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# A new method for straightening DNA molecules for optical restriction mapping

**Hiroki Yokota, Forrester Johnson, Hongbo Lu, R. Maxwell Robinson, Anna M. Belu[1], Michael D. Garrison[1], Buddy D. Ratner[1], Barbara J. Trask and David L. Miller[2,*]**

Department of Molecular Biotechnology and [1]Center for Bioengineering, University of Washington, Seattle, WA 98195, USA and [2]Department of Electrical Engineering, The Pennsylvania State University, 121 Electrical Engineering East Building, University Park, PA 16802, USA

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## ABSTRACT

**We have developed an improved method of straightening DNA molecules for use in optical restriction mapping. The DNA was straightened on 3-aminopropyltriethoxysilane-coated glass slides using surface tension generated by a moving meniscus. In our method the meniscus motion was controlled mechanically, which provides advantages of speed and uniformity of the straightened molecules. Variation in the affinity of the silanized surfaces for DNA was compensated by precoating the slide with single-stranded non-target blocking DNA. A small amount of $MgCl_2$ added to the DNA suspension increased the DNA-surface affinity and was necessary for efficient restriction enzyme digestion of the straightened surface-bound DNA. By adjusting the amounts of blocking DNA and $MgCl_2$, we prepared slides that contained many straight parallel DNA molecules. Straightened λ phage DNA (48 kb) bound to a slide surface was digested by *Eco*RI restriction endonuclease, and the resulting restriction fragments were imaged by fluorescence microscopy using a CCD camera. The observed fragment lengths showed excellent agreement with their predicted lengths.**

## INTRODUCTION

The past several years have been marked by significant developments in DNA sequence localization techniques that involve the direct optical observation of individual DNA molecules. Improvements in resolution from ~3 Mb to 1–3 kb have been made by the use of techniques that uncoil and straighten DNA before analysis. (1–5). High-resolution mapping is exemplified by the process of 'optical restriction mapping' developed by Schwartz and colleagues (6–9). In this approach, Schwartz's group used flow forces to straighten and attach YAC and bacteriophage DNA to glass coverslips for direct observation of restriction enzyme digestion. A straight DNA molecule, stained with a fluorescing dye, can be seen under the fluorescence microscope as a bright straight line. Its length can be determined optically to an accuracy of better than a micron, which corresponds to ~3 kb. By measuring the position of the cut sites of surface-bound individual molecules or the fluorescence intensity of the fragments, an 'optical' restriction map could be generated rapidly. Optical mapping holds great promise for replacing gel electrophoresis in restriction fragment fingerprinting because both the order and the lengths of the restriction fragments are evident. In addition, the range of sizes that can be accurately quantified greatly exceeds the range that can be analyzed in a single agarose gel.

Optical mapping is most practical if the number of analyzable (i.e., intact, straight and digestible) molecules per slide is high. The accuracy of an optical map depends on reproducible and consistent straightening of each DNA molecule, so that there is a small variation in length per base-pair within each molecule and between molecules. Several methods for straightening DNA molecules have been reported. Chromatin can be straightened, albeit with a high degree of variability along and among molecules, by allowing the contents of lysed cells to flow down or spread across a glass slide (2,3). A variety of approaches to anchor and then straighten DNA molecules have also been devised (10–13).

Remarkably straight DNA has been achieved recently by the use of a moving liquid/air interface (meniscus) (5,14). Motion of the meniscus was generated by the evaporation of a DNA containing solution between a slide and coverslip. DNA molecules in the liquid assume a random-coil configuration, but some of those near to a suitably treated glass slide will attach to it by their ends. Fewer attach at internal positions, for reasons not understood. As the meniscus recedes, forces associated with the liquid surface tension uncoil the DNA molecules, and straightened molecules are deposited on the drying surface.

We report here a new method of producing straight, surface-attached DNA molecules that make suitable targets for optical restriction mapping. Our method uses mechanical movement of a meniscus to produce straight molecules more rapidly and reliably than the liquid evaporation technique used by Bensimon *et al.* (15). In this new procedure, a 2–5 µl volume of DNA solution is dispensed at the intersection of a straight-edged coverslip and a slide. A motor then pulls the coverslip and solution across the slide surface at a constant velocity of a few

*To whom correspondence should be addressed. Tel: +1 814 865 2730; Fax: +1 814 865 7065; Email: dlm15@psu.edu

centimeters per minute, and straightened DNA is deposited parallel to the direction of meniscus motion.

