Journal of Chromatography, 559 (1991) 237–246
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 519

Low-cost, high-sensitivity laser-induced fluorescence detection for DNA sequencing by capillary gel electrophoresis

DA YONG CHEN, HAROLD P. SWERDLOW[a], HEATHER R. HARKE, JIAN ZHONG ZHANG and NORMAN J. DOVICHI*

Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2 (Canada)

ABSTRACT

A low cost, 0.75-mW helium neon laser, operating in the green region at 534.5 nm, is used to excite fluorescence from tetramethylrhodamine isothiocyanate-labelled DNA fragments that have been separated by capillary gel electrophoresis. The detection limit (3σ) for the dye is 500 ymol [1 yoctomole (1 ymol) = 10^{-24} mol] or 300 analyte molecules in capillary zone electrophoresis; the detection limit for labeled primer separated by capillary gel electrophoresis is 2 zmol [1 zeptomole (1 zmol) = 10^{-21} mol]. The Richardson–Tabor peak-height encoded sequencing technique is used to prepare DNA sequencing samples. In 6% T, 5% C acrylamide, 7 *M* urea gels, sequencing rates of 300 bases/hour are produced at an electric field strength of 200 V/cm; unfortunately, the data are plagued by compressions. These compressions are eliminated with addition of 20% formamide to the sequencing gel; the gel runs slowly and sequencing data are generated at a rate of about 70 bases/hour.

INTRODUCTION

In 1977, Sanger *et al.* [1] reported a powerful technique for DNA sequencing based on the dideoxynucleotide chain-terminating reaction. A set of radioactively labeled DNA fragments is generated in four reactions and separated in adjacent lanes of a high-resolution polyacrylamide gel. Detection is by use of autoradiography. The sequence is interpreted from a series of alternating bands in the lanes corresponding to the terminal base.

An advance in sequencing technology occurred in 1986–87 when Smith *et al.* [2], Ansorge *et al.* [3] and Prober *et al.* [4] reported DNA sequencers that replaced the radioactive labels and autoradiography in Sanger *et al.*'s method with fluorescent labels and laser-based detection. In Smith *et al.*'s and Prober *et al.*'s automated sequencers, one of four fluorophores is associated with the terminating dideoxynucleotide either through the use of four separate dideoxynucleotide reactions with four

[a] Visiting scholar from the Department of Human Genetics, University of Utah, Salt Lake City, UT 84132, USA.

0021-9673/91/$03.50

labeled primers that are pooled before use [2] or through the use of fluorescently labeled dideoxynucleotides in a single reaction mixture [4]. In the method of Ansorge *et al.* [3], a single fluorescent label is used with each dideoxynucleotide chain terminating reaction and the products are run on separate lanes of a slab gel. These automated fluorescence sequencers have been commercialized by Applied Biosystems, Pharmacia, and DuPont; as an example, the Applied Biosystems instrument runs sixteen lanes simultaneously on a slab gel to produce sequencing rates of 75 bases/h per lane or 1200 bases/h per slab. Similar sequencing rates are produced by the other instruments. Sequence may be determined, by use of computer algorithms, to about 450 bases, and is limited by the resolution of the gel.

In 1990, Tabor and Richardson [5], and independently Ansorge *et al.* [6] reported a sequencing technique based on a single fluorophore; by varying the amount of dideoxynucleotide in a single reaction mixture, the terminal base is identified with a particular fluorescence peak height during separation in a single lane of an acrylamide gel. This technique relies on uniform labeling of the reaction product through the use of the manganese–T7 polymerase reaction. In the work that has been published, separation is by slab-gel electrophoresis, a fluorescein-labeled primer is excited with an argon ion laser at 488 nm and emission is detected in a single spectral band.

