Two-dimensional conformation-dependent electrophoresis (2D-CDE) to separate DNA fragments containing unmatched bulge from complex DNA samples

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ABSTRACT

DNA fragments containing mispaired and modified bases, bulges, lesions and specific sequences have altered conformation. Methods for separating complex samples of DNA fragments based on conformation but independent of length have many applications, including (i) separation of mismatched or unmatched DNA fragments from those perfectly matched; (ii) simultaneous, diagnostic, mismatch scanning of multiple fragments; (iii) isolation of damaged DNA fragments from undamaged fragments; and (iv) estimation of reannealing efficiency of complex DNA samples. We developed a two-dimensional conformation-dependent electrophoresis (2D-CDE) method for separating DNA fragments based on length and conformation in the first dimension and only on length in the second dimension. Differences in migration velocity due to conformation were minimized during second dimension electrophoresis by introducing an intercalator. To test the method, we constructed 298 bp DNA fragments containing cytosine bulges ranging from 1 to 5 nt. Bulge-containing DNA fragments had reduced migration velocity in the first dimension due to altered conformation. After 2D-CDE, bulge-containing DNA fragments had migrated in front of an arc comprising heterogeneous fragments with regular conformation. This simple and robust method could be used in both analytical and preparative applications involving complex DNA samples.

INTRODUCTION

Unusual secondary structures in linear DNA fragments often change the conformation of the entire molecule. Such changes in conformation frequently result in curvature of DNA fragments. Curvature has been detected, for example, in DNA fragments containing mispaired bases (1), insertion/deletion bulges (2), UV lesions (3,4), base adducts (5), base methylation (6), A-tracts (7), GC-rich sequences (8), cross-links (9), DNA hairpins or cruciforms (10), slipped-strand DNA (11), nicks (12) and nucleotide gaps (13).

Polyacrylamide gel electrophoresis (PAGE) allows separation of DNA fragments based on both length and conformation, while agarose gel electrophoresis separates DNA fragments essentially only according to length. Migration velocity of DNA fragments in PAGE decreases as the square of the degree of curvature in a DNA fragment (14). This quantitative relationship between curvature and migration velocity of DNA fragments in PAGE has been used experimentally to measure curving of DNA (15,16).

Some methods to screen for mutations or polymorphisms are based on conformation-dependent separation in PAGE. In heteroduplex analysis (HA), the migration velocity of mismatched DNA fragments (altered conformation) and perfectly matched DNA fragments (normal conformation) are compared (17). Conformation-sensitive gel electrophoresis (CSGE) is a variation of heteroduplex analysis where mildly denaturing solvents are used to enhance the tendency of single-base mismatches to produce conformational changes (1).

Use of one-dimensional techniques such as HA and CSGE is limited to situations where DNA fragments to be tested are of known length. In addition, only simple DNA samples containing one or, at most, only a few different DNA fragments can be tested in each lane. If the sample contained many DNA fragments of different lengths, it would be impossible to identify which fragments show conformation-dependent migration since individual bands would not resolve.

Methods for conformation-dependent separation of DNA fragments from a complex sample of DNA fragments of different lengths are of great interest. They could be used for (i) physical separation of mismatched and perfectly...
Here we describe a two-dimensional conformation-dependent electrophoresis (2D-CDE). By exploiting interactions between intercalators and curved DNA, we achieved separation of DNA fragments based on differences in conformation but independent of their length. 2D-CDE utilized the same gel matrix in both dimensions, thus eliminating troublesome transfer between two different matrices. Migration of DNA fragments with normal conformation was primarily determined by their relative lengths in both dimensions, resulting in an arc of DNA fragments lying diagonally through the gel. DNA fragments with altered conformation migrated at a relatively slower rate in the first dimension compared with the second dimension. They were therefore displaced in front of the arc after the second dimension electrophoresis, allowing their easy identification and isolation. Using 2D-CDE, we were able to separate DNA fragments containing two different unusual secondary structures, i.e. bulges and A-tracts, from a complex mixture of heterogeneous DNA fragments.

**MATERIALS AND METHODS**

**Oligonucleotides**

HPLC-purified oligonucleotides (Table 1) were purchased from TAG Copenhagen and stored in 100 µl of H2O at −20°C.

**Construction of tripartite DNA fragments**

We constructed six different 298 bp DNA fragments using the method outlined in Figure 1. One DNA fragment was perfectly matched. Five fragments contained one defined cytosine bulge, 1–5 nt in size.

