METHODS: A Companion to Methods in Enzymology
Vol. 3, No. 2, October, pp. 91–97, 1991

Two-Dimensional Agarose Gel Electrophoresis of Restriction-Digested Genomic DNA

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Two-dimensional agarose gel electrophoresis of genomic restriction endonuclease digestion products can physically separate fragments that comigrate in conventional agarose gels. To ensure that the results of each gel are reproducible, duplicate gels are prepared. The gels may be analyzed by Southern hybridization, or restriction fragments of known size may be preparatively extracted from agarose plugs. This method can be used to compare the digestion patterns of specific sequences from different organisms in a population, different cell types, or different developmental states. © 1991 Academic Press, Inc.

The need to separate large numbers of unique DNA fragments has prompted the development of techniques that enhance the resolution of standard electrophoretic separations. By fractionating genomic DNA fragments in multiple stages, it has become possible to increase the resolving power of agarose gel electrophoresis. Nucleic acid species that comigrate in single-dimension gels can be separated by two-dimensional gel (2D) electrophoresis. Conventional double digestion and electrophoresis of genomic DNA may not resolve individual components of all multicopy sequences because the internal structure of these repeats may be highly conserved. Individual members of multicopy sequence families may be resolved by sequentially digesting and electrophoresing genomic DNA onto a two-dimensional grid. The origin of fragments that migrate off the major diagonal in the second dimension can be extrapolated from each of the first-dimension digestion products. The restriction maps of individual off-diagonal fragments are a unique feature of the 2D gel system, since this information may not be accessible from double digests separated in conventional gels.

The method involves incubating an agarose gel slab containing restricted genomic DNA with a second enzyme to redigest DNA fragments *in situ* (Fig. 1). The gel is then embedded perpendicular to the initial direction and electrophoresed. Fragments that are not cleaved in the second digestion migrate along a diagonal that bisects the gel and that extends from the largest to the smallest fragments in the initial digest. Digestion products released by the second enzyme are distributed throughout the lower half of the gel grid. The gel may then be analyzed by Southern hybridization, or specific digestion products can be recovered by direct purification from agarose blocks. Such fragments can then be directly cloned or amplified by the polymerase chain reaction (P. Rogan, P. Lemkin, A. Klar, J. Singh, and J. Strathern, in preparation).

Two-dimensional electrophoresis has been used to separate DNA fragments from small genomes so that each fragment migrates to a unique position on the gel grid. This has made it possible to identify subtle genomic rearrangements in the *Escherichia coli* genome (1, 2). This is not currently feasible for complex genomes, where too many fragments are generated to achieve this level of resolution. In such cases, 2D Southern hybridization with probes that recognize multiple fragments may be used to study subsets of these genomes.

The precise copy number of multicopy genomic sequences and the physical maps surrounding family members can be deduced from 2D agarose gels. Large-scale physical maps of a family of mammalian genes were constructed using probes that cross-react with several family members (3). Populations of different individuals can also be studied with polymorphic restriction enzymes using multicopy probes. This technique has demonstrated that most or all of the known murine *t* locus haplotypes are recent descendants of a single ancestral chromosome (4). Individual variable number tandem repeat loci on different human chromosomes have been similarly studied to determine the segregation properties and the informativeness of each marker (5).

Some aspects of gene regulation have been studied by digesting genomic DNA with restriction enzymes whose recognition sequences are congruent with known biolog-

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1046-2023/91 $3.00

ical specificities. Pairs of restriction enzymes that exhibit different sensitivities to methylation can be used to distinguish modified from unmodified genomic domains adjacent to interspersed, endogenous proviral sequences in the murine genome (6). The observed methylation patterns were generally conserved in different tissues from the same individual, although some tissue specificity was detected. Since DNA methylation is thought to play an important role in maintaining specific patterns of gene expression in eukaryotic cells (7), those differences revealed by 2D gel electrophoresis may be indicative of the expression profile of these sequence elements in different cell types.

