Localisation of the endpoints of deletions in the 5' region of the Duchenne gene using a sequence isolated by chromosome jumping

---

Susan J.Kenrick, Terry J.Smith, Sarah England, Francis Collins¹ and Kay E.Davies

---

Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK
and ¹Department of Internal Medicine and Human Genetics, Howard Hughes Medical Institute,
University of Michigan, Ann Arbor, MI 48109, USA

Received November 27, 1987; Revised and Accepted December 29, 1987

ABSTRACT

We have used chromosome jumping technology to move from within a large intron sequence in the Duchenne muscular dystrophy (DMD) gene to a region adjacent to exons of the gene. The single copy jump clone, HH1, was used to characterise deletions in patients previously shown to be deleted for DNA markers in the 5' end of the gene. 12 out of 15 such patients have breakpoints which lie between HH1 and the genomic locus J-47. Thus the vast majority of the deletions in these patients have proximal breakpoints in a similar region distal to the 5' end of the gene. HH1 was mapped with respect to the X;1 translocation in a DMD female and was shown to lie at least 80 kb from the starting point of the chromosome jump, HIP25.

INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked muscle-wasting disorder affecting about 1 in 3,000 boys¹. The high incidence of this recessive disorder is accounted for by a mutation rate of about 10^{-4} (reference 2). A location for the disease locus at Xp21 was first suggested by the discovery of female DMD patients with balanced X;autosome translocations³. This location was confirmed by linkage studies using restriction fragment length polymorphisms (RFLPs)^{4,5} and the finding of DMD patients deleted for part of Xp21^{6,7}. Other studies indicated that Becker muscular dystrophy (BMD), a milder form of the disease, is an allelic variant of DMD⁸.

Even though the biochemical basis for DMD is unknown, genomic sequences from within the locus have been isolated. pERT87 and HIP25 were isolated from subtractive hybridisation experiments using an individual with a large deletion of Xp21^{9,10}, and XJ1.1 represents the X chromosome site of the breakpoint in a DMD female with an X;21 translocation¹¹. Conserved sequences from the pERT87 region were used to identify a large messenger RNA in fetal muscle cells¹². Recently, cDNA sequences corresponding to parts of this mRNA have been isolated^{13,14,15,16,17,18} and the entire fetal cDNA found to contain at least 60 exons and represent a messenger RNA of 14 kb. Mapping sections of this cDNA to the genome has shown that the expressed sequences constitute less than one hundredth of the genomic sequence

indicating that, if this exon to intron ratio holds true for the whole gene, it is over a megabase in size. Large scale restriction maps generated by pulsed-field gel electrophoresis (PFGE) also indicate that the region affected by mutation in DMD covers at least 2 megabase pairs^{19,20,21,22}.

The distribution of mutations across the DMD locus has been the subject of recent scrutiny. The breakpoints for translocations leading to DMD in females are spread across the whole region³, whereas there is some evidence that deletion breakpoints, although they occur across the whole gene, may be clustered^{14,16}. More than 50% of DMD or BMD patients carry a deletion for all or part of the DMD gene, although to date no correlation has been found between the extent or position of a deletion and the severity of the disease. Because of the size of the gene and the large intron to exon size ratio, large areas of the gene exist for which no unique sequences are yet available. The generation of more probes within the DMD locus would greatly assist a more detailed analysis of the mutations causing the disease. One way to obtain new sequences is by chromosome walking from existing probes, and indeed the regions around pERT87 and XJ1.1 have been expanded by this method^{13,17}.

Sequences approximately 100 kb from existing probes can be generated using the technique of chromosome jumping²³. This method has been used successfully to isolate a sequence 50-240 kb from the metG probe, a marker closely linked to the cystic fibrosis (CF) gene²⁴, and recently a DNA segment approximately 200kb from the G8 marker which is near to the Huntingtons Chorea locus (Collins, unpublished).

