Reverse Southern hybridization

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ABSTRACT

A DNA oligomer 25 nucleotides long which contained an HMT (4'-hydroxymethyl-4,5',8-trimethylpsoralen) furan side monoadduct to thymidine at a 5'-TpA-3' site was used as a probe for the polylinker sequence present in single-stranded M13 mp19 DNA and in double-stranded pUC 19 DNA. Hybridization and photofixation were carried out simultaneously in solution under conditions approximating the melting temperature of the probe-target hybrid. Use of probe concentrations greater than 10^{-8} M permitted hybridization times of a few minutes. Irradiation with near ultraviolet light converted the HMT monoadduct present in hybrid complexes into an interstrand crosslink. Efficient photofixation removed hybrid from the equilibrium distribution and resulted in the formation of additional probe-target complexes. After removal of excess probe by centrifugation through a semi-permeable membrane (Centricon-30), samples were electrophoresed through an alkaline agarose gel which was analyzed by autoradiography. When using an HMT-modified 25-mer probe end-labeled with 3,000 Ci/mmole 32P, 0.015 ng (3.8 x 10^6 copies) of M13 DNA could be detected. With this same probe 10 μg of denatured human DNA (corresponding to 3.0 x 10^6 copies) did not give a signal.

INTRODUCTION

Several years ago Southern developed a widely used technique for hybridizing a labeled probe to fractionated patterns of DNA immobilized on a membrane support (1). This approach has several drawbacks including long hybridization times and low levels of target coverage. In this communication we report the use of a model crosslinkable DNA oligonucleotide probe which permits hybridization to be conducted in solution prior to a denaturing electrophoretic separation. This Reverse Southern procedure eliminates the transfer and fixation steps of the original protocol, is much more rapid, and achieves high levels of target coverage with negligible background.

The ability to electrophorese a probe-target hybrid in a denaturing gel requires the existence of a covalent crosslink between the two strands. DNA probes which contain a furan side HMT monoadduct at a TpA site are ideal crosslinking agents (2). The presence of the psoralen monoadduct is not believed to interfere with base pairing and modified probes behave similarly to their unmodified counterparts as regards the kinetics and specificity of hybridization (3) and the stability of the hybrid (4). Crosslinkage of the probe-target hybrid is conveniently effected by irradiation with 320-400 nm light. The resultant complex has little
absorption in the near ultraviolet and does not undergo further photochemistry. The crosslinkage reaction is absolutely dependent upon the monoadduct residing in a base paired helix and in fact provides an additional tier of discrimination. Once formed, the crosslink is stable to a variety of denaturing agents including high temperature, alkali, formamide, and urea.

Use of crosslinkable oligomeric probes permits hybridization to be carried out in solution using high concentrations of probe. It is recommended that both annealing and photofixation be carried out concurrently at the melting temperature of the respective probe-target hybrid (5). At that temperature discrimination between correct and incorrect sequences is optimal, secondary structure in the target is minimized, and kinetics are rapid. Photofixation of the hybrid drives the equilibrium to give high target coverage. This process is referred to as photochemical pumping. With denatured double-stranded DNA, photofixation also prevents displacement of the probe by subsequent target renaturation. Once hybridization and photofixation have been effected, removal of excess probe as well as gel electrophoresis can be carried out under denaturing conditions, leading to a minimization of background. In this communication, we describe several experiments conducted with a HMT-monoadducted 25-mer which illustrate the above points.

MATERIALS AND METHODS

HMT-Monoadducted 25-mer

Preparation of this oligonucleotide will be described in detail elsewhere (3). Briefly, a few μg of the 13-mer, 5'-GCTCGGTACCCCGG-3', were partially kinased with a limiting amount of 3,000 Cj/m mole γ-32P-ATP. After heat denaturation of the kinase, the 13-mer was hybridized to a complementary 8-mer (5'-GGGTACCG-3') and irradiated with 320-400 nm light at 4°C in the presence of 30 μg/ml HMT (HRI Associates), 100 mM NaCl, 10 mM MgCl2, and 10 mM Tris-HCl, pH 7.0. The HMT crosslinked 13-mer/8-mer complex was isolated by electrophoresis on a 0.4 mm thick 7 M urea-20% polyacrylamide gel and photoreversed by a brief exposure to a short wavelength ultraviolet lamp. HMT-monoadducted 13-mer [5'-GCTCGGT(HMT)ACCCCGG-3'] was isolated by electrophoresis on another thin denaturing 20% polyacrylamide gel. The modified 13-mer was converted to a 25-mer by ligating a 12-mer (5'-CAGTGAATTGA-3') onto the 5' end. The oligomers were oriented for T4 DNA ligase action by annealing to a complementary template oligomer (5'-ATCCCGGGTACCGAGCTCGAA-3'). Overall yield starting out with 1 mCi of 32P-end labeled 13-mer was approximately 107 cpn of HMT-modified 25-mer. Two-thirds of the modified 25-mer contained a furan side HMT monoadduct and one-third contained a pyrone side HMT monoadduct; only the former is competent to go on to crosslink (6).

