis was complete (2-3hr) chloroform was added and cell debris was removed by centrifugation at 12,000xg for 10 min. Phage were then pelleted by centrifugation at 45,000 xg for 1 hr, treated with 10 µg/ml DNase and RNase for 2 hr at room temperature, and finally purified by a CsCl equilibrium gradient (12) followed by a CsCl step gradient (18).
Introduction of the λSam7 mutation into a hybrid phage containing histone DNA. Phage λ c1857 Sam7 (3x10^9 pfu) and the hybrid phage λh22 (7 x 10^8 pfu) were mixed with 1 ml of E.coli 803 supIII cells which had been grown at 37°C in L-broth plus 0.5% maltose to 4 x 10^8 cells/ml. After 15 min adsorption at 41°C the infected cells were diluted 10^-5 in L-broth containing 1% glucose. After 30 min the temperature was lowered to 37°C and incubation was continued for a further 90 min. Chloroform was then added and 0.1 ml samples of a 10^-2 dilution of the lysate were plated on a background of 803 supIII cells. The plates were incubated overnight at 32°C. From these, 200 clear plaques were picked and analysed for the Sam7 phenotype as described in Results and Discussion.

Nomenclature. Recombinant phage are permanently recorded by terms showing the vector genotype, the origin and identity of the inserted DNA, and an isolation number that refers to a plaque-purified clone, for example λb538 redB113 imm^434 (X1 5S)-10 and λb538 redB113 imm^434 (Pm histone)-22. In this paper these are abbreviated to λ5S-10 and λh22 respectively. When the P.miliaris histone DNA fragment from the latter recombinant is cloned in the Sam7 version of the λ598 vector, the hybrid phage is referred to as λSam7h22.

RESULTS AND DISCUSSION

Isolation of 5S DNA from Xenopus laevis. A 5S DNA fraction from X.laevis was purified by equilibrium centrifugation in two kinds of gradients; three gradient steps were employed in all. In the first, actinomycin-CsCl (Figure 1a), 5S DNA was found in three regions of the gradient, the majority being on the heavy side of main band DNA and in the main band DNA itself. It is possible that these two 5S DNA fractions are identical and that the 5S RNA hybridization to main band DNA reflects trapping of the high density 5S DNA component due to the high DNA concentrations (70-100 µg/ml) used in these gradients. An additional, presumably different, 5S DNA fraction was found on the light side of bulk X.laevis DNA (Figure 1a) in a region where some tDNAs
Figure 1. Isolation and HindIII digestion of X. laevis DNA.

The three gradients are: (a) first, and (b) second actinomycin-CsCl, and (c) final Ag⁺/Cs₂SO₄. Samples of DNA from each fraction were denatured, immobilized on filters, and hybridized with an excess of ³H 5S RNA (-----) as described in ref.13 (-----) absorbance at 260 nm. The brackets indicate fractions pooled for further purification. (d) HindIII digest of 0.5μg of 5S DNA from Ag⁺/Cs₂SO₄ fractions 17-20; the same pattern was observed for fractions 27-29. (e) HindIII digest of 1.2μg of PM2 DNA; the fragment lengths in kb are electronmicroscope measurements (19). Electrophoresis was through 2% agarose tube gels (1 x 11 cm) at 12V for 15 hr; gels were stained with ethidium bromide and photographed as described in ref.13.
are located (13). For this report we have concerned ourselves with
the 5S DNA of high density in actinomycin-CsCl. This fraction was
further purified and conveniently concentrated by a second actino-
mycin-CsCl gradient (Figure 1b). A final centrifugation in a
Ag⁺/Cs₂SO₄ gradient yielded coincident profiles of DNA absorbance
and 5S RNA hybridization in two regions of the gradient (Figure
1c). Electrophoretic separation of complete HindIII digests of
DNA from both regions gave the pattern of ethidium fluorescence
shown in Figure 1d.

The average length of the 5S DNA fragments is about 0.7 kb
but the band width is much greater than that of viral DNA frag-
ments of defined length (Figure 1e), which suggests a variable
spacing between HindIII sites in the 5S DNA. In both respects
the 5S DNA appears to be identical to X.laevis oocyte-type 5S
DNA (8) previously isolated by a somewhat different procedure (4).
This has not been verified by sequence analysis, nor is it known
whether both 5S DNA regions of the final Ag⁺/Cs₂SO₄ gradient are
identical. The heavier region (shown by brackets in Figure 1c)
was used for the following experiments. Very little of this DNA
was resistant to HindIII or was converted to fragments whose
lengths differed very greatly from 0.7 kb (Figure 1d), thus indi-
cating a high degree of purity for this 5S DNA fraction.

