Automated analysis of multiplex microsatellites

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Abstract
The use of automated DNA fragment analysis with the Applied Biosystems 672 Genescanner system was evaluated in a routine diagnostic setting. The aim of the study was to compare automated fragment detection and analysis with conventional methods. For cystic fibrosis analysis the ΔF508 mutation in exon 10 of the cystic fibrosis transmembrane regulator (CFTR) gene was multiplexed with two intragenic microsatellites. The analysis of the Prader-Willi/Angelman region of chromosome 15 used a panel of five microsatellites. For dystrophin, seven microsatellites covering the entire dystrophin gene were co-amplified. Automated analysis was faster and more accurate than analysis using radiolabelled products with sequencing gels, although some inconsistencies in the sizing of microsatellite alleles were seen.

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Since their initial description^{1,2} microsatellites, particularly those containing dinucleotide repeats, have become widely used as genetic markers for routine clinical molecular genetics³ and their use is likely to increase as their central role in genome mapping is now established.⁴ Microsatellites are usually detected by high resolution electrophoresis of radiolabelled

DNA amplification products followed by autoradiography. Unfortunately many dinucleotide repeat microsatellites can be difficult to resolve even using denaturing sequencing gel conditions. This is because often more than one band per allele is seen on a gel ("band ladder"), making it difficult to assign alleles to particular bands. For clinical molecular genetic applications, alleles are usually scored within a pedigree, or in some cases with reference to a size standard in an adjacent lane. By the judicious selection of product sizes it is possible to resolve three or more different microsatellites on the same gel. The development of automated DNA sizing using four colour fluorescent labelling offers an alternative means of microsatellite analysis.^{5,6} There are several advantages with this approach: a non-radioactive detection system, semiquantitative product analysis, the option of internal lane size standards, high order multiplex amplification, and electronic analysis and data storage. This study reports a comparison between conventional autoradiography and the Applied Biosystems 672 Genescanner in a diagnostic setting for the molecular genetic analysis of cystic fibrosis, Prader-Willi and Angelman syndromes (PWAS), and Duchenne muscular dystrophy.

Methods
PATIENTS
All cases were referred from the Yorkshire Re-

Table 1 Primers used in this study

Marker	Size (approx)	Sequence (5'-3')
CFTR multiplex (59°C annealing)		
CFTR F508	96 or 99	6-FAM GTT TTC CTG GAT TAT GCC TGG GCA C GTT GGC ATG CTT TGA TGA CGC TTC
CFTR IVS-8	170-190	6-FAM ACT AAG ATA TTT GCC CAT TAT CAA GT TCT ATC TCA TGT TAA TGC T
CFTR IVS-17b	200-260	6-FAM GAC AAT CTG TGT GCA TCG 5' GCT GCA TTC TAT AGG TTA TC 3'
Prader-Willi/Angelman syndrome multiplex (55°C annealing)		
D15S10	170-6	6-FAM GAC AGC TGA ACG TAG TTA AAG GTA ACA CTA TGA ATT GTT AGT G
GABRB3	160-80	JOE-CTC TTG TTC CTG TTG CTT TCA ATA CAC CAC TGT GCT AGT AGA TTC AGC TC
D15S11	170-190	JOE-GAC ATG AAC AGA GGT AAA TTG GTG G GCT CTC TAA GAT CAC TGG ATA GG
GABRA5	230-60	JOE-GAT GAC TTA CCC ACC TTT ATT C GTA GAA TTT CCC TGT AAA GGC AC
D15S113	120-30	6-FAM CAT GTA CTG TTT TAT CCC TGT GGC CTG CTG CTT ATA CTC TTT CTC TAT TC
Dystrophin multiplex (59°C annealing)		
5' dys-I	172-184	TAMRA GTT AAC AAA ATG TCC TTC AGT TCT ATC C ACT GTA AAT GAA ATT GTT TTC TAA GTG CC
5' dys-II	216-230	TAMRA ATT ATG AAA CTA TAA GGA ATA ACT CAT TTA GC TCT TGA TAT ATA GGG ATT ATT TGT GTT TGT TAT AC
STR 44	184-204	FAM TC CAA CAT TGG AAA TCA CAT TTC AA TCA TCA CAA ATA GAT GTT TCA CAG
STR 45	160-184	JOE GAG GCT ATA ATT CTT TAA CTT TGG C CTC TTT CCC TCT TTA TTC ATG TTA C
STR 49	232-256	JOE CG TTT ACC AGC TCA AAA TCT CAA C CAT ATG ATA CGA TTC GTG TTT TGC
STR 50	238-254	FAM AAG GTT CCT CCA GTA ACA GAT TTG G TAT GCT ACA TAG TAT GTC CTC AGA C
3'-CA	127-139	TAMRA GGA TGC AAA ACA ATG CGC TGC CTC GAA AGA TTG TAA ACT AAA GTG TGC