It is critical to control the properties of the slide surface in order to obtain straightened DNA reproducibly. If DNA binds too easily to the surface, the number of retained molecules will be high, but we have found that the fraction of straight molecules will be low. If DNA binds infrequently to the surface, the number of retained molecules will be low, but the fraction of straight molecules will be high. Treatment of the glass surface with silane compounds terminated with appropriate functional groups ('silanization') is known to increase DNA–slide affinity (8,16). The DNA–slide affinity is, however, sensitive to small variations in silanization process parameters such as cleanliness of the glass surface, pressure of the silane vapor, and the temperature during silanization. We have observed unacceptable variation among our silanized slides and have also observed a decrease in DNA–slide affinity with storage over a few months.

Because many batches of our silanized slides had an initial DNA–surface affinity that was too high for effective DNA straightening, we investigated the effects of precoating the slide with non-target, single-stranded DNA (blocking DNA) or nucleotides (dNTP) to reduce the affinity to an acceptable value. We also added $MgCl_2$ to the DNA solution during straightening to examine its effect on the conformation and retention of DNA molecules, and on the digestion rate of surface-bound DNA by restriction enzymes.

## MATERIALS AND METHODS

Our procedure for straightening and optically mapping DNA is shown schematically in Figure 1.

### Slide silanization

We cleaned microscope glass slides (3" × l" with frosted end, Richard-Allan Scientific) by immersing them in 2:1 $HNO_3$:HCl solution for 3 h at room temperature. Slides were rinsed extensively in deionized water (Millipore Ultra-pure water system) and dried with $N_2$ gas. Cleaned slides were silanized by exposing them in air to 5 µl of 3-aminopropyltriethoxysilane (APTES, A3648, Sigma) in a 3000 ml glass chamber at 100°C for 30 min. Silanized slides were stored in ambient air at room temperature for as long as several months.

### Slide surface characterization

Characterization of slide surfaces was done with atomic force microscopy (AFM) and electron spectroscopy for chemical analysis (ESCA). AFM was performed on a Nanoscope II scanning probe microscope (Digital Instruments, Santa Barbara, CA), with a V-shaped cantilever and standard square pyramidal silicon nitride tips. The tip was rastered relative to the sample in contact mode under ambient conditions at constant force (~10 nN). RMS surface roughness values were obtained from each quadrant of a 3 $\mu m^2$ image and averaged. X, y and z positioning of the sample was verified to ±5% using a calibration grid supplied by the manufacturer.

ESCA was performed on a Surface Science Instrument (SSI, Mountain View, CA) Xprobe ESCA instrument, utilizing an aluminum $K_\alpha$1,2 monochromatized X-ray source and a hemispherical energy analyzer. This instrument permits the investigation of the outermost 2–10 nm of up to 0.7 $mm^2$ surface area. Analysis

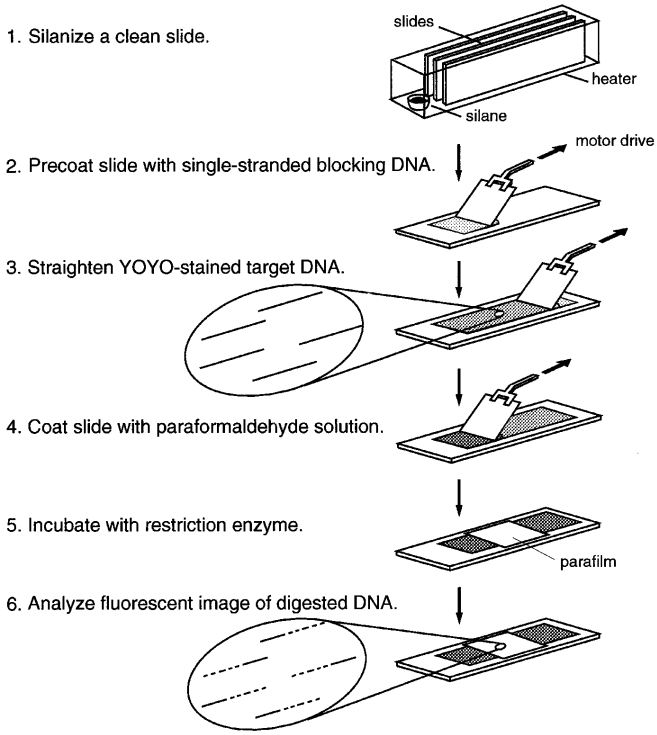


**Figure 1.** A schematic diagram illustrating the procedure for straightening and optical mapping single DNA molecules. For details, see Materials and Methods.