**Formation of right and left end parts.** Two DNA fragments were PCR amplified from plasmid pBR322 (GenBank accession no. J01749). A 545 bp PCR product was amplified using forward and reverse left end part (F-LEP and R-LEP) primers. A 154 bp PCR product was amplified using forward and reverse right end part (F-REP and R-REP) primers. Each reaction (100 µl) contained 1 ng of pBR322, 0.2 µM of each primer, 200 µM of each dNTP, 7 U of KlenTaq 1 polymerase (AB peptides), 0.05 U of PfuI polymerase (Stratagene) and 1× RDA buffer (25). PCR conditions for both reactions were a hot start at 84°C by adding dNTPs, initial denaturation at 94°C for 5 min, 94°C for 1 min, 58°C for 1 min, and 68°C for 2 min for

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### Table 1. Oligonucleotides and primers used in the study

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Sequence 5’ to 3’ and location</th>
<th>5’ modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward-LFP</td>
<td>Forward primer for PCR product used for amplification of left end part of tripartite DNA fragments&lt;sup&gt;a&lt;/sup&gt;</td>
<td>TAACGGATTCACACTCTGCAAG (1298–1319)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>None or Cy5</td>
</tr>
<tr>
<td>Reverse-LFP</td>
<td>Reverse primer for PCR product used for amplification of left end part of tripartite DNA fragments</td>
<td>CGATACGGTTATCTGATAGT (1842–1823)</td>
<td>None</td>
</tr>
<tr>
<td>Forward-RFP</td>
<td>Forward primer for PCR product used for amplification of right end part of tripartite DNA fragments</td>
<td>CGACTACGGATCATGGAAG (339–359)</td>
<td>None</td>
</tr>
<tr>
<td>Reverse-RFP</td>
<td>Reverse primer for PCR product used for amplification of right end part of tripartite DNA fragments</td>
<td>TCATCAGCGGAAGTGG (495–475)</td>
<td>None</td>
</tr>
<tr>
<td>TMP</td>
<td>Top middle part. Complementary to TMP</td>
<td>TCGGCAGACATGAC (2948–2924)</td>
<td>Cy5</td>
</tr>
<tr>
<td>BMP</td>
<td>Bottom middle part. Complementary to TMP</td>
<td>CTCCTGGAACAGATGATCTGGAAGTCTCTG</td>
<td>Phosphate</td>
</tr>
<tr>
<td>BMP 1C</td>
<td>BMP containing one extra cytosine at position 16</td>
<td>CTCCTGGAACAGATGATCTGGAAGTCTCTG</td>
<td>Phosphate</td>
</tr>
<tr>
<td>BMP 2C</td>
<td>BMP containing two extra cytosines at position 16</td>
<td>CTCCTGGAACAGATGATCTGGAAGTCTCTG</td>
<td>Phosphate</td>
</tr>
<tr>
<td>BMP 3C</td>
<td>BMP containing three extra cytosines at position 16</td>
<td>CTCCTGGAACAGATGATCTGGAAGTCTCTG</td>
<td>Phosphate</td>
</tr>
<tr>
<td>BMP 4C</td>
<td>BMP containing four extra cytosines at position 16</td>
<td>CTCCTGGAACAGATGATCTGGAAGTCTCTG</td>
<td>Phosphate</td>
</tr>
<tr>
<td>BMP 5C</td>
<td>BMP containing five extra cytosines at position 16</td>
<td>CTCCTGGAACAGATGATCTGGAAGTCTCTG</td>
<td>Phosphate</td>
</tr>
<tr>
<td>[Forward 1-Cy5]</td>
<td>Forward primer for formation of a series of perfectly matched DNA fragments</td>
<td>GGTGAAACCTGCAATGAC (2092–2115)</td>
<td>Cy5</td>
</tr>
<tr>
<td>[Reverse 1-Cy5 155]</td>
<td>Reverse primer for formation of 155 bp perfectly matched DNA fragment</td>
<td>TACACCGCTTCTTAGCTAGTGCAC (2246–2223)</td>
<td>Cy5</td>
</tr>
<tr>
<td>[Reverse 1-Cy5 346]</td>
<td>Reverse primer for formation of 346 bp perfectly matched DNA fragment</td>
<td>CGTATTACGGCTTCTTAGCTAGTGCAC (2437–2415)</td>
<td>Cy5</td>
</tr>
<tr>
<td>[Reverse 1-Cy5 543]</td>
<td>Reverse primer for formation of 543 bp perfectly matched DNA fragment</td>
<td>GGGGAAACCGTTCTTAGCTAGTGCAC (2634–2610)</td>
<td>Cy5</td>
</tr>
<tr>
<td>[Reverse 1-Cy5 857]</td>
<td>Reverse primer for formation of 857 bp perfectly matched DNA fragment</td>
<td>CCATTTGAAAACCTGCTAGTGCAC (2948–2924)</td>
<td>Cy5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Construction of tripartite DNA fragments is explained in Figure 1.