Gel-filled capillaries have attracted interest for DNA sequencing because their high surface-to-volume ratio provides excellent heat transport properties, allowing the use of very high electric field strengths. Early work with capillary-dimension electrophoresis media was performed by Edström [7,8], who described the use of very fine cellulose fibers (5 μm diameter) for the electrophoresis of nucleic acids from single cells at an electric field strength of 125 V/cm. In 1965, Matioli and Niewisch [9] studied hemoglobin from single cells on fine polyacrylamide fibers of 50 μm diameter. Grossbach [10] reported in 1974 the use of 50-μm diameter gel-filled glass capillaries. Larger scale capillary polyacrylamide gel electrophoresis was reported by Neuhoff *et al.* [11] in 1970, who used 5-μl capillaries for the study of ribonucleic acid polymerase. In 1983, Hjerten [12] reported the use of a 150-μm I.D. capillary polyacrylamide electrophoresis separation of several samples, including monomers through pentamers of bovine serum albumin. More recently, Karger and co-workers [13–16] have reported capillary polyacrylamide gel electrophoresis for the separation of proteins, chiral amino acids, polyadenisoic acid fragments and fluorescently labeled products of a single DNA sequencing reaction. Several patents have been issued that describe the use of a bifunctional reagent to bind polyacrylamide chemically to the capillary surface [17–19]. Swerdlow and Gesteland [20] described DNA sequence analysis by use of capillary polyacrylamide gel electrophoresis with on-column fluorescence detection. The separation of a single reaction mixture in gel-filled capillaries was reported by Drossman *et al.* [21]. When operating at 400 V/cm, the system produced sequencing rates of 1000 bases/hour after elution of the primer, a *ca.* 25 times higher sequencing rate per lane than that produced by conventional slab gel electrophoresis. Luckey *et al.* [22] reported a four-color sequencing system based on the reaction products in the ABI sequencing system.

Laser-induced fluorescence detection in slab-gel electrophoresis requires low detection limits, typically in the order of 1–100 amol of each fragment [2,3]. This detection performance is complicated for both Smith *et al.*'s [2] and Prober *et al.*'s [4] techniques because the fluorescence signal must be measured in several spectral chan-

nels. However, the detection requirement in capillary gel electrophoresis is more severe. Based on a comparison of the cross-section of a capillary with the cross-section of a loading well in a slab gel, Drossman *et al.* [21] estimated that the sample loading in capillary gel electrophoresis will be in the order of 1–10 amol. Detection limits in the zeptomole range (1 zeptomole = 10^{-21} mol = 600 molecules)[a] are necessary for DNA sequencing by capillary gel electrophoresis. This task is complicated further by the requirement that the detection volume be less than 10^{-10} l to preserve the separation efficiency of the nano-scale electrophoresis technique. Swerdlow and Gesteland's [20] system used an on-column laser-induced fluorescence detection with detection limits of 200 zmol of fluorescently labeled product. Smith and co-workers [21,22] described the use of one- and four-channel on-column fluorescence detectors; both the single-and four-channel detector produced detection limits of about 100 zmol of labeled fragment. We have reported [24] the use of a post-column fluorescence detector in the gel electrophoresis separation of the products of a single chain-terminating reaction; the detection limit was 10 zmol.

This research group has developed three DNA sequencers based on capillary gel electrophoresis [25]. The first of these sequencers is based on the Applied Biosystems set of four fluorescently labeled sequencing primers. An on-column detector produced a detection limit of 200 zmol for each primer. Sequencing rates of 300 bases/h are produced at an electric field strength of 150 V/cm for samples up to 550 bases in length. The second detector is based on the DuPont set of four fluorescently labeled dideoxynucleotides. A sheath-flow cuvette post-column detector produced a detection limit of 20 zmol for labeled dideoxynucleotide triphosphate. Sequencing rates of 1000 bases/h are produced at an electric field of 465 V/cm. The third of these sequencers is based on the Richardson–Tabor–Ansorge sequencing method.

This paper describes our detector for the single color sequencer based on the Richardson–Tabor–Ansorge sequencing method. Details are also given on the separation of fluorescently labeled DNA fragments in acrylamide–urea gels and acrylamide–urea–formamide gels.