Construction of hybrid phages containing X.laevis 5S DNA.
Fragments from a complete HindIII digest of 5S DNA were mixed
with HindIII-cleaved λ598 DNA and the single-stranded comple-
mentary ends were joined by T4 polynucleotide ligase. Since the
5S DNA was available in μg quantities, several ligase reactions
were done to determine the efficiency of hybrid phage formation
at various ratios of 5S DNA to λ598 DNA. The infectious recombi-
nant DNA molecules from each ligase reaction were putatively
identified by their ability to form clear plaques after trans-
fection of E.coli. Positive identification was then made by
hybridization of the phage DNA with labelled 5S RNA.

The transfection efficiency of intact λ598 DNA on the C600
rec B C^- host was usually about 500 turbid plaques per ng, but
occasionally was as much as 10-fold lower. In all cases, the
incidence of spontaneous clear plaque formation was 0.1% or less. Digestion of \( \lambda 598 \) DNA with HindIII reduced the transfection efficiency to about 1% while treatment of the digest with poly-nucleotide ligase restored infectivity to about 10% of the value for the intact DNA. The corresponding values were 10-fold lower on the \( 803 \text{ rec A}^- \) strain. In addition, it was often difficult to distinguish between clear and turbid plaques on the latter host. For these reasons the \( C600 \text{ rec B C}^- \) strain was routinely used for transfection and for plaque purification. The isolated clones were then propagated on \( 803 \text{ rec A}^- \) cells to minimize the chance of recombination.

Addition of increasing amounts of HindIII-cleaved 5S DNA to the ligase reactions had two effects: a gradual decline in transfection efficiency and a concomitant increase in the percentage of clear plaques formed (up to 18%, sample 4 of Table 1). Both effects are presumably due to competition of the 5S DNA fragments for the left and right arms of the vector DNA, thus producing not only infectious recombinant molecules but also a variety of noninfectious molecules amongst which would be, for example, hybrids of two identical vector arms and one or more 5S DNA fragment. At a very high 5S DNA input, both the infectivity and the incidence of clear plaque formation were reduced (compare samples 4 and 5, Table 1). The high concentration of ends would strongly favour the ligation of the 5S DNA fragments into concatemers (20) which, even if inserted, might have produced molecules too long to be packaged into virus particles. Alternatively, since concatemer formation in turn reduces the concentration of ends, any fragments with one damaged end would have increasingly inhibited the reaction. A third possibility, that the 5S DNA was first ligated into linear concatemers and then circularized, is unlikely because the ligase reactions were incubated for only 30 min (at 14°C).

**Identification of hybrid phages containing \( X.laevis \) 5S DNA.** Three clear plaques were chosen at random from the transfection plates of each ligase reaction that contained 5S DNA (samples 2-5, Table 1). After plaque purification, plate stocks were made
TABLE I. Efficiency of hybrid phage formation after ligation at various ratios of 5S DNA to λ598 DNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>5S DNA HindIII ends</th>
<th>λ598 DNA HindIII ends</th>
<th>Plaques/μg of λ598 DNA</th>
<th>Clear Plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 (no 5S DNA added)</td>
<td>&gt;5000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>4100</td>
<td>0.8%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>2800</td>
<td>6.6%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12.6</td>
<td>2500</td>
<td>18.1%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>38.0</td>
<td>1900</td>
<td>12.6%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>12.6 (no ligase added)</td>
<td>900</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations of HindIII ends were calculated assuming a length of 38.1 kb for λ598 DNA (82% of λ+ and an average length of 0.7 kb for X. laevis 5S DNA (Figure 1). The number of plaques/transfection plate varied from 55 (sample 6) to >350 (sample 1).

