Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3919–3923, May 1990
Genetics

Enhanced resolution of DNA restriction fragments: A procedure by two-dimensional electrophoresis and double-labeling

MING YI, LO-CHUN AU*, NORIO ICHIKAWA, AND PAUL O. P. TS'O†

Division of Biophysics, The Johns Hopkins University, School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205

Communicated by Ronald W. Estabrook, February 21, 1990 (received for review December 5, 1989)

ABSTRACT **A probe-free method was developed to detect DNA rearrangement in bacteria based on the electrophoretic separation of twice-digested restriction fragments of genomic DNA into a two-dimensional (2-D) pattern. The first restriction enzyme digestion was done in solution, followed by electrophoresis of the restriction fragments in one dimension. A second restriction enzyme digestion was carried out *in situ* in the gel, followed by electrophoresis in a second dimension perpendicular to the first electrophoresis. The 2-D pattern provides for the resolution of 300–400 spots, which are defined and indexed by an "*x,y*" coordinate system with size markers. This approach has greatly increased the resolution power over conventional one-dimensional (1-D) electrophoresis. To study DNA rearrangement, a 2-D pattern from a test strain was compared with the 2-D pattern from a reference strain. After the first digestion, genomic DNA fragments from the test strain were labeled with ^{35}S, while those from the reference strain were labeled with ^{35}P. This was done to utilize the difference in the energy emission of ^{35}S and ^{32}P isotopes for autoradiography when two x-ray films were exposed simultaneously on top of the gel after the 2-D electrophoresis. The irradiation from the decay of ^{35}S exposed only the lower film, whereas the irradiation from the decay of ^{32}P exposed both the lower and upper films. Different DNA fragments existed in the test DNA compared with the reference DNA can be identified unambiguously by the differential two 2-D patterns produced on two films upon exposure to the ^{35}S and ^{32}P fragments in the same gel. An appropriate photographic procedure further simplified the process, allowing only the difference in DNA fragments between these two patterns to be shown in the map. We have utilized the difference map obtained from *Escherichia coli* strains HB101 and HB101 (λ) genomic DNA to show the incorporation of one copy of phage λ DNA without the use of a λ DNA probe. This is the same test system that was used previously.**

Advances in the science and technology of restriction enzymes afforded an unique approach to a sequence-specific fragmentation of genomic DNA. Much has been learned about the genome based on studies of DNA restriction fragments. To have a systematic description of the complete organization of the genome, a prerequisite for the nucleotide sequencing of the entire genome, a new approach is needed to resolve and to identify all of the restriction fragments obtained from a genome. The recent success in providing the complete restriction map of the entire *Escherichia coli* genome is an outstanding example of such an investigation (1).

One obvious approach is to develop a two-dimensional (2-D) pattern for resolving all of the restriction fragments; a 2-D pattern certainly will have a higher resolution capability than current one-dimensional (1-D) electrophoretic separation. Several attempts have been made in this direction utilizing phage λ DNA incorporation into the bacterial genome as a model system for investigation (2–4). In this paper, an approach has been developed based on the electrophoretic separation of the twice-digested restriction fragments of genomic DNA into a 2-D pattern. The 2-D pattern provides a resolution of 300–400 spots that are defined by an "*x,y*" coordinate system with size markers. This is the first aspect of this communication.

The second aspect of this paper concerns the monitoring of genome rearrangement without using a probe. How to monitor unknown genome rearrangement is still a great technical challenge even though the possible importance of genomic DNA rearrangement in adaption, development, and carcinogenesis has been generally recognized. Common genome rearrangement includes deletion, insertion, translocation, switching, and amplification. Unprogrammed rearrangement and programmed rearrangement are two categories in this process (5).

An effective method has been developed recently in our laboratory (2) for monitoring the difference between the genomic DNA of two strains of *E. coli* [*E. coli* HB101 and *E. coli* HB101 (λ)]. In this method, a mixture of labeled DNA restriction fragments of the test strain [HB101 (λ)] and unlabeled DNA restriction fragments from the reference strain (HB101) was electrophoresed in one dimension. The DNA fragments after electrophoresis were alkali-denatured, followed by hybridization *in situ* in the gel. The labeled rearranged DNA fragments from the test cells rehybridized much more slowly since they had no counterpart in the driver DNA from the reference strain, which was 100- to 1000-fold in excess. In the single-stranded conformation, the rearranged but unreannealed DNA fragments moved faster in the second-dimension electrophoresis (perpendicular to the first dimension) and were revealed after autoradiography.