<sup>b</sup>Numbers in parentheses refer to position in pBR322 (GenBank accession no. J01749).

<sup>c</sup>C nucleotide(s) in a bulge are underlined.
Formation of middle parts. Double-stranded middle parts (MPs) were formed by annealing 100 pmol of each of the six different bottom middle parts (BMPs) (Table 1) and 120 pmol of the top middle part (TMP) in 20 µl of 0.3× SSC (1× 150 mM NaCl and 15 mM Na-citrate pH 7.0). The reaction was kept at 85°C for 2 min and then allowed to cool to room temperature at the rate of 1.2°C per min.

Tripartite ligation
LEP, each of the six different MPs and REP (2–10 pmol) were ligated in 50 µl for 16 h at 16°C using 400 U of T4 DNA ligase (New England Biolabs). Products were either gel isolated to remove shorter bipartite products or GFX purified.

Formation of Cy5-labeled perfectly matched DNA fragments
DNA fragments (155, 357, 543 and 857 bp) were amplified from pBR322 using a forward Cy5 primer and one of the four reverse Cy5 primers (Table 1). Reactions were identical to the end part PCR described above, except that annealing was at 56°C.

Migration velocity
Migration velocity was evaluated in PAGE, MDE and CSGE in 7 × 8 × 0.1 cm gels using the Mini-Protean II system (Bio-Rad) or in 18 × 24 × 0.1 cm gels using the Hoefer SE 660 system (Amersham Biosciences). Gels were stained for 10 min in electrophoresis buffer containing 1 µg/ml EtBr.

Polyacrylamide gels (6–15%) were made from a 29:1 acrylamide: bisacrylamide mixture (Amersham Bioscience) in 0.5× TBE. Electrophoresis was carried out at room temperature in 0.5× TBE at 20 mA until bromophenol blue (BB) migrated to the lower edge of the gel.

MDE gels (FMC BioProducts) were made from a 0.75× MDE solution and 0.6× TBE. Brief electrophoresis was performed at room temperature in 0.6× TBE at 20 mA for 5 min to load samples into the gel, followed by incubation for 10 min in 0.6× TBE with or without 1 µg/ml of EtBr. After incubation, gels were run in a Pharmacia Multiphor system (Amersham Bioscience) at 5, 20 or 35°C in the same direction as before at 5 mA in 0.6× TBE until BB migrated to the edge.

CSGE gels (10%) were prepared as previously described (26). Electrophoresis was carried out at 35°C in a 0.5× TTE buffer at 10 W until BB migrated two-thirds of the gel.

The migration velocities of DNA fragments were compared using the Conformation factor (C-factor) defined as the apparent migration velocity of perfectly matched DNA fragments divided by the apparent migration velocity of bulge-containing DNA fragments of the same length.

2D-CDE
First dimension. PAGE, MDE and CSGE gels were prepared as described above. A mixture of DNA fragments in ficoll (for CSGE) or glycerol gel-loading buffer was loaded in the second lane of each gel. The sample volume was 10–20 µl and the amount of DNA was 100–1000 ng. Vertical electrophoresis was then carried out and gels subsequently incubated with shaking for 10 min in 100 ml of the appropriate electrophoresis buffer containing 1 µg/ml EtBr.

Second dimension. Electrophoresis was carried out in a Multiphor system perpendicular to the first dimension at 5 W and 20°C using paper electrode wicks until BB migrated to the edge of the gel. The same buffer system was used as for the first dimension except that it contained 1 µg/ml of EtBr.

Fluorescent-labeled DNA fragments were analyzed using the fluorescence-scanning mode of a Typhoon 8610 variable mode imager (Amersham Biosciences).

Two-dimensional temperature-dependent electrophoresis
The same gel and buffer were used as previously described (19). The first dimension was carried out as described above.
Separation and enrichment of DNA fragments containing unmatched bulge from a complex mixture of perfectly matched DNA fragments

λ Phage DNA (dam+, dcm−) was purchased from MBI Fermentas. λ Phage DNA (10 μg) was digested for 60 min with 30 U of NdeII (Roche) at 37°C.