EXPERIMENTAL

Zone electrophoresis

A 48 cm × 10 μm I.D. capillary (Polymicro Technologies, Phoenix, AZ, USA) was used for the separation. The buffer was 5 m*M* borate (pH 9.0)–10 m*M* sodium dodecyl sulfate (SDS); the SDS was added to eliminate peak tailing in the electropherogram. Samples were injected electrokinetically for 5 s at 1 kV, corresponding to 30 pl of sample. The electropherogram proceeded at a potential of 30 000 V. The photomultiplier was operated at 1200 V and a 0.1-s time constant electronic filter was used to condition the data. The sheath stream flow-rate was 0.24 ml/h. The data were displayed on a strip-chart recorder. The standard deviation of the background signal was obtained by recording 100 data points at 1-s intervals; the order of magnitude

[a] Zepto (2) = 10^{-21} and yocto (4) = 10^{-24} were accepted as prefixes by the Comité International des Poids et Mesures in October 1990 to be put forward to the Conférence Générale des Poids et Mesures for adoption in October 1991 [23].

difference between the sampling rate and the electronic time constant ensures that there is minimal correlation between successive data points.

DNA sample

A nested set of fluorescently labeled DNA fragments was kindly provided by C. Fuller of the United States Biochemical Corporation. The sequencing reaction was carried out in 40 m*M* 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.5)–50 m*M* NaCl–10 m*M* $MnCl_2$–15 m*M* sodium isocitrate. Dye-labeled primer (Applied Biosystems 21M13 TAMRA, 1.6 p*M*) was annealed to 1 μg of M13mp18 single-stranded DNA at 65°C for 2 min followed by slow cooling. A mixture of deoxy- and dideoxynucleotide triphosphates was added to give an average nucleotide ratio (dNTP/ddNTP) of 1200:1 with 7-deaza-2′-deoxyguanosine-5′-triphosphate used in place of dGTP. The ratios of nucleotides were adjusted to yield a nominal peak-height ratio of 8:4:2:1 for T, G, C and A, respectively. The mixture was warmed to 37°C and 6 units of Sequenase Version 2.0 and 0.006 units of pyrophosphatase were added. Incubation was continued at 37°C for 30 min, after which the DNA was precipitated with ethanol. The sample was resuspended in 2 μl of formamide containing trace EDTA and injected at 150 V/cm for 30 s. After injection, the sample was replaced with a fresh vial of 1 × TBE [1.08% tromethamine (Tris), 0.55% boric acid, 0.07% EDTA in 100 ml water]. The separation proceeded at 150 V/cm. The sheath stream was 1 × TBE at a flow-rate of 0.16 ml/h.

Gel electrophoresis

When the gel-filled capillary was operated at high electric field strength, the gel migrated about 100 μm from the detection (positive electrode) end of the capillary. This migration is different from the well known migration of the gel from the injection (negative electrode) end of the capillary caused by electroosmosis. The migration from the detection end, presumably due to electrostriction, is undesirable because the gel can block the laser beam which is positioned a few micrometers beyond the tip of the capillary. To eliminate this migration, the gel was covalently bound to the wall of the detection tip of the capillary; the last 5 mm of the detection end of the capillary was dipped for a few seconds into a 0.5% (v/v) solution of γ-methacryloxypropyltrimethoxysilane in a water–acetic acid solvent (1:1). The gel was poured within 15 min of treatment of the capillary tip.

Two gels were used in this study. A 6%T, 5%C[a] polyacrylamide–7 *M* urea gel was covalently cross-linked to the last 5 mm of the detection end of the capillary of the 31 cm × 50 μm I.D. × 190 μm O.D. capillary; this gel was operated at 200 V/cm. A highly denaturing 6%T, 5%C polyacrylamide–7 *M* urea–20% formamide gel was covalently cross-linked to the last 5 mm of the 31 cm × 50 μm I.D. × 190 μm O.D. capillary; this gel was operated at 150 V/cm.