In this paper, we have developed another approach for monitoring a DNA rearrangement in *E. coli*, which is again used as the model system for a genomic size of approximately 5 megabases. In this approach, the 2D pattern from the test strain (DNA fragments labeled with ^{35}S) has been compared with the 2-D pattern from the reference strain (DNA fragments labeled with ^{32}P). Based on the difference in the radiation energy of the β particles emitted during the decay of the two isotopes, the appropriate x-ray film exposure arrangement, and a negative–positive photographic technique, a difference map was constructed that exhibits only the DNA fragments that are different in the two 2-D patterns. The application and the future direction of this approach is also discussed.

MATERIALS AND METHODS

Bacteria Strains. *E. coli* HB101 (*recA*, *hsdR*^{b}, *hsdM*^{b}) is a hybrid strain of *E. coli* K-12 × *E. coli* B (6). *E. coli* HB101

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: 1-D and 2-D, one- and two-dimensional.

*Present Address: Department of Medical Research, Veterans General Hospital, Taipei, Taiwan, Republic of China.
†To whom reprint requests should be sent.

(λ), a lysogenic strain of HB101, was picked up from a single-cell colony that originally came from a turbid plaque caused by the infection of HB101 with λ phage. These strains were used and described previously from our laboratory (2).

Extraction of High Molecular Weight DNA. The procedure was identical to that published earlier (2).

Restriction Enzyme Digestion. High molecular weight bacterial DNA obtained as described (2) was digested with *Eco*RI (2.5 units/μg of DNA) in reaction buffer (100 mM Tris, pH 7.75/50 mM NaCl/10 mM $MgCl_2$) at 37°C overnight. The DNA solution was extracted with same volume of phenol once and the same volume of chloroform twice. One-ninth volume of 3 M sodium acetate (pH 7.0) was added to the extracted solution. The DNA fragments in the solution were precipitated by the addition of 2.5 volumes of 95% alcohol, and the solution was kept at −20°C overnight. The DNA precipitate was centrifuged, rinsed twice with 70% ethanol and once with 95% ethanol, and redissolved in TE buffer (10 mM Tris/1 mM EDTA, pH 8.0) after being dried.

DNA Labeling. The DNA fragments of the test cells and reference cells obtained after the first enzymic digestion were labeled by replacement synthesis. The DNA fragments of the test strain were labeled with [α-^{35}S]dCTP (1000–1500 Ci/mmol; NEN DuPont, Wilmington, DE; 1 Ci = 37 GBq) with T4 DNA polymerase (Bethesda Research Laboratories), according to a published procedure (7). The reaction mixture (22.5 μl) contained 1 μg of DNA, 33 mM Tris acetate (pH 7.9), 66 mM potassium acetate, 10 mM magnesium acetate, 0.1 mg of bovine serum albumin (BSA) per ml, and 0.625 units of T4 DNA polymerase. After a 25-min exonuclease reaction at 37°C, 13.2 pmol of [α-^{35}S]dCTP and 1 μl each of 2 mM dATP, dGTP, and dTTP were added to the mixture. The duration for resynthesis was 60 min at 37°C. The DNA fragments of reference strain were labeled with [α-^{32}P]dCTP (400 Ci/mmol, DuPont/NEN) by using the same enzyme and protocol.

The labeled DNA fragments were purified by column chromatography using a Nensorb 20 cartridge (NEN) according to the supplier's manual.