can be performed at high (25 eV pass energy) or low (150 eV pass energy) resolution. SSI data analysis software was used to calculate the elemental compositions from the peak areas and to peak fit the high resolution spectra. Typical pressures in the analysis chamber during spectral acquisition were $10^{-9}$ Torr.

### DNA preparation

λ Phage DNA (cat. no. 25250-010, GibcoBRL, Gaithersburg, MD) was suspended at 450 ng/µl in 10 mM Tris–HCl (pH 7.4), 5 mM NaCl, and 0.1 mM EDTA. A working DNA suspension was prepared (50 µl), containing 0.2 ng/µl λ DNA, 0.2 µM of the DNA dye YOYO-1 (Y-3601, Molecular Probes Inc., Eugene OR), and 0–2 mM $MgCl_2$, together with the remnants of stock ingredients (final concentration 4.4 µM Tris–HCl, 2.2 µM NaCl and 0.044 µM EDTA).

### DNA straightening

A motor-driven droplet-spreading apparatus was used to straighten target DNA. Prior to straightening the target (λ phage) DNA, 5 µl of blocking DNA (heat denatured human Cot-1 DNA cat. no. 15279-011, GibcoBRL, at concentrations ranging up to 100 ng/µl) or dNTP (an equal mixture of dATP, dCTP, dGTP and dTTP at concentrations ranging up to 1 mM each), was spread on the silane-functionalized glass slide. A droplet of this blocking DNA was applied at the intersection of a functionalized slide and an 18 mm square #1 coverglass (Corning). Wetting of the slide and coverslip surfaces caused a wedge-shaped meniscus to spread across the width of the slide. The coverslip, attached to the motor-drive and tilted at 45° to the slide surface, was dragged

across the slide surface at 3 cm/min. Five µl of λ (target) DNA suspension was then applied on the glass slide in the same way.

### Restriction endonuclease digestion

Prior to endonuclease digestion, 5 µl of a 1% paraformaldehyde (cat. no. 19200, Electron Microscopy Sciences, Fort Washington, PA) solution in water was applied over the straightened DNA. The straightened surface-bound λ DNA was then digested by *Eco*RI restriction endonuclease (cat. no. 15202-120, GibcoBRL). Twenty µl of a solution containing ~10 U *Eco*RI in 1× restriction buffer (final concentration 50 mM Tris–HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithioerythritol) was dispensed onto the DNA-coated glass slide, and the solution was covered by a 22 mm square of parafilm. The slide was incubated for 1 h in the dark at 37°C. The parafilm was then gently removed, and ~5 µl of a glycerol- and phenyldiamine-based antifade solution (17) was applied under a glass coverslip.

### Optical image analysis

YOYO-l-stained double-stranded target DNA was imaged by an aircooled 1035×1316 pixel CCD camera (model TEA/CCD-1517-K/1, Princeton Instruments, Inc., Trenton, NJ) through a Zeiss Axiophot fluorescence microscope equipped with a 100×, 1.3 NA Plan Neofluor objective. The width of one pixel corresponded to 0.065 µm in the specimen. Exposure times were 5 s. CCD-images were analyzed using IP Lab Spectrum software (version 2.5.7, Signal Analysis Corporation, Vienna, VA). From 5 to 30 images, each covering a 67 µm × 86 µm field of view, were analyzed to derive estimates of the relative retention of molecules and the fraction that was straight. A molecule was designated as 'straight' if a rectangle of aspect ratio ~20:1 would completely enclose its image.

## RESULTS

### Mechanically-driven meniscus motion for rapid DNA straightening

We have found that the moving-meniscus method of straightening DNA first reported by Bensimon *et al.* (14) can be successfully applied at a meniscus speed as large as several cm per minute. At this speed, hydrodynamic forces in the droplet remain too low to break DNA molecules. Our meniscus motion is much faster than the evaporation-driven motion of Bensimon's method.

