Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene

(*Drosophila melanogaster* DNA/recombinant DNA molecules/plasmids/18–28S rRNA genes/autoradiography)

MICHAEL GRUNSTEIN* AND DAVID S. HOGNESS†

Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Communicated by A. D. Kaiser, August 4, 1975

ABSTRACT   A method has been developed whereby a very large number of colonies of *Escherichia coli* carrying different hybrid plasmids can be rapidly screened to determine which hybrid plasmids contain a specified DNA sequence or genes. The colonies to be screened are formed on nitrocellulose filters, and, after a reference set of these colonies has been prepared by replica plating, are lysed and their DNA is denatured and fixed to the filter *in situ*. The resulting DNA-prints of the colonies are then hybridized to a radioactive RNA that defines the sequence or gene of interest, and the result of this hybridization is assayed by autoradiography. Colonies whose DNA-prints exhibit hybridization can then be picked from the reference plate. We have used this method to isolate clones of ColE1 hybrid plasmids that contain *Drosophila melanogaster* genes for 18 and 28S rRNAs. In principle, the method can be used to isolate any gene whose base sequence is represented in an available RNA.

Segments of DNA from *Drosophila melanogaster* chromosomes (Dm segments) can be isolated by cloning hybrid DNA molecules that consist of a Dm segment inserted into the circular DNA of an *Escherichia coli* plasmid. We have previously reported on the use of such cloned segments in the analysis of DNA sequence arrangements in the *D. melanogaster* genome (1–3). However, that analysis has been limited by our inability to isolate cloned Dm segments that contain a specified DNA sequence or gene. In this article we describe a procedure that permits the isolation of such specific Dm segments, and which can be extended to DNA segments from any organism.

Experimental Plan. Consider an experiment in which the Dm segments in a random set are individually inserted into a given *E. coli* plasmid. Transformation of *E. coli* by these hybrid plasmids to a phenotype conferred by genes in the parental plasmid will yield colonies that individually contain a single cloned Dm segment (1–3). If these segments are randomly distributed and exhibit a mean length of 10,000 base pairs, or 10 kb, then we expect that about one colony in 16,000 will contain a particular nonrepetitive *D. melanogaster* DNA sequence the length of a typical structural gene, i.e., 1–2 kb. Hence, the goal is to devise a screening procedure whereby one can rapidly determine which colony in thousands contains such a sequence.

The screening procedure that we have developed is designed to detect sequences that can hybridize with a given

radioactive RNA. In this procedure the colonies to be screened are first grown on nitrocellulose filters that have been placed on the surface of agar petri plates prior to inoculation. A reference set of these colonies is then obtained by replica plating (4) to additional agar plates that are stored at 2–4°C. The colonies on the filter are lysed and their DNAs are denatured and fixed to the filter *in situ* to form a "DNA-print" of each colony. The defining, labeled RNA is hybridized to this DNA and the result of the hybridization is monitored by autoradiography on x-ray film. The colony whose DNA-print exhibits hybridization with the defining RNA can then be picked from the reference set.

The characteristics of this procedure and its application to the isolation of hybrid plasmids containing the *D. melanogaster* genes for '18' and '28'S rRNAs are described in this paper.

MATERIALS AND METHODS

Bacteria. *E. coli* K12 strains HB101, HB101 [pDm103], and C600 [pSC101] are those used previously (plasmids are indicated in brackets) (3). Strain W3110 has been described (5), and W3110 [ColE1] was obtained from D. R. Helinski.

DNAs, Complementary RNAs (cRNAs), and Enzymes. pDm103 (3) and ColE1 (6) DNAs were generously provided by D. M. Glover and D. J. Finnegan, respectively, and were prepared from HB101 [pDm103] and W3110 [ColE1] according to the indicated references, except that the ColE1 was amplified by overnight incubation of W3110 [ColE1] in the presence of chloramphenicol (7) prior to lysis. ^{32}P- and ^{3}H-labeled cRNAs were transcribed *in vitro* from these DNAs with *E. coli* RNA polymerase (8), as described by Wensink *et al.* (1). The RNA polymerase was prepared according to the indicated reference, and was the generous gift of W. Wickner. Pancreatic ribonuclease and proteinase K were obtained from Worthington Biochemical Corp. and E. Merck Laboratories, respectively.

