

## TECHNICAL NOTE

## The rapid analysis of dystrophin gene deletions shows variable electrophoretic mobility

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### Abstract

**The introduction of PCR technology to the molecular diagnosis of genetic diseases has increased the speed and range of DNA tests available. Previous analyses of dystrophin gene mutations were time consuming, taking weeks to complete, and used radioisotopic methods. Further developments in DNA amplification and post-amplification techniques have made conventional tube PCR redundant. The rapid methodologies described enable the efficient screening of large populations for genetic disorders, although precautions must be taken when analysing the PCR products.**

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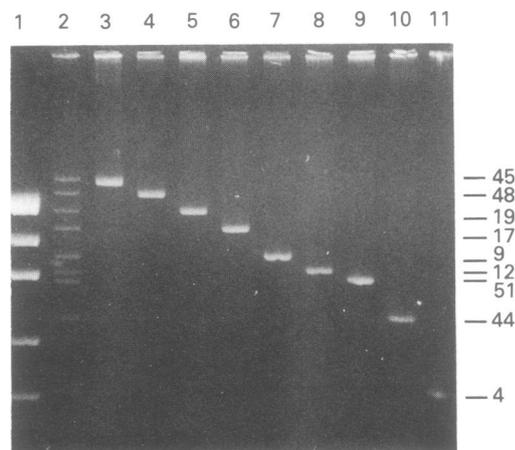
Duchenne and Becker muscular dystrophies (MD) represent the most common and serious heritable neuromuscular disorders. Deletion mutations of the dystrophin gene, consisting of approximately 70 exons which span over 2 megabases of chromosome Xp, are described in 60% of reported MD cases.<sup>1</sup> Rapid analysis of the exon composition of a MD patient has been made possible by the polymerase chain reaction (PCR). Multiplex PCR, using 38 primers to amplify 19 different exons, can now detect 98% of dystrophin gene deletions previously analysed by Southern transfer and cDNA probing.<sup>2,3</sup> This has reduced prenatal diagnosis time from several weeks to a few days.

We describe a modification of traditional PCR technology and the analysis of amplification products which enables a one hour diagnosis of dystrophin gene deletions in either extracted DNA or fetus related tissues.

Small volume PCR (10 µl) with fast heat transfer between reaction and heating block, known as capillary PCR, allows 30 cycles of amplification in approximately 30 minutes. A 10 minute procedure for crude DNA extraction from amniotic fluid cells or chorionic villus biopsy involves heating the sample to 95°C in non-ionic detergent (0.1% Triton X-100) and 0.1 mol/l NaOH before neutralisation.<sup>4</sup> Sufficient PCR template is obtained from 10 µl of whole blood or 1 mg of chorionic villus or amniotic cells. The amplification reaction contains 3 pmol of each primer and requires 0.5 U of *Taq* polymerase. The 19

primer sets are divided into a nine product reaction (multiplex A)<sup>2</sup> and a 10 product reaction, which includes a previously described control exon<sup>3</sup> (multiplex B). High levels of MgCl<sub>2</sub>, up to 5 mmol/l, have been added to the reaction and assist the amplification of crudely extracted template. Cycling parameters for capillary PCR are as follows: initial denaturation, three minutes at 94°C; cycle denaturation, five seconds at 92°C; annealing, 10 seconds at 56°C; extension, 20 seconds for 10 cycles and 40 seconds for a further 12 cycles at 65°C. Samples undergo 22 cycles of amplification to allow for the presence of up to 5% maternal contamination in the prenatal biopsy.

The entire multiplex PCR and individual exon products were electrophoresed on either a 3% nusieve agarose gel in TBE buffer and ethidium bromide or a discontinuous polyacrylamide (PAGE) gel in TAE buffer (figs 1 and 2). The polyacrylamide gradient is made with 8%, 19:1 crosslinking (acrylamide:bisacrylamide) at the bottom and 10%, 37:1 crosslinking at the top of the gel. The presence of ethidium bromide and the use of borate or acetate electrophoresis buffers does not alter relative band mobilities. Polyacrylamide gels were 8 cm long and subjected to 400 V (150 mA) for approximately 20 minutes to resolve all exon PCR products.



**Figure 1** Analysis of muscular dystrophy multiplex PCR on 3% nusieve agarose/TBE/EtBr. Lane 1, pUC19 *Hpa*II digest. Lane 2, multiplex A.<sup>2</sup> Lanes 3 to 11, exon amplicons 45 (547 bp), 48 (506 bp), 19 (459 bp), 17 (416 bp), 9 (360 bp), 12 (331 bp), 51 (319 bp), 44 (268 bp), and 4 (196 bp) respectively.

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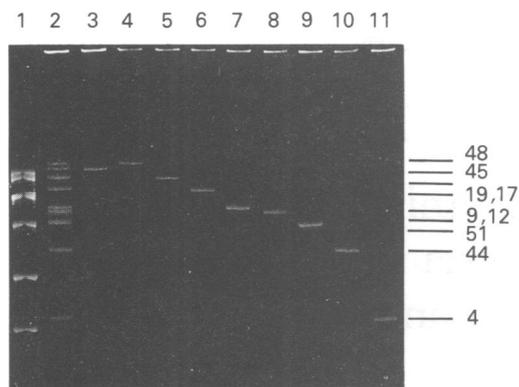


Figure 2 Analysis of muscular dystrophy multiplex PCR on discontinuous polyacrylamide gel (8%, 19:1 crosslinking with 10%, 37:1 stacking gel/TAE). Lane 1, pUC19 HpaII digest. Lane 2, multiplex A. Lanes 3 to 11, exon amplicons 45, 48, 19, 17, 9, 12, 51, 44, and 4 respectively.

The incidence of non-contiguous genetic deletions in MD is extremely low. Using the multiplex primer set described here as A,<sup>2</sup> a much higher frequency of apparently non-contiguous deletions has been recognised in our laboratory. These results are explained by the relative electrophoretic mobility of exons 45 (547 base pairs (bp)) and 48 (506 bp) in agarose and PAGE. We have found that the perceived molecular size of the exon 45 amplicon decreases by approximately 40 bp in PAGE as compared to its size in agarose. This change in electrophoretic retardation enables the exon 45 band to run lower than the exon 48 band (fig 2), opposite to the observed order in an agarose gel (fig 1). Polyacrylamide was

initially chosen as a gel matrix in this application for the benefits of cost, speed of resolution, densitometry scanning, and alternative silver staining for low product quantities. The comparison of PCR product patterns from the original, agarose based data with the PAGE pattern may lead to the misassignment of MD exon deletions if this anomaly is not taken into account. The amplicon for exon 12 in this multiplex increases its apparent molecular size by about 20 bp when analysed on PAGE but this does not alter the band order. No changes in the patterns of electrophoretic mobility between gel media occur with the multiplex B.

Structural analysis of the PCR product of exon 45 indicates that there are no obvious extraordinary tertiary structures which could explain the band shift. This phenomenon could possibly be related to the separating properties of the cross linked acrylamide matrix as compared to an agarose gel matrix. It may therefore be important that PCR DNA fragment diagnoses of this type be approached cautiously when selecting an analysis medium.

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