We have used a simple motor-driven linear motion apparatus to move the meniscus formed at the intersection of a silanized slide and an untreated glass coverslip (Fig. 1). The constant speed of the straight-edged meniscus formed between the slide and coverslip results in the uniform deposition of a large number of DNA molecules over most of the slide. Varying the menicus speed in the range from 0.5 cm/min to 5 cm/min did not affect the population of DNA molecules significantly (data not shown), and so the speed of the meniscus was fixed at 3 cm/min.

### Characterization of APTES-treated slide surfaces

AFM and ESCA were used to characterize the surface of three batches of silanized slides to test for parameters that might correlate with optimal DNA stretching. Table 1 shows the qualitative suitability of slides for DNA retention and stretching as determined by fluorescence microscopy, the percent surface nitrogen detected by ESCA, and the RMS surface roughness measured by AFM. Slide batch A50 had been stored in air for ~10 months prior to characterization, while batches A208 and A209 had been stored less than a month. AFM images of the silanized glass surfaces revealed a large number of sub-micron island-like features that were markedly different in morphology and height from any features on the glass or silicon controls (data not shown). The largest number of these features appeared to be present on batch A209, less on A208, but very few on A50. We observed that the slide batch with the largest number of islands (A209) had the highest affinity for DNA, which led to its unsuitability for DNA without compensation. No direct correlation between the RMS surface roughness and the stretching suitability was noted.

**Table 1.** Slide surface properties and suitability for DNA stretching

| Slide silanization batch | Suitability without blocking DNA or $MgCl_2$ | | ESCA surface nitrogen (atomic %) | rms roughness (nm) |
|---|---|---|---|---|
| | retention | stretching | | |
| control glass | 0 | ++ | 1.1 | 0.8 ± 0.3 |
| control silicon | 0 | not observed | not observed | 1.0 ± 0.3 |
| A50 | + | +++ | 3.5 | 0.5 ± 0.3 |
| A208 | ++ | ++ | 2.2 | 19.8 ± 1.0 |
| A209 | +++ | 0 | 5.5 | 15.8 ± 0.7 |

The elemental composition of the samples as measured by ESCA revealed surface species of carbon, oxygen, nitrogen, silicon, sulfur, sodium and trace amounts of chlorine, calcium and magnesium. The concentration of these species varied from sample to sample, especially the trace salts. Increased surface nitrogen was noted on the silanized glass, consistent with the formation of an amino-silane overlayer. However, no correlation was seen between the amount of nitrogen and the stretching suitability. The large variation in surface morphology and chemical composition of the samples prepared under the same preparatory conditions suggested that further treatment was necessary to allow reproducible stretching of DNA molecules. Below, we demonstrate the use of blocking DNA to compensate for surface variability.

### Adjustment of APTES-treated slide surface properties with single-stranded blocking DNA or dNTP and $MgCl_2$

We found that it is possible to straighten a few λ DNA molecules by moving a meniscus across a clean glass slide that has not been chemically functionalized. The retention of DNA molecules is poor, however, and the straightened DNA is easily washed off in an aqueous solution (data not shown). In order to improve retention, we coated glass slides with APTES by exposing the slides to silane vapor as described in Materials and Methods. We also tested a second silane compound, 7-octenyltrichlorosilane, but APTES was used for further studies because it resulted in a higher retention of DNA molecules (data not shown).

The improved retention of DNA molecules on the APTES-silanized surface resulted in the DNA assuming a variety of conformations including some straightened molecules, but many that were folded, looped and irregularly coiled (Fig. 2A). Precoating the slide after silane functionalization with single-stranded 'blocking' DNA or dNTP reduces the retention of DNA on the slide surface, but increases the number of straight DNA molecules. Figure 2B illustrates the conformation of straightened