CHOOSING RESTRICTION ENDONUCLEASE AND PROBE COMBINATIONS

Due to the high degree of complexity of eukaryotic genomes and the physical limitations of gel electrophoresis systems, it is difficult, if not impossible, to find experimental conditions that separate each fragment from all other fragments in a genomic digest. The compromise is to choose restriction enzymes that generate DNA fragments distributed over the largest physical distance in the gel matrix.

Agarose gel matrices are suitable for separating a broad range of sizes; however, the migration of nucleic acids in a constant, unidirectional electric field shows an approximately logarithmic dependence on the length of the molecule. This results in decreased resolution for DNA fragments of high molecular weight. The range of fragment lengths that demonstrates the greatest physical separation is affected by the agarose gel density, the chemical constitution of the gel matrix, the running buffer, and the temperature. These conditions can be adjusted to produce optimal separations from 100 bp up to 20 kb.

The collection of unique fragments should be separated over as large an area as possible. Since the agarose gel density is easily adjusted, it is more critical to find restriction endonucleases that generate approximately a uniform distribution of fragment sizes. Although it is difficult to predict a priori which restriction enzymes will generate a broad distribution of different-sized cleavage products, the sizes and number of fragments that are released from genomic digestion can be used to empirically predict the mean and variance of the distribution of enzyme recognition sites in the genome. The sizes of digested genomic DNAs that have been end-labeled with Klenow DNA polymerase (or if the enzyme produces a 3′ recessed end, T4 polynucleotide kinase (8)) can be calibrated against appropriate DNA size markers. Optical density measurements of autoradiographic films produced from these gels reveal the average size and the distribution of fragment sizes in these digests. Gel slabs containing end-labeled fragments can also be treated with other enzymes

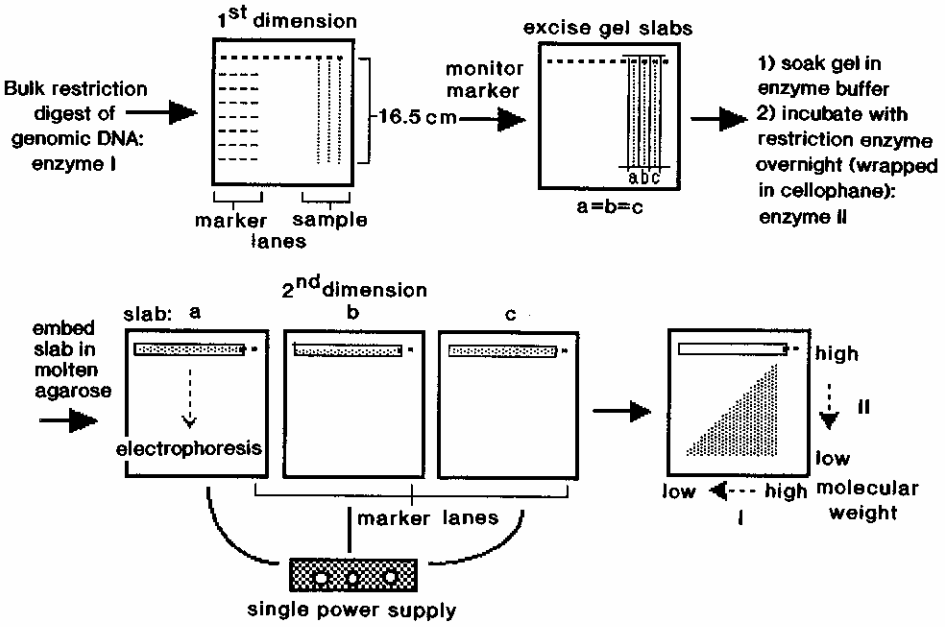

FIG. 1. Method of preparing two-dimensional agarose gels. Genomic DNA is digested with enzyme I and electrophoresed. Gel slabs containing identical genomic samples are dissected and incubated with enzyme II. Duplicate slabs are reoriented and run in a second gel perpendicular to the original direction of electrophoresis. The resultant gels may be transferred to nylon filters for Southern analysis or stored for subsequent preparative extraction of doubly digested genomic fragments.

to determine the distribution of doubly digested restriction fragments.