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gional Genetics Service, with the exception of some of the Prader-Willi/Angelman syndrome cases which were from the Genetics Services of the Northern and South West Thames Regions.

PRIMER SYNTHESIS

Unlabelled and 6-FAM (a fluorescein derivative) labelled oligonucleotides were synthesised using an Applied Biosystems 391 PCR-Mate on a 40 nM scale using 6-FAM amidite. After deprotection and column elution, the oligonucleotide was ethanol precipitated, redissolved in distilled water, and stored at -20°C until use. The other fluorescent dye labelled oligonucleotides (JOE (green), FAM (blue), and TAMRA (yellow)) were supplied by British Biotechnology Ltd as HPLC purified solids. The sequence of each primer is shown in table 1. Three multiplex systems were examined: a CFTR region which included primers flanking F508,⁷ IVS-8 (CA)_n, and IVS-17b(TA)_n^{8,9} using 6-FAM labelled primers, a set of five (CA)_n markers from 15q11-q12 close to the Prader-Willi and Angelman syndrome critical regions¹⁰⁻¹³ using 6-FAM and JOE labelled primers, and a set of seven (CA)_n markers from the dystrophin locus using FAM, JOE, and TAMRA labelled primers.¹⁴⁻¹⁶ In each case the Red channel of the Genescanner was reserved for size standards (Rox 2500, Applied Biosystems).

DNA AMPLIFICATION

Amplification reactions were in 20 μl containing 0.5 U *Taq* polymerase, 200 $\mu\text{mol/l}$ dNTPs, 50 mmol/l KCl, 10 mmol/l Tris-Cl pH 8.5, 1.5 mmol/l MgCl₂, 0.01% triton X-100. For the incorporation of radioactivity, ³²P dATP (1 μCi /reaction at 1000 Ci/mmol, Amersham) was used and the concentration of unlabelled dATP was reduced to 2.5 $\mu\text{mol/l}$. Each reaction contained approximately 100 ng of human DNA and between 20 and 50 pmol of each primer for radiolabelling, or 2 to 5 pmol for fluorescence labelling.

Multiplex amplification conditions were established by comparing a range of annealing temperatures for each primer pair and selecting the highest temperature at which all primer pairs yielded a product. An initial multiplex reaction was then carried out using equimolar primer concentrations. Based on the relative yields of products in this assay, concentrations of individual primer pairs were then adjusted to produce as near as possible equivalent yields. Cycling conditions were similar for all primer sets; an initial denaturation at 93°C for five minutes, during which the reaction mixtures were combined under oil to achieve "hot start" conditions. This was followed by 22 cycles (fluorescent primers) or 27 cycles (radiolabelled products) of 95°C for 30 seconds and $55-60^{\circ}\text{C}$ for one minute using a Hybaid Omnigene Thermal Cycler with tube temperature control. A final extension for three minutes was carried out at 60°C before stopping the reactions by adding an equal volume of deionised formamide.