Denatured Human DNA

Human placental DNA (Sigma; 200 μg) was restricted overnight with 2,000 units of Eco R1 in a 2 ml volume. The reaction mixture was phenol extracted and then concentrated to
120 µl and the solvent exchanged to 2 mM Tris-HCl, pH 7.0, 0.1 mM EDTA using Centricon-30's (Amicon). The solution was further reduced in volume to 12 µl in a Speedvac (Savant) resulting in a DNA concentration of 10 µg/µl. The DNA was denatured by heating at 100°C for 5 min followed by quick cooling in ice.

Dot Blot Hybridization

Standard methodology was followed (7-9). DNA was denatured by incubation in 0.5 M NaOH for 10 min at 37°C. Following denaturation, the solution was cooled to 1-2°C in an ice bath and neutralized to pH 10-12 by the addition of an equal volume of 2.0 M ammonium acetate pH 7.0. Samples were transferred to either nitrocellulose or nylon membranes using a Schleicher & Schuell "Minifold" micro-sample filtration manifold. After transfer, nitrocellulose membranes (BA85 from Schleicher & Schuell and Hybond-C from Amersham) were allowed to air dry and then placed in a vacuum oven for 4 hr at 80°C while nylon membranes (Nyttran from Schleicher & Schuell and Hybond-N from Amersham) were irradiated on an ultraviolet transilluminator (West Coast Scientific, Model WC-510) for 5 min while still wet and then allowed to air dry. All blots were stored desiccated at 4°C.

Prehybridization was carried out in 0.9 M NaCl, 0.09 M Tris-HCl, pH 7.5, 5 mM EDTA, 0.1% SDS, 0.5% Nonidet P-40, and 100 µg/ml "homochromatography mix" (randomly cleaved tRNA; 10) for 1-3 hr at the hybridization temperature. After the addition of probe, hybridization was allowed to continue 24 hr unless otherwise indicated in the aforementioned hybridization buffer. One or more wash steps were carried out in aqueous formamide, in 1X SSPE (0.18 M NaCl, 0.01 Na₂HPO₄, pH 7.0, 1 mM EDTA) or in 6X SSC (0.9 M NaCl, 0.03 M sodium citrate, pH 7.0). Blots were visualized by autoradiography and quantified by excising the dots from the membrane with a hole punch and counting them in the presence of scintillation fluid.

Solution Hybridization and Photofixation

Hybridizations were conducted in 100 mM NaCl, 10 mM Tris-HCl, pH 7.0, 1 mM EDTA (1X NTE) containing 35% deionized formamide at 45°C. Siliconized 1.5 ml Eppendorf tubes were used to hold 5-10 µl volumes per sample. Crosslinkage was carried out under identical conditions with 400 mW/cm² of 320-400 nm light. The light source (Model A5000, Photon Technology International) contained a 0.5 kW Hg/Xe arc lamp positioned within an elliptical mirror. Broad band 320-400 nm light was obtained using a 9 cm water filter, a 0.6 cm pyrex filter, and a 9 cm aqueous 1.7% cobaltous nitrate--2% sodium chloride filter (11).

Removal of Excess Probe

Two washing protocols based on the use of Centricon-30 cartridges (Amicon) were developed. For quantitative removal of free probe under denaturing conditions, each photofixed sample was diluted with 0.5 ml of hot (65°C) 0.05% SDS, 0.1 M phosphate, pH 4.0, 50% formamide and centrifuged 10 min at 6400 rpm in an SS34 rotor. This procedure
was repeated a total of eight times. To liberate entrapped target nucleic acid from the membrane, the Centricon-30 cartridge was placed in a sonicator bath (Branson, Model 221) for 3 min both midway through and at the conclusion of the washing protocol. The retentate was combined with two 0.5 ml rinses of the membrane and counted by Cerenkov. Counts represented crosslinked probe-target hybrid free of excess probe.