The probability of cleaving a fragment produced in the initial digest with the second-dimension enzyme depends on the length of the first-dimension fragment and the frequency of its recognition sequence in the genome. Therefore, larger fragments are more likely than smaller ones to generate second-dimension cleavage products. This can result in a tendency to concentrate double digestion products in one quadrant of the gel. This effect can be minimized by cleaving with an enzyme that generates a distribution of fragments that, on average, contain at least one second-dimension restriction site, but that are not likely to contain several such sites. Digestion of *Saccharomyces cerevisiae* genomic DNA with a pair of enzymes recognizing a degenerate 6-nucleotide sequence released fragment sizes in the first dimension up to 6 kb in length; Fig. 2). The fragments detected with a reiterated sequence probe are cleaved once or twice in the second-dimension digest and are well resolved in 1% agarose gels. Other enzymes with degenerate 6-nucleotide specificities produced similar distributions of fragment sizes (results not shown). However, since the lengths of resultant digestion products are a complex function of the genome size, composition, and organization, it is difficult to predict a priori which restriction enzymes will generate suitable fragment size distributions.

The probe selected for 2D Southern analysis may be dictated by the experimental objectives, i.e., to study genomic organization or methylation patterns in a specific region of the genome. Probes derived from a single chromosomal domain can produce patterns that range from extremely simple to those resembling sequences with a broad genomic distribution. Cloned single-copy sequences generally detect a discrete set of genomic fragments; the number of fragments that hybridize depends primarily on the carrying capacity of the cloning vector. Large genomic inserts derived from complex genomes are more likely to contain reiterated sequence elements with an interspersed rather than a localized distribution. To restrict the analysis to sequences originating from a specific chromosomal domain, signals arising from repeat elements can be