We have previously reported a conserved sequence, HIP25, located in the 5' end of the DMD locus, which is deleted in 10% of DMD patients and lies within 10 kb of XJ1.1¹⁰. This conserved sequence does not identify a unique mRNA expressed in human muscle and a chromosome walk in the region failed to identify part of the DMD gene sequence. This region is now known to correspond to one of the largest introns in the gene (>100 kb)¹⁷.

In an attempt to isolate coding sequences of the gene and to further characterise the DMD locus, we used HIP25 as a starting point for chromosome jumping, resulting in the isolation of a new unique sequence, HH1. The jump from HIP25 crosses the breakpoint for the X;1 female translocation indicating that HH1 represents a jump of more than 80 kb. Furthermore, the vast majority of the proximal deletion breakpoints in patients deleted for HIP25 and XJ1.1 lie between HH1 and the probe J-47.

EXPERIMENTAL METHODS

Cell Lines and DNA probes

Cell lines were produced from blood of DMD patients and normal controls using Epstein Barr virus.

The probes from the pERT87 region, J-Bir, J-MD, J-47 and pERT84 were provided by Dr L. Kunkel. XJ1.1 was a gift from Professor R. Worton. HIP25 was previously isolated in our laboratory¹⁰.

Deletion Analysis

DNA extraction, Southern blotting, hybridisation of radioactive probes and autoradiography was conducted as previously described²¹. All probes were labelled using the random primer kit (Boehringer/Mannheim) to at least 10^8 cpm/ μ g.

Hybridisation of phage inserts under competition was carried out according to Porteous et al²⁵ using a 2000 fold excess of cold sonicated human DNA to compete out repetitive DNA in labelled probes.

Jumping Library

The jumping library Fc3A obtained from Francis Collins, was designed to allow jumps of 80-130 kb and is described in Collins et al²⁴. For its construction, human lymphoblastoid DNA was digested partially by Mbo I. The fraction of molecules sized between 80 and 130 kb were ligated to form large circles in the presence of Bam HI-ended supF genes as a selectable marker. The religated circles were digested to completion with Eco RI and cloned into the Eco RI site of Ch3A lacXba (gift of F. Blattner). This vector has a capacity for inserts of 0-12 kb. The library was plated and subsequently grown on MC1061, as this host is supF⁻ and therefore only clones containing the supF gene will grow. Individual surviving clones should contain two DNA sequences 80-130 kb apart in the genome, in opposite orientation, and bracketing the supF gene. The original library contained 3×10^6 clones and one of four amplified fractions, representing 7.5×10^5 clones, was used for this study.

Library Screening

6×10^5 clones were screened using the method of Benton and Davis²⁶ with hybrid nylon filters (Amersham). Hybridisation was carried out as detailed for deletion analysis and all filters were washed to high stringency (0.1xSSC, 0.1% SDS). Positive plaques were picked and phage eluted into SM buffer²⁷. Plaques containing single clones were obtained following secondary and tertiary screening.

Phage and plasmid DNA extraction

Phage and plasmid DNA were prepared as described by Maniatis²⁷. Inserts were examined after restriction of phage DNA with Eco RI and separated on 0.8% agarose gels.

Subcloning phage inserts

Eco RI inserts were subcloned into the plasmid vector pBRΔAva, which has no Ava I site. As the supF gene contains an Ava I site this allowed convenient mapping of the insert. Selection for clones carrying a supF-containing insert was conducted by transforming the plasmid into host CARD-15 which has a lacZam mutation. When grown on MacConkey's agar plus Ampicillin supF carrying colonies were identified as purple as opposed to pink colonies. The presence of the supF gene was confirmed by its hybridisation to Eco RI-digested DNA from positive colonies using Southern blotting.

Preparation of the supF gene

SupF gene DNA was prepared from plasmid pTBsupF which contains 7 tandem copies of the Bam HI ended supF gene cloned into pBR328 (F.C unpublished). The pTBsupF host, CARD-15 was grown on MacConkey's agar containing ampicillin and plasmid DNA prepared as described above. The DNA was digested with Bam HI and the 219 bp supF fragment purified on 1.4% agarose.