The products were then either stored at

-20°C until use, or heated to 80°C and loaded (5 μl for radiolabelled products, 1–1.5 μl for fluorescent products) onto denaturing polyacrylamide gels (6%) for electrophoresis at 60 W on 40 cm gels (radiolabelled) or 40 W on 12 or 24 cm well-to-read (the distance between the wells and the laser scanning) gels (fluorescent). High temperature gels were run using a temperature controlled sequencing rig (Hybaid) at 65°C .

ANALYSIS OF RESULTS

Autoradiographs were scored manually in allele size order (allele 1 = largest) for each microsatellite within each pedigree. All samples were run in duplicate. The mobility of fluorescent peaks was compared to internal lane standards (Rox 2500) using Genescan 1.1 software, and sizes in base calculated automatically using the third order least squares approximation included in the software. The mean size of duplicate samples was rounded up to the nearest whole number and used to designate the allele size in base pairs.

Results

OPTIMISING THE MULTIPLEX ASSAYS

Initial studies (not shown) gave optimal results

Figure 1 CFTR multiplex. Allele sizes are written in base length, sized using Genescan software as described in the Methods. Three base deletions detected using exon 10 primers are scored as ΔF508 (although other, rare deletions are not formally excluded). Normal sized products are scored as wtF508 (wild type), although point mutations and allele dropout are not formally excluded. The affected child in this pedigree has a carrier mother with an unidentified mutation in the CFTR gene. His father is deleted at F508 on one chromosome. The high risk maternal chromosome (wild type at F508) can be identified as having a 200 base IVS17b microsatellite.

Figure 2 Dystrophin multiplex. Multiplex amplification of seven microsatellites using equimolar primer concentrations resulted in uneven yields. The relative yields could be manipulated by increasing primer concentrations for the weakly amplified products to give a more robust signal.

with fluorescent primer concentrations reduced by between 5 and 10 fold (2–5 pmol of each

primer in a 20 µl reaction) and cycle numbers of between 22 and 24. In general, larger fragments were less efficiently amplified, although with some exceptions (for example, IVS17b in the CFTR multiplex) (fig 1). In the dystrophin multiplex, the 5' CA-II microsatellite gave the weakest signal, probably owing to a combination of the use of TAMRA (which gives a less intense signal than FAM and JOE) as the primer label and the intrinsic lower efficiency of this amplification reaction. We were able to partially compensate for this by increasing the relative primer concentration for 5' CA-II (fig 2).

RESOLUTION OF PRODUCTS
Most microsatellites have had primers designed to amplify products of between 100 and 300 bp and with careful selection of different sized products it is possible to resolve three sets of non-overlapping microsatellites on the same autoradiograph. Even so it is often difficult to determine small size variations in larger products. Using single colour labelling with an internal lane standard of a different colour gave results equivalent to autoradiography (fig 3) with the additional benefits of allele size estimates in bases and improved resolution of larger microsatellites. In attempts to improve the resolution of radiolabelled microsatellites, end labelled primers and high temperature gels were investigated, as well as the two phase amplification protocol described previously.¹⁷ Although both methods helped to reduce the number of extra bands (fig 4) they were still not eliminated completely. Shadow bands were also visible using the Genescanner but they were not very pronounced and major bands were easy to identify. Even so, heterozygotes with alleles of similar size were sometimes difficult to score. Generally this became easier as operators became familiar with the characteristic profile of each microsatellite.

Figure 3 Dystrophin multiplex: comparison of autoradiographic results with Genescan results. Lane 1 in each autoradiograph is a standard, followed by a pedigree consisting of the mother, the father, their son, affected by Duchenne muscular dystrophy, and his sister, who is at risk of carrying a dystrophin deletion. The table compares the results obtained from manual scoring of the autoradiographs with the results generated by the Genescan 672 software. The at risk carrier was heterozygous at STR 45, 49, and 50, all of which were deleted in her brother. A new allele size was seen in her STR 45 marker, scored as 1 on the autoradiograph and as 183 bases using the Genescan software. Since the other six microsatellites were consistent with true paternity, this new size probably represents a new mutation.