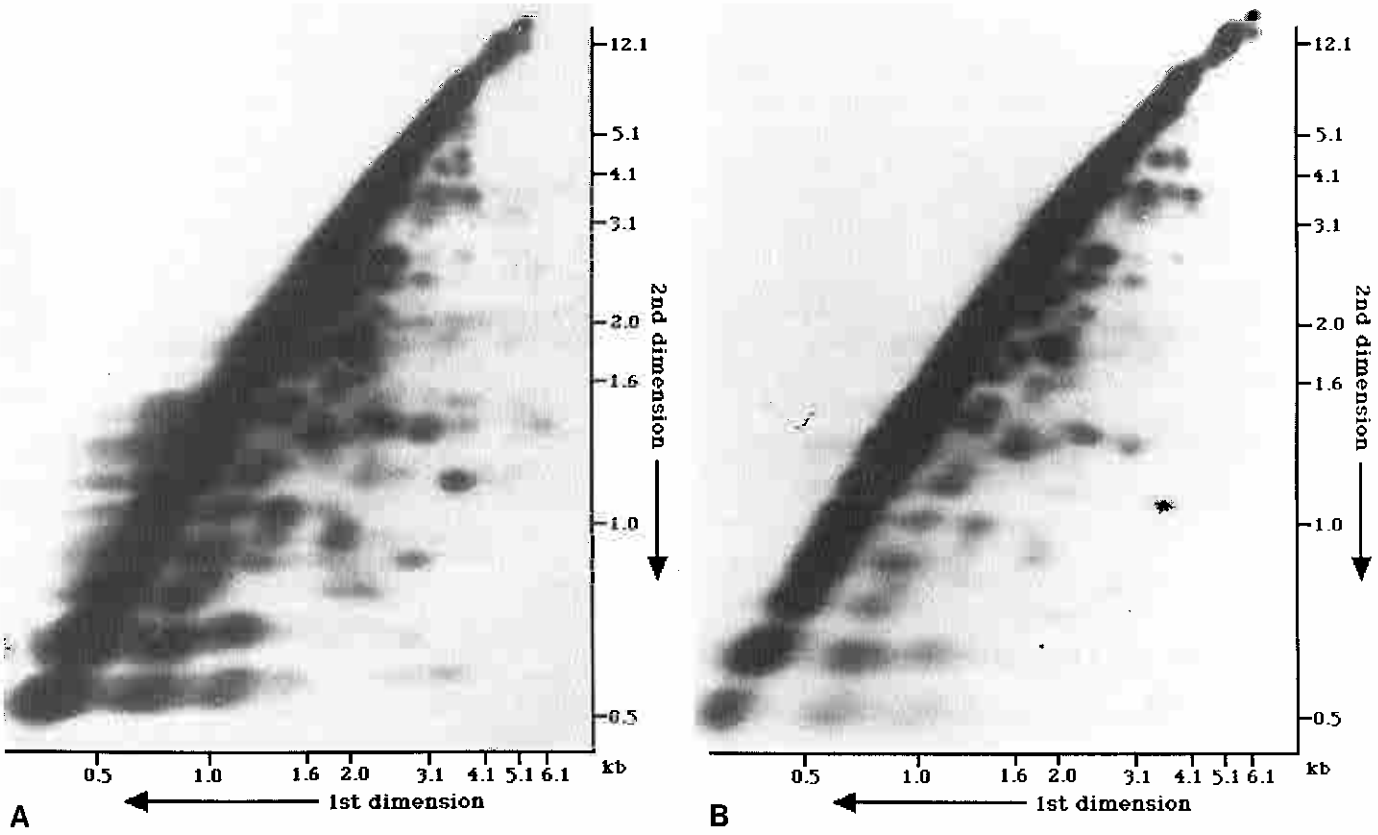

FIG. 2. Southern hybridization of duplicate two-dimensional genomic blots. *Saccharomyces cerevisiae* genomic DNA was prepared from a strain expressing the *E. coli dam* methyltransferase gene (LP2649-1A: *MATα, can1, leu2-3, leu2-112, ura3-52, rad3-2,* from L. Prakash). DNA was digested initially with *Hin*cII and with *Mfl* I, an enzyme that contains the dam recognition site but is inhibited by adenine methylation. Methylated sites were cleaved by an isoschizomer that is insensitive to adenine methylation, *Bst*YI, prior to running of the second dimension of the gel. The gels were blotted to nylon filters and hybridized to a cloned probe, pLTRΔ9, containing a member of the solo δ reiterated sequence family (17). A and B are duplicate gels, which were prepared according to the scheme shown in Fig. 1.

quenched by competitive prehybridization with unlabeled genomic DNA.

The outcome of 2D Southern analyses with reiterated sequence probes is not as predictable as that of studies performed with single-copy sequences because measurements of the genomic representation of these sequences may be imprecise. Conserved restriction sites within and adjacent to the repeat elements can be helpful in selecting enzymes for optimal separation of different family members. However, repeated sequence elements may exhibit sequence heterogeneity that affects the efficiency of hybridization at some cognate loci. Thus, the number of detectable fragments may be controlled by adjusting the stringency of the hybridization and washing conditions. This parameter may be critical in limiting the complexity of the hybridization pattern so that the resolution threshold of the gel system is not exceeded.

