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Gene dosage in capillary electrophoresis: pre-natal diagnosis of Down's syndrome

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Abstract

Modern proposals for pre-natal genetic analysis of Down's syndrome consist in isolating DNA from amniotic cells and amplifying a highly polymorphic small tandem repeat region of the chromosome 21-specific D21S11 marker. The polymerase-chain-reaction-amplified fragments are typically 5′-end labelled with a green or blue fluorescent reporter and data acquisition occurs on-lane in DNA sequencing gel-slabs and equipment. The following patterns are expected: for normal individuals, 1 peak or two peaks in a 1:1 ratio. In the case of trisomy 21, the following patterns are found: either three peaks in a 1:1:1 ratio or a two-peak profile with a 2:1 gene ratio. We have developed a capillary electrophoretic system, offering precise diagnostic value by exploiting the intrinsic DNA absorbance at 254 nm. The separation occurs in capillaries coated with an extremely stable and hydrophilic layer of poly(N-acroyloyl amino ethoxy ethanol) and filled with a background electrolyte consisting of 89 m*M* Tris-borate, 2 m*M* EDTA, 2.5 μM ethidium bromide and 8% short-chain, low-viscosity, replaceable, liquid, linear, sieving polyacrylamide. The technique offers high reproducibility and precise on-line, automated peak acquisition and quantitation.

Keywords: Capillary electrophoresis; Down's syndrome; Gene dosage; DNA

1. Introduction

Free trisomy 21 accounts for about 95% of all cases of Down's syndrome [1], the leading cause of genetically-inherited mental retardation. This birth defect is one of the most common genetic diseases, with an incidence of about 1 in 700 births [2], with an exponential increase in pregnancies in women over 35 years of age [3]. Prior to birth, Down's syndrome can be detected by amniocentesis or chorionic villus sampling and karyotyping, an expensive, labour-intensive and time-consuming process. Thus, a less expensive screening test indicating the presence of chromosomal abnormalities would be highly desirable.

In 1991, Lubin et al. [4] described a quantita-

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tive polymerase-chain-reaction (PCR) amplification process for detecting X-chromosome dosage differences in patients with sex chromosomal aneuploidies. By assuming that, within the exponential phase of PCR amplification, the amount of specific DNA produced is proportional to the quantity of initial target sequences [5], this procedure was modified by Mansfield [6] for detecting trisomies 21 and 18 and the triple-X syndrome. The assay is based on extracting DNA either from blood or from amniotic cells and in amplifying a highly polymorphic (90% heterozygosity), chromosome 21-specific D21S11 marker [7]. Three classes of hypervariable repeats are typically found throughout the genome: variable number tandem repeats (VNTR), or minisatellites [8], small tandem repeats (STR) with repeat elements of 3–6 bases in length [9] and CA-repeats or microsatellites that have a 2-base repeat length [10]. Of these repeat types, the STR markers are by far the most amenable to quantitative analysis. Based on these findings, Mansfield [6] and Perti et al. [11], have recently proposed a rapid molecular method for pre-natal diagnosis of Down's syndrome. In their protocol, the PCR fragments are 5'-end-labelled with green or blue fluorescent markers and analysis is performed in 6%T polyacrylamide gel-slabs in automated DNA sequencing instruments.

Capillary zone electrophoresis (CZE) is rapidly emerging as a highly competitive technique, especially in the field of DNA analysis. A few reviews [12–14] have already described the analytical properties of CZE for separation of oligo- and poly-nucleotides. CZE appears to be an easy, precise and fast method for the analysis and detection of DNA restriction fragments (up to 2 kilo base pairs, kbp) and of PCR products [14]. Among the advantages: (a) the amount of DNA necessary for a single CZE run is about 5 ng, two orders of magnitude lower than the ca. 500 ng DNA per lane needed in slab gel operations; (b) no toxic compounds are necessary for DNA detection; (c) automated, fast data acquisition and evaluation. After the pioneering work of Heiger et al. [15] who proposed, already in 1990, the possibility of using very low (barely 0.5%C) and even zero crosslinked polyacrylamides (i.e., liquid sieving polymers!) for separation of DNA restriction fragments, the technique has indeed evolved towards the use of un-crosslinked, replenishable matrices, notably of the liquid, linear polyacrylamide type. Among some of the important breakthroughs which have rendered the technique easy to operate and highly reliable: (a) the introduction of a novel polymeric coating for the inner capillary surface, an acrylamido derivative (N-acryloyl amino ethoxy ethanol) combining a unique hydrophilicity with extreme hydrolytic stability [16,17]; (b) a novel method for producing low-viscosity, short-chain length polyacrylamides as liquid sieving polymers, allowing replenishing and restoring of the starting conditions after each run [18]. We report in the present paper the use of this novel methodology for the diagnosis of Down's syndrome.