Colony hybridization

Formation of the Filter and Reference Sets of Colonies. Colonies are formed on Millipore HA filters (0.45 μm pores) that have been washed three times in boiling H_2O (1 min per wash), placed between sheets of absorbant paper, autoclaved at 120° for 10 min, and dried for 10 min in the autoclave. The filter is then placed on an L-agar petri plate (1) and the desired bacteria are transferred to the filter surface either by spreading or using sterile toothpicks to obtain ≤7 colonies per cm² after incubation of the filter-plate at 37°. The reference set is produced by replica plating of the colonies that develop on the filter to L-agar plates and is stored at 2–4°.

Abbreviations: kb (kilobases), 1000 bases or base pairs in single- or double-stranded nucleic acids, respectively; Dm, a segment of *Drosophila melanogaster* DNA; cDm and pDm, hybrid plasmids consisting of a Dm segment inserted into ColE1 and pSC101 DNAs, respectively; SSC = 0.15 M NaCl, 0.015 M sodium citrate; cRNA, RNA complementary to DNA; rDNA, DNA coding for ribosomal RNA.

* Present address: Molecular Biology Institute and Department of Biology, University of California, Los Angeles, Calif. 90024.
† To whom reprint requests should be sent.

FIG. 1. Apparatus for treatment of colonies on filters. To wet the underside of the filter, solutions are introduced through ports (a) or (b), while the tube connected to the vacuum port is clamped off. Solutions are removed through the vacuum port which is connected to a water aspirator. Other procedures are described in the text. ", inches (2.54 cm); o.d., outside diameter; P.V.C., polyvinyl chloride.

Lysis, DNA Denaturation, and Fixation. To prevent movement of the bacteria or DNA from their colonial sites during lysis, denaturation and fixation, the solutions used to effect these reactions are applied to the underside of the filter and allowed to diffuse into the colony. The apparatus shown in Fig. 1 has been designed for this purpose. The filter is lifted from the agar plate and placed on the perforated disc that is set in a plastic cylinder which has ports cut into it to introduce solutions sequentially to the underside of the filter and to apply vacuum. Unless otherwise indicated, all operations are carried out at room temperature (20–25°).

Lysis and DNA denaturation are effected by introducing 0.5 N NaOH beneath the filter until it barely floats. After 7 min the NaOH is slowly removed with a minimum of vacuum, and replaced by 1.0 M Tris·HCl (pH 7.4) for 1 min. This solution is replaced with the same buffer, after which the pH of the solution in contact with the filter should be approximately neutral. The last wash is replaced by 1.5 M NaCl, 0.5 M Tris·HCl (pH 7.4), which is removed after 5 min. The stainless steel collar is then placed over the filter, and full vacuum is applied for approximately 2 min until the colonial residues assume a dry appearance. At this point there is less danger of movement from the colonial site and the remaining solutions can be layered on the upper side of the filter.

A 2 mg/ml solution of proteinase K in 1 × SSC (0.15 M NaCl, 0.015 M sodium citrate) is added to just cover the filter. After 15 min, it is removed by vacuum filtration, and 95% ethanol (1 ml/cm² of filter) is similarly passed through the filter. After five washes effected by passing chloroform through the filter (2 ml/cm² per wash), the filter is removed from the apparatus, dipped into 0.3 M NaCl to remove loose cellular debris, and baked at 80° *in vacuo* for 2 hr.