Since the complex mixture of fragments produced by total genomic digestion exceeds the resolution of the gel matrix, a representative subset of the genome may be surveyed by sampling a fraction of it in each experiment (P. Rogan, P. Lemkin, A. Klar, J. Singh, and J. Strathern, in preparation). Southern analysis with reiterated sequence probes surveys only those restriction sites adjacent to these elements. As many as 300–500 different fragments can be distinguished in a single experiment. By selecting probes from families of interspersed, repeated elements with sequence heterogeneity, the fragments that are detected have an unbiased distribution. The density of hybridizing fragments can be controlled by varying the hybridization and washing conditions. Probes that exclude internal restriction enzyme recognition sites in their consensus sequences can be selected so that each fragment corresponds to the genomic environment around a single repeat element. After autoradiography, filters can be stripped and reprobed with another sequence derived from a different reiterated sequence family. The ubiquity and the distribution of repeated elements and the iterative application of this procedure can overcome limiting resolution of the 2D gel system, and a large number of restriction sites throughout the genome can be independently sampled.

PREPARATION OF TWO-DIMENSIONAL AGAROSE GELS

The production of high-quality 2D Southern blots depends on determining the optimum conditions that detect fragments homologous to the probe sequence, maximizing DNA fragment resolution, and ensuring that gels are reproducible. As in conventional Southern analysis, sequence detection is a function of the specific activity and the concentration of the labeled probe as well as the quantity of the target sequence on the nylon membrane. Optimal separations are achieved by matching the density and size of the agarose gel matrix to the distribution of restriction fragment sizes. Direct comparison of autoradiographs of different gels is generally possible when all of the gels in an experiment are prepared in parallel. However, the preparation of each gel involves several steps, each of which may contribute to experimental variation. It is necessary to analyze duplicate gels with the same combinations of digested genomic DNA and probes. Therefore, the following protocol anticipates the simultaneous production of several gels in the same experiment.

Electrophoresis of the First Dimension

Genomic DNA is initially digested in bulk (>100 μg) in quantities that are sufficient to run multiple gels. If necessary, digestion reactions can be tested for completeness by conventional Southern analysis with probes that generate known digestion patterns. In the first dimension, multiple 20-μg samples are loaded in adjacent lanes on a single 1% agarose gel. The highest grade of agarose available (with a low content of sulfonated polysaccharides) is used, since the subsequent restriction digest is carried out within the gel matrix. The agarose gel density used should optimally resolve the majority of the DNA fragments produced by enzymatic digestion. An agarose concentration of >0.9% is preferred to minimize breakage of the gel resulting from subsequent manipulations. A higher agarose density or a different type of agarose may be required to prevent the slab from melting, if the activity of restriction enzyme used prior to second-dimension electrophoresis has an optimal thermal profile above 50°C.

The detection of single-copy genomic sequences is enhanced by maximizing the quantity of DNA loaded as long as the capacity of the gel matrix to resolve different-sized fragments is not exceeded. The goal of achieving the highest possible hybridization signals is offset by the desire to minimize the dispersion of spots in the second electrophoretic separation. To generate the smallest possible gel cross section without overloading the gel (reducing electrophoretic resolution), a thick comb producing a narrow lane width is used (2 mm wide × 4 mm long × 2–3 mm deep). Loss of resolution due to thermal effects can be minimized by running the gel in either TBE (Tris–borate–EDTA) or TAE (Tris–acetate–EDTA) buffer at 4°C at low voltage (3–4 V/cm). Inversion of the electric field has also been shown to produce well-defined spot patterns for fragments smaller than 40 kb (6). Ethidium bromide (EtBr) should not be included in either the gel or the running buffer, since it may inhibit digestion by the second restriction enzyme. The distance that the front migrates is limited to 16.5 cm, so that the slab can be reoriented along the comb axis in the second dimension (this also allows for a size marker lane). The dimensions of the system used to transfer DNA in the gel to a nylon support may also place limits the length of both the first- and the second-dimension runs.