2. Experimental

2.1. Reagents

Acrylamide, N,N'-methylene bisacrylamide, tris(hydroxymethyl aminomethane), ammonium peroxodisulphate, and N,N,N'N'-tetramethylethylendiamine (TEMED) were obtained from Bio-Rad Labs (Hercules, CA, USA), while the marker V DNA size standard was from Boehringer. All the reagents for PCR were from GeneAmp., Perkin-Elmer. Bind silane [3-(trimethoxysilyl)propylmethacrylate] and ethylendiaminetetraacetic acid (EDTA), boric acid and acetic acid were purchased from Aldrich (Steinheim, Germany). The Centricon 30 membranes were from Amersham (Buckinghamshire, UK). Fused-silica capillaries (75 μm I.D., 375 μm O.D.) were from Polymicro Technologies (Phoenix, AZ, USA).

2.2. DNA isolation and PCR amplification of D21S11

DNA was extracted from ca. 2 ml of whole blood samples of human donors or from 2 ml of amniotic fluid (uncultured cells) by the standard phenol–chloroform extraction procedure. DNA

concentration was adjusted to 5 ng/μl. For amplification of the STR region D21S11, the VS17T#3 and VS17T#4 primers, as described by Sherma and Litt [7], were used. PCR was performed in a total volume of 25 μl containing 5 ng of genomic DNA, 0.5 μ*M* of each primer, 50 m*M* KCl, 10 m*M* Tris-HCl buffer, pH 8.3, 1.5 m*M* $MgCl_2$, 0.01% (w/v) gelatin, 200 μ*M* of each dNTP and 0.5 unit Taq polymerase (Perkin-Elmer/Cetus). Initial denaturation was at 95°C for 5 min followed by an initial annealing temperature of 62°C for 1 min, decreased by 1°C/cycle for the first 10 cycles and held constant at 52°C for 30 s for the remaining 20 cycles. For all cycles, denaturation was for 1 min at 95°C and extension was for 1 min at 72°C.

2.3. Capillary zone electrophoresis in polymer networks

Capillary zone electrophoresis (CZE) analyses were performed with the Quanta 4000E capillary Ion Analyzer from Millipore (Milford, MA, USA). A 37 cm × 75 μm I.D. capillary, coated by the Hjertén protocol [19], but utilizing the novel, hydrolytically stable, N-acryloyl amino ethoxy ethanol monomer, was used [16]. The separation buffer consisted of 89 m*M* Tris, 89 m*M* boric acid and 2 m*M* EDTA (TBE), pH 8.3, containing 2.5 μ*M* ethidium bromide and 8%T [%T: (g acrylamide + g Bis)/100 ml solution] acrylamide linear viscous polymer (in the absence of crosslinker), polymerized at 70°C in presence of a chain transfer agent, and dissolved in TBE buffer, after dialysis and lyophilization [18]. The capillary was refilled with fresh sieving buffer solution after each run. The samples and standards were loaded electrophoretically by applying 100 V/cm for 15–20 s depending on sample concentration. Separations were performed at 100 V/cm, a typical run lasting for 50–55 min. Ultraviolet absorbance was monitored at 254 nm. The peaks were quantified by integration with the Water's Quanta Millennium program. In order to obtain a detectable UV peak, the amplified fragments were desalted and concentrated prior to CZE injection with Centricon membranes. In order to check for the fragment length, a marker V fragment standard sequence was used both in gel-slabs and in the CZE experiments.

2.4. Polyacrylamide gel-slab electrophoresis

The PCR-amplified samples were electrophoresed in a 10%T, 4%C (%C: g Bis/100 g total monomers) polyacrylamide gel-slab, 1 mm thick and 40 cm long, in 89 m*M* Tris-acetate, 2 m*M* EDTA buffer, pH 8.3, run overnight at 8 V/cm. At the end of the run, the gel was silver stained [20].