Restriction Enzyme Digestion of DNA for PFGE

Agarose blocks containing large molecular weight human DNA from transformed lymphocytes were prepared and digestion conducted as described in Kenwick et al²¹. Sperm were treated in the same way as lymphocytes, except that 10mM dithiothreitol was included in the lysis solution and cell density was adjusted to account for the haploid chromosome number. The enzymes Xho I, Sfi I, Mlu I and Sma I were obtained from New England Biolabs.

Gel Running and Apparatus

Pulsed-field gel analysis was carried out using a commercial apparatus from LKB with gel preparation and running conditions as described in Kenwick et al²¹.

RESULTS AND DISCUSSION

Isolation of jump clone HH1

A chromosome walk in an X chromosome phage library initiated from HIP25 resulted in the isolation of a 1 kb unique Hind III sequence 5' to HIP25. This probe, HIPW, gave a cleaner hybridisation signal to genomic DNA than HIP25 and was used in place of the latter to screen Fc3A, a jumping library constructed to allow jumps of 80-130 kb (see methods). Two positive clones

Figure 1

Southern blot showing hybridisation of the 8 kb Eco RI insert from phage clone FcH1 to Eco RI-digested DNA from a control male, lane 1, patient 1400, lane 2 and a control female, lane 3.

(FcH1 and FcH2) were identified in a total of 6×10^5 phage plaques. DNA isolated from these phage was digested with Eco RI to excise the inserts, and analysed on agarose gels. FcH1 possesses inserts of 8 kb and 2.5 kb and FcH2 carries an insert of 6 kb. HIPW hybridised to the 8 kb insert of FcH1 and to the single 6 kb insert of FcH2. The supF gene was found to hybridise to the same fragments indicating that these represent real junction fragments and that the 2.5 kb fragment is a non-junction sequence ligated with the phage during library construction. Previous analyses of the library have indicated that 10% of the recombinant clones contain more than one Eco RI insert. These supF-positive inserts were cloned into the plasmid vector pBRΔAva for further analysis. SupF-positive clones were selected using the host CARD-15 and MacConkey's agar.

In order to determine whether the junction fragments contained DNA from the appropriate region of the X chromosome, the 8 kb and 6 kb inserts from FcH1 and FcH2 were hybridised under competition to Southern blots prepared using Eco RI-digested DNA from male and female human controls and from a DMD patient who carries a large deletion in Xp21 (patient 1400, SS of Wilcox et al²⁸). Under these conditions we were able to determine that the 8 kb insert from FcH1 contains at least two unique sequences from Xp21: one that recognises the same 4 kb Eco RI fragment as the probe used to screen the library, HIPW, and a second that identifies a 13 kb band (Fig.1). Both bands are absent in the deletion patient (lane 2). These represent independent unique sequences on either side of the junctional supF gene as

Figure 2

Hybridisation of HH1 (a) and J-47 (b) to a Southern blot of Pst I-digested DNA from a control male, lanes 1 and 3, DMD patient 1426, lane 2, patient 1406, lane 4, a control female, lane 5.

no Eco RI sites exist within the 8 kb insert. The insert from FcH2 was found to hybridise in a highly repetitive fashion and was not studied further. The 8 kb insert from FcH1 was subcloned into pBRΔAva. The subclone pFcH1 was restriction mapped and a unique Bgl II fragment of 3.5 kb was found to identify the 13 kb Eco RI band shown in Fig.1. Southern blotting was used to show that this fragment was on the other side of the supF gene to that containing HIPW. This new unique sequence, designated HH1, should be 80-130 kb from HIP25 within the DMD locus.

