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Plasmid Detection and Sizing in Single Colony Lysates

Abstract. *A simple and contained procedure for the rapid assay of the presence and size of plasmids similar to Col E1 is described. Bacteria are picked from an agar plate with a toothpick, lysed with dodecyl sulfate and heat, and placed directly on an agarose gel for electrophoresis.*

Bacteria often contain extrachromosomal genetic elements in the form of small circles of DNA called plasmids. One of the two classes of DNA cloning vehicle used in genetic engineering is the plasmid; the other class is phage or virus. The most useful plasmids for DNA cloning are those with the Col E1 replicon, since these plasmids are present in a relatively high number of copies (20 to 100) per cell, and they can be further amplified (increased in number) in the presence of chloramphenicol until they constitute 45 percent of the cellular DNA (*1*–*3*). In manipulating DNA on plasmids it is often desirable or necessary to determine the size of the plasmids. Potentially dangerous plasmids should not be cultured or manipulated more than necessary while their properties are being determined. I have devised a procedure which can determine the presence and size of the Col E1 class of DNA plasmids by analyzing a small amount of bacteria which are picked from an agar plate, and then immediately killed and lysed by heating with detergent.

Bacteria containing various plasmids were grown as small (0.5 cm^2) streaks on an agar plate with any suitable growth medium (legend to Fig. 1). About 1 mm^3 of cells was picked up from each streak or large colony with the flat end of a small toothpick and suspended in 0.2 ml of electrophoresis buffer (0.16*M* tris-acetate, *p*H 8.3, 0.08*M* sodium acetate, 8 m*M* Na_2EDTA) by mixing with a Vortex stirrer. One drop (30 to 50 μl) of lysis mixture (5 percent sodium dodecyl sulfate, 50 percent glycerol, 0.1*M* Na_2EDTA, 0.2 percent xylene cyanol) was then added with mixing. After heating at 70°C for 10 minutes, the lysate was sheared slightly by taking it up in a drawn capillary, and 40 μl was then placed directly from the capillary into the well of a 1 percent agarose slab gel. Electrophoresis was carried out slowly (1 to 2 volt/cm) for 9 hours; after this time the bromphenol blue dye in a control slot had migrated 5 cm (*4*). Agarose gels were run in the horizontal conformation described by Shinnick *et al.* (*5*). Two parallel rows of 15 samples could be conveniently analyzed on a slab gel (20 by 20 cm). High-quality photographs were necessary for visualization of the plasmid DNA bands (see legend to Fig. 1).

An example toothpick assay is shown in Fig. 1. Several classes of stable nucleic acid are partially resolved on the gel, including transfer RNA (tRNA) and 16*S* and 23*S* ribosomal RNA (rRNA). The RNA bands were overexposed in order to visualize the faint plasmid bands. Host chromosomal DNA just entered the gel and fluorescing material of unknown composition remained at the origin. The plasmids migrated as faint bands between the rRNA and bacterial DNA.

Plasmids with the Col E1 replicon (mini E1, Col E1, and pMB9) have 20 to 30 copies per cell (*1*) and can be readily visualized in this crude assay. Plasmid pSC201 [pSC101 with a temperature-sensitive replicon (*6*)], with one to two copies per cell (*7*) can just be visualized at the limit of detection with this technique, but not reproducibly. It is not visible in Fig. 1. Plasmids pWB91, -2, -3, and -4 are mini E1 plasmids carrying a portion of the histidine operon from *Salmonella typhimurium* (*8*). They are reproducibly more easily detected than the parent plasmid in this assay. Evidently their replication is in some way activated (*8*).

The predominant plasmid band for each sample (except the lysed Col E1 strain) is the supercoiled form of the plasmid. In some of the toothpick assays, and in the slots heavily loaded with purified DNA, the nicked (relaxed) form of the DNA circles can be seen as a band with less mobility than the supercoils. The distance traveled by the supercoils (measured from Fig. 1) is proportional to the log of their size (*9*) at least over the range 3,300 to 18,000 base pairs.

The toothpick lysate of a Col E1–carrying strain which is thy^- shows a background which may be due to DNA dam-

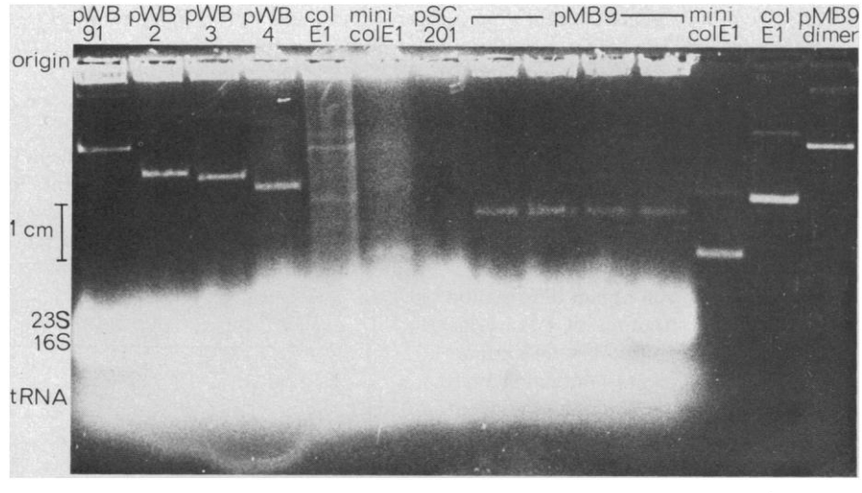

Fig. 1. Sample toothpick assays. The lysis and assay were performed as described. After electrophoresis, the gel was stained by soaking in ethidium bromide (0.5 μg/ml) and then placed on an exposed (black) sheet of x-ray film as a photographic background. The gel was photographed under shortwave ultraviolet illumination through an orange filter and with Tri-X 35-mm film. Polaroid type 57 (ASA 3000) film was not suitable. The *Escherichia coli* background for mini Col E1, pWB91, pWB2, pWB3, and pWB4 was TA2043 (*sm^r*, *his-gnd* deletion) (*11*), the background for Col E1 was JC411 (thy^-) (*12*), and pMB9 (*13*) was in W3110 (*14*). Bacteria were grown in rich agar with added thymidine. Tetracycline (20 μg/ml) was added to the medium for pMB9 and pSC201. In the right-hand three samples there were 50 ng of purified plasmid DNA prepared as described (*15*).