Procedure for Detecting Genome Rearrangement. ^{35}S-labeled DNA fragments (2 μg; approximately 1.9×10^7 cpm) from the test strain were mixed with 0.1 μg of ^{32}P-labeled DNA fragments (approximately 5.2×10^5 cpm) from the reference strain. The mixture was loaded onto a horizontal 25 × 20 × 0.46 cm slab gel containing 1% ultrapure agarose (Bethesda Research Laboratories). The sample well was 0.6 cm wide and 1.0 mm thick. Electrophoresis was performed at room temperature in TPE buffer (0.08 M Tris phosphate/0.008 M EDTA, pH 7.7) at 53 V for 21 hr. After the first-dimension electrophoresis, the DNA-containing portion of the gel was cut into a 17 × 0.8 cm strip, and the DNA fragments were digested in the gel with *Bam*HI as the second restriction enzyme as follows: the gel strip was first equilibrated with *Bam*HI reaction buffer (0.1 M Tris, pH 7.7/0.1 M NaCl/10 mM $MgCl_2$) containing 250 μg of BSA per ml by shaking 1 hr at room temperature. The gel strip was put into a 20 × 1.0 cm tube (total volume, 15 ml) that contained *Bam*HI reaction buffer containing 250 μg of BSA and 133 units of *Bam*HI per ml. The tube was gently rotated at 37°C for 20 hr. After the *Bam*HI *in situ* digestion, the gel strip was equilibrated with 1× TPE buffer and then fused with a 1% agarose gel slab by adding melted agarose to fill up the gap. The second-dimensional electrophoresis, perpendicular to the first one, was carried out at 70 V and 4°C for 23 hr in 1× TPE buffer. The DNA slab gel was then dried in a gel dryer with a Whatman 3 MM paper underneath the gel. The