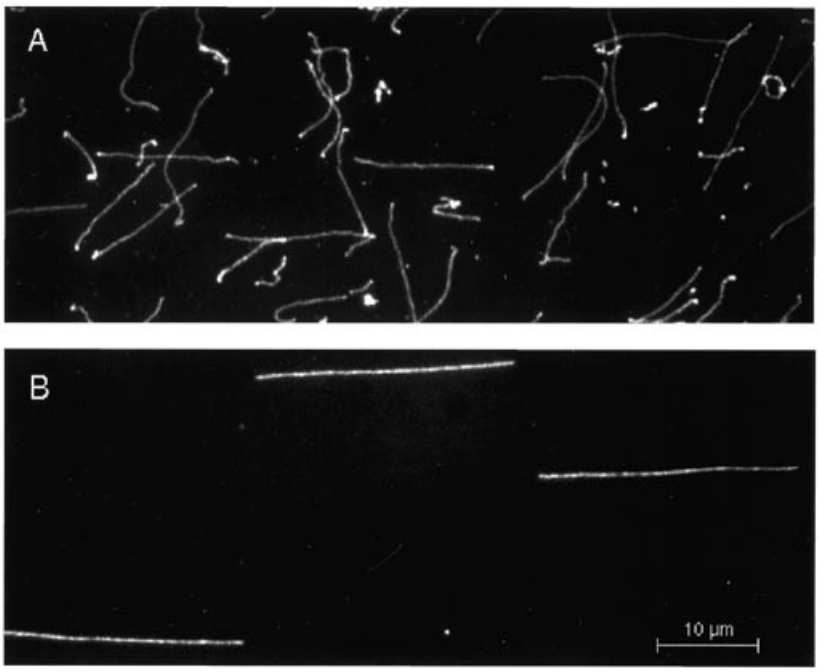


**Figure 2.** Effects of blocking DNA precoating and $MgCl_2$ on straightening λ phage DNA (48 kb). λ DNA was deposited on an APTES-functionalized glass slide from batch A209 after precoating single-stranded human Cot-1 DNA. Double-stranded λ DNA was stained with YOYO-1, but the single-stranded blocking DNA was not stained by this dye. DNA images were collected by a CCD camera mounted on a Zeiss Axiophot fluorescence microscope using a 5 s exposure time. (**A**) Poorly-straightened λ DNA when no Cot-1 DNA was applied on the surface prior to straightening. (**B**) Well-straightened λ DNA on a silanized glass slide from the same slide batch precoated with 100 ng/µl Cot-1 DNA.

DNA molecules obtained on slides (silanization batch A209) coated with heat-denatured (i.e., single-stranded) human Cot-1 DNA. The number of retained molecules decreased (Fig. 3A), but the fraction of straightened molecules increased markedly (not shown) with increasing amounts of blocking DNA. The number of straightened molecules depends on both the amount of blocking DNA applied and the presence of $MgCl_2$ in the DNA solution (Fig. 3B). The highest number and fraction of straightened DNA molecules occurred for this batch of silanized slides at 100 ng/µl blocking DNA with the use of $MgCl_2$. The slides used for these experiments had a relatively high affinity for DNA prior to the addition of blocking DNA. Other slides with a lower initial affinity needed less blocking DNA to optimize the number of straightened molecules. A mixture of dNTP can be used in place of Cot-1 DNA.

The addition of $MgCl_2$ to the DNA buffer prior to straightening also increased the retention of DNA on slides precoated with blocking DNA at concentrations of 10 and 100 ng/µl (Fig. 3A) while maintaining the trend of increasing percentage of straight molecules with increasing concentration of blocking DNA. An elevated retention of DNA in the presence of $MgCl_2$ was observed for slides functionalized by either silane. The addition of $MgCl_2$ also resulted in slightly shorter straightened DNA (length reduction ~10% for 2 mM $MgCl_2$ compared with no $MgCl_2$) and a wavy conformation on a micron scale (not shown) in contrast with the smooth conformation of DNA straightened without $MgCl_2$ (Fig. 2B).

## Restriction endonuclease digestion of straightened surface-attached λ DNA

We found DNA straightened by our method to be a good substrate for optical mapping. λ DNA cut by the restriction endonuclease *Eco*RI yields six fragments of size 21, 4.9, 5.6, 7.4, 5.8 and 3.5 kb, in this order. The observed restriction fragments from two preparations are shown in Figure 4. In Figure 4A, λ DNA was straightened on a slide from batch A208 with low initial DNA affinity and which therefore required no precoating with blocking DNA to reduce the affinity to an acceptable value. On slide preparation A209 (Fig. 4B), the slide initially had excessive DNA affinity and therefore required precoating with 100 ng/µl blocking DNA. For both preparations, 0.5 mM $MgCl_2$ was added to the λ DNA suspension.