Detector

A 0.75-mW helium–neon laser beam (543.5 nm) (Melles Griot, Ontario, Canada) was focused with a 5× microscope objective about 200 μm below the exit of the

[a] %T = g acrylamide + g Bis per 100 ml of solution; %C = %Bis in T.

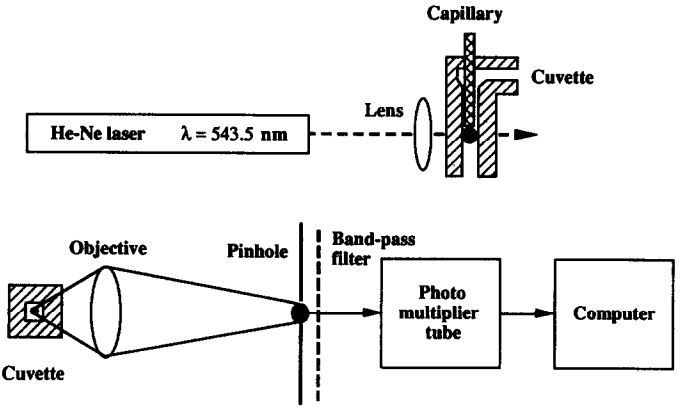

Fig. 1. Optical diagram. See text for description of the instrument.

capillary with a 200-μm square flow chamber and 2-mm thick windows (Fig. 1). Fluorescence was collected at right-angles with an 18×, 0.45 numerical aperture objective (Melles Griot) and imaged onto a 0.8-mm diameter pinhole. A single interference filter (590 nm center, 40 nm band pass, Model 590DF35; Omega Optical, VT, USA) was used to block scattered laser light. Fluorescence was detected with a Hamamatsu (CA, USA) R1477 photomultiplier tube, operated at 1000 V and cooled to −25°C with a Products for Research (MA, USA) photomultiplier (PMT) cooler. The PMT tube output was passed through a 1-s RC low-pass filter, digitized by a computer and treated with a 21-point quadratic–cubic polynomial filter before display. A similar optical system was used for an on-column fluorescence detector, except that an OG-570 colored glass filter (Melles Griot) was added to the collection optics to decrease the background signal. Data were recorded at 0.4-s intervals for conventional gel data and 0.5-s intervals for formamide data.

RESULTS AND DISCUSSION

Detection

The high-sensitivity capillary gel electrophoresis detector is based on a post-column sheath flow cuvette. In this detector, similar to that used in flow cytometry, the sample flows as a narrow stream in the center of a 200-μm square flow chamber, surrounded by a sheath stream consisting of conducting buffer. The high optical quality windows of the cuvette produce at least two orders of magnitude less light scatter than does an on-column detector. Also, by transferring the analyte to the moving sheath stream, the linear velocity, and hence the illumination time of the analyte, is independent of fragment length. The extent of photobleaching, which depends on illumination time, is constant for all analytes. We have reported the use of a single spectral channel fluorescence detector based on the sheath flow cuvette for capillary zone electrophoresis separation of zeptomole amounts of fluorescently labeled amino acids and for capillary gel electrophoresis separation of zeptomole amounts of the products of sequencing reactions [24–28]; Keller and co-workers

[29,30] have reported high-sensitivity detection for neat solutions of highly fluorescent dyes. Recently, Keller's group [31] reported, and Mathies' group [32] confirmed, detection of single phycoerythrin molecules in neat solution with a single spectral channel fluorescence detector.

The argon ion laser (488, 514.5 nm) is used by most investigators for excitation of fluorescence in capillary electrophoresis. However, the laser is rather expensive (*ca.* US$ 10 000) and requires forced air cooling. An interesting alternative excitation source for fluorescence excitation is the helium–neon laser (the so-called Gre–Ne laser) operating in the green region at 543.5 nm. The laser is inexpensive (*ca.* US$ 1000) and features the same construction as the conventional red helium–neon laser. Six conventional red helium–neon lasers have been in operation in this laboratory for 6–8 years with no tube failures; the green helium–neon laser should have a similar lifetime. The laser produces a low output power (0.75 mW) with excellent spatial coherence and good noise characteristics. Finally, the beam is easily focused to a 10-μm radius spot for fluorescence applications.