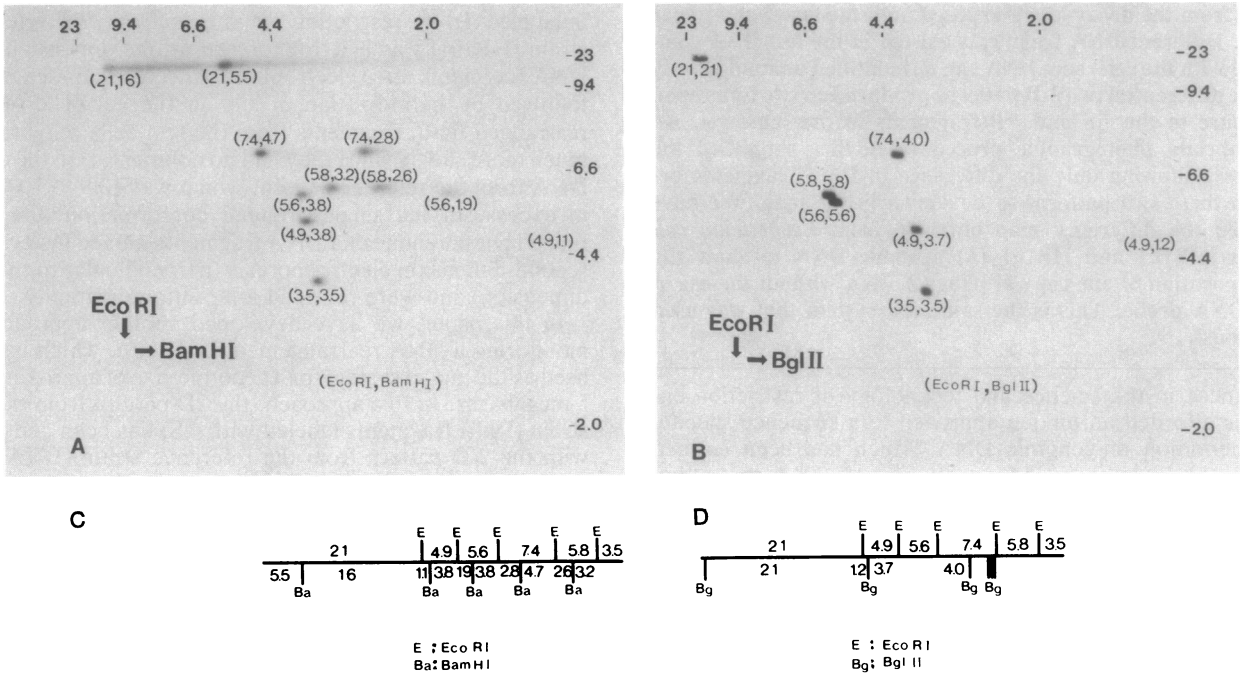

FIG. 1. 2-D electrophoresis mapping of restriction fragments of λ phage DNA digested by *Eco*RI/*Bam*HI (*A*) and by *Eco*RI/*Bgl* II (*B*). The mixture containing 0.048 μg of λ phage DNA *Eco*RI fragments labeled by [α-^{35}S]dCTP and 3.4 μg of unlabeled *E. coli* HB101 DNA *Eco*RI fragments (genomic ratio, 1:1) was electrophoresed in the first dimension at 53 V for 21 hr at room temperature. The *in situ* digestion was done in the gel with *Bam*HI (*A*) or with *Bgl* II (*B*). The second-dimension electrophoresis was carried out at 70 V for 23 hr at 4°C. The vertical downward arrows denote the first dimension, and the horizontal arrows denote the second dimension. (*C*) Restriction map of λ phage DNA obtained from *Eco*RI/*Bam*HI digestion. The "*x,y*" coordinates of each fragment in the 2-D map is given by their respective mobility in the 2-D electrophoresis map representing their molecular sizes. For example, fragment (8,5) denotes that this DNA moves with the same mobility of the 8-kb fragment in the first dimension and then moves with same mobility of the 5-kb fragment in the second dimension (See Table 1). The molecular size coordinates can be given to two figures of significance from the linear restriction map of λ phage DNA shown in *C* and *D*. The coordinates of these fragments are now shown in *A* and *B*.

Table 1. Comparison of coordinates of the DNA restriction fragments obtained from 2-D electrophoresis maps of restriction fragments of HB101 (λ) and from the linear restriction map of λ phage DNA

Enzyme combination	*x,y* coordinates in kb of restriction fragments	
	2-D map of HB101 (λ)	Linear restriction map of λ phage
*Eco*RI/*Bam*HI	8,5	HB101 (λ)*
	8,2	HB101 (λ),1.9
	7,5	7.4, 4.7
	7,3	7.4, 2.8
	6,3	5.8, 3.2
	6,3	5.8, 2.6
	5,4	HB101 (λ)*
	5,4	4.9, 3.8
*Eco*RI/*Bgl* II	7,4	7.4, 4.0
	5,4	4.9, 3.7
	5,1	4.9, 1.2

*The size of the fragment depends on the restriction enzymes cutting site on the bacterial portion of the HB101 (λ) DNA.

orientation of the gel was precisely marked by the addition of ^{32}P radioactivity to the four corners of the 3 MM paper. Autoradiography was carried out with two x-ray films (Kodak SB) on the top of the dried gel with a black paper between these two films. The film was covered with one piece of aluminum foil, and the whole assembly was put in a cassette. The films were exposed at −80°C for 2–3 days before development.

Photography. To cancel out the common (or shared) spots in the two films (the upper film exposed only to ^{32}P fragments and the lower film exposed to both the ^{32}P fragments and ^{35}S fragments), a negative–positive masking procedure was used. To prepare a negative from the lower autoradiogram (exposed to both ^{35}S and ^{32}P), a 1.5-sec room light exposure was given to a sandwich consisting (from top to bottom) of a plane of glass, the lower film, a Kodalith film (emulsion side up), and a piece of black paper. To prepare a positive of the upper autoradiogram (exposed only to ^{32}P), again a brief light exposure was given to a sandwich consisting (from top to bottom) of a plane of glass, the upper film, a LPD 7 film (emulsion side up), and a piece of a black paper. Then the negative film (Kodalith on top) and the positive film (LPD 7 at bottom) were taped together in a precise register to make an Ektapan negative. This negative, in turn, was used to make a print on regular photographic paper.

RESULTS

2-D electrophoretic mapping of the restriction fragments of λ phage DNA digested by a combination of *Eco*RI/*Bam*HI and by a combination of *Eco*RI/*Bgl* II are shown in Fig. 1 *A* and *B*, respectively. These maps are useful as guides to interpret the maps shown subsequently in Figs. 2 and 4. The linear restriction maps of λ DNA based on DNA sequence data are also shown in Fig. 1 *C* and *D* to provide an explanation for the coordinates in the experimental maps. We have identified all of these DNA fragments in 2-D electrophoretic mapping experiments in which λ DNA *Eco*RI fragments were mixed with the *E. coli* DNA *Eco*RI fragments (1:1 ratio) and then digested by *Bam*HI and *Bgl* II (data not shown).

All of the λ phage DNA *Eco*RI/*Bam*HI and *Eco*RI/*Bgl* II fragments can be denoted by a set of coordinates [in kilobase pair (kb) units]. The coordinate consists of the two molecular size parameters shown in the 2-D electrophoresis map (see the legend in Figs. 2 and 4). These coordinates, obtained experimentally, should be related to the molecular sizes predicted from the liner restriction maps of λ DNA after digestion with *Eco*RI/*Bam*HI (Fig. 1*C*) and *Eco*RI/*Bgl* II (Fig. 1*D*). The comparison between the values from experimental maps and those from linear restriction mapping based on sequence information is shown in Table 1. Unlabeled *E. coli Eco*RI fragments were added (genome ratio 1:1) in this set to create an identical experimental condition to the latter part of the experiment. It was found that the DNA fragments in the gel could be digested readily by restriction enzymes in the presence of BSA in the digestion buffer (8).