3. Results

In the analysis of Down's syndrome, via polymorphic STR allelic markers, one expects the results to fall in the following categories: (a) for normal individuals, the homozygote would exhibit a single, double intensity peak, whereas the heterozygote would be resolved into two, equal intensity peaks (1:1 ratio). Trisomic individuals, on the contrary, are expected to fall into two major groups: those with three STR peaks of similar intensities (1:1:1 ratio; i.e., three different STR alleles) or those with two peaks with a ratio of 2:1 (i.e., two copies of an identical STR allele and one of a different allele; however, in rare cases of homozygosity, a single peak might be obtained [11]; in such cases, only karyotyping will give a correct diagnosis). Fig. 1 shows a silver-stained, gel-slab electrophoretic run of a series of PCR-amplified samples. Samples 2 to 5 represent normal individuals, one homozygous (lane 2, a single, double-intensity peak) and the other heterozygous (double peaks of equal intensity). Sample 1 represents a trisomic patient, with two bands having a 2:1 intensity ratio. Lane 6 contains the Marker V DNA size standards.

Fig. 2 shows the same analysis performed by CZE in sieving liquid polymers (8% short-chain, low-viscosity linear polyacrylamide). Panel 2A shows the tracing of sample 2 (homozygous, normal individual), exhibiting indeed a single peak. Figs. 2B and C represent two heterozygous, normal individuals with two peaks of a

Fig. 1. Polyacrylamide gel slab electrophoresis of PCR-amplified markers of Down's syndrome. Gel: 10%T, 4%C, in 89 m*M* Tris-acetate, 2 m*M* EDTA buffer, pH 8.3. Run: overnight at 8 V/cm, room temperature. Staining with silver nitrate. Lane 1: trisomy 21 (2 peaks, 2:1 ratio); lane 2: healthy, homozygous individual (1 peak); lanes 3–5: healthy, heterozygous individuals (2 peaks, 1:1 ratio); lane 6: Marker V DNA standards. All samples are post-natal, with DNA isolated from whole blood. The vertical arrow indicates the migration direction (the cathode being at the top). Note that this picture represents only a small portion of the gel slab, centered on the 184–267 bp region of the DNA size standards (the lowest band in track 6 representing the unresolved 123/124 bp doublet).

ratio very close to unity. In all cases, the early eluting peaks (from 20 to 35 min), represent excess primers (and primer–dimers), whereas the diagnostic fragments have elution times in the 45–47 min window.

In Fig. 3, a sample from a normal, heterozygous individual, has been run admixed with the Marker V DNA size standards. The PCR fragments, amplified according to [7], should in principle contain either one or both the E7 (224 bp in length) and E8 (220 bp in length) alleles. It is in fact seen that their size falls indeed between the 213 bp and 234 bp standards.

Fig. 4 shows the profile of sample 1 from Fig. 1, i.e. of a trisomic individual. Integration of the two peaks gives indeed the expected 2:1 ratio, corresponding to a Down's syndrome diagnosis (as confirmed by independent cytogenetic analysis).

All the samples analysed so far were from DNA isolated from whole blood of healthy or affected individuals. In genetic analysis it is of utmost importance to be able to perform also correct pre-natal diagnosis in pregnancies at risk. Fig. 5 shows the same CZE analysis of Fig. 4 performed on amniotic fluid samples. The fetus, due to the characteristic 2:1 gene dosage, was diagnosed as affected by Down's syndrome, as independently confirmed by cytogenetic analysis, which indicated a case of trisomy 21.

As stated in the introduction, another possibility, in trisomy 21, is the appearance of three peaks, with a characteristic 1:1:1 ratio. Fig. 6 shows indeed such a pattern, in a case we had to evaluate for a pregnancy at risk. The diagnosis was confirmed by both, karyotyping and gel-slab electrophoresis (not shown). Although performed at present on a relatively small total sample size (15 normal and 5 trisomic individuals, 5 Down's fetuses), our CZE system would appear to offer easy, efficient and unambiguous post- and pre-natal diagnosis of Down's syndrome.