Mapping HH1 within the DMD locus

To determine its physical location, HH1 and a number of other genomic sequences from within the DMD locus were hybridised to Pst I digested DNA from a series of DMD and BMD patients with deletions in Xp21 (for probe references see reference 29). HH1 identifies three Pst I genomic bands of 1 kb, 4.4 kb and 14.5 kb which are different from that identified by HIPW. An example of this analysis is shown in Fig. 2 where a) and b) show hybridisation of HH1 and J-47 to control and patient DNA samples. The 4.5 kb Pst I band identified by J-47 is distinct from that seen by HH1 as evidenced by the differential hybridisation of these probes to the patient DNA in lane 4. Table 1 details the results of the analysis for all of our patients that had previously been found to be deleted for HIP25. The

Figure 3

Southern blot showing hybridisation of HH1 to Pst I DNA from control male, lane 1, and DMD patients 1421, lane 2, 1424, lane 3, 1425, lane 4, 1429, lane 5

physical order of the probes is shown at the top of the table. HH1 was found to be deleted in 14 out of 15 of these unrelated patients, the exception being 1424. In this patient, the 14.5 kb Pst I band was absent and a novel band at 3.5kb was apparent (Fig.3, lane 3). A changed band was also detected in this DNA for the enzyme Hind III, indicating that HH1 lies near a breakpoint for this deletion, i.e. between pert87-30 and pert87-15 or between J-MD and J-47. The absence of a hybridisation signal for patients with deletions extending from XJ1.1 to J-MD (inclusive), that is patients 1486, 1487, 1488, 1489, 1516 and 1517, shows that HH1 must lie in the interval J-MD to J-47. Patients previously found to be only deleted for sequences distal to HIP25 were all found to be positive for HH1 (the patients in lanes 2, 4 and 5 of Fig. 5 are of this category). A useful consequence of determining the direction of the jump is that it is possible to orientate the sequences HH1 and HIPW with respect to the gene.

It is interesting to note that 12 out of the 15 patient deletions that

Table 1

Deletion of DNA markers (top row) in DMD patients (left hand column). O, absent; ●, present.

←Xpter	J-B1r	pERT 87-					XJ1:1	HIP 25	J-MD	HHI	J-47	pERT 84	X-cen →
		41	30	15	8	1							
1426	●	○	○	○	○	○	○	○	○	○	○	○	
1424	●	●	●	○	○	○	○	○	○	A	●	●	
1338	●	●	○	○	○	○	○	○	○	○	●	●	
1399	●	●	●	○	○	○	○	○	○	○	●	●	
1406	●	○	○	○	○	○	○	○	○	○	●	●	
1423	●	●	●	○	○	○	○	○	○	○	●	●	
1431	○	○	○	○	○	○	○	○	○	○	○	●	
1461	●	○	○	○	○	○	○	○	○	○	●	●	
1462	●	●	○	○	○	○	○	○	○	○	●	●	
1486	●	●	●	●	●	●	○	○	○	○	●	●	
1487	●	●	●	●	●	●	○	○	○	○	●	●	
1488	●	●	●	●	●	●	○	○	○	○	●	●	
1489	●	●	●	●	●	●	○	○	○	○	●	●	
1516	●	●	●	●	●	●	○	○	○	○	●	●	
1517	●	●	●	●	●	●	○	○	○	○	●	●	

Figure 4
PFGE analysis of Xho-1 digested DNA using probes J-MD (a), HH1 (b), J-47 (c). Lanes 1 to 5 show hybridisation to a series of male DNAs digested to different extents with Xho I. The position of the steps of a 50 kb Lambda ladder used to size the DNA fragments is shown to the left of each figure by black dots.

Figure 5

PFGE analysis of Sfi I-digested DNA from control males, lanes 1 and 2, and the X:1 translocation DMD patient lane 3. Figures a) and b) show hybridisation to HH1 and pERT84 respectively.

include HIP25 have the proximal breakpoint between HH1 and J-47, whereas, the distal breakpoint is more variable in location. The significance of this result will depend on the relative distance between HH1 and J-47 compared to the size of the whole region. An assessment of the distance between these probes using restriction enzymes with hexameric consensus sequences was inconclusive as HH1 and J-47 do not share restriction fragments for at least 6 enzymes (Pst I, Bam HI, Eco RI, Eco RV, Hind III, and Bgl II).