from each independent clone and the phage DNA recovered. When DNA samples from each were denatured, immobilized on nitrocellulose filters and hybridized with X. laevis 5H-5S RNA, 10 of the 12 clones showed positive hybridization (Table 2). Moreover, DNA from several clones showed very substantial hybridization, suggesting that they contain more than one 5S RNA gene. That this is due to the insertion of multiple fragments of 5S DNA, rather than the presence of multiple copies of 5S RNA genes on a single fragment, is indicated by the electrophoretic separation of HindIII digests of the 10 positive clones (Figure 2). Each clone contained two high molecular weight fragments, representing the left and right arms of the vector, and at least one extra fragment within the size range of 5S DNA repeat lengths. In some clones, for example λ5S-10, the number of bands detected in the gel was fewer than the number of 5S RNA genes detected by hybridization. However, densitometer tracings showed that in these cases some bands were present at twice molar amounts, and hence that two fragments of very similar or identical length had been inserted. These inserted concatemers represent in vitro ligation products rather than partial digestion products because the 5S DNA fragments were derived from a complete HindIII digest. The gel analyses and hybridization results have been correlated to estimate the number of 5S DNA fragments inserted into each hybrid.
As expected, the frequency of multiple insertions increased with higher ratios of 5S DNA to vector DNA. The \lambda598 vector, which is 82% of \lambda+ length (1), could theoretically accept about 12 5S DNA fragments. However, even at the highest ratio, no phage approached this number of inserts. Hence, damage to the HindIII cohesive ends, rather than insertion of very long concatemers, appears to be the more likely explanation for the reduced frequency of clear plaques found in this sample.

TABLE 2. Identification of hybrid phages containing 5S DNA fragments by RNA excess hybridization

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>(^3)H 5S RNA hybridized (cpm ± 15%)</th>
<th>Number of 5S DNA fragments/phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>\lambda598</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Ligase sample 2: \lambda5S-1</td>
<td>315</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>355</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Ligase sample 3: \lambda5S-4</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>705</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>340</td>
<td>1</td>
</tr>
<tr>
<td>Ligase sample 4: \lambda5S-7</td>
<td>335</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>320</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>375</td>
<td>1</td>
</tr>
<tr>
<td>Ligase sample 5: \lambda5S-10</td>
<td>1040</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>345</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>1375</td>
<td>4</td>
</tr>
</tbody>
</table>

Samples of 1 \(\mu\)g of DNA were denatured, immobilized on 13mm diameter nitrocellulose filters, hybridized with 0.6 \(\mu\)g/ml \(^3\)H 5S RNA (240,000 cpm/\(\mu\)g) for 2 hr in 6 x SSC/50% formamide at 61°C, washed and treated with RNase as described in ref.21. The values represent the average of three determinations. No machine background has been subtracted.

Construction and identification of hybrid phages containing Psammochinus miliaris 6 kb histone DNA repeat units. While histone coding sequences may be enriched some 130-fold by repeated actinomycin-CsCl gradient centrifugation (5), the 6 kb histone DNA band is readily detected by agarose gel electrophoresis.
Figure 2. Agarose gel electrophoresis of HindIII digests of DNA from hybrid phages containing X. laevis 5S DNA.

About 0.8 μg of DNA from each indicated clone was digested with HindIII (except λ55-10 DNA, of which only about 0.35 μg was inadvertently used). Slot (a) is an EcoRI digest of 0.6 μg of 029 DNA; the fragment lengths (22) are shown in kb. Electrophoresis was through a 1.5% agarose slab gel (16x16x0.3cm) at 70 V for 4 hr; the gel was stained and photographed as described in ref. 6.

After HindIII digestion of 20-fold purified material (6; see also slot f of Figure 3). Excision of this band, followed by CsCl gradient centrifugation, considerably enhances the purity of the DNA. A small amount of 6 kb DNA prepared in this way, estimated to be approximately 50ng, was incubated with 320ng of HindIII-cleaved λ598 DNA and T4 ligase, and the products were used for transfection. Despite the low input of histone DNA, the ratio of 6 kb DNA to λ598 DNA HindIII ends was estimated to be approximately 1:1 which, from the 5S DNA results (Table 1), led us to expect a low but significant percentage of clear plaques. In the
Figure 3. Analyses of HindIII digests of DNA from hybrid phages containing P. miliaris histone DNA.