Tetramethylrhodamine isothiocyanate (TRITC) is well suited for application in laser-induced fluorescence detection. The molecule has a molar absorptivity of about 85 000 l mol^{-1} cm^{-1} at the green helium–neon laser wavelength. Keller [33] reported that the molecule has a fluorescence quantum yield of 15% and a photodestruction yield of 5×10^{-6}; under conditions of complete photobleaching, the molecule is expected to produce about 30 000 fluorescent photons, a factor of four greater signal than produced by fluorescein [34].

A sheath flow cuvette is used as a post-column fluorescence detector to minimize background light scatter. Further reduction in the background signal comes from the relatively long excitation wavelength and low-power excitation beam (750 μW). With this detector, the major contribution to the background signal was dark current produced by the photomultiplier tube. This contribution to the background signal is minimized by cooling the photomultiplier tube to -25°C.

The detection limit of this detector for TRITC was determined in a zone electrophoresis system. The relative standard deviation ($n = 5$) in peak height of a 1.28×10^{-10} *M* TRITC solution was 10%. Fig. 2 shows a zone electropherogram of a 4-zmol injection of analyte corresponding to 2300 analyte molecules. A linear calibration curve ($r = 0.986$) was constructed from $6.4 \cdot 10^{-11}$ to $2.56 \cdot 10^{-10}$ *M* TRITC. The detection limit, three standard deviations above the background signal, was 300 analyte molecules or 500 ymol (1 yoctomole = 1 ymol = 10^{-24} mol) [23]. Note that the data were not conditioned beyond that provided by the 0.1-s time-constant filter,

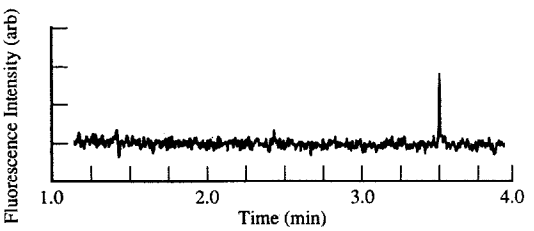

Fig. 2. Capillary zone electropherogram of 4 zmol (2300 molecules) of tetramethylrhodamine isothiocyanate.

appropriate digital filtering would undoubtedly improve the detection performance. Also, the use of a higher power laser would undoubtedly improve the detection limits further.

The major components used in the experiment (laser, high-voltage power supply, optical breadboard, sheath flow cuvette, photomultiplier tube power supply, and PMT cooler) have a total cost of about US$ 8000. It is possible to construct a simple, modest cost instrument with excellent analytical performance.

In our one-color DNA sequencer, a tetramethylrhodamine-labeled primer is excited by a green helium–neon laser (543.5 nm). The standard deviation in the background signal for this system corresponds to 700 ymol of labeled primer introduced onto the capillary; detection limits are, by definition, a factor of three higher (1200 molecules). The difference between these detection limits and the zone electrophoresis system probably reflects the low sample migration rate produced in the gel electrophoresis system; detection limits are degraded by dilution of the sample in the cuvette during the transit time from the capillary exit to the illumination volume. Detection limits improve for higher molecular weight fragments that undergo less diffusion in the cuvette and are more concentrated in the illumination volume. As a result, the detection limit for the system improves for larger DNA fragments that are typically produced in lower concentration compared with early eluting fragments. These detection limits, produced by a very low power laser, are associated with the excellent spectral properties of the tetramethylrhodamine fluorophore and reflect the simple detector design required by the single-color sequencing technique.