We found that the addition of $MgCl_2$ to the DNA buffer prior to straightening was necessary for *Eco*RI activity on surface-bound DNA. The smallest concentration tested, 0.5 mM, led to enzyme activity sufficient to cleave most of the straightened DNA during the 1 h incubation. DNA straightened with no $MgCl_2$ in the straightening buffer were seldom cleaved during the same incubation time, despite the presence of $Mg^{++}$ ions in the standard restriction buffer. We determined the cutting efficiency for a 1 h digestion by averaging the percentage of cleaved cut sites per DNA molecule (the observed number of cuts out of the five possible cuts) on a separate slide batch using 0, 0.5 and 1.0 mM $MgCl_2$. The blocking DNA precoating was adjusted for each $MgCl_2$ concentration to give good DNA straightening. With no $MgCl_2$ added to the λ DNA suspension (no blocking DNA precoating of the slide), the cutting efficiency was 17% (n = 50, mean cut number per molecule = 0.86, SD = 1.0). When 0.5 mM $MgCl_2$ was added to the DNA suspension (slide precoated with 50 ng/µl blocking DNA), the cutting efficiency was 73% (n = 50, mean cut number per molecule = 3.64, SD = 0.92). With 1 mM $MgCl_2$ added to the DNA suspension (slide precoated with

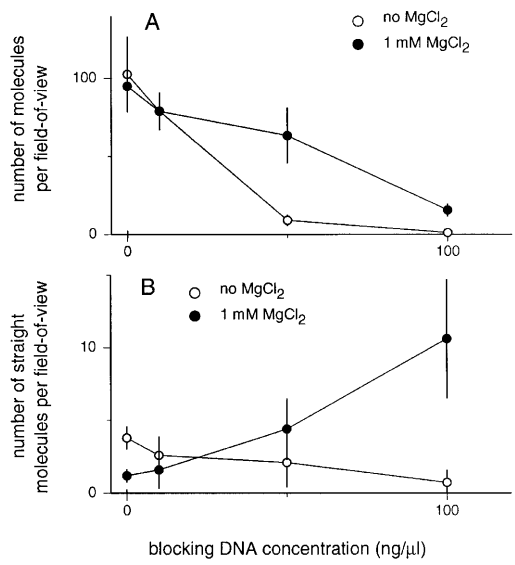


**Figure 3.** (**A**) Effect of blocking DNA and $MgCl_2$ on DNA retention on slides from silanization batch A209. The mean number of deposited DNA molecules per field of view was calculated from up to 30 CCD images at 0, 10, 50 and 100 ng/μl blocking DNA. Open circles correspond to the absence of $MgCl_2$ in the λ DNA suspension, and closed circles correspond to 1 mM $MgCl_2$. Error bars represent the standard deviation. At 100% retention, on average 131 molecules would be deposited in the field of view. (**B**) Effect of blocking DNA and $MgCl_2$ on DNA straightening. We determined the number of straightened DNA molecules which were fixed to the surface. The number of straightened DNA molecules increased for the sample with $MgCl_2$. The percentage of straightened DNA molecules increased as the concentration of blocking DNA increased both with and without $MgCl_2$ in the λ DNA suspension (not shown).

100 ng/μl blocking DNA), the cutting efficiency was 45% (n = 50, mean cut number per molecule = 2.24, SD = 1.36).

We also found that mild fixation of the DNA to the slide prior to enzyme digestion was necessary to avoid random loss of restriction fragments. Applying a 1% paraformaldehyde solution over the straightened DNA helped to maintain attachment of the restriction fragments to the slide. Figure 4C shows a gallery of digested DNA molecules from slide preparation A209.

For each slide preparation, five molecules, each containing all six restriction fragments, were analyzed. The mean fragment length was measured between the centers of cut-site gaps in the optical image, and was converted to kb by equating the sum of the fragment lengths to 48 kb. There is a good linear relationship between the observed and predicted lengths for all fragment sizes, despite the small sample size. The slope of the linear best fit between the observed and predicted fragment sizes was 0.996 and 0.997 and the square of the correlation coefficient ($r^2$) was 0.994 and 0.997 for preparations A208 and A209, respectively (Fig. 4A and B).