Detection of the incorporated λ phage DNA in the genome of HB101 (λ) after *Eco*RI/*Bam*HI digestion is shown in Fig. 2. The HB101 (λ) bacteria containing a single copy of λ phage DNA in its genome was used as the source of test DNA and was labeled by ^{35}S. The HB101 strain was used as the source of the reference DNA and was labeled by ^{32}P after first digestion. After first-dimension electrophoresis, the DNA fragments were digested by *Bam*HI *in situ*, and the DNA gel strip was electrophoresed in the second dimension perpendicular to the first electrophoresis. Autoradiography was

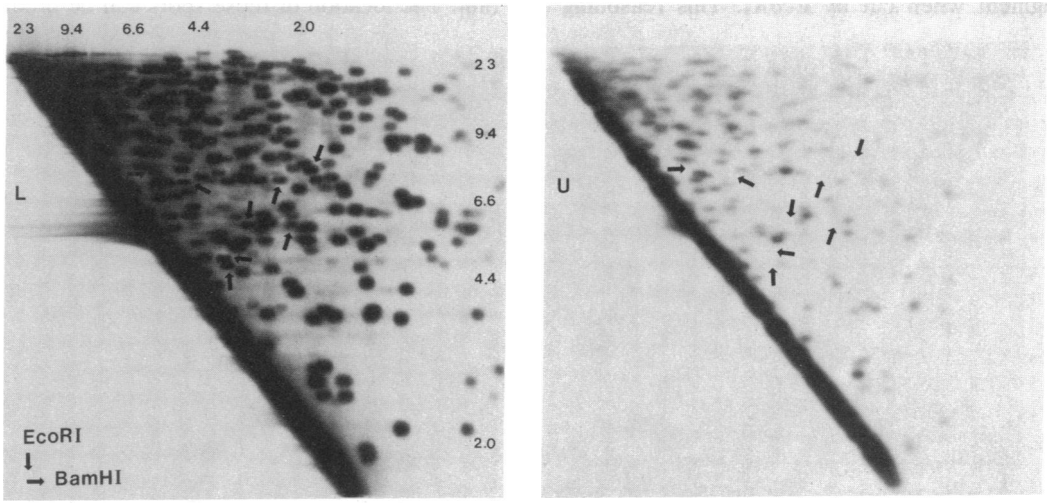

FIG. 2. Detection of the incorporation of λ phage DNA (single copy) in the strain of HB101 (λ) bacteria. The 2-D map of the DNA restriction fragments was obtained after *Eco*RI/*Bam*HI digestion with the test DNA containing 2 μg of HB101 (λ) *Eco*RI fragments labeled with [α-^{35}S]dCTP and with the reference DNA containing 0.1 μg of HB101 *Eco*RI fragments labeled with [α-^{32}P]dCTP. The exposure of the upper (U) and lower (L) films by the gel is described in *Materials and Methods*. (*Left*) Lower film shows the presence of eight ^{35}S fragments (8,5; 8,2; 7,5; 7,3; 6,3; 6,3; 5,4; and 5,4) as indicated by the arrows. (*Right*) Upper film shows the absence of the eight ^{35}S fragments.

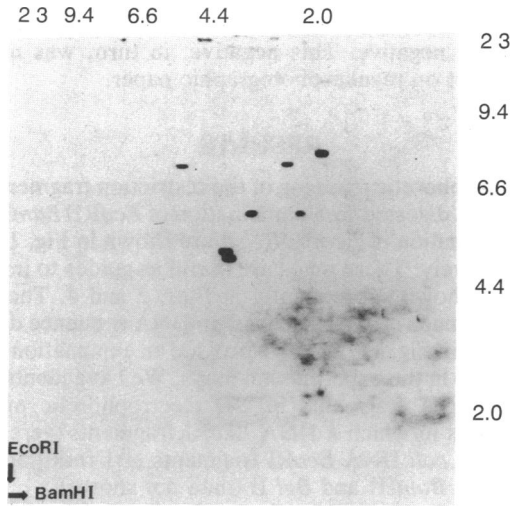

FIG. 3. The 2-D difference map of the incorporated λ phage DNA in the strain of HB101 (λ) bacteria after *Eco*RI/*Bam*HI digestion. The locations of all eight fragments are shown by the negative–positive photographic procedure in canceling out the common (or shared) spots in the upper (*Right*) and lower (*Left*) films of the 2-D gel shown in Fig. 2.