Several identical lanes containing DNA size markers are run to ensure that the front migrates exactly the same distance in each of the gels and to calibrate fragment sizes upon completion of the experiment. Markers should exhibit a uniform size distribution so that fragment lengths can be interpolated from genomic digests. The progress of the run is monitored by excising a single marker lane at various times and staining it with EtBr. The markers are photographed with a fluorescent ruler. Fragment sizes can be estimated by comparing the Southern blot with the photograph. To minimize the possibility of contamination between lanes, those containing marker DNA are well separated from those containing genomic digests. When different genomic DNA sources are compared in the same experiment, each digestion is run in a different electrophoresis unit connected in parallel to a single power supply. The distance between anode and cathode should be the same to ensure that DNA separations carried out in different gel boxes migrate the same distance.

Restriction Endonuclease Digestion in Agarose Gel Slabs

Restriction endonuclease digestion of DNA embedded within agarose matrices has proven to be a valuable tool in the physical mapping of chromosomes (9). Since diffusion occurs more slowly within agarose than in liquid reactions, higher restriction enzyme concentrations and longer incubation times are needed to achieve complete digestion. However, the distinct geometry of the agarose gel slab in the 2D gel required several modifications of this procedure.

The gel is dissected into individual lanes with a scalpel prior to digestion or, for ease of handling, a contiguous set of identical adjacent lanes may be excised as a single piece (and sliced after the reaction is complete). The edges of each gel slice should be linear and coincide with the boundary of the gel lanes, since inaccuracy at this stage can result in aberrant DNA migration or stoichiometry of different-sized fragments. Extraneous agarose segments that do not contain DNA should be removed to minimize the gel volume in the second restriction digestion.

The gel slab is prepared for *in situ* digestion by incubation in restriction enzyme buffer at 4°C in the absence of enzyme. One or more identical gel lanes (either presliced or a single slab composed of several identical samples) are weighed, mixed with 3 vol of buffer, and sealed in cellophane wrap for 2 h. The buffer is changed twice, incubated, and aspirated. The enzyme is prepared in 0.5 gel vol of buffer and distributed over the gel, which is then rewrapped in several layers of cellophane and incubated overnight on a rocking platform. Additions of 0.1–0.2 unit of enzyme/μl gel volume were found to be sufficient for complete digestion of the embedded DNA. Reaction conditions are carried out as specified by the manufacturer. The high cost of some restriction enzymes may make it necessary to titrate the minimum quantity needed to achieve complete digestion *in situ* or, if it is possible, to reverse the order of first and second enzymatic digestions. Other considerations, such as the instability of some restriction enzymes (e.g., *Sma*I), may limit their application for restriction digestion in agarose gels, since the rate of cleavage is limited by the rate of diffusion of the enzyme into the matrix. For many specificities, however, stable isoschizomers are often available, and sometimes at a lower cost.

Electrophoresis of the Second Dimension

To separate genomic fragments cleaved by digestion in the agarose gel from those that do not contain internal restriction sites, the gel slab is reoriented perpendicular to the initial direction of electrophoresis and embedded in a second horizontal gel. The gel slab is positioned at a predefined location marked on the gel mold. The gel slab is biased to one side of the mold to allow for a marker DNA lane. It should be situated so that the electric field traverses the minimum cross section of the gel. The surface of the gel mold directly beneath the slab should be somewhat abrasive so that the slab adheres to this surface while the second gel is poured. The liquid agarose is kept slightly above the gelling temperature (50°C) and carefully poured around the slab. The top of the gel slab should be equal to the height of the second gel.

The size of the slab cross section exposed to the electric field can affect the bandwidths of DNA fragments as they migrate through the second gel. If the density of the second gel is greater than that of the gel slab, the result is a concentrating or stacking effect for DNA fragments at the interface between the two agarose matrices (10). When fragments detected in both dimensions are of similar size, a higher gel density for the second dimension will improve bandwidths at the expense of reduced fragment resolution. First- and second-dimension gels of similar density are used when the range of fragment sizes spans several kilobase pairs, although this results in more diffuse spot patterns after Southern blotting (Fig. 2). These spot shapes can be identified and segmented by an automated image detection filter (11).