Hybridization and ³²P-Autoradiography or ³H-Fluorography. The dry filter is moistened with a 5 × SSC, 50% formamide solution containing the labeled RNA, using 10–15 μl/cm² of filter. The filter is covered with mineral oil, incubated for 16 hr at 37° to allow hybridization, and then washed for 10 min in a beaker containing chloroform that is gently agitated on a shaking platform. Two more identical chloroform washes are followed by 10 min washes in 6 × SSC, 2 × SSC, and 2 × SSC containing 20 μg/ml of pancreatic ribonuclease. If the RNA is ³²P-labeled, the filter is blotted to remove excess liquid, covered with Saran Wrap, and placed under Kodak RPS/54 x-ray film for autoradiography. If the RNA is ³H-labeled, the filter is dried for 30 min at 80° *in vacuo*, and 40 μl of 7% 2,5-diphenyloxazole (PPO) in ether is applied per cm² of filter. The dry filter is then placed under x-ray film for fluorography at −82° (9).

RESULTS

Colony hybridization distinguishes between [ColE1]⁺ and [ColE1]⁻ bacteria

We have turned increasingly toward the use of the colicinogenic plasmid, ColE1, as a cloning vector because one can obtain much higher cellular concentrations of its hybrids (7) than is the case for the tetracycline resistance plasmid, pSC101, which we used previously (1–3). The first test system for colony hybridization therefore consisted of ³²P-labeled cRNA made by transcription of ColE1 DNA *in vitro* with *E. coli* RNA polymerase, and *E. coli* containing or not containing ColE1, i.e., [ColE1]⁺ or [ColE1]⁻ bacteria.

Fig. 2A shows the autoradiographic response obtained after hybridization of [³²P]cRNA to the DNA-prints of [ColE1]⁺ and [ColE1]⁻ colonies formed on nitrocellulose fil-

Biochemistry: Grunstein and Hogness

Proc. Nat. Acad. Sci. USA 72 (1975) 3963
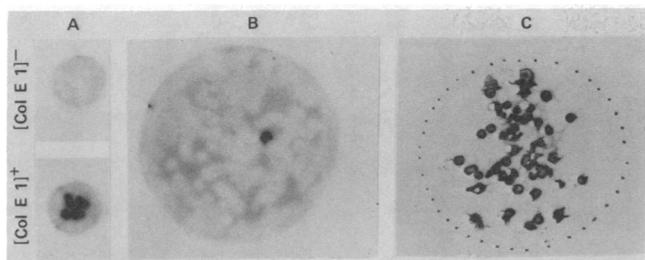
FIG. 2. Hybridization of ColE1 cRNA to [ColE1]⁻ and [ColE1]⁺ colonies. The procedures for colony hybridization, autoradiography, and fluorography are described in *Materials and Methods*, as are the W3310 and W3110 [ColE1] *E. coli* strains used to form the [ColE1]⁻ and [ColE1]⁺ colonies, respectively. (A) 1×10^5 cpm of [^{32}P]cRNA (5×10^7 cpm/μg) were applied to each 13-mm filter (area $= 1.3$ cm^2) in a 20 μl volume. After hybridization, the DNA-prints of [ColE1]⁺ colonies contained an average of 1.8×10^2 cpm per colony, which is 30-fold greater than the background radiation from an equivalent area on the filter. Exposure time = 45 min. (B) A mixture of [ColE1]⁺ and [ColE1]⁻ bacteria in a 1:100 ratio was spread on a 47-mm filter (area = 17.3 cm^2) to obtain a total of 1 to 2×10^2 colonies per filter; 5×10^5 cpm of [^{32}P]cRNA (3×10^7 cpm/μg) in 250 μl were applied to the filter. Exposure time = 4 hr. (C) A 1:1 mixture of [ColE1]⁺ and [ColE1]⁻ bacteria was spread on a 47-mm filter to obtain a total of 93 colonies, of which 52 gave the A⁺ response seen in the figure; 1×10^6 cpm of [^{3}H]cRNA (2×10^7 cpm/μg) in 200 μl were applied to the filter. Exposure time = 24 hr.

ters. The positive response given by the [ColE1]⁺ colonies is abbreviated by A⁺ and the negative response of [ColE1]⁻ colonies by A⁻. Colonies obtained by spreading mixtures of [ColE1]⁺ and [ColE1]⁻ bacteria in different ratios gave the expected frequencies of A⁺ and A⁻ responses. Fig. 2B shows the result obtained when [ColE1]⁺/[ColE1]⁻ = 1/100.