An on-column fluorescence detector was constructed for gel electrophoresis; a window was produced by removing a portion of the polyimide coating of the capillary before the capillary was filled with gel. We noticed that a fan of scattered light was generated in the plane perpendicular to the capillary axis. This fan was attenuated significantly if the capillary was tilted by more than *ca.* 15° with respect to the laser beam. The attenuation in scatter intensity does not appear to be associated with Brewster's angle; only one component of the unpolarized laser beam would be extinguished at Brewster's angle. Also, the scattered light remained attenuated if the capillary was tilted by more than 15°, again inconsistent with Brewster's angle. Instead, reflection and refraction at the curved surface of the capillary dominate the background signal; tilting the capillary presumably directs the scattered light out of the field of view of the collection optics. The background light scatter signal does not appear to originate in the gel; similar background signals were observed for a buffer-filled and gel-filled capillary. Instead, the light scatter appears to originate from the walls of the capillary.

The detection limit for the on-column detector was an order of magnitude poorer that the sheath flow cuvette results. The two detectors used identical lasers, focusing optics, collection optics, electrophoresis potential, and PMT tube. Because of the high background signal produced by the capillary, it was necessary to use additional spectral filtering and a different spatial mask in the collection optics for the on-column detector. The additional OG-570 filter produces a three orders of magnitude decrease in background signal at the laser excitation wavelength; assuming that autofluorescence of the filter did not contribute significantly to the background signal, and realizing that the standard deviation of a shot-noise limited background signal increases with the square root of the signal intensity, it appears that the back-

ground signal due to the on-column detector is at least five orders of magnitude higher than that of the post-column detector based on the sheath flow cuvette.

Separation

By varying the ratio of dideoxynucleotides used in the chain extention reaction, the identity of the terminal dideoxynucleotide is encoded in peak height (Fig. 3). In any local neighbourhood, peaks associated with T will be higher than peaks associated with G, which in turn are larger than those for C and A. Unfortunately, in our standard sequencing gel, significant compressions are produced with this fluorescently labeled primer. A sequence of GGGTACCG, corresponding to fragments 59–66 nucleotides in length, is severely compressed. This compression appears to be associated with the low temperature at which the separation proceeds; data generated on a commercial slab gel sequencer showed no compression.

A sequencing gel was modified by addition of 20% (v/v) of formamide; the data generated on this gel show only a slight sequencing error for fragments 63–65 nucleotides in length (Fig. 4). However, this modified gel produces a very low sequencing rate of 70 bases/h at an electric field strength of 150 V/cm. We assume that operation of conventional gels at a temperature above ambient will eliminate the compressions, providing higher sequencing rates.

To obtain accurate base determination with this sequencing method, it is necessary to employ very careful enzymology; minor variations in peak amplitude can lead to poor discrimination in base determination. Pauses in the sequencing reaction can produce ghost peaks that contaminate the peak amplitude. Ghost peaks add the greatest proportional error to the lowest amplitude peaks. It seems that a carefully