age. This DNA damage is not seen in a thy$^+$ background, but the isogenic experiment has not been done. This DNA damage is reflected in the distribution of plasmid forms. The predominant plasmid band is the linear form, which has a mobility between that of the supercoil and nicked circle forms.

This procedure is a contained way of measuring plasmid size, since no large volumes or aerosols need be risked by preparing significant amounts of DNA. If care is taken that all bacteria contact the lysing sodium dodecyl sulfate, all cells are killed in the immediate heat step—that is, there were no detectable survivors out of 10^9 cells (*10*). Telford *et al.* (*10a*) have independently developed a similar screening procedure.

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9. Plasmid sizes used were taken from the literature assuming 1510 base pairs per 10^6 daltons. For example, Col E1, 6300 base pairs (*1*); mini Col E1, 3300 base pairs (*1–3*); pMB9, 5300 base pairs (*13*).
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Isolation of Eukaryotic DNA Fragments Containing Structural Genes and the Adjacent Sequences

Abstract. *In* Drosophila melanogaster *structural genes are located close to moderately reiterared sequences. One of the clones obtained contains the DNA related to intercalary heterochromatin of* D. melanogaster. *These are individual differences in the distribution of genetic material in polytenic chromosomes of different stocks of* D. melanogaster. *The techniques that allow isolation of DNA fragments containing structural genes at the beginning, in the middle, or the end of the coding strand have been elaborated.*

One of the main areas where the genetic engineering (*1–3*) could be studied is the investigation of the organization of genetic material in eukaryotic cells. Working only with amplified homogeneous DNA, one can obtain information concerning the structure of eukaryotic genes. For this purpose, it is very important to have fragments containing both the structural gene and the adjacent DNA sequences.

Two general strategies could be used to isolate such structures. First is the amplification of random DNA fragments in "shotgun" experiments (cloning with unselected fragments from the total genome) followed by detection of the interesting clones by hybridization with mRNA. The second includes the preliminary enrichment of DNA used for amplification with the fragments containing structural genes.

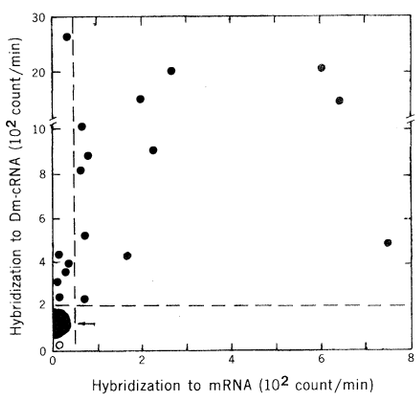

Fig. 1 Hybridization of poly(A)$^+$ mRNA and Dm cRNA with DNA samples obtained from 62 clones. The arrow indicates the position of 44 points. (•) DNA of clones; (○) *E. coli* DNA. The vertical dotted line separates clones, whose DNA does not hybridize to mRNA; the horizontal dotted line cuts the clones containing only unique DNA sequences.

The first approach was used in studies on the *Drosophila melanogaster* genome both by Hogness and co-workers (*3, 4*) and by ourselves (*5*).

For amplification of the Eco RI fragment of *D. melanogaster* DNA, λgt–λC bacteriophage (*1*) was used. The C fragment was replaced by *Drosophila* DNA and, after transfection of *Escherichia coli* strain 802 rk$^-$mk$^+$ with this recombinant DNA, a number of clones were obtained.

The DNA was isolated from groups containing ten clones each and those groups containing DNA hybridizable with messenger RNA (mRNA) of *Drosophila* were selected. Then each clone of the group was studied separately. In this way seven clones were obtained, the DNA of which efficiently hybridized to mRNA prepared from cultured *Drosophila* cells. In other words, these clones contained structural genes that were expressed in the cultured cells. Among them, three clones (NN 118m, 225, and 234) bind the highest percentage of mRNA (about 0.1 percent).

Except in the hybridization with mRNA, the DNA of clones was tested for hybridization with complementary RNA (cRNA) transcribed from total DNA of *D. melanogaster* (Dm cRNA). It was shown in separate experiments that cRNA was transcribed more or less randomly from the whole DNA. Therefore only the clone containing DNA sequences represented in the genome in many copies could bind a significant amount of cRNA. In all cases, when DNA from the clone hybridized with mRNA, it also hybridized with Dm cRNA (see Fig. 1). Even low binding of mRNA always coexists with the increased binding of Dm cRNA. The size of DNA fragments of *Drosophila* in the clones containing structural genes varied from 2 to 4 million daltons, whereas the average size of structural gene seemed to be of about 1.5×10^6. Thus, at least in many cases, the structural gene in *D. melanogaster* may be closely linked with the repetitive DNA sequences. Another interpretation of the above results is that in all cases the structural genes themselves are repetitive. To check these two possibilities we performed experiments with one of the clones (λgt–Dm 225). The DNA prepared from it was hybridized with either mRNA or Dm cRNA in the presence of an excess of unlabeled mRNA. The addition of a competitor sig-