A more precise measure of the specificity of colony hybridization of mixtures is given by the following experiment in which a 1:1 mixture of [ColE1]⁺ and [ColE1]⁻ bacteria was spread on a filter to yield 31 colonies. Hybridization and autoradiography revealed that 16 were A⁺ and 15 A⁻. Bac-
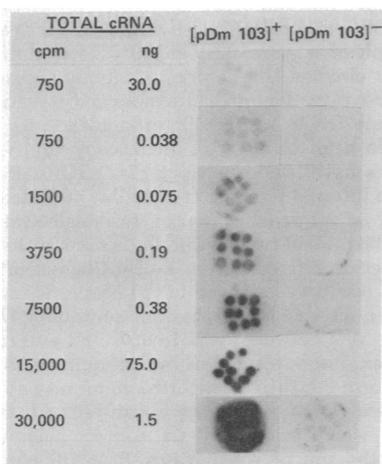
FIG. 3. Hybridization of different amounts of pDm103 [^{32}P]cRNA to [pDm103]⁺ and [pDm103]⁻ colonies. Colonies were obtained by transferring HB101 [pDm103] or HB101 bacteria, respectively, to 13-mm filters with toothpicks. In the experiments where ≤1.5 ng of cRNA were applied per filter, the specific activity = 2×10^7 cpm/μg. The lower specific activities used for the other two experiments were obtained by mixing this cRNA with unlabeled pDm103 cRNA. The weak response observed for [pDm103]⁻ colonies could result either from *E. coli* DNA impurities in the pDm103 DNA preparations used to prepare the [^{32}P]cRNA, or from some similarity of sequence in pDm103 and *E. coli* DNAs.

teria from each of the corresponding colonies on the agar replica plate were then tested for colicin production according to an overlay technique described by Finnegan and Willets (10). All 16 A⁺ colonies were colicin-positive (i.e., [ColE1]⁺); all 15 A⁻ colonies were colicin-negative and therefore presumed to be [ColE1]⁻.

Fig. 2A and B show that the position of A⁻ colonies can be detected on the autoradiograph because of the higher background radiation from the filter itself. While this background radiation is convenient for the direct visualization of A⁻ colonies and is not critical to the observation of the A⁺ response obtained with cRNAs, it may become an important factor with other RNAs if they give a weaker A⁺ response. Our observations indicate that the level of this background varies with the preparation of labeled RNA and, possibly, with the batch of filters, but we have not examined such factors in detail.

Fig. 2C shows that the colony hybridization procedure can be adapted to ^{3}H-labeled cRNA by impregnating the filter with 2,5-diphenyloxazole after hybridization and prior to placement on the x-ray film (*Materials and Methods*). Of the 93 colonies obtained by spreading a 1:1 mixture of [ColE1]⁺ and [ColE1]⁻ bacteria, 52 were A⁺ and 41 A⁻. We estimate from the extent of the A⁺ response that this ^{3}H-fluorography is about one-twentieth as efficient as the ^{32}P-autoradiography.

The autoradiographic response is proportional to the total radioactivity of the applied cRNA and insensitive to its specific activity

We next examined the dependence of the A⁺ response on the total and the specific radioactivity of the applied cRNA. In this case, the ^{32}P-labeled cRNA was transcribed *in vitro* from a hybrid plasmid called pDm103, and hybridized to DNA-prints of colonies that either contained this hybrid, [pDm103]⁺, or did not, [pDm103]⁻. The pDm103 hybrid was formed between pSC101 plasmid DNA (9 kb) and a segment of *D. melanogaster* DNA (Dm103; 17 kb) that contains the gene for '18' and '28'S rRNAs (3).