## DISCUSSION

We have developed a new method for straightening DNA molecules and binding them to a surface for optical restriction mapping. The reproducible generation of an *Eco*RI restriction map of λ DNA demonstrates the method. Features that differentiate our method and results from those previously described by others include the use of a mechanically-moved meniscus to rapidly straighten the DNA, and regulation of the surface retention and straightness of DNA molecules by a precoat of single-stranded blocking DNA or dNTP and the addition of $MgCl_2$ to the solution of double-stranded target DNA.

Our method can theoretically straighten DNA of any size, because the straightening force generated by surface tension is independent of the length of the DNA molecule (15), and the meniscus speed can be limited to minimize hydrodynamic forces that might break the DNA. In addition to the 48 kb λ phage DNA shown here, we have successfully straightened yeast chromosomes of ~300 kb (H.Y. and D.M., unpublished). Furthermore, since the motion of the meniscus is driven by a linear motor at a constant speed of a few cm/min, hundreds of DNA molecules are straightened in a few minutes. In contrast, the evaporation approach used by Bensimon *et al.* (15) and Weier *et al.* (5) typically takes several hours, and even longer in a humid environment.

For our method, each restriction fragment which results from enzyme digestion typically remains straight and attached to the slide, allowing its length to be accurately determined from its image. Even small segments, <6.5 kb in length, have image lengths which are proportional to their length in kb, as seen by the good fit for the shorter fragments in Figure 4. This is similar to the work of Meng *et al.* (7) on polylysine-treated glass surfaces, who reported that fragment lengths estimated on the basis of restriction fragment image size was consistent over their entire range from 0.8 to 28 kb. In contrast, lengths determined from fluorescent intensity were systematically undersized for short fragments <5.1 kb long in their work.

Our technique allows us to start with slides which have been functionalized to have too high an affinity for DNA to give good straightening and then to adjust the affinity downward with blocking DNA to a point where the surface concentration of straightened DNA molecules is maximized. In this way, we can compensate for batch-to-batch variations in slide preparation. The result is a field of DNA molecules which are uniformly distributed across the slide and aligned parallel to each other. These features makes them attractive targets for automated imaging and pattern recognition. Such automation would make it possible to collect information from many DNA molecules and restriction fragments in a very short time. The large sample number combined with the spatial information available with optical mapping would allow the accurate reconstruction of long maps even in the face of incomplete digestion.

There is as yet no detailed model of the chemistry and microscopic physical arrangement of the DNA-surface attachment process and of enzyme action on cut sites in surface-attached DNA. Therefore, our approach to DNA stretching and surface attachment for optical mapping has been empirical. We have observed an uncontrolled variation in the DNA binding properties of silanized surfaces. We speculate that the chemical state of the surface nitrogen may play a role in DNA binding, because a comparison of the high resolution C1s spectra indicated a possible correlation between the amount of amide functionality (rather than amine) and stretching suitability. However this result requires further investigation. Here we show that by varying the concentration of single-stranded DNA or dNTP in precoating the silanized slide, surface properties can be modified so that double-stranded target DNA can be efficiently straightened. We have observed that APTES-silanized slides gradually lose affinity for DNA molecules over a period of several months. Adjusting the concentration of blocking DNA is one solution to control the temporal variation in affinity as well as the variation among