4. Discussion

4.1. CZE as a novel diagnostic tool

As a tool for the analysis of PCR-amplified products, for screening of genetics diseases and for forensic analysis, CZE is rapidly coming of age. A number of examples can be given: thus Kleparnik et al. [21] have used CZE for analysis of bacteriophage restriction fragments. McCord et al. [22,23] and Butler et al. [24,25] performed rapid screening of the short tandem repeat HUMTH01 locus (located on human chromosome 11), which consists of the tetrameric repeat AATG; Schwartz et al. [26] have analyzed PCR products aimed at the detection of the AIDS (HIV-1) virus in blood. Arakawa et al. [27,28] have diagnosed medium-chain acyl-coenzyme A dehydrogenase deficiency, by distinguishing a 175-bp mutant allele from a 202-bp normal DNA fragment. Del Principe et al. [29] proposed CZE analysis of the PCR product of the DXS 164 locus in the dystrophin gene. Gelfi et al. [30–33] have analysed a number of genetic diseases by

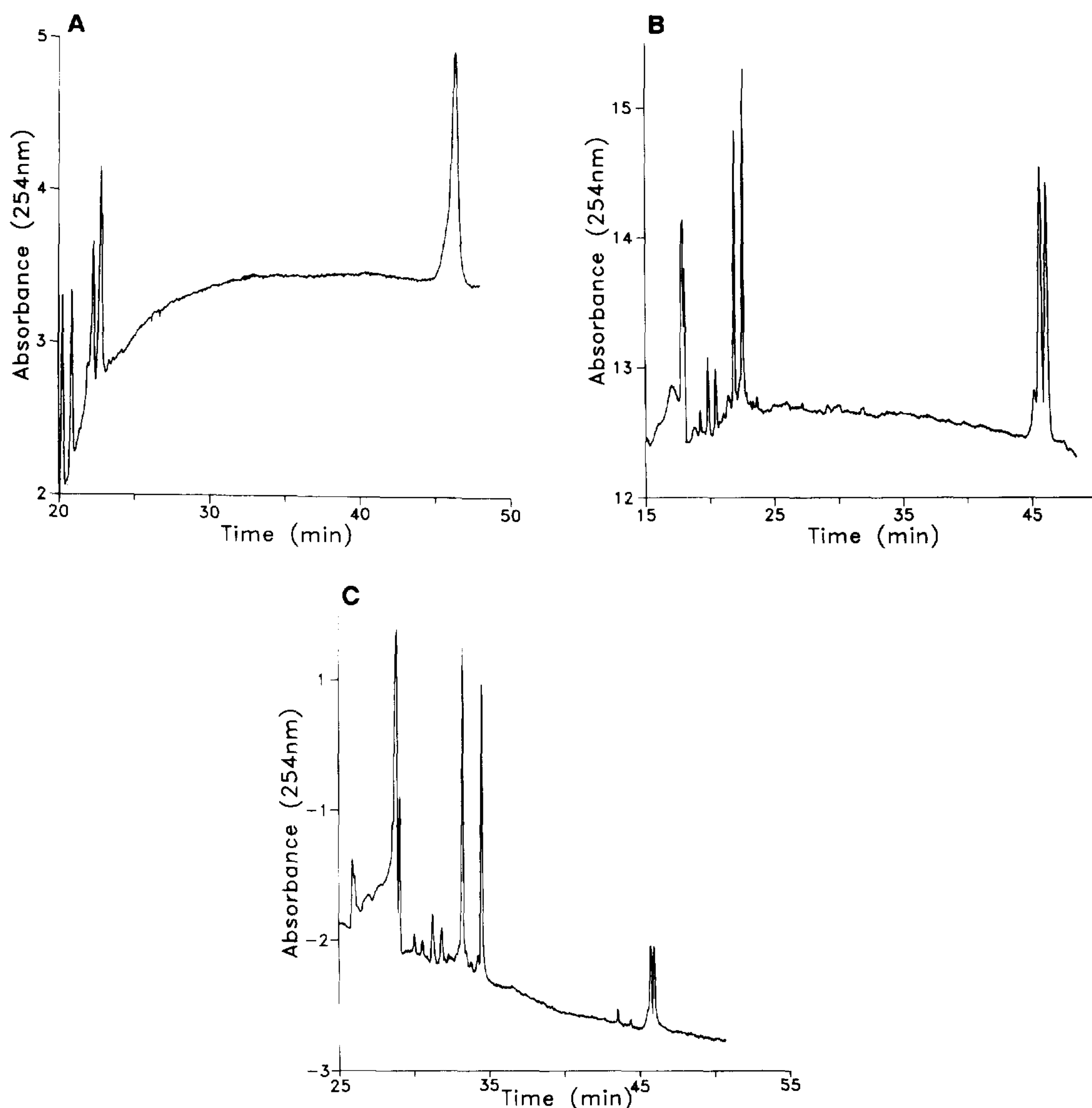

Fig. 2. CZE of PCR-amplified markers of Down's syndrome. Capillaries: 75 μm I.D., 37 cm length, coated with poly(N-acryolyl amino ethoxy ethanol). Background electrolyte: 89 m*M* Tris-borate, 2 m*M* EDTA, pH 8.3, containing 8% short-chain, low-viscosity, liquid sieving polyacrylamide and 2.5 μ*M* ethidium bromide. Sample injection: 15–20 s, at 100 V/cm. Run: 100 V/cm, room temperature. (A) Homozygous, healthy individual (lane 2 in Fig. 1); (B, C) heterozygous, healthy individuals (lanes 3 and 5 in Fig. 1). Note in this last case the two-peak pattern with a 1:1 gene ratio. All peaks eluting between 20 and 35 min represent primers and primer dimers.