Fig. 3 shows that the autoradiographic response obtained when pDm103 [^{32}P]cDNA was hybridized to 13-mm filters containing [pDm103]⁺ colonies is roughly proportional to the total radioactivity. It is clearly insensitive to the mass of cRNA containing that radioactivity, i.e., to its specific activity. For example, the response to 750 cpm of [^{32}P]cRNA is approximately the same whether contained in 0.038 ng or in 30 ng. Similarly the response to 15,000 cpm contained in 75 ng is intermediate between that to 7,500 cpm and 30,000 cpm, although the last two samples contained only 0.38 and 1.5 ng, respectively. This would suggest that the RNA·DNA hybridization is occurring under conditions of DNA excess even when 75 ng of pDm103 cRNA are applied per 13 mm filter. However, we have calculated that there is only some 2 ng of pDm103 DNA per colony [i.e., (2×10^7 cells per colony) $\times$ (4 pDm103 per cell) 2.9×10^{-8} ng DNA per pDm103]. This value is based on our observation of 2×10^7 cells per 1 mm colony and the presence of 4 pDm103 per cell in liquid culture (3). Evidently only a small fraction of the applied cRNA can react with the DNA-prints on the filter even though the reaction is occurring ostensibly in DNA excess. A similar result was observed when ColE1 cRNA was hybridized to [ColE1]⁺ colonies (legend, Fig 2A). Of 2 ng cRNA applied to each filter only 0.004 ng (i.e., 0.2%) hybridized per [ColE1]⁺ colony. A 1 mm [ColE1]⁺ colony is estimated to contain 3–4 ng of ColE1 DNA.

A simple explanation of these results is obtained if one as-
sumes that most or all of the cRNA in the small fraction of
the RNA solution which wets a DNA-print will hybridize,
and that the remainder of the cRNA will not hybridize at a
significant rate, due perhaps to its slow diffusion through the
nitrocellulose, or because of other barriers. Thus a DNA-
print from a 1-mm colony, which occupies 0.6% of the area
of a 13-mm filter, would be expected to hybridize ≤0.6% of
the applied RNA, an expectation that is compatible with the
0.2% observed. For a given ratio of colony to filter area, the
fraction of applied cRNA that hybridizes to a DNA-print, in
conditions of local DNA excess, would therefore be constant
and independent of the total applied cRNA over a wide
range of values.

Colony hybridization with cRNA to pDm103 provides a screen for cDm plasmids containing *D. melanogaster* rDNA

Hybrid plasmids consisting of a Dm segment inserted into
ColE1 DNA are called cDm plasmids, as distinguished from
pDm plasmids where the Dm segment has been inserted
into pSC101. In this section we describe two applications of
colony hybridization that result in the isolation of cDm plas-
mids that contain DNA from the repeating gene-spacer units
for '18–28'S rRNAs (i.e., rDNA) in *D. melanogaster* (3). In
the first application, [32P]cRNA to pDm103 was used to iso-
late clones of cDm103 plasmids; i.e., plasmids in which the
Dm103 segment is inserted into ColE1 DNA at its single
*Eco*RI endonuclease cleavage site (7). In the second applica-
tion, the same [32P]cRNA was used to screen a large set of
random cDm clones for rDNA. cRNA formed by transcrip-
tion of the entire pDm103 DNA can be used for these pur-
poses since we have demonstrated that pSC101 and ColE1
sequences do not interact to give a significant A⁺ response
(data not shown).