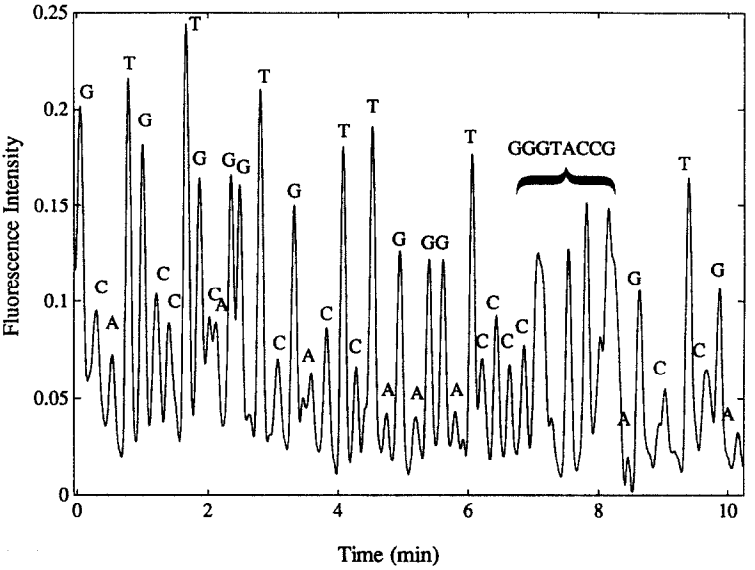

Fig. 3. Capillary gel electropherogram of M13mp18 reaction fragments: 6% T, 5% C–7 *M* urea acrylamide gel; fragments 28–73 nucleotides in length are shown. Time is arbitrarily set to zero for the fragment 27 nucleotides in length.

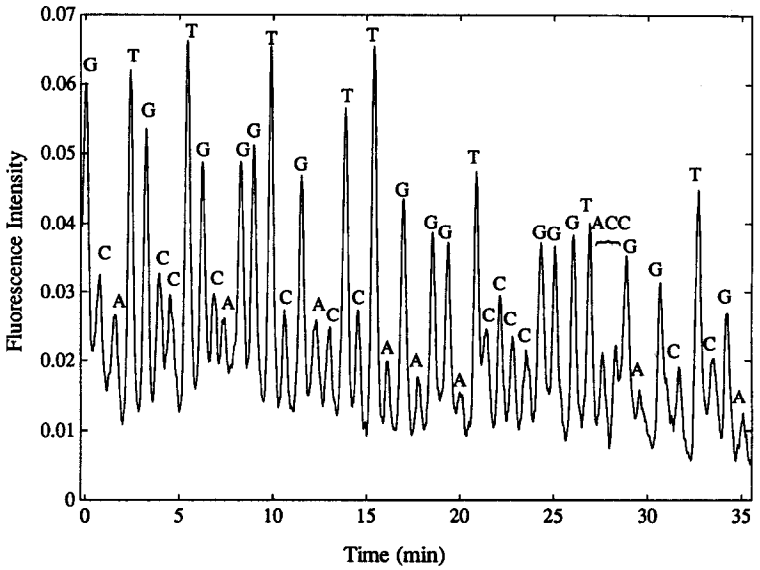

Fig. 4. Capillary gel electropherogram of M13mp18 reaction fragments: 6% T, 5% C–7 *M* urea–20% formamide acrylamide gel; fragments 28–73 nucleotides in length are shown. Time is arbitrarily set to zero for the fragment 27 nucleotides in length.

designed sequencing kit, optimized for a particular sequencing template, must be used to obtain accurate sequencing data from this amplitude modulated sequencing protocol.

ACKNOWLEDGEMENTS

This work was funded by the US Department of Energy–Human Genome Initiative (grant DE-FG02-91E R61123), Natural Sciences and Engineering Research Council of Canada, Department of Chemistry of the University of Alberta, Pharmacia/LKB, Waters Division of Millipore, and Beckman. Support by D.O.E. does not constitute an endorsement of the views expressed in this article. The DNA sample was kindly provided by C. Fuller of United States Biochemical Corporation. H.P.S. acknowledges a predoctoral fellowship from the National Science Foundation and H.R.H. acknowledges a fellowship from the Alberta Heritage Fund for Medical Research. J.E. Bertie of the University of Alberta kindly loaned the photomultiplier cooler.

REFERENCES

1 F. Sanger, S. Nicklen and A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.*, 74 (1977) 5463.
2 L. M. Smith, J. Z. Sanders, R. J. Kaiser, P. Hughes, C. Dodd, C. R. Connell, C. Heiner, S. B. H. Kent and L. E. Hood, *Nature (London)*, 321 (1986) 674.
3 W. Ansorge, B. S. Sproat, J. Stegemann and C. Schwager, *J. Biochem. Biophys. Methods*, 13 (1986) 315.
4 J. M. Prober, G. L. Trainor, R. J. Dam, F. W. Hobbs, C. W. Robertson, R. J. Zagursky, A. J. Cocuzza, M. A. Jensen and K. Baumeister, *Science (Washington, D.C.)*, 238 (1987) 336.