CZE in liquid polyacrylamides: the most common ΔF508 deletion in cystic fibrosis (CF, a 3-bp deletion removing a Phe at position 508 of the predicted CFTR protein), as well as other more rare mutations in the CF chromosome. Additionally, they have separated two main allelic forms, one exameric (111 bp) and one heptameric (115 bp) of a tetranucleotide (GATT) repeat polymorphism, useful for prenatal diagnosis and CF-carrier detection; moreover, the same CZE technique was applied to the screening of a rare disease, congenital adrenal hyperplasia. Kuypers

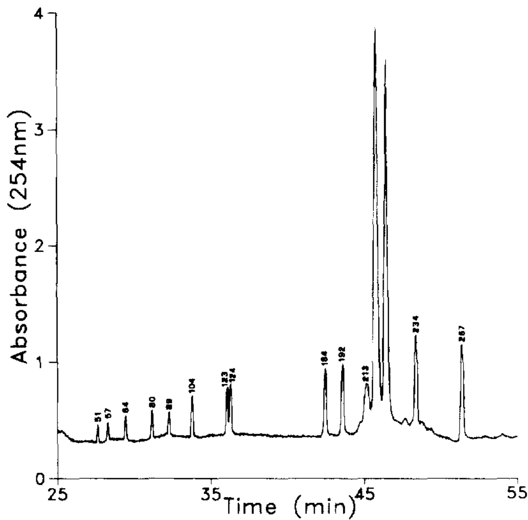

Fig. 3. CZE of PCR-amplified markers of Down's syndrome. All conditions as in Fig. 2, except that the analyte (lane 3 in Fig. 1) has been mixed with Marker V DNA standards for evaluating the size of the amplified fragments.

et al. [34,35] also recommended CZE for detection of mutations in PCR-products: in one application, they detected a mutation in the p53 gene, a tumour suppressor gene known to be frequently mutated in malignant cells; in another report, they quantified residual lymphoma cells carrying

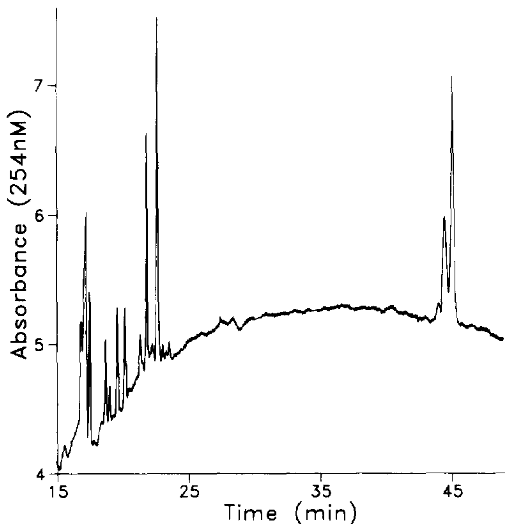

Fig. 4. CZE of PCR-amplified markers of Down's syndrome for post-natal diagnosis. The analyte represents lane 1 of Fig. 1. Note the characteristic 2-peak profile with a 2:1 gene expression ratio.