Cleavage of circular pDm103 DNA with the *Eco*RI re-
striction endonuclease yields intact Dm103 segments and
linear pSC101 DNA (3). In cooperation with D. M. Glover,
we treated a mixture of *Eco*RI-cleaved pDm103 and ColE1
DNAs with *E. coli* ligase under previously described condi-
tions (3), and then transformed colicin-sensitive *E. coli* to
colicin E1 immunity with this mixture of ligated DNAs (11).
Since the *Eco*RI termini of the linear ColE1, pSC101, and
Dm103 molecules can be randomly joined by the ligase, any
of the following circular products of this ligation may be
present in the colonies of transformants: (*i*) recyclized ColE1
(monomers, dimers, etc), (*ii*) molecules containing one
ColE1 and one pSC101 segment [abbreviated by (c)₁(p)₁],
(*iii*) (c)₁(Dm103)₁ molecules, i.e., the desired cDm103 plas-
mids, or (*iv*) rarer more complex combinations, such as
(c)₁(p)₁(Dm103)₁, which contain one or more copies of
ColE1.

Forty-eight of the transformants were screened for the
presence of either pSC101 or Dm103 segments by colony
hybridization with [32P]cRNA to pDm103 (Fig. 4A), and for
the presence of the pSC101 segment by testing for resistance
to tetracycline. Of the eight A⁺ transformants shown in Fig.
4A, six were tetracycline resistant and probably contain
(c)₁(p)₁ plasmids. They were not examined further. The re-
maining two (indicated by 1 and 2 in Fig. 4A) were tetracy-
cline sensitive, and were assumed to contain cDm103 plas-
mids; they were designated cDm103/1 and cDm103/2, re-
spectively.

Proof of this assumption was obtained by electron micro-
scopic examination of the plasmids isolated from the two
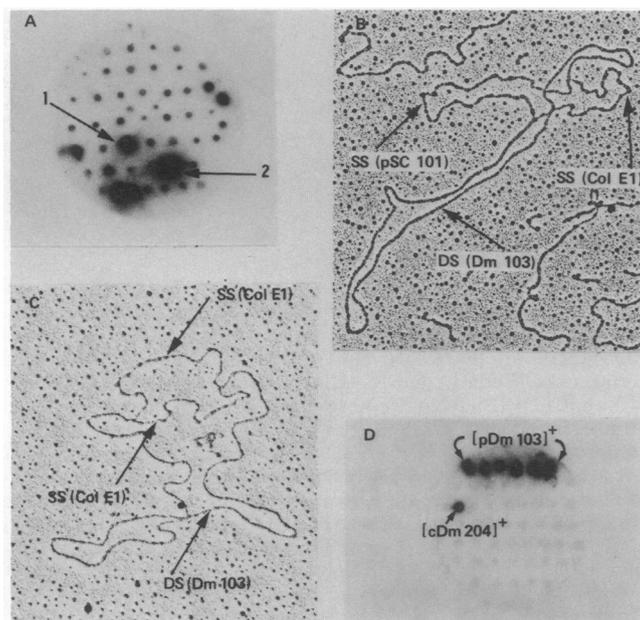
FIG. 4. (A) The screen for cDm103 hybrids. 5 μg of pDm103
DNA and 0.25 μg of ColE1 DNA were cleaved to completion with
*Eco*RI endonuclease (in 0.120 ml of 0.1 M Tris·HCl, pH 7.5, 0.01 M
MgSO₄), heated for 5 min at 65° to inactivate the enzyme and
brought to 4°. The DNAs were then incubated at 14° with DNA li-
gase (14 μg/ml) in 0.1 M Tris·HCl, pH 7.5, as well as a reaction
buffer consisting of 0.1 mM DPN, 1 mM EDTA, 10 mM
(NH₄)₂SO₄, 10 mM MgSO₄ with 100 μg/ml of bovine serum albu-
min for 120 min in a total volume of 0.140 ml. The solution was
then diluted 3-fold with the same reaction buffer and incubated
for 36 hr at 14° in the presence of ligase (10 μg/ml). The ligated
mixture of *Eco*RI-cleaved pDm103 and ColE1 DNAs (see *text*)
was used to transform HB101 to colicin E1 immunity as described
previously (11). Each of 48 transformants were transfered by
toothpick to a 47-mm filter for colony hybridization (*Materials
and Methods*), and to L-agar plates containing 15 μg of tetracy-
cline per ml. 5 × 10⁵ cpm of pDm103 [32P]cRNA (2 × 10⁷ cpm/μg)
were used for the colony hybridization, which after a 6-hr exposure
yielded the above autoradiograph. The colonies marked 1 and 2
contain cDm103/1 and cDm103/2 hybrids, respectively. (B) Elec-
tron micrograph of a pDm103·cDm103/2 heteroduplex. pDm103
and cDm103/2 circular DNAs were randomly nicked (broken in
one strand) by x-rays. The procedures for denaturation and rena-
turation of these DNAs to form heteroduplexes, for spreading in
40% formamide prior to electron microscopy, and for measuring
contour lengths have been described (1). pSC101 (9.2 kb; ref. 1)
was used as an internal reference for double-stranded lengths (DS
in the figure); no reference was used for single-stranded lengths
(SS), as only the ratio of two SS-lengths is used in the analysis (see
text). (C) Electron micrograph of a cDm103/1·cDm103/2 hetero-
duplex. The procedures are given in (B) above. See *text* for expla-
nation. (D) The screen for cDm hybrids containing *D. melanogas-
ter* rDNA. Hybrids between *Eco*RI-cut ColE1 and randomly bro-
ken Dm segments were formed as indicated in the *text*, and then
used to transform HB101 to colicin E1 immunity as in (A) above.
300 independent transformants were transferred to six 47-mm fil-
ters, each of which contained six control colonies of HB101
[pDm103] at the top of the pattern. 5 × 10⁵ cpm of pDm103
[32P]cRNA (2 × 10⁷ cpm/μg) was applied per filter for the colony
hybridization. The autoradiograph in the figure resulted from one
of the six filters after a 5-hr exposure, and shows one of the five
rDNA hybrids (cDm204) identified by this screening procedure.