About 0.5μg of DNA from each indicated clone was digested with HindIII. Slot f is a HindIII digest of 0.5μg of the 20-fold enriched DNA from which the 6 kb histone DNA band was recovered for ligation. Electrophoresis was through a 1% agarose slab gel (10 x 8 x 0.3cm) at 70V for 1 hr (6). DNA bands from each slot were denatured, transferred to nitrocellulose filters (23), and the strips hybridized with total 9S histone mRNA (15,000 cpm/20μl/cm² filter) and, in the case of λh22, with five individual (15) histone mRNAs (5,000 cpm/20μl/cm² filter) for 4 hr at 65°C in 2 x SSC, 0.1% SDS. The strips were washed with 2 x SSC, treated with RNase, dried and fluorographed as described in ref. 6.
event, the transfection efficiency was higher than in the 5S DNA experiments, at 12 pfu/ng of λ598 DNA, and 2% of the plaques were clear. A total of 72 clear plaques were obtained, all of which were plaque purified.

1 ml cultures of 40 of these clones were made, total DNA was extracted from each, and the DNA samples were hybridized with $^{3}$H-uridine labelled 9S histone mRNA from P.miliaris. Positive hybridization was found for 38 out of the 40. Five of the positive clones were analyzed further. Plate stocks were made from each and the phage DNA recovered. After HindIII digestion, each was shown by electrophoresis (Figure 3) to contain the λ598 vector arms and only a single 6 kb DNA fragment. Hybrid phage with two 6 kb inserts were not detected, presumably because such recombinant molecules (108% of λ length) would be too long to be packaged. In addition, their formation would not be favoured by the low ratio of 6 kb DNA to λ598 DNA used in the ligase reaction. When DNA from the gel shown in Figure 3 was denatured and transferred to nitrocellulose strips by the Southern technique (23), the subsequent 9S histone mRNA hybridization was solely confined to the 6 kb DNA band from each clone. Moreover, the 6 kb DNA band from one clone examined, λh22, hybridized to each of five individual, highly purified mRNAs coding for the histone proteins H4, H2A, H2B, H3 and H1 (Figure 3). By these criteria, the cloned histone DNA appears to be representative of the cellular histone DNA previously characterized (6).

Construction of a high yielding hybrid phage containing P.miliaris histone DNA. λ+ phage when grown in wild type E.coli hosts usually produces lysates with titres of 2-3x10$^{10}$ pfu/ml, corresponding to a burst size of about 100. The hybrid phages just described carry a red$^{-}$ mutation and they have been grown on rec$^{-}$ hosts to reduce the frequency of recombinational events in the inserted DNA. Each of these mutations lowers phage yield by a factor of 2-3. Consequently we have obtained lysate titres of 2-5 x 10$^{9}$ pfu/ml and yields of only 100-250μg of recombinant DNA per litre.
The Sam7 mutation in phage λ both inhibits host lysis and permits the intracellular accumulation of as many as 1000 phage per cell (9). To improve yields this mutation has been crossed into a hybrid phage carrying a fragment of histone DNA, λh22, as shown diagrammatically in Figure 4.

![Diagram of phage phage λ and hybrid λSam7h22 with HindIII sites](image)

**Figure 4.** Construction of λSam7h22 (λh22 redB113 imm434 Sam7 (Pm histone)-22) HindIII sites (1, 12) are shown by arrows for the parent phages (a) λh22 and (b) c1857 Sam7. Of 200 phage that formed clear plaques on a supIII+ host, 10% failed to lyse supIII− cells and therefore contained both the histone DNA insert and Sam7. Type I recombinants contain HindIII site 6, whereas recombinants of type II lack this site (depicted by an X) which was eliminated in the original λ598 vector by genetic exchange between λ and Φ80 in the region of the Q gene (1,12).

Recombinants that contained the histone DNA insert and Sam7 formed clear plaques on supIII+ cells but would not plate on supIII− cells. These recombinants were of two types (Figure 4). For ease of recovery of the inserted histone DNA it is desirable that HindIII site 6 be absent. Hence, DNA from several of the recombinants was screened by gel electrophoresis after HindIII digestion. Of the six examined, one lacked site 6. This phage, referred to as λSam7h22, yields 2.5-4 mg of recombinant DNA per litre.

2630
The Sam7 version of the λ598 vector was recovered from λSam7h22 by digestion with HindIII, followed by ligation and transfection. Numerous turbid plaques were obtained on the supIII⁺ host, representing the λ598 Sam7 vector from which the histone DNA had been excised. One has been selected for further use. In addition to its value for new cloning experiments, DNA fragments previously cloned in λ598 can be transferred to the Sam7 version by HindIII cleavage of the respective DNAs, followed by ligation, transfection, and a selection for clear plaques that plate only on supIII⁺ cells.

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