The second Centricon-30 wash procedure was used prior to gel electrophoresis to remove 98-99% of free probe from photofixed samples under nondenaturing conditions. In this workup the samples were diluted with 2 ml of 0.05% SDS, 0.1 M sodium phosphate, pH 4.0 and centrifuged as before for 10 min. This was repeated a second time after which the sample was diluted with 1 ml of 0.05% SDS, 1 mM sodium phosphate, pH 4.0 for a final centrifugation. At this stage 200 µl of water was added to the retentate and the solution briefly vortexed. The diluted retentate was recovered as usual except that 100 µl of the 1 mM phosphate wash solution was spun through the reverse side of the membrane to liberate entrapped hybrid. Each sample had a final volume of ~350 µl.

**Gel Electrophoresis**

Photofixed hybridization samples containing less than $10^5$ cpm of free probe were supplemented with 6-8 µg of digested tRNA (homochromatography mix), up to 100 ng of M13 carrier DNA, supplemental deionized formamide to give a total formamide volume of 10 µl, and indicator bromphenol blue and xylene cyanol dyes. The samples were taken to water dryness in a Speedvac and briefly heated at 37°C prior to loading onto a 5.5 x 8.0 cm 1% alkaline agarose gel. Gels were electrophoresed for 2-2.5 hr at 40 V in 30 mM NaOH, 1 mM EDTA. Samples which contained 10 µg of denatured human DNA were loaded in 25 µl volumes onto a 11.2 x 14 cm 1% alkaline agarose gel which was run at 30 V for 15 hr with continuous circulation of running solution between the electrode chambers. For autoradiography, the gels were dried down onto filter paper (Hoefer, Model SE540 gel dryer) and exposed to Kodak XAR-5 X-ray film. Intensifying screens (Dupont, cronex lightning plus) were used where indicated.

**Data Analysis**

To facilitate comparison of individual experiments, the data were processed to eliminate the effect of $^{32}$P decay on the concentration and specific activity of the probe. Thus the kinetic and cycling data correspond to results which would have been obtained using 3,000 Cj/mmole probe. We emphasize that the target coverage data reported here are subject to possible errors in terms of the absolute amount of target present at the time of reaction, the actual specific activity of the probe at the reference date, and the extent to which probe-target hybrid is lost during workup. Although coverage values are internally consistent within an experiment, there is considerable variance in values between experiments. Cerenkov cpm were multiplied by a factor of two to obtain dpm.
RESULTS AND DISCUSSION

Immobilization of Target DNA

Both dot blot and Southern blot hybridizations utilize single-stranded target, usually fixed onto a nitrocellulose or nylon membrane. The efficiency with which nucleic acid is transferred and immobilized onto a solid support and the degree to which it is retained during the hybridization and wash steps are important factors in determining the signal. Any comparison of target coverage using membrane versus solution hybridization formats must take into consideration the above factors. Therefore, we have quantified the efficiencies of fixation and retention of labelled pBR322 DNA on both nitrocellulose and nylon membranes using commonly accepted protocols in model dot blot hybridizations.

The data in Table 1 indicate that approximately 50% of the nick-translated plasmid DNA was retained by nitrocellulose following baking while 90-100% was bound to nylon following ultraviolet irradiation with a 305 nm light. After hybridization, however, the overall loss from nitrocellulose was somewhat less than from nylon (retention of 10-11% versus 7-9%). This trend held following a wash step with 6-8% of the labelled DNA remaining on nitrocellulose and only 2-3% remaining on nylon. Thus nylon gives a higher initial loading but a lower overall retention following hybridization and washing. Better results were obtained using 2.0 μg of calf thymus DNA (photochemically labelled with tritiated 5-methylisopsoralen) as the target. In this case 72% of the DNA was retained on nitrocellulose following fixation, 23% following a mock hybridization (1 hr at 45ºC in hybridization buffer containing 25% Table 1. Immobilization of pBR322 DNA on Nitrocellulose and Nylon Membranes.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Following fixation</th>
<th>%Immobilized* Following hybridization</th>
<th>Following washing</th>
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<tr>
<td>Nitrocellulose</td>
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<tr>
<td>BA85</td>
<td>52</td>
<td>11</td>
<td>8</td>
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<tr>
<td>Hybond-C</td>
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<td>Hybond-N</td>
<td>98</td>
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Nick-translated pBR322 DNA was immobilized to nitrocellulose or nylon in the presence of 20 ng of carrier M13 mp19 viral DNA. Annealing was conducted at 40ºC for 36 hr in hybridization buffer and was followed by a 5 min wash in 6X SSC at 100ºC. Duplicate samples were run for each point.