5 S. Tabor and C. C. Richardson, *J. Biol. Chem.*, 265 (1990) 8322.
6 W. Ansorge, J. Zimmermann, C. Schwager, J. Stegemann, H. Erfle and H. Voss, *Nucleic Acids Res.*, 18 (1990) 3419.
7 J. E. Edström, *Nature (London)*, 172 (1953)) 809.
8 J. E. Edström, *Biochim. Biophys. Acta*, 22 (1956) 378.
9 G. T. Matioli and H. B. Niewisch, *Science (Washington, D.C.)*, 150 (1965) 1824.
10 U. Grossbach, in R. C. Allen and H. R. Maurer (Editors) *Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel*, Walter de Gruyter, Berlin, 1974, p. 207.
11 V. Neuhoff, W. B. Schill and H. Sternbach, *Biochem. J.*, 117 (1970) 623.
12 S. Hjerten, *J. Chromatogr.*, 270 (1983) 1.
13 A. S. Cohen and B. L. Karger, *J. Chromatogr.*, 397 (1987) 409.
14 A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg and B. L. Karger, *J. Chromatogr.*, 488 (1988) 41.
15 A. S. Cohen, D. R. Najarian, A. Paulus, A. Guttman, J. A. Smith and B. L. Karger, *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 9660.
16 A. S. Cohen, D. R. Najarian and B. L. Karger, *J. Chromatogr.*, 516 (1990) 49.
17 B. L. Karger and A. S. Cohen, *US Pat.*, 4 865 706, 1989.
18 B. L. Karger and A. S. Cohen, *US Pat.*, 4 865 707, 1989.
19 P. F. Bente and J. Myerson, *US Pat.*, 4 810 456, 1989.
20 H. Swerdlow and R. Gesteland, *Nucleic Acids Res.*, 18 (1990) 1415.
21 H. Drossman, J. A. Luckey, A. J. Kostichka, J. D'Cunha and L. M. Smith, *Anal. Chem.*, 62 (1990) 900.
22 J. A. Luckey, H. Drossman, A. J. Kostichka, D. A. Mead, J. D'Cunha, T. B. Norris and L. M. Smith, *Nucleic Acids Res.*, 18 (1990) 4417.
23 G. R. Freeman, personal communication.
24 H. Swerdlow, S. Wu, H. Harke and N. J. Dovichi, *J. Chromatogr.*, 516 (1990) 61.
25 H. Swerdlow, J. Z. Zhang, D. Y. Chen, H. R. Harke, R. Grey, S. Wu, C. Fuller and N. J. Dovichi, *Anal. Chem.*, submitted for publication.
26 Y. F. Cheng and N. J. Dovichi, *Science (Washington, D.C.)*, 242 (1988) 562.
27 S. Wu and N. J. Dovichi, *J. Chromatogr.*, 480 (1989) 141.
28 Y. F. Cheng, S. Wu, D. Y. Chen and N. J. Dovichi, *Anal. Chem.*, 62 (1990) 496.
29 N. J. Dovichi, J. C. Martin, J. C. Jett and R. A. Keller, *Science (Washington, D.C.)*, 219 (1983) 845.
30 N. J. Dovichi, J. C. Martin, J. C. Jett, M. Trkula and R. A. Keller, *Anal. Chem.*, 56 (1984) 348.
31 D. C. Nguyen, R. A. Keller, J. H. Jett and J. C. Martin, *Anal. Chem.*, 59 (1987) 2158.
32 K. Peck, L. Stryer, A. N. Glazer and R. A. Mathies, *Proc. Natl. Acad. Sci. U.S.A.* 86 (1989) 4087.
33 R. Keller, paper presented at *OE-LASE '91, 23 January, 1991*, paper 1435-27.
34 T. Hirshfeld, *Appl. Opt.*, 15 (1976) 3135.