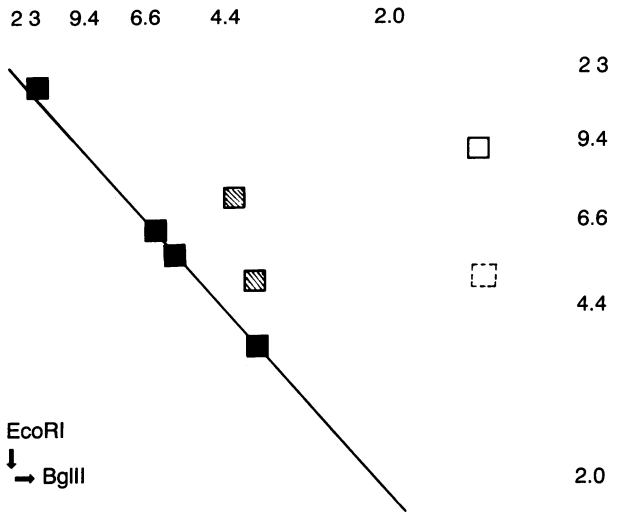

FIG. 5. This schematic drawing represents the composite information derived from: (*i*) the 2-D λ phage DNA fragment map obtained after *Eco*RI/*Bgl* II digestion shown in Fig. 1*B*; (*ii*) the 2-D maps of DNA restriction fragments containing both HB101 (λ) as test DNA and HB101 as reference DNA shown in the upper and lower film of Fig. 4; (*iii*) 2-D difference map of incorporated λ DNA after the photographic canceling procedure described in Fig. 4 (data not shown). ■, Incorporated λ DNA *Eco*RI restriction fragments having no second restriction enzyme (*Bgl* II) cutting site (the diagonal dark band shown in Fig. 4); ▧, The incorporated λ DNA fragments revealed after the photography canceling procedure; □, Artifact spot shown after the photography canceling procedure due to insufficient darkening of this spot in the upper film. (This artifact spot can be removed by a longer exposure to the upper film or by differential photographic manipulation of the upper film.); □, Faint spot of incorporated λ DNA fragment, which can be detected by longer exposure to the lower film or by differential photographic manipulation of the lower film.

carried out after the second electrophoresis. The 2-D map of the DNA restriction fragments from both HB101 (λ) labeled with ^{35}S and HB101 labeled with ^{32}P are shown in the lower (L) film (Fig. 2 *Left*). The upper (U) film only shows the 2-D map of ^{32}P-labeled HB101 fragments (Fig. 2 *Right*). When comparing the lower film with the upper film, 8 spots (indicated by the arrows in the figures) are found among the 300–400 spots that exist only on the lower film but not the upper film. Application of the negative–positive photographic procedure to cancel out the common (or shared) spots in the lower and upper films (exposed to the 2-D gel) results in a difference map (Fig. 3) that clearly depicts the 8 unshared spots (representing eight ^{35}S-labeled DNA fragments) present only in HB101 (λ) strain but absent in HB101. Because the integration site of λ DNA in the *E. coli* genome DNA, att site, is located in the middle of the 5.6-kb DNA fragment obtained by *Eco*RI digestion, the size of the two flanking fragments arising from cleavage of this 5.6-kb λ fragment depends on the size of *E. coli* DNA attached to each end of the fragment when cut by *Eco*RI. This reasoning provides the explanation why some λ DNA spots in the HB101 (λ) 2-D map are located at the places different from those on the λ phage DNA restriction map (Table 1).