*Defined as the % cpm remaining of the total cpm originally applied to the membrane.
formamidine), and 16% following a denaturing wash (25 min at 45°C in aqueous 66% formamidine).

Our data indicate that only 10-20% of the target nucleic acid remains on the filter in a typical dot blot protocol. Because of these losses, target coverage in most membrane based hybridization assays is at best only 10-20% of the theoretical value. With oligonucleotide probes, actual coverage may approach only 1-3% based upon target input. For example, when 25 ng of M13 mp19 viral DNA was probed with 1 x 10^-8 M unmodified 25-mer in a conventional dot blot assay (annealed for 24 hr at 45°C C in hybridization buffer containing 25% formamidine followed by two 20 min washes at 45°C C in 1X SSPE), a signal corresponding to only 0.8% coverage of the input target or 8.0% of the retained target was obtained. This result indicates that the majority of the retained M13 target sequences were inaccessible to the probe. This inaccessibility reflects a combination of factors including fixation induced damage to the target DNA, interaction of the target with the membrane, and the existence of secondary structure in the immobilized target.

The loss of immobilized nucleic acid from a filter has been observed by others (12-15) and has prompted the development of alternative fixation procedures. For example, with chemically activated paper, the nucleic acid is covalently bound. However, the binding capacity of these matrices is relatively low and the fixation procedure is more complicated than with other types of filters (16). Furthermore, transfer is not necessarily complete (17). Simpler procedures (18,19) which irreversibly bind the target nucleic acid to nylon or Whatman 541 paper should give improved signals when used in blot hybridization.

**Solution Hybridization to M13 mp19 DNA**

Secondary structure in single-stranded target nucleic acid can be a significant barrier to solution hybridization with oligonucleotide probes (3,20,21). This structure can be minimized by conducting the hybridization at or near the melting temperature of the probe-target hybrid. If the oligomer contains a crosslinkable furan side HMT monoaduct the hybrid can be photofixed and removed from the equilibrium distribution. Reestablishment of equilibrium generates additional probe-target hybrid which can also be photofixed. Provided the kinetics of hybridization and crosslinkage are sufficiently fast, continuous irradiation can generate high levels of target coverage through "photochemical pumping." These same conditions also reduce background.

Figure 1 shows the formation of a crosslinked hybrid between single-stranded M13 DNA and HMT-modified 25-mer under conditions which met the criteria for efficient photochemical pumping. Both hybridization and photofixation were carried out concurrently in solution near the melting temperature of the probe-target hybrid. We estimate that temperature to be 48°C in 1X NTE-35% formamidine for 6.5 x 10^-8 M probe (3,5). In this particular experiment the half-life for hybridization was predicted to be 7 sec based on a binary rate constant of 1.5 x 10^6 M^-1 sec^-1 (3). Calibration of the light source had previously indicated
Figure 1. Continuous photochemical pumping of the hybridization equilibrium between HMT-modified 25-mer and single-stranded M13 mp19 DNA. Annealing and photofixation were carried out at 45°C in 1X NTE with 35% formamide. Irradiation was preceded by a 1 min preincubation at 45°C. Aliquots of 5 µl were removed after the indicated times of irradiation for analysis by Centricon-30. [M13 DNA] = 2.08 x 10^{-9} M. [Probe] = 6.48 x 10^{-8} M. Each data point corresponds to the signal given by 25 ng M13 DNA.

that crosslinkage of existing probe-target complexes proceeded with a half-life of just a few seconds and that single-stranded HMT-monoadducted probe photoreversed with a half-life of 15 min. The biphasic curve obtained in Figure 1 is believed to reflect hybridization of the probe first to readily accessible target sequences and then to those sequences which are masked by secondary structure. After 8 min of irradiation, 57% of the target polylinker sequences were crosslinked to the 25-mer. This level of coverage represents approximately a three fold enhancement of the equilibrium value for target coverage by the furan side HMT-monoadducted probe in the absence of photochemical pumping (3).