Digested λ phage was labeled by extension of overhangs [10 mg of DNA, 5 U of Klenow fragment (Fermentas), 100 mM unlabeled dNTPs (Fermentas), 100 mM Cy-labeled dCTP (Amersham Biosciences)] for 15 min at 30°C. The reaction was quenched by addition of 1 μl of 0.5 M EDTA. Labeling reactions were purified using a GFX PCR and Gel band purification kit.

Cy5 NdeII-digested λ phage DNA (800 ng) was mixed with a Cy5 tripartite DNA fragment containing 5C bulge (5 ng) and 3C bulge (5 ng). The DNA pool was separated using 2D-CDE in a 9% polyacrylamide gel (7 × 8 × 0.1 cm) and fluorescence scanned as described in ‘2D-CDE’. Using ImageQuant 5.1 (Amersham Biosciences), a multi-segmented string was aligned along the length of the arc of perfectly matched DNA. The width of the string was chosen to match the width of the DNA in the arc. Copies of the string were moved horizontally in steps of one-string width. A real size picture of the gel showing the parallel strings was placed underneath and the gel sliced, using the string boundaries as reference. Each slice was crushed in an Eppendorf tube, 400 μl of 0.05× TE was added, and DNA was eluted overnight at 4°C on a shaker (600 r.p.m.). The collected supernatant was reduced to ~20 μl using Savant Speedvac and analyzed on a 2% agarose gel.

RESULTS

Construction of 298 bp DNA fragments each containing a specific cytosine bulge near its center

To develop a method for conformation-dependent separation, it was necessary to construct long DNA fragments (~300 bp) with specific secondary structures that affect the conformation of the entire molecule. A semi-synthetic strategy for construction of tripartite DNA fragments (298 bp) was designed (Fig. 1). We constructed five fragments containing one defined cytosine bulge at the center of the fragment with bulge size ranging from 1 to 5 nt (1C–5C). An identical perfectly matched DNA fragment was prepared as a control. We evaluated purity and length of all fragments with agarose electrophoresis (Fig. 2A). As expected, all DNA fragments migrated at the same rate independently of the bulge size.

Migration velocity in PAGE of tripartite DNA fragments containing an unmatched bulge

DNA fragments containing a bulge have reduced migration velocity in PAGE compared with perfectly matched DNA fragments of the same length, and the reduction is dependent on the bulge size (2). Migration velocities of tripartite DNA fragments were evaluated in PAGE to validate formation of specific cytosine bulges (Fig. 2B). The DNA fragment containing the 1C bulge migrated slightly more slowly than the perfectly matched fragment. We identified a strong inverse correlation between bulge size in fragments containing 2–5 cytosine bulges and migration velocity in gels (Fig. 2B and Table 2). To enhance the migration differences between perfectly matched DNA fragments and DNA fragments containing bulges, we used an MDE gel matrix. Higher C-factors were obtained in MDE gels for DNA fragments containing large bulges (Table 2).

To define the effect of temperature and intercalators on altered migration, three DNA fragments (perfectly matched, 2C and 4C bulge) were analyzed in an MDE gel at three constant temperatures (4, 20 or 35°C), both with and without EtBr. Calculated C-factors revealed that the altered migration velocity of bulge-containing DNA fragments was almost independent of temperature (Table 3). In contrast, the effect of a bulge on migration velocity was strongly reduced by addition of the intercalator. In the presence of EtBr, bulge-containing DNA fragments migrated more consistently based on their length and with much less dependence on the size of the bulge. The difference in migration velocity, whether EtBr was present or not, was most pronounced for the fragment containing the larger 4C bulge.

These results allowed us to define conditions where DNA fragments either showed conformation-dependent migration,
or not, in a single gel matrix. Gels without EtBr allowed separation according to both length and conformation of each DNA fragment. Gels containing EtBr allowed separation mainly according to length. By combining these two conditions in 2D gel electrophoresis, one should be able to achieve conformation-dependent separation of DNA fragments containing an unusual conformation from a complex sample of heterogeneous DNA fragments.