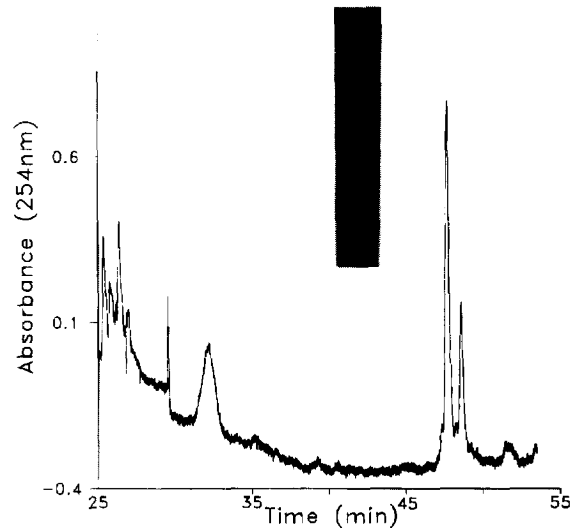

Fig. 5. CZE of PCR-amplified markers of Down's syndrome for pre-natal diagnosis. All conditions as in Fig. 2, except that the analyte is a pre-natal sample, with DNA isolated from amniotic cells. The insert shows the same analysis performed in slab gels (left lane: DNA markers; right lane: amplified STRs; vertical arrow head: migration direction). Note the characteristic 2-peak profile with a 2:1 gene expression ratio.

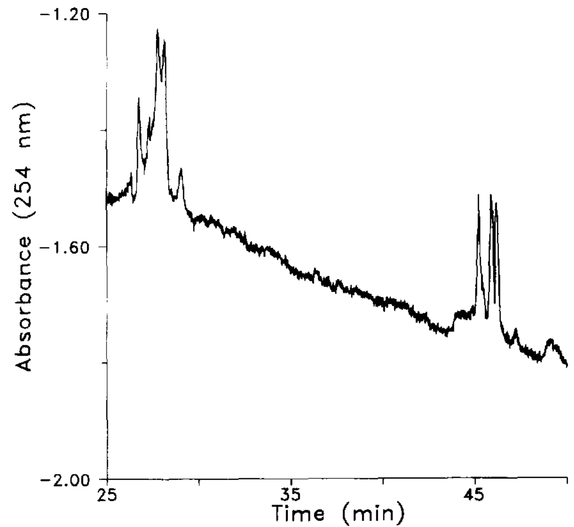

Fig. 6. CZE of PCR-amplified markers of Down's syndrome for pre-natal diagnosis. All conditions as in Fig. 2, except that the analyte is a pre-natal sample, with DNA isolated from amniotic cells. Note, in this particular sample, the characteristic 3-peak profile with a 1:1:1 gene expression ratio (45–47 min migration window).

a translocation between chromosomes 14 and 18 in patients' peripheral blood samples amplified by competitive PCR. Nesi et al. [36] have also proposed CZE in linear polyacrylamides for screening of Kennedy's disease, an X-linked motoneuronal disorder associated with an increase in the number of $(CAG)_n$ triplet repeats in the first exon of the Androgen receptor gene. Rossomando et al. [37] have also utilized CZE for the separation and quantitation of reverse transcriptase PCR products from polio virus. Srinivasan et al. [38,39] have applied CZE to the analysis of PCR-DNAs from three different genetic loci: apolipoprotein B, VNTR locus D1S80 and mitochondrial DNA. Wenz [40] has shown that CZE can be a quite useful technique in detecting dsDNA exhibiting an anomalous migration (bent or curved DNA). Williams et al. [41], in the screening of PCR products, additionally suggest two different methods for desalting the DNA samples prior to electrokinetic injection: in one approach, a droplet of analyte is deposited on a Millipore VS 0.025-μm membrane floating on water; in the other, standard approach, larger volumes are subjected to ultrafiltration/centrifugation using the Millipore Ultrafree 30 filter. At the end of this brief excursus, we note that analysis of PCR products has also been proposed by Gelfi et al. [42] for the screening of dystrophin gene exons in the Duchenne and Becker muscular dystrophy (DMD/BMD) conditions. For the simultaneous screening of both pathologies, one needs to amplify and resolve a total of 18 exons, ranging in size from 113 to 547 bp. So far, nobody has succeeded in separating simultaneously, within a single electrophoretic track, all 18 fragments; here, CZE showed a distinct advantage over gel-slab separations, in which some bands were always missing from the entire population of 18 zones.