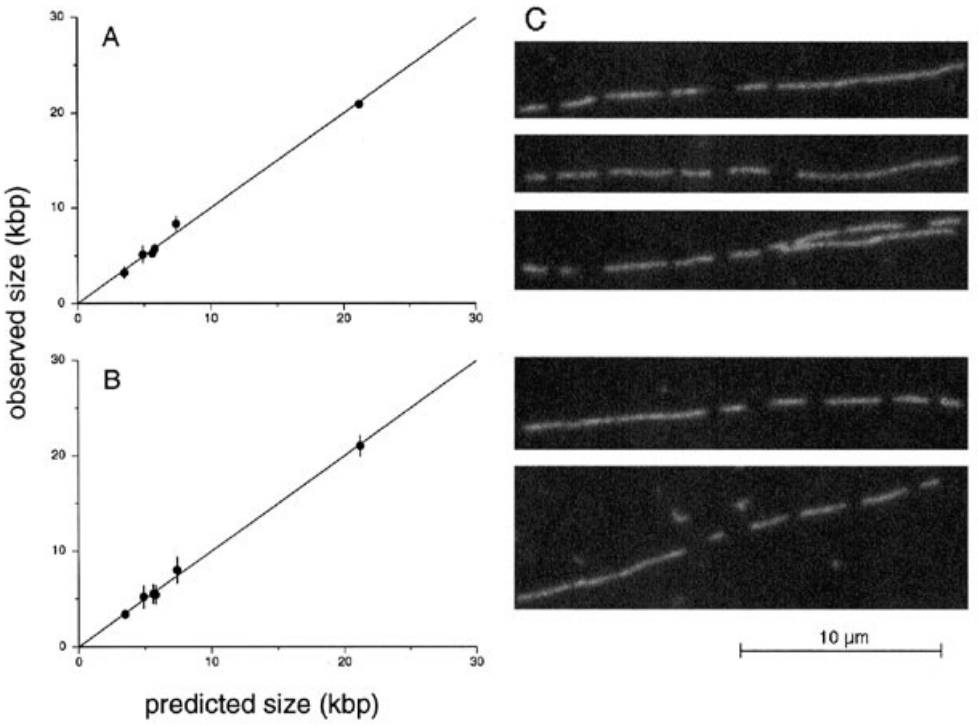


**Figure 4.** *Eco*RI endonuclease restriction fragments of straightened surface-attached λ DNA. The stretched DNA was digested by *Eco*RI, and CCD images were collected with 5 s exposure time. (**A**) Comparison of the observed fragment size to the predicted size (batch A208). Fragment sizes were determined based on the center-to-center distance of restriction cut sites. Error bars represent standard deviation from five molecules. The slope for the best fit is 0.996 with $r^2 = 0.994$. (**B**) Reproducible correlation between the observed size and the predicted size using a different slide preparation (batch A209). The slope for the best fit is 0.997 with $r^2 = 0.997$. (**C**) Gallery of five DNA molecules digested by *Eco*RI (batch A209). A complete digestion would generate six restriction fragments of 21.2, 4.9, 5.6, 7.4, 5.8 and 3.5 kb in size in order.

preparations. We show also that the binding of DNA to the slide can be further modified by the addition of $MgCl_2$.

Although the variables for best DNA straightening must be adjusted empirically for each batch of silanized slides, we recommend the following conditions as a good starting point: silanize clean glass slides using APTES for 30 min at 100°C, precoat silanized slides with single-stranded DNA at 100 ng/ml, straighten target DNA at 0.2 ng/ml in 0.5 mM $MgCl_2$, and postcoat 1% paraformaldehyde solution over straightened DNA prior to enzyme digestion. After observing the results of this starting-point preparation, one can then adjust the concentration of single-stranded blocking DNA to optimize the number of straight molecules on subsequent slides from the same silanization batch. A higher concentration will decrease the total number of attached molecules, but increase the fraction of straight molecules. Although we used denatured human Cot-1 DNA as blocking DNA because of its availability in our laboratory and its size range (>50% of human Cot-1 DNA runs as a broad band between 72 and 310 bp according to the suppliers), other cheaper sources of single-stranded DNA (e.g., sonicated denatured herring-sperm DNA, tRNA, dNTPs) should also be suitable.

Although we have found conditions for enzyme cutting which seem to be reliable from slide to slide within a batch of slides once the surface affinity has been adjusted by blocking DNA to give good straightening, it may be possible to improve the cutting efficiency further by optimizing the balance of blocking DNA, $MgCl_2$ and paraformaldehyde. Both $MgCl_2$ and the DNA-binding properties of the slide, which can be modified by blocking DNA, affect the degree of extension of the target DNA molecules. The degree of extension and the microscopic conformation of the surface-bound DNA may be factors which affect restriction enzyme activity. We observed that DNA straightened on our surfaces without the use of $MgCl_2$ is not readily cut with *Eco*RI and that the more easily-cut DNA straightened with $MgCl_2$ was less straight on a micron scale than DNA straightened without $MgCl_2$. Because the degree of extension and the conformation of the DNA is probably related to the spacing of DNA-surface bonds, and because these bonds may affect enzyme recognition of cut sites, it is reasonable to expect extension and conformation to influence the efficiency of cutting.

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