Figure 4 Improved resolution of microsatellites using heated gels and end labelled primers. STR 45 (lower bands) and STR 50 (upper bands) from the dystrophin gene were co-amplified using either (A) labelling by incorporation followed by resolution on a conventional 6 mol/l urea sequencing gel, (B) running the same product on a temperature controlled gel with 6 mol/l urea at 60°C, or (C) using one primer end labelled followed by resolution on a heated gel as in (B). The STR 45 microsatellite was resolved best using end labelled primers and very stringent denaturing conditions (C).

REPRODUCIBILITY OF RESULTS
Variation in size estimates obtained using 24 cm and 12 cm gels was more pronounced than within each gel type. In 24 cm (but not the 12 cm) gel formats it was necessary to define the size standards manually because of peak splitting. In the course of this study we found that by using the third order least squares approximation and taking the mean size estimated from two gel lanes always gave values with reproducibility of within one base provided that the same gel size was used. Table 2 shows data on the automated sizing of the IVS-17b microsatellite of the same sample over 10 runs using a 12 cm gel. We observed cases in which the PCR product size varied by almost

exactly one base. The D15S113 product was particularly prone to generating this artefact (fig 5). The anomalous sized alleles in D15S113 products were seen in amplification products from the same person when the annealing temperature was changed to 57°C from 55°C. The change in allele size was seen only in D15S113, not in any other of the four microsatellites multiplexed in the same reaction.

DIAGNOSTIC USE OF THE ASSAYS
Cystic fibrosis

The most frequent CFTR mutation in the Yorkshire Region is ΔF508, accounting for over 82% of the mutations seen on CF chromosomes. Screening for known mutations in addition to ΔF508 does little to modify prior risks for this population since the three next most common mutations combined account for less than 3% of CFTR mutations tested so far. Further studies aimed at identifying the CFTR mutations in our local population are planned. At present it is more cost effective to establish high risk chromosomes by linkage to intragenic microsatellites. The microsatellite approach provides the additional benefit of detecting maternal contamination of chorionic biopsies in most cases.¹⁷

Prader-Willi/Angelman syndrome
In a comparison between manual radioactive and automated fluorescent detection systems

Table 2 Reproducibility of size estimates of the CFTR IVS-17b(TA)_n microsatellite. The size estimates shown are from the same person analysed on different occasions over a six month period. Samples from this heterozygote (251–259) were run in duplicate. By taking mean values the range of sizes observed was within 1 base. In practice sufficiently accurate results can usually be obtained without running duplicates

Worksheet	Track 1	Track 2	Mean	Track 1	Track 2	Mean
335	251-28	251-23	251-26	259-07	259-21	259-14
341	251-28	251-37	251-33	259-28	259-25	259-27
35	251-58	251-36	251-47	259-42	259-35	259-39
51	251-24	251-36	251-30	259-26	259-27	259-27
70	250-99	251-11	251-05	259-12	259-00	259-06
100	251-12	251-19	251-16	259-21	259-30	259-26
112	251-35	251-16	251-26	259-58	259-17	259-38
123	250-95	250-89	250-92	258-91	258-94	258-93
156	251-63	251-64	251-64	259-70	259-96	259-83
173	251-58	251-62	251-60	259-75	259-81	259-78
Mean	251-30	251-29	251-30	259-33	259-33	259-33
SD	0-21	0-28	0-24	0-48	0-42	0-45
Max	251-63	251-64	251-64	259-75	259-96	259-83
Min	250-95	250-89	250-92	258-91	258-94	258-93

Figure 5 Variation in the product size of the D15S113 microsatellite. A one base size variation was seen with the D15S113 microsatellite in the same person, although four other microsatellites in the same multiplex were not affected. The ratio of the peak heights between the two alleles was also changed. Amplification conditions were identical except that the sample with the smaller alleles had been annealed at 57°C instead of 55°C.

at three loci in nine pedigrees (28 persons) no discrepancies were seen (table 3). Paternal 15q11–q13 deletions or unimaternal disomy were detected in 40% ($n=20$) of the putative Prader-Willi syndrome cases using the five microsatellites described. Only one of nine possible Angelman syndrome cases showed a detectable loss of maternal markers. Uncertain diagnosis of some of the PWAS cases may account for the low numbers of deletions detected, and further studies (including methylation analysis) are in progress.