Large scale physical maps, which encompass the probes listed in Table 1 have been produced using pulsed-field gel electrophoresis in conjunction with enzymes that cut mammalian DNA infrequently^{19,20,21,22}, but no data is available on the distance between probes J-MD and J-47 other than that they are included on a common Sfi I fragment of 700 kb. We have attempted to determine more accurately the distance between the sequences J-MD, HH1 and J-47 by using Xho I, Sma I and Mlu I, enzymes that generally give rise to fragments of 25 kb to 400 kb. Bands for the latter two enzymes were observed using sperm but not lymphoblastoid DNA, a result that probably reflects a degree of methylation in this region that differs between cell types.

J-MD and HH1 share common Xho I bands of 100 kb, 150 kb and 200 kb (Fig. 4) and a Sma I band of 125 kb (data not shown) and therefore probably

Figure 6

Pst I digested patient DNA hybridised to a Hinc II/Eco R I fragment of the cDNA Cf27. lanes 1 and 14, female and male controls respectively, lanes 2 to 13 patients 1517 (2), 1487 (3), 1489 (4), 1516 (5), 1488 (6), 1462 (7), 1486 (8), 1461 (9), 1406 (10), 1423 (11), 1399 (12), 1388 (13). Patients 1487 and 1516 suffer from BMD.

lie within 100 kb of each other. HH1 and J-47 are on consecutive Xho I fragments of 100 kb and 160 kb respectively and identify common partial bands of 270 kb and 320 kb, the latter partial also being recognised by J-MD (Fig. 4). The interval containing most of the deletion endpoints in this region ie. that between HH1 and J-47 has a maximum size therefore of 270 kb.

In a cosmid walk around XJ1.1, this sequence, and therefore also HIP25, has recently been shown to lie approximately 120 kb proximal to the distal end of a 700 kb Sfi I fragment³⁰. We have previously demonstrated that the X;1 translocation breakpoint in a DMD female lies approximately 200 kb proximal to the same Sfi I site²¹. In this patient HH1 hybridises to the 550 kb Sfi I fragment of the derivative X chromosome detected also by pERT84 (Fig.5), as opposed to the 200 kb fragment from the derivative chromosome 1 which is detected by HIP25²¹. This implies that HH1 is proximal to the translocation breakpoint and that a distance of more than 80 kb has been jumped from HIPW (and therefore HIP25) to HH1. J-MD identifies the same 200 kb altered band as HIP25 (data not shown). HH1 and J-MD therefore bracket the translocation breakpoint within a distance of 100 kb.

Recently we reported an exon map using cDNA sequences isolated from the 5' end of the DMD gene¹⁸. Hybridisation of HH1 to this cDNA revealed that HH1 itself does not detect coding sequences. However a Pst I band in a phage clone extending distal from HH1 cross-hybridised with a cDNA clone (Cf27, ref 18). This 14.5 kb Pst I band is seen in Southern blots by both HH1 and the cDNA clone and is changed in patient 1424 (see Table I). This

Figure 7

A cDNA map of the interval between J-MD and J-47 in the 5' region of the DMD gene. Exons (a-f) are represented by solid blocks and the sizes of the Pst I fragments they detect are indicated below. The order of exon c relative to d and exon e relative to f has not been possible to determine using our deletion patients. The Hinc II/Eco R1 cDNA fragment used to examine deletion breakpoints includes the 4 exons c,d,e and f. HH1 and the nearest 3' exon detect the same 14.5 kb Pst I band.

maps this exon of the cDNA between HH1 and J-MD, and enables us to distinguish its location from that of other exons mapping between HIP25 and J-47. The updated version of this cDNA map is presented in Fig. 7.