2D-CDE

We tested this hypothesis using a mixture of DNA fragments with normal or altered conformation in a small MDE™ gel. The mixture included an HaeIII digest of φX174 DNA (11 perfectly matched DNA fragments) and a 298 bp DNA fragment containing a 3C bulge. One DNA fragment (281 bp) from the φX174 digest is known to show sequence-induced altered migration at 20°C (27) and should serve as an internal control for conformation-dependent separation. Two DNA fragments clearly migrated in front of the arc after 2D-CDE (Fig. 3). The slower migrating band was the 3C DNA fragment and the faster migrating band was the 281 bp DNA fragment from HaeIII-digested φX174. By increasing the time of second dimension electrophoresis, further separation was achieved (Fig. 3B–D).

We also ran two-dimensional electrophoresis on identical DNA mixtures where the only difference between dimensions was temperature (20°C in the first and 35, 50, 60 or 65°C in the second dimension). No separation was detected for the 3C bulge DNA fragment, but the 281 bp A-tract DNA fragment migrated in front of the arc under temperatures up to 60°C (data not shown). This result further confirmed the temperature dependency of the curvature associated with A-tracks.

2D-CDE was also performed in the large gel format using 10% PAGE. This system allowed separation of 2C–5C DNA fragments from perfectly matched DNA fragments (data not shown).

To further increase the resolution of 2D-CDE, we used CSGE matrix and buffers in a large gel format (1). Using this approach, we were able to separate tripartite and dipartite DNA fragments containing 1C–5C bulges from a mixture of perfectly matched DNA fragments (Fig. 4).

We adapted 2D-CDE for using fluorescent detection to increase the sensitivity and versatility of the method. This required a washing step after 2D-CDE to reduce background fluorescent detection from the intercalator. By using double-dye fluorescent detection we were able to visualize the separation of perfectly matched DNA fragments and 3C–5C DNA fragments (Fig. 5).

Table 3. Effect of temperature and EtBr intercalator on C-factors of bulge-containing DNA fragments

<table>
<thead>
<tr>
<th>Size of bulge in DNA fragment</th>
<th>4°C − EtBr</th>
<th>4°C + EtBr</th>
<th>20°C − EtBr</th>
<th>20°C + EtBr</th>
<th>35°C − EtBr</th>
<th>35°C + EtBr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>1.1</td>
<td>1.0</td>
<td>1.3</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>1.1</td>
<td>2.4</td>
<td>1.2</td>
<td>2.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Figure 3. Separation by 2D-CDE of a bulge-containing DNA (298 bp) and a curved DNA fragment (281 bp) due to an A-tract from perfectly matched HaeIII fragments of RF φX174. (A) First dimension electrophoresis (20°C in 0.75× MDE matrix) to separate DNA fragments according to both their length and conformation. (B–D) Second dimension electrophoresis perpendicular to the first dimension was carried out after addition of EtBr to separate mainly according to the length of the DNA fragments. Interim time points of 30 min (B), 45 min (C) and 60 min (D). Two DNA fragments were displaced in front of the perfectly matched DNA fragments. The DNA fragment labeled 1 contained a 3C bulge. The DNA fragment labeled 2 was a 281 bp DNA fragment from the HaeIII digest of the RF φX174 containing an A-tract.
Isolation of DNA fragments containing bulges from complex DNA samples

We also tested whether DNA fragments containing bulges can be efficiently separated from λ phage DNA. Digested λ phage DNA was mixed with 3C and 5C DNA fragment in an equimolar concentration. The mixture was separated using 2D-CDE in a small gel format (Fig. 6). The bulge-containing DNA fragments were displaced in front of the arc of digested λ phage DNA fragments. Quantitative analysis of the gel revealed several hundred-fold enrichment for both 3C and 5C DNA fragments (Fig. 7). To further demonstrate the potential of the system, we gel-isolated DNA from the arc and DNA displaced in front of the arc. These samples were analysed with agarose gel electrophoresis (Fig. 8). As expected, no DNA was detected in the segment excised from behind the arc, λ DNA was detected in the arc, and only bulge-containing DNA fragments in the gel segments excised from in front of the arc. These results showed the efficiency of the method for preparatory work.

DISCUSSION

In this paper, we describe a novel physicochemical method, 2D-CDE. The method allows separation of DNA fragments according to their conformation but independent of their length. The method should be a powerful tool for various tasks where length-independent separation and isolation of DNA fragments with altered conformation is needed. It is easy to carry out and requires only limited labor. Due to its simplicity, the method is reproducible and robust. The method does not require a specific instrument. All reagents are inexpensive and commonly used in the biosciences.