It is noteworthy that the coverage obtained with continuous irradiation is similar to that obtained when irradiation is broken up into 0.5 min periods interspersed by 30 min dark periods (Figure 2). The similarity of coverage suggests that 40% of the target M13 sequences are involved in stable secondary structure interactions. Brief exposure to high temperature would be expected to redistribute some of these sequences into more accessible conformations capable of interacting with the probe.

Solution Hybridization to pUC 19 DNA

An additional factor comes into play when an oligomeric probe is hybridized to denatured double-stranded DNA in solution. Unlike DNA immobilized on a membrane,
Figure 2. Non-continuous photochemical pumping of the hybridization equilibrium between HMT-modified 25-mer and single-stranded M13 mp19 DNA. Annealing and photofixation were carried out at 45° C in 1X NTE with 35% formamide. Each cycle consisted of a 0.5 min light period followed by a 30 min dark period. Aliquots of 5 μl were withdrawn after each irradiation for analysis by Centricon-30. [M13 DNA] = 2.08 x 10^{-9} M. [Probe] = 6.38 x 10^{-8} M. Each data point corresponds to the signal given by 25 ng M13 DNA.

Figure 3. Photochemical entrapment of the hybrid between HMT-modified 25-mer and denatured double-stranded pUC 19 DNA. Annealing and photofixation were carried out at 45° C in 1X NTE with 35% formamide. In the first cycle, the reaction mixture was immersed in a 100° C water bath for 3 min, followed by a quick cooling to 45° C and a 2 min irradiation period. In subsequent cycles the 100° C heat jump was reduced to 1 min. After each irradiation an aliquot of 5 μl was removed for analysis by Centricon-30. [pUC DNA] = 1.13 x 10^{-9} M. [Probe] = 6.84 x 10^{-9} M. Each data point corresponds to the signal given by 10 ng pUC DNA.

Watson and Crick strands can renature in solution. Should this occur, the oligomeric probe would be displaced from the target sequence by the longer complementary target strand. Utilization of short crosslinkable probes at a concentration one hundred fold greater than the
Figure 4. Detection of single-stranded M13 mp19 DNA with a high concentration of HMT-modified 25-mer probe. A concentration series of M13 DNA was hybridized and photofixed to HMT-modified 25-mer by irradiating each sample for 8 min at 45°C in 1X NTE with 35% formamide. The samples were then processed through Centricon-30 and electrophoresed through a 1% alkaline agarose gel. The autoradiogram was obtained after a 4 day exposure at -70°C with no screen. The M13 concentration series consisted of (lane 1) 5.0 ng, (lane 2) 2.5 ng, (lane 3) 1.0 ng, (lane 4) 0.50 ng, (lane 5) 0.25 ng, (lane 6) 0.10 ng, (lane 7) 0.05 ng, and (lane 8) 0.025 ng, each in a 5 μl volume. [Probe] = 6.56 x 10^-8 M. Target coverage was approximately 60%.

target concentration should lead to rapid formation of the desired metastable hybrid which in turn can be photochemically trapped by crosslinkage (5).

Hybridization of HMT-modified 25-mer to denatured FIII pUC 19 DNA provided a test for the efficiency of photochemical entrapment. In Figure 3 the formation of probe-target hybrid is plotted versus the number of irradiation cycles. In this experiment each 2 min irradiation period was preceded by a brief heat denaturation step. The irradiation was conducted near the melting temperature for the probe-target hybrid using a sixty fold molar excess of 25-mer. After five such cycles target coverage approached 15%. It is not clear why hybridization reached a plateau after three cycles. An attractive explanation is that repeated heat denaturations, which resulted in a gradual loss of water, increased the formamide concentration to a level which prevented the 25-mer from hybridizing.