We have previously shown that a Hinc II/Eco R1 cDNA fragment (fragment 4 of ref. 18) detects 4 exons represented by Pst I bands of 2.5 kb, 5 kb, 9.8 kb and 15 kb. A comparison of the deletion pattern in patients for HH1 and these exons revealed that at least two exons, those detecting 2.5 kb and 5 kb Pst I bands, must lie between HH1 and J-47. The Hinc II/Eco R1 fragment was therefore used to examine the breakpoints in the patients described here. Figure 6 shows the result of this analysis. The 2.5 kb and 9.8 kb Pst I fragments are usually very faint and can not be detected at all on this autoradiograph, although the deletion pattern for the 2.5 kb band has been shown to mimic that for the 5 kb band¹⁸. Nevertheless, we can see that the 5 kb band is present in six patients DNA and absent in six others, whereas, the 15 kb band is present in all samples. We can therefore conclude from figure 6 that the breakpoints of these patients do not lie within the same sequence.

A novel band of about 20 kb is seen for patient 1487 which may represent a deletion junction fragment detected by one of these two exons.

In summary, we have successfully used a chromosome jumping library to explore the DMD locus and move from a sequence within a large intron in the gene to a more proximal region. Pulsed field gel electrophoresis studies indicate that the jump clone (HH1) lies at least 80 kb from the starting point. This sequence defines a region of less than 270 kb in which many of the proximal endpoints of deletions in patients lie. The significance of this result remains to be determined but the endpoints are not tightly clustered within a short stretch of DNA sequence. Recently Heilig et al³⁰ reported a cluster of three deletion breakpoints within a distance of 20 kb

as part of a cosmid walk in a 5' direction from the sequence XJ1.1. The relationship of these two breakpoint regions has yet to be determined. HH1 has also enabled us to refine the cDNA exon map and further analysis using portions of the cDNA shows that the deletion endpoints can be divided by exons in this region.

ACKNOWLEDGEMENTS

We are grateful to our clinical colleagues for samples, Jeffrey Cole and Mitch Drumm for assistance in construction of the hopping library. Helen Blaber for typing of the manuscript, and Sue Forrest and Mark Patterson for helpful discussions. We would like to thank the Medical Research Council, the Muscular Dystrophy Group of Great Britain, the Muscular Dystrophy Association, USA, the National Institute Grant : GM34960 and the Howard Hughes Medical Institute for financial support. TJS is a recipient of an SERC Instant Award.