Another 2-D map obtained from the comparison of *E. coli* HB101 (λ) as test DNA and the HB101 strain as the reference DNA was obtained with *Bgl* II as the second restriction enzyme (Fig. 4). In this *Eco*RI/*Bgl* II map, three spots were revealed that existed only in the lower film but not the upper film. The location of these spots can be predicted from the

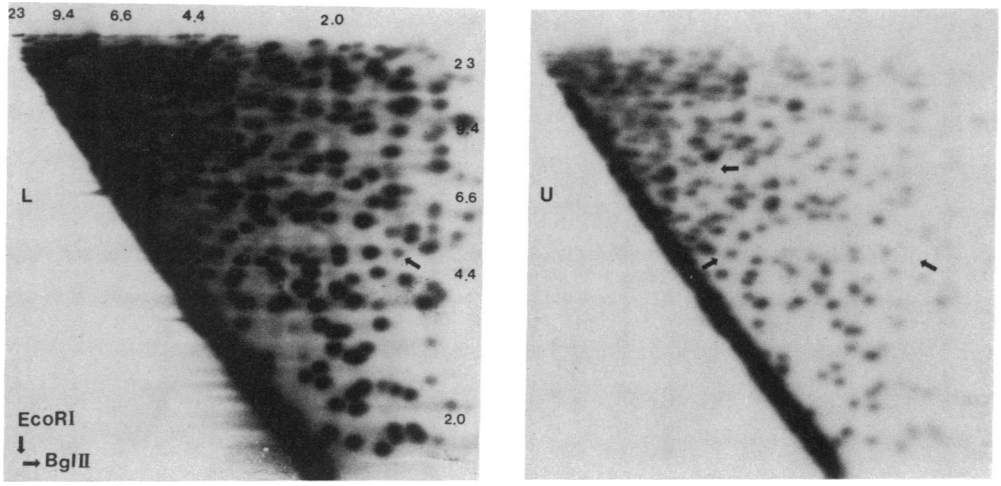

FIG. 4. Detection of the incorporation of λ phage DNA (single copy) in the strain of HB101 (λ). The 2-D map of the DNA restriction fragments was obtained after *Eco*RI/*Bgl* II digestion with the test DNA containing 2 μg of HB101 (λ) *Eco*RI fragments labeled with [α-^{35}S]dCTP, with the reference DNA containing 0.1 μg of HB101 *Eco*RI fragments labeled with [α-^{32}P]dCTP. The 2-D electrophoresis gel was then exposed to a lower film (exposed to both ^{35}S and ^{32}P) and to an upper film (exposed only to ^{32}P) as described in *Materials and Methods*.

linear restriction map shown in Fig. 1*D*. It should be noted that these spots obtained from the combination of *Eco*RI/*Bgl* II are different from those obtained from the combination of *Eco*RI/*Bam*HI. Other issues of this experiment will be discussed below (Fig. 5).

DISCUSSION

The first objective of this investigation was to increase substantially the resolution of the DNA restriction fragments obtained from the bacterial genome (or any genome of several megabases in size). This goal has been reached by the development of a two-restriction-enzyme digestion procedure coupled with a 2-D electrophoretic separation. The key step in this approach is a second restriction enzyme digestion carried out in the gel after the 1-D electrophoretic separation of the first enzymic digest which was done in solution. The final 2-D patterns shown in Figs. 2 and 4 provide a resolution of 300–400 spots, which are defined by an "*x,y*" coordinate system representing the molecular sizes of these fragments calibrated with size markers. This approach has greatly increased the resolution power over the conventional 1-D electrophoresis. Indeed, by comparing the two 2-D patterns obtained from *E. coli* HB101 genome and from *E. coli* HB101 (λ) genome, the single copy of incorporated λ phage DNA in the *E. coli* genome was identified without the use of λ DNA as probe.

The second objective of this investigation was to enhance significantly the ease and certainty of identifying the differences between the DNA restriction fragments obtained from two similar (but not identical) genomes. To this end, we have utilized the differential energy emission of two isotopes, and the related difference in their degree of penetration as a means to expose two layers of x-ray film placed on top of a gel after electrophoresis; β-irradiation from ^{35}S can only expose the lower film, which is directly on top of the dried gel, whereas the β-irradiation from ^{32}P can expose both the lower and the upper film. In these experiments, the DNA restriction fragments from the test cell were labeled by ^{35}S, and the DNA fragments from the reference cells were labeled by ^{32}P after the first restriction enzyme digestion. By comparing these two exposed films from the same gel (which contains both test DNA fragments and reference DNA fragments), one can readily locate the differences in the DNA fragments from these two sources since spots can be detected that are only in the lower film but not in the upper film (Figs. 2 and 4). By the use of an appropriate photographic procedure, a difference map was constructed that only shows the difference in DNA fragments between these two 2-D patterns (Fig. 3). Since this procedure requires no DNA probes, it can be used to monitor any unknown DNA rearrangement in a genome of about 5 megabase in size.