We have attempted to hybridize the HMT modified 25-mer to denatured FI pUC 19 DNA without success. This DNA, like other supercoiled DNAs, is irreversibly denatured by transient exposure to high pH (22). The irreversibility of this process has been attributed to the loss of register between two interlocked strands. This interpretation is consistent with our
Figure 5. Detection of single-stranded M13 mpl9 DNA in the presence of denatured human DNA with a high concentration of HMT-modified 25-mer probe. A concentration series of M13 DNA, each in the presence of 10 µg of denatured Eco R1 restricted human DNA, was hybridized and photofixed to HMT-modified 25-mer by irradiating each sample for 4.0 min at 45°C in 1X NTE with 35% formamide. The samples were processed as in Figure 5. The autoradiogram was obtained after a 3 day exposure at -70°C with an intensifying screen. The M13 concentration series consisted of (lane 2) 1.0 ng, (lane 3) 0.25 ng, (lane 4) 0.50 ng, (lane 5) 0.10 ng, and (lane 6) 0 ng, each in a 5 µl volume containing 10 µg of human DNA. Lane 1 is a control which contains 1.0 µg of M13 DNA photofixed to probe in the absence of any human DNA, 10 µg of which was added prior to electrophoresis. [Probe] = 7.6 x 10^-6 M. Estimated target coverage was 50%.

results. Secondary and tertiary interactions in denatured form I DNA are heightened by proximity effects thereby making the target sequence totally inaccessible to the 25-mer.

Reverse Southern Hybridization

The sensitivity of the Reverse Southern protocol utilizing M13 viral DNA as a target is demonstrated in Figure 4. In this experiment the target DNA was hybridized and photofixed to HMT-modified 25-mer near the melting temperature of the probe-target hybrid. The resultant autoradiogram shows a faster moving band which is single-stranded linear DNA and a slower moving band which is single-stranded circular DNA. After four days of autoradiography
0.015 ng of phage DNA could be detected. This corresponds to 3.8 x 10^6 copies of the target sequence and, at 60% coverage, represents approximately 10 cpm. The sensitivity obtained with the crosslinkable 25-mer probe in a Reverse Southern protocol is approximately seven fold greater than that obtained with a similarly labeled unmodified oligomer when used in a slot blot format with immobilized M13 DNA (9). No signal was obtained when an M13 mp8 viral DNA concentration series was employed as the target, thus confirming the specificity of hybridization with the HMT-modified 25-mer (data not shown).

As a test of the discrimination possible with a Reverse Southern, HMT-modified 25-mer was hybridized and photofixed as described above to a concentration series of M13 mp19 DNA, each in the presence of 10 µg of Eco R1 restricted and heat denatured human DNA. An autoradiogram of the denaturing gel is shown in Figure 5. With a 3 day exposure it was possible to detect 0.1 ng (2.5 x 10^7 molecules) of target M13. Based on the relative masses of viral to human DNA present at the time of hybridization, this corresponds approximately to the detection of a gene present eight times in a sample of human genomic DNA. The absence of signal when probe was hybridized to human DNA alone (Figure 5, lane 6) indicates that human DNA does not contain a complement to the 25-mer probe and that the Reverse Southern protocol generates a minimum level of background due to incorrect hybridization or to nonspecific binding. Signal present near the top of the gel is M13 dependent and probably represents entrapment of labeled viral DNA in polymeric human sequences at the well.

The prototype Reverse Southern protocol described here has obvious advantages in time, sensitivity, and convenience over traditional methodology. By employing a totally denaturing electrophoretic scheme and a short oligomeric probe, it is anticipated that the presence of an attached oligomer on a large single-stranded restriction fragment will not affect its electrophoretic mobility; thus that parameter will provide accurate molecular weight information above a certain size range. A related approach based on the stability of DNA-RNA hybrids has recently been reported (23). However, it does not employ a totally denaturing gel and may not give accurate molecular weight estimates. The oligomer restriction method originated by Saiki et al. (24,25) permits solution hybridization with short probes. Analysis consists of the restriction of probe-target hybrid followed by electrophoretic detection of the cleaved probe. This novel approach shares many of the advantages we have observed with crosslinkable probes but it does not provide information on the size of the target. The direct-gel hybridization technique described by Schinnick et al. (26) for long probes and by Studencki and Wallace (27) for short probes eliminates the transfer and fixation steps of a traditional Southern blot by carrying out hybridization in the gel. With oligonucleotide probes, however, secondary and tertiary structure in the target nucleic acid can significantly reduce the sensitivity of this method (27).

In addition to the advantages described earlier, crosslinkable oligomeric probes may
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facilitate the post-electrophoretic processing of labeled bands. For instance, a crosslinked probe can not only serve as a primer for complementary strand synthesis but can also be photoreversed off the target by 254 nm light. Once it becomes possible to directly synthesize DNA oligomers containing a furan side psoralen monoadduct, routine hybridizations which make use of probes of known sequence will be able to take advantage of the Reverse Southern protocol.

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