Duplicate gels as well as those containing different sources of genomic DNA are electrophoresed in TEA buffer at low voltage and are set up in a parallel configuration from a single power supply. EtBr can be added to the gel to monitor the second-dimension digestion and to record the positions of the size markers. The sizes of fragments detected by Southern hybridization are easier to calibrate if the distance that the front migrates in each dimension is the same. When the run is complete, the region of DNA fluorescence will be bounded by a diagonal that bisects the gel from the high to low molecular weight size ranges. If the gel will be used for preparative genomic DNA purification, it may be wrapped in cellophane and stored at 4°C for several weeks. After Southern hybridization has identified genomic fragments of interest, cor-

responding plugs may be retrieved for cloning or amplification from duplicate gels that have been stored in this manner.

The gels are processed according to a standard Southern analysis protocol (8), except that DNA is mechanically transferred to a nylon support using a positive pressure apparatus (Stratagene). This modification not only expedites the processing of gels, but also appears to efficiently remove most of the nucleic acids from the gel (as judged by post-transfer EtBr staining). Nucleic acids are crosslinked to nylon supports with a consistent dose of ultraviolet radiation (1200 J/m^2 (12)); this allows multiple probes to be sequentially hybridized to the same filter. If possible, all filters in a single experiment should be simultaneously hybridized with probe, washed, and exposed to X-ray film. This will ensure that corresponding signals from different genomic DNA sources can be compared directly. The choice of film can also be critical, since more sensitive emulsions (high ASA, e.g., X-Omat R; Kodak) can reveal light spots at the expense of a higher background. Although this effect can be corrected by image processing, the dose response of the film and, consequently, the absorbance calibration curve may be nonlinear. X-ray films with linear response over a wide dynamic range (such as Cronex; Dupont) may be more suitable when changes in the spot intensity, rather than their presence or absence, are measured.

FUTURE TECHNOLOGICAL ENHANCEMENTS

The potential applications of 2D agarose gel technology in genome analysis have only begun to be tapped. To make this methodology accessible to more investigators, improvements that should result in greater fragment resolution, reproducibility, and a more fail-safe, convenient protocol can be incorporated. For example, inversion of the electric field as well as a larger gel format may result in greater physical separation of larger DNA fragments (13). It may also be possible to incorporate a discontinuous buffer system in the second dimension of electrophoresis, which could result in sharper definition of spots due to a stacking effect at the inception of the run (14). To ensure that each gel in the same experiment is exposed to the same electric field, multiple 2D gels could be run in the same electrophoresis apparatus by layering the gel molds on top of one another. To dissect the first-dimension gel into individual lanes more easily and with greater consistency, a slicing tool with parallel blades could be customized to fit the gel mold. These ideas represent only a small fraction of the potential methodological improvements. It is hoped that the recognition that 2D agarose gel electrophoresis offers another approach for the analysis of large genomes will stimulate such efforts.

POTENTIAL APPLICATIONS OF 2D GEL TECHNOLOGY

In addition to the physical separation of genomic fragments and polymorphic loci and the identification of chromosomal rearrangements, 2D gel electrophoresis technology can be applied to the study of gene regulation and to the identification of candidate genes. Restriction enzymes that preferentially recognize and cleave sequences contained within genomic transcription templates can be selected.

Transcribed DNA sequences can be specifically tagged by expressing a DNA methylase in *S. cerevisiae*. Synthetic methylation has been analyzed throughout the genome with pairs of isochizomers that differ only in their ability to cleave modified DNA recognition sites (Fig. 2; P. Rogan, P. Lemkin, A. Klar, J. Singh, and J. Strathern, in preparation). Genomic DNA cleaved with a methylation-sensitive enzyme (*Mfl*I) in the first dimension has been redigested with an enzyme refractory to methylation (*Bst*YI) and separated perpendicular to the original direction of fragment separation. Fragments containing unmethylated restriction sites fall on the diagonal of the gel, while methylated cleavage products are released from larger fragments on the diagonal and are found below it. A retroviral-like long terminal repeated sequence with an interspersed genomic distribution identifies approximately 70 methylated fragments in *MAT*α yeast cells. Several of these digestion products are present only in specific mating types and may represent genes expressed in a cell-type-specific manner (P. Rogan, P. Lemkin, A. Klar, J. Singh, and J. Strathern, in preparation).