Dystrophin

The data generated were essentially the same as those seen in previous studies using dystrophin microsatellites,¹⁸ and the test has been used to determine the carrier status of at risk females in three families (12 persons) so far. No discrepancies between autoradiographic and fluorescent microsatellite data were seen. In one case a new allele size was seen (an increase in STR45 from 181 to 183 bases), thought to be the result of a new mutation. This new allele size was also seen by autoradiography (fig 3).

Discussion

The findings reported here show that fluorescent microsatellite detection using the Applied Biosystems 672 Genescanner was comparable or better than radiolabelling in terms of accuracy and sensitivity. However, substantial costs are incurred in both equipment and consumables for fluorescent systems. These are offset by increased efficiency using multiplex assays. The dystrophin multiplex containing seven microsatellites can be amplified and analysed within four hours, each sample requiring only one reaction tube. The same test carried out using autoradiography would require three separate reactions per sample followed by an exposure time of at least 16 hours.

The CFTR multiplex described enables informative prenatal testing to be carried out in almost every instance with the additional be-

nefit of exclusion of maternal contamination of chorionic villus biopsies. Although only one colour was used for the CFTR primers, the use of internal lane markers of a different colour allowed accurate size estimates of amplification products to be made (table 2). This is clearly more satisfactory than the arbitrary scoring of alleles.

By using two colours it was possible to multiplex five microsatellites mapped to chromosome 15q11–q13, providing a one tube test for deletions and disomy in Prader-Willi and Angelman syndromes. The inclusion of D15S97 in this multiplex should make the test even more informative.

The highest order multiplex carried out was the use of seven microsatellites at the dystrophin locus. Because of the large physical size and high recombination frequency at the dystrophin locus, linkage analysis and risk assignment can be difficult. The microsatellite markers which have been described at the 5' end of the gene, the four intragenic markers, and the 3' untranslated region are useful for identifying high risk chromosomes and recombination events for carrier testing. The intragenic markers have the additional benefit of being within regions in which deletions are known to occur. These markers can be used as a panel of highly informative linked polymorphisms and sometimes to provide direct information on the carrier status of at risk females. Null alleles in affected males are readily detectable, indicating that lane to lane contamination is not a major problem in automated analysis. The dystrophin multiplex could be expanded to include additional microsatellites.

Although some shadow bands are seen in fluorescent microsatellite images, the reduced number of amplification cycles (22 to 24 cycles) and end labelling of primers minimises this effect. As expected, no anomalous fragment sizes are seen in non-microsatellite amplification products such as $\Delta F508$. This is consistent with the laddering effect being dependent on the repeat sequence. Some improvements in resolution of the autoradiographs were obtained by using end la-

Table 3 Comparison of autoradiography with Genescan 672 data at GABRB3, D15S11, and GABRA5. Allele sizes in this series were found to be equivalent when determined manually from autoradiographic data or by Gene 672 software from fluorescent samples. Sizes were rounded up to the nearest whole number