The problem in utilizing this approach is briefly outlined in Fig. 5. The first requirement is that during the second-dimension electrophoresis, the rearranged (or newly incorporated) DNA restriction fragments have to migrate away from the base line of the first-dimension electrophoresis into the open space of the gel to resolve and identify the DNA fragments (see Figs. 2 and 4); however, some fragments do not move away from the base line of the first-dimension electrophoresis. These DNA restriction fragments obtained from the first digestion (*Eco*RI in these experiments) do not contain restriction sites for the second restriction enzyme (*Bam*HI and *Bgl* II in these experiments). This means that DNA restriction fragments from the first restriction enzyme digestion, containing the rearranged (or incorporated) DNA segment, must have a restriction site for the second restriction enzyme in order for the rearranged DNA segment to be separable from the base line. This situation is illustrated in Fig. 5. Among the *Eco*RI DNA restriction fragments that contain the incorporated λ phage DNA segment, three fragments contain *Bgl* II restriction sites and four fragments contain no *Bgl* II restriction sites or contain a *Bgl* II restriction site at the very end. As the result of this *Bgl* II site distribution, the four *Eco*RI fragments, containing no *Bgl* II site or containing only an in-end *Bgl* II site, remained at the base line, and only three *Eco*RI fragments containing a *Bgl* II site were cleaved and migrated out into the 2-D pattern. This is the reason why eight spots are found in the difference map obtained from an *Eco*RI/*Bam*HI combination and only three spots are found in the difference map obtained from an *Eco*RI/*Bgl* II combination (Table 1). Clearly for the identification of all unknown DNA rearrangements (or DNA incorporation), a battery of second restriction enzymes has to be used to ensure that all of the *Eco*RI DNA fragments are cleaved at least by one of the second restriction enzymes before one can obtain a 2-D pattern containing the rearranged DNA segment. Currently, our study indicates that five restriction enzymes would be sufficient to ensure all of the *Eco*RI DNA fragments from the *E. coli* genome would be cleaved at least once by one of these five enzymes (data not shown). It also means that five sets of 2-D patterns or five difference maps from five combinations of restriction enzymes (*Eco*RI plus one of the five) would be sufficient to cover all of the unknown changes in *E. coli* genome.

Since the labeling of the fragments is done by the replacement procedure, the spots corresponding to the small DNA fragments invariably would be lighter in density in the x-ray film than the spots corresponding to the large DNA fragments, which contain more radioactivity; therefore, there is unevenness in the intensity of these spots in the 2-D pattern. To compensate for such an unevenness in the intensity of all the spots, different photographic exposure has to be used to cover different regions of the 2-D pattern. This means that the overall difference map has to be constructed as a composite from two or more 2-D difference maps, as represented in Fig. 5. In such a reconstructed composite difference map, artifacts due to photography also can be identified easily from the original 2-D electrophoretic pattern.

The most serious limitation of the application of this procedure clearly is the size of the 2-D electrophoretic gel pattern. It is hoped that with a larger gel, 1000–1500 spots can be sufficiently resolved in a 2-D pattern. With this advance, the genomes of about 10 megabases in size can be analyzed and compared in terms of restriction fragments. Part of the early development of this approach has been described by Au in his thesis dissertation (8).

This work was supported in part by DE-FG02-88ER60636 grant from the U.S. Department of Energy, Office of Health and Environmental Research (Washington, DC).

1. Kohara, Y., Akiyama, K. & Isono, K. (1987) *Cell* **50**, 495–508.
2. Au, L. C. & Ts'o, P. O. P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5507–5511.
3. Fisher, S. G. & Lerman, L. S. (1979) *Cell* **16**, 191–200.
4. Yee, T. & Inouye, M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2723–2327.
5. Simon, M. & Herskowitz, I. (1985) *Genome Rearrangement* (Liss, New York).
6. Boyer, H. W. & Roulland-Dussoix, D. (1969) *J. Mol. Biol.* **41**, 459–472.
7. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
8. Au, L. C. (1988) Dissertation (The Johns Hopkins University, Baltimore).