4.2. Gene dosage

This latter aspect is, however, still in its infancy. Only a few reports exist at present on the use of CZE for a quantitative assay of gene expression. So far, only the two most common cases (not requiring quantitation) have been solved: (a) in the case of small deletions, the resolution and detection of a normal chain of a given length, vs. a shorter DNA fragment; (b) in the case of point mutations, the detection of a characteristic four-zone pattern, due to two homo- and two hetero-duplexes, obtained by partial denaturation in denaturing gradients (thermal gradients, so far, in CZE) [43,44]. In the present case, gene dosage requires precise quantitation of gene expression and correct assessment of peak ratios. In gel-slab operations, this is usually achieved either by post- or pre-electrophoretic labelling procedures. Peak quantitation for correct diagnosis of Down's syndrome has been performed by Mansfield [6] and by Perti et al. [11] via pre-labelling of primers with fluorescent dyes and automatic peak integration in, e.g., the Applied Biosystems' DNA sequencer. In CZE, very few reports are available. As stated above, Kuypers et al. [35] have attempted quantitation of residual lymphoma cells carrying a translocation between chromosomes 14 and 18 in patients' peripheral blood samples. Lu et al. [45] also reported quantitative analysis of PCR products from HIV-1 DNA: their CZE technique allowed a linear range over three orders of magnitude in DNA concentrations and offered a sensitivity of detection of as little as 200 to 500 000 viral particles per ml of serum. As a third report, Gelfi et al. [46], in the diagnosis of human ovarian carcinomas (epithelial and endometrial tumours), attempted quantitation of the amount of mRNA coding for the basic fibroblast growth factor (bFGF) by reverse transcription PCR in a competitive PCR assay. In the last case, however, only semi-quantitative data were obtained. We feel that the present report is the first demonstration of rigorous gene dosage by exploiting the natural UV absorbance of the amplified DNA fragments, in the absence of either pre- or post-electrophoretic labelling.