REFERENCES

1. Emery, A.E.H. (1987) Oxford Monographs on Medical Genetics No 15. Duchenne Muscular Dystrophy, Oxford University Press, Oxford.
2. Moser, H. (1984) Hum. Genet. 66: 17-40.
3. Boyd, Y. (1987) Ann. Hum. Genet. 51: 13-26.
4. Murray, J.M., Davies, K.E., Harper, P.S., Meredith, L., Mueller, C.R. and Williamson, R. (1982) Nature 300: 69-71.
5. Davies, K.E., Pearson, P.L., Harper, P.S., Murray, J.M., O'Brien, T., Sarfarazi, M. and Williamson, R. (1983) Nuc. Acid. Res. 11: 2303-2312.
6. Francke, U., Och, H.D., de Martinville, B., Giacolone, J., Lindgren, V., Distech, C., Pagon, R.A., Hofker, M.H., van Ommen, G.-J., Pearson, P.L. and Wedgewood, R.J. (1985) Am. J. Hum. Genet. 37: 250-267.
7. De Martinville, B., Kunkel, L.M., Bruns, G., Morle, F., Koenig, M., Mandel, J.L., Horwich, A., Latt, S.A., Gusella, J.F., Housman, D. and Francke, U. (1985) Am. J. Hum. Genet. 37: 235-249.
8. Kingston, H.M., Sarfarazi, M., Thomas, N.S.T. and Harper, P.S. (1984) Hum. Genet. 67: 6-17.
9. Kunkel, L.M., Monaco, A.P., Middlesworth, W., Ochs, H.D. and Latt, S.A. (1985) Proc. Natl. Acad. Sci. USA. 82: 4778-4782.
10. Smith, T.J., Wilson, L., Kenwrick, S.J., Forrest, S.M., Speer, A., Coutelle, Ch. and Davies, K.E. (1987) Nucl. Acid. Res. 15: 2167-2174.
11. Ray, P.N., Belfall, B., Duff, C., Logan, C., Kean, V., Thompson, M.W., Sylvester, J.E., Gorski, J.L., Schmickel, R.D. and Worton, R.G. (1985) Nature 318: 672-675.
12. Monaco, A.P., Bertelson, C.J., Middlesworth, W., Colletti, C-A., Aldridge, J., Fischbeck, K.H., Bartlett, R., Pericak-Vance, M.A., Roses, A.D. and Kunkel, L.M. (1985) Nature 316: 842-845.
13. Monaco, A.P., Neve, R.L., Colletti-Feener, C., Bertelson, C.J., Kurnit, D.M. and Kunkel, L.M. (1986) Nature 323: 646-650.
14. Koenig, M., Hoffman, E.P., Bertelson, C.J., Monaco, A.P., Feener, C. and Kunkel, L.M. (1987) Cell 50: 509-517.
15. Cross, G.S., Speer, A., Rosenthal, A., Forrest, S.M., Smith, T.J., Edwards, Y., Flint, T., Hill, D. and Davies, K.E. (1987) EMBO J. 6: 3277-3283.
16. Forrest, S.M., Cross, G.S., Speer, A., Gardner-Medwin, D. and Davies, K.E. (1987) Nature 329: 638-640.
17. Burghes, H.M., Logan, C., Hu, X., Belfall, B., Worton, R.G., and Ray, P.N. (1987) Nature 328: 434-437.
18. Smith, T.J., Forrest, S.M., Cross, G.S., and Davies, K.E. (1987) Nucl. Acid. Res. 15: 9761-9769.
19. Burmeister, M. and Lehrach, H. (1986) Nature 324: 582-585.
20. Van Ommen, G.J.B., Verkerk, J.M.H., Hofker, M.H., Monaco, A.P., Kunkel, L.M., Ray, P., Worton, R., Wieringa, B., Bakker, E. and Pearson, P.L. (1986) Cell 47: 499-504.

21. Kenwrick, S., Patterson, M.N., Speer, A., Fischbeck, K. and Davies, K. (1987) *Cell* 48: 351-357.
22. Van Dunnen, J.T., Bakker, E., Breteler, E.G.K., Pearson, P.L. and Van Ommen, G.J.B. (1987) *Nature* 329: 640-642.
23. Collins, F.S. and Weissman, S.M. (1984) *Proc. Natl. Acad. Sci. USA* 81: 6812-6816.
24. Collins, F.S., Drumm, M.C., Cole, J.C., Lockwood, W.K., Vande Woude, G.F. and Iannuzzi, M.C. (1987) *Science* 235: 1046-1049.
25. Porteous, D.J., Morton, J.E.N., Cranston, G., Fletcher, J., Mitchel, A., van Heyningen, V., Fantes, J.A., Boyd, P.A. and Hastie, N.D. (1986) *Mol. Cell. Biol.* 6: 2223-2232.
26. Benton, W.D. and Davis, R.W. (1977) *Science* 196: 180-182.
27. Maniatis, T., Frisch, E.F. and Sambrook, J. (1982) Cold Spring Harbour Laboratory, USA.
28. Wilcox, D.E., Cooke, A., Colgan, J., Boyd, E., Aitken, D.A., Sinclair, L., Glasgow, L., Stephenson, J.B.P. and Ferguson-Smith, M.A. (1986) *Hum. Genet.* 73: 175-180.
29. Monaco, A.P. and Kunkel, L.M. (1987) *Trends Genet.* 3: 33.
30. Heilig, R., Lemaire, C., Mandel, J-L. (1987) *Nucl. Acid. Res.* 15: 9129-9142.