In mammalian genomes, CpG dinucleotides are substantially underrepresented and are often located 5′ to functional genes (15). Since the compositions of coding sequences and intergenic DNA are quite different, the choice of restriction enzyme can be intentionally biased toward preferential cleavage in expressed regions of the genome. 2D electrophoresis could separate and enrich for expressed gene candidates with pairs of enzymes capable of cleaving only unmethylated CpG-containing restriction sites. Since the distance between CpG islands exceeds the resolution of the gel system, fragments of the appropriate size in the first dimension will be generated by selecting an enzyme that cleaves frequently within intergenic domains.

It may also be possible to exploit 2D gel electrophoresis to enrich for expressed genes containing specific regulatory sequences. *cis*-Acting regulatory elements frequently contain palindromic sequences (16). Genomic fragments that terminate in a particular regulatory motif could be produced by cleavage (in the first dimension) with a restriction enzyme that has an overlapping recognition site. Although this will also produce some irrelevant fragments,

cleavage (in the second dimension) with an enzyme recognizing hypomethylated, CpG-enriched sequences should generate off-diagonal fragments, some of which should contain gene candidates proximate to *cis*-acting, regulatory elements.

ACKNOWLEDGMENTS

P.K.R. thanks Drs. Michael Chorney and Roger Ladda for their valuable comments. This research was sponsored in part by the National Cancer Institute, DHHA, under Contract N01-CO-74101 with ABL. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

1. Au, L. C., and Tso, P. O. P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5507–5511.
2. Yi, M., Au, L. C., Ichikawa, N., and Tso, P. O. P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3919–3923.
3. Walter, M. A., and Cox, D. W. (1989) *Genomics* **5,** 157–159.
4. Silver, L. M., *et al.* (1987) *Mol. Biol. Evol.* **4,** 473–482.
5. Armour, J. A., Wong, Z., Wilson, V., Royle, N. J., and Jeffreys, A. J. (1989) *Nucleic Acids Res.* **17,** 492–535.
6. Mietz, J., and Kuff, E. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2269–2273.
7. Keshet, I., Lieman-Horowitz, J., and Cedar, H. (1986) *Cell* **44,** 535–543.
8. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) *Molecular Cloning,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
9. Burke, D., Carle, G., and Olson, M. (1987) *Science* **236,** 606–612.
10. Woodhead, J. L., Bhave, N., and Malcolm, A. D. B. (1981) *Eur. J. Biochem.* **115,** 293–296.
11. Lemkin, P., and Rogan, P. (1991) *Appl. Theor. Electrophoresis,* in press.
12. Church, G., and Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
13. Young, D. A., Zoris, B. P., Maytin, E. D., and Colbert, R. A. (1983) *in* Methods in Enzymology (Hirs, C. H. W., and Timasheff, S. N., Eds.), Vol. 91, pp. 190–214, Academic Press, New York.
14. Orban, L., and Chrambach, A. (1990) *Electrophoresis* **12,** 233–240.
15. Brown, W., and Bird, A. (1986) *Nature* **327,** 336–338.
16. Benoist, C., and Mathis, D. (1990) *Annu. Rev. Immunol.* **8,** 681–704.
17. Weinstock, K., Mastrangelo, M. F., Burkett, T. J., Garfinkel, D. J., and Strathern, J. N. (1990) *Mol. Cell. Biol.* **10,** 2882–2893.