transformants, and of heteroduplexes formed between
pDm103 and cDm103/2, and between cDm103/1 and
cDm103/2. The mean lengths ±SD (*n* = 18) of cDm103/1
and cDm103/2 are 23.0 (±1.2) kb and 21.7 (±1.5) kb, re-
spectively. The sum of the lengths of Dm103 (17 kb) and

ColE1 (6 kb; ref. 7) is 23 kb, in reasonable agreement with these values.

A heteroduplex formed between pDml03 and cDml03/2 is shown in Fig. 4B. It consists of a 17 kb double-stranded element whose ends are connected by each of two single-stranded elements that exhibit a length ratio of 1.5. This is the structure expected if cDml03/2 consists of a Dml03 segment inserted at the EcoRI cleavage site of ColE1; i.e., the double-stranded element represents the paired Dml03 segments of the two plasmid strands, and the larger and smaller single-stranded elements represent the pSC101 and ColE1 segments respectively (expected length ratio = 9 kb/6 kb = 1.5).

The heteroduplex formed between cDml03/1 and cDml03/2 consists of a 17 kb duplex whose ends are connected by two single-stranded elements of equal length (Fig. 4C). The simplest explanation of this structure is that the Dml03 segments were oppositely inserted into ColE1 during formation of cDml03/1 and cDml03/2. If the Dml03 segments in the single strands of two such oppositely oriented plasmids pair to create a 17 kb duplex element, then the two single-stranded ColE1 segments would contain identical rather than complementary base sequences, and could not pair.

The last experiment consists in screening hundreds of different [cDm]+ colonies for rDNA. The [cDm]+ colonies were obtained by transformation of colicin-sensitive E. coli to immunity with a heterogeneous population of cDm molecules constructed from EcoRI-cleaved ColE1 and random Dm segments (obtained by shear breakage) by the poly(dA)·poly(dT) joining method (1). These transformants were provided by D. J. Finnegan and G. Rubin. They were individually transferred by toothpick to six 47-mm nitrocellulose filters, each filter containing about 50 independent transformants. Colony hybridization with pDml03 [^{32}P]cRNA indicated no A+ colonies on three filters, 1 A+ colony on two filters, and 3 A+ colonies on one filter. The autoradiograph of one of the two filters containing a single A+ colony, cDm204, is given in Fig. 4D (the top row of A+ colonies on the filter are [pDml03]+ controls). When each of the 5 A+ colonies was retested by repeating this colony hybridization on subclones, such subclones were consistently A+.