In principle, this method can be used to separate DNA fragments that have conformational changes that can be eliminated by addition of an intercalator. We showed that 2D-CDE can be used to separate and enrich DNA fragments containing bulges from complex samples of perfectly matched DNA.
DNA fragments (~50–2000 bp). 2D-CDE can also be used to separate DNA fragments containing an A-tract from a complex DNA mixture. The method may also separate DNA fragments containing other local sequences and secondary structures that are known to affect conformation of DNA, including UV lesions, GGCC sequences, cross-links, slipped-strand DNA, nicks and nucleotide gaps.

The resolution of 2D-CDE will vary according to the conditions used. A gel matrix such as the MDE allowed better conformation-dependent separation than a regular polyacrylamide gel matrix. In our experience, the highest resolution was achieved using CSGE conditions and a gel matrix in the first dimension. We were able to separate DNA fragments containing one nucleotide bulge using CSGE conditions from a mixture of perfectly matched fragments ranging in length from 155 to 857 bp. We have also detected an increased difference in migration of a DNA fragment containing single base differences, but the increase was not sufficient for two-dimensional separation (data not shown). We do not exclude the possibility of length-independent separation of single-base mismatches that we are currently pursuing is to combine enzyme treatment of DNA pools with 2D-CDE.

In this paper, we have shown that bulges as large as 5 nt can be separated using the 2D-CDE. For efficient separation of DNA fragment-containing microsatellite repeats from human DNA, it is important to be able to resolve larger bulges (typically 10–20 nt) The largest bulge we have tested to date was 18 nt in a 657 bp DNA fragment. This fragment separated well in 2D-CDE (unpublished results).

To eliminate or decrease differences in conformation between DNA fragments after the first dimension electrophoresis, we exploited interactions between an intercalator and DNA. Insertion of intercalating molecules causes elongation of the DNA duplex and local unwinding of the base pairs. Values for unwinding have been reported for many intercalators. They constitute an indirect measurement of conformation alterations of DNA (28). We reviewed previously reported values of 19 different intercalators where values ranged from 8 to 80° (29,30). We chose to use EtBr, which has an intermediate unwinding value of 26°. EtBr intercalates double-stranded nucleic acids with limited sequence specificity with the exception of poly(dA)–poly(dT) (31). It has been documented that EtBr shows higher affinity for double-stranded DNA-containing bulges than their perfectly matched counterparts (32–34).
Suppression of altered migration by adding EtBr into the gel matrix was achieved for all tested bulge-containing DNA fragments. We believe that intercalators showing unwinding values similar to or higher than EtBr are also potential candidates for various applications of 2D-CDE.

The effect of temperature on migration velocity was also tested. We found that relative migration of bulge-containing fragments compared with perfectly matched DNA fragment was essentially independent of temperature. The temperature independency of migration for bulge-containing DNA was confirmed further by analyses of a two-dimensional system similar to the one described by Mizuno (19) (unpublished results). This system uses temperature as a variable between dimensions and was reported to be efficient for separation of DNA fragments containing A-tracts from a complex DNA pool. Although in our experiments the temperature was raised up to 60°C in the second dimension, no separation was achieved for bulge-containing DNA. With the same system setup, it was enough to use 35°C in the second dimension to allow separation of DNA fragments containing A-tracts. We suggest that elevated temperature, e.g. 35°C, in the first dimension could be used to avoid 2D-CDE separation of DNA fragments with A-tracts (35).

 Genome mismatch scanning (GMS) was developed to circumvent the need to screen markers one at a time to identify genomic regions that are identical by descent (IBD). In theory, the method assays every polymorphic base between two genomes. Enrichment of perfectly matched heterohybrids is a key step in GMS. When GMS was applied successfully to yeast, DNA enrichment was achieved using the Escherichia coli mismatch repair proteins mut H, L and S (36). GMS has not been applied efficiently to the human genome and it has had limited application in genetics. 2D-CDE is a candidate method for such mismatch scanning due to its simplicity and robustness. Further, the current setup of 2D-CDE allows specific enrichment of bulge-containing fragments that are formed between highly polymorphic sequences such as microsatellite repeats. Bulge-containing heteroduplexes are presumably frequently co-isolated with the perfectly matched heterohybrids using the mut HLS system since mut S only interacts with single-base mismatches and bulges up to 4 nt (37). Larger bulges are not recognized.

2D-CDE can be used for various preparative and analytical tasks where separation according to conformation but independent of length can be exploited. An application we are currently pursuing is the separation of polymorphic DNA sequences from the human genome. We are also applying 2D-CDE for separation of DNA containing UV lesions from intact DNA and for insertion/deletion variation scanning in cDNA.

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