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References

[1] C. Valenti, E.J. Shutta and T. Kehaty, Lancet, ii (1968) 220–221.
[2] E.B. Hook, Obst. Gyn., 58 (1981) 282–285.
[3] E.B. Hook, Am. J. Hum. Genet., 35 (1983) 1307–1313.
[4] M.B. Lubin, J.D. Elashoff, S.W. Wang, J.T. Rotter and H. Toyoda, Mol. Cell Probes, 5 (1991) 307–312.
[5] F. Ferre, PCR Methods Appl., 2 (1992) 1–9.
[6] E.S. Mansfield, Hum. Mol. Genet., 2 (1993) 43–50.
[7] V. Sherma and M. Litt, Hum. Mol. Genet., 1 (1992) 67–73.
[8] Y. Nakamura, Y. leppert, P.O'Connell, R. Wolff, T. Holm, M. Culver and C. Martin, Science, 235 (1987) 1616–1622.
[9] G. Zuliani and H.H. Hobb, Am. J. Hum. Genet., 46 (1990) 963–969.
[10] J.L. Weber and P.E. May, Am. J. Hum. Genet., 44 (1989) 388–396.
[11] B. Perti, S.C. Yau, J. Sherlock, A.F. Davies, C.G. Mathew and M. Adinolfi, Lancet, 343 (1994) 1197–1198.
[12] A.S. Cohen, D.L. Smisek and P. Keohavong, Trends Anal. Chem., 12 (1993) 195–202.
[13] Y. Baba, J. Chromatogr., 618 (1993) 41–55.
[14] P.G. Righetti and C. Gelfi, in P.G. Righetti (Editor), Capillary Electrophoresis in Analytical Biochemistry, CRC Press, Boca Raton, FL, 1995, pp. 431–476.
[15] D.N. Heiger, A.S. Cohen and B.L. Karger, J. Chromatogr., 516 (1990) 33–44.
[16] M. Chiari, C. Micheletti, M. Nesi, M. Fazio and P.G. Righetti, Electrophoresis, 15 (1994) 177–186.
[17] M. Chiari, M. Nesi and P.G. Righetti, Electrophoresis, 15 (1994) 616–622.
[18] C. Gelfi, A. Orsi, F. Leoncini and P.G. Righetti, J. Chromatogr. A, 689 (1995) 97–105.
[19] S. Hjertén, J. Chromatogr., 347 (1985) 191–198.
[20] T. Rabilloud, Electrophoresis, 11 (1990) 785–794.
[21] K. Kleparnik, Z. Mala, J. Doskar, S. Rosypal and P. Bocek, Electrophoresis, 16 (1995) 366–376.
[22] B.R. McCord, D.L. McClure and J.M. Jung, J. Chromatogr. A, 652 (1993) 75–82.
[23] B.R. McCord, J.M. Jung and E.A. Holleran, J. Liq. Chromatogr., 16 (1993) 963–981.
[24] J.M. Butler, B.R. McCord, J.M. Jung, M.R. Wilson, B. Budowle and R.O. Allen, J. Chromatogr. B, 658 (1994) 271–280.
[25] J.M. Butler, B.R. McCord, J.M. Jung and R.O. Allen, Biotechniques, 17 (1994) 1062–1070.
[26] H.E. Schwartz, K. Ulfelder, F.J. Sunzeri, M.P. Busch and R.G. Brownlee, J. Chromatogr., 559 (1991) 267–284.
[27] H. Arakawa, K. Uetanaka, M. Maeda and A. Tsuji, J. Chromatogr. A, 664 (1994) 89–98.
[28] H. Arakawa, K. Uetanaka, M. Maeda, A. Tsuji, Y. Matsubara and K. Narisawa, J. Chromatogr. A, 680 (1994) 517–523.
[29] D. Del Principe, M.P. Iampieri, D. Germani, A. Menichelli, G. Novelli and B Dallapiccola, J. Chromatogr., 638 (1993) 277–282.
[30] C. Gelfi, A. Orsi, P.G. Righetti, V. Brancolini, L. Cremonesi and M. Ferrari, Electrophoresis, 15 (1994) 640–643.
[31] C. Gelfi, A. Orsi, P.G. Righetti, M. Zanussi, P. Carrera and M. Ferrari, J. Chromatogr. B, 657 (1994) 201–205.
[32] C. Gelfi, P.G. Righetti, V. Brancolini, L. Cremonesi and M. Ferrari, Clin. Chem., 40 (1994) 1603–1605.
[33] C. Gelfi, P.G. Righetti, C. Magnani, L. Cremonesi and M. Ferrari, Clin. Chim. Acta, 229 (1994) 181–189.
[34] A.W.H.M. Kuypers, P.M.W. Willems, M.J. van der Schans, P.C.M. Linssen, H.M.C. Wessels, C.H.M.M. de Bruijin, F.M. Everaerts and E.J.B.M. Mensink, J. Chromatogr., 621 (1993) 149–156.
[35] A.W.H.M. Kuypers, J.P.P. Meijerink, T.F.C.M. Smetsers, P.C.M. Linssen and E.J.B.M. Mensink, J. Chromatogr. B, 660 (1994) 271–277.
[36] M. Nesi, P.G. Righetti, M.C. Patrosso, A. Ferlini and M. Chiari, Electrophoresis, 15 (1994) 644–646.
[37] E.F. Rossomando, L. White and K.J. Ulfelder, J. Chromatogr. B, 656 (1994) 159–168.
[38] K. Srinivasan, J.E. Girard, P. Williams, R.K. Roby, V.W. Weedn, S.C. Morris, M.C. Kline and D.J. Reeder, J. Chromatogr. A, 652 (1993) 83–92.
[39] K. Srinivasan, S.C. Morris, J.E. Girard, M.C. Kline and D.J. Reeder, Appl. Theor. Electr., 3 (1993) 235–239.
[40] H.M. Wenz, Nucleic Acid Res., 22 (1994) 4002–4006.
[41] P.E. Williams, M.A. Marino, S.A. Del Rio, L.A. Turni and J.M. Devaney, J. Chromatogr. A, 680 (1994) 525–540.
[42] C. Gelfi, A. Orsi, F. Leoncini, P.G. Righetti, I. Spiga and M. Ferrari, BioTechniques, 19 (1995) 254–263.
[43] C. Gelfi, P.G. Righetti, L. Cremonesi and M. Ferrari, Electrophoresis, 15 (1994) 1506–1511.
[44] K. Khrapko, J.S. Hanekamp, W.G. Thilly, A. Belenkii, F. Foret and B.L. Karger, Nucl. Acid. Res., 22 (1994) 364–369.
[45] W. Lu, D.S. Han, J. Yuan and J.M. Andrieu, Nature, 368 (1994) 269–271.
[46] C. Gelfi, F. Leoncini, P.G. Righetti, L. Cremonesi, A.M. di Blasio, C. Carniti and M. Vignali, Electrophoresis, 16 (1995) 780–783.