Since pSC101 and ColE1 sequences do not interact to give an A+ response, we presume that the cDm plasmids in these 5 A+ colonies contain sequences present in Dml03; i.e., they contain rDNA from D. melanogaster. Indeed, D. M. Glover and R. L. White (personal communication) have shown recently that the 28 kb Dm segment in cDm204 contains the same arrangement of '18'-'28'S and spacer sequences as is found in Dml03.

DISCUSSION

In principle, colony hybridization of cloned hybrid plasmids can be used to isolate any gene, or other DNA segment, whose base sequence is represented in an available RNA. We used cRNA to pDml03 for the isolation of cDm plasmids containing rDNA. However, as we have observed that [pDml03]+ colonies give an adequate A+ response with ^{3}H-

labeled '18' plus '28'S rRNAs isolated from D. melanogaster cell cultures (3), the isolation could have been accomplished with these rRNAs. For rRNA the genes are repeated hundreds of times per genome, and this is the reason that we were able to isolate several hybrids containing rDNA by screening only a few hundred colonies.

By contrast, we calculate that it would be necessary to screen approximately 50,000 hybrid clones to have a 95% chance of finding a hybrid containing a nonrepeated structural gene of typical length from D. melanogaster. From the data given in Fig. 3 and assuming 24-hr exposures, we estimate that this would require a total of approximately 4 × 10^6 cpm of [^{32}P]mRNA (specific activity ≥ 4 × 10^5 cpm/μg) applied to about one hundred thirty-five 82-mm filters. Thus a screen of this size is quite feasible. The isolation of nonrepeated genes from larger genomes would, of course, proportionately increase the number of colonies to be screened and hence the total required radioactivity.

An important advantage of colony hybridization is that it facilitates containment of any potentially hazardous hybrid plasmids that may be cloned in such large screening operations. By confining the reproductive state of the hybrid-clones to colonies, the probability of escape is reduced over that for liquid cultures because the number of bacteria per clone is generally smaller and aerosols or accidental spills are less likely. Furthermore the screening operation can be confined to small, controllable areas.

1. Wensink, P. C., Finnegan, D. J., Donelson, J. E. & Hogness, D. S. (1974) Cell 3, 315–325.
2. Hogness, D. S., Wensink, P. C., Glover, D. M., White, R. L., Finnegan, D. J. & Donelson, J. E. (1975) in The Eukaryote Chromosome, eds. Peacock, W. J. & Brock, R. D. (Australian National University Press, Canberra), in press.
3. Glover, D. M., White, R. L., Finnegan, D. J. & Hogness, D. S. (1975) Cell 5, 149–157.
4. Hayes, W. (1965) in The Genetics of Bacteria and Their Viruses (John Wiley & Sons, New York), pp. 185–188.
5. Lederberg, E. M. (1960) Symp. Soc. Gen. Microbiol. 10, 115–131.
6. Katz, L., Kingsbury, D. K. & Helinski, D. R. (1973) J. Bacteriol. 114, 577–591.
7. Hershfield, V., Boyer, H. W., Yanofsky, C., Levett, M. A. & Helinski, D. R. (1974) Proc. Nat. Acad. Sci. USA 71, 3455–3459.
8. Berg, D., Barrett, K. & Chamberlin, M. (1971) in Methods in Enzymology, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol XXI, pp. 506–519.
9. Randerath, k. (1970) Anal. Biochem. 34, 188–205.
10. Finnegan, D. J. & Willetts (1972) Mol. Gen. Genet. 119, 57–66.
11. Glover, D. (1975) in New Techniques in Biophysics and Cell Biology, eds. Pain, R. & Smith, B. (John Wiley & Sons, New York), in press.