Pedigree	Sample No	GABRB3		D15S11		GABRA5	
		1	2	1	2	1	2
3160	92215	184	192	244	244	283	289
		2	1	2	2	2	1
	92216	180	184	244	244	283	283
		3	2	2	2	2	2
	92217	180	192	244	246	283	289
		3	1	2	1	2	1
BA	921221	180	180	243	243	284	286
		3	3	1	1	2	1
	921222	180	191	243	243	284	286
		3	2	1	1	2	1
	921223	180	198	243	243	282	286
		3	1	1	1	3	1
WI	920154	180	180	244	244	282	287
		3	3	3	3	3	1
	920155	180	192	244	260	279	287
		3	1	3	1	4	1
	920153	180	184	244	252	282	284
		3	2	3	2	3	2
1114	92353	192	192	244	244	283	283
		1	1	3	3	2	2
	92360	180	180	244	254	283	283
		2	2	3	1	2	2
	92361	192	192	244	250	283	286
		1	1	3	2	2	1
1292	92350	180	180	249	251	280	282
		2	2	3	2	2	1
	92352	180	184	243	260	280	282
		2	1	4	1	2	1
	92351	180	180	249	260	282	282
		2	2	3	1	1	1
	92578	180	180	249	259	282	282
		2	2	3	1	1	1
1321	92434	180	192	243	260	278	286
		3	2	2	1		
	92433	180	192	243	260	278	286
		3	2	2	1		
	92432	180	196	243	260	278	286
		3	1	2	1		
1610	92885	180	182	250	252	283	283
		4	3	2	1	2	2
	92884	180	198	250	252	283	285
		4	1	2	1	2	1
	92883	182	192	243	250	283	285
		3	2	3	2	2	1
1911	91838	180	188	244	244	287	289
		4	3	2	2	2	1
	92128	180	188	244	244	287	289
		4	3	2	2	2	1
	92129	194	196	244	250	283	287
		2	1	2	1	3	2
3136	92262	180	180	244	246	285	287
		3	3	2	1	3	2
	92263	180	192	244	244	283	285
		3	1	2	2	4	3
	92261	180	186	244	246	287	289
		3	2	2	1	2	1

belled primers and by heating the gel, but even with these modifications the resolution was no higher than that seen using the fluorescent detection system. More accurate size analysis of microsatellites has shown an artefactual change in D15S113 and other microsatellite product sizes. Some microsatellite markers are especially prone to this artefact, and there is a danger that results could be misinterpreted because of it. We have also observed that amplification products are one base shorter when amplified using enzymes with 3'-5' exonuclease activity, so it is possible that this artefact is caused by the terminal transferase-like activity of *Taq* polymerase.

Fluorescent primers appear to be stable over many months at -20°C. While unlabelled primers can be stored in ammonium hydroxide solution which maintains them in liquid form at -20°C, fluorescent primers are not stable in ammonia. To avoid exposing primers to repeated freeze-thaw cycles, we have found that

primer pools can be stored in 50% glycerol at concentrations of between 2 and 5 pmol/μl for each primer, requiring the addition of about 1 μl for a 20 μl reaction. Because the cost of Applied Biosystems fluorescent dyes is so high, the use of generic microsatellite primers that anchor to the 5' end of a CA repeat is being investigated. These would then enable the use of unlabelled primers for microsatellite amplification followed by labelling using the generic primers as nested primers. This approach could be readily adapted to the recently described three stage linear amplification process for the detection of microsatellites.¹⁹

In conclusion, automated microsatellite analysis using Genescan 672 software was found to be suitable for diagnostic applications. Samples run in duplicate gave robust size calling, generally within 1 base accuracy. Checking of results can be carried out quickly using Genescan software, and long term storage of the data is possible using read-write CD-ROM disks. Although the capital outlay is considerable, the ability to automate sequencing, microsatellite analysis, and the detection of unknown mutations²⁰ offers considerable flexibility for clinical molecular genetic applications with speed and safety advantages.

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- 1 Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 1989;44:388-96.
- 2 Litt M, Luty JA. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am J Hum Genet* 1989;44:397-401.
- 3 Taylor GR, Noble JS, Hall JL, Stewart AD, Mueller RF. Hypervariable microsatellite for genetic diagnosis. *Lancet* 1989;ii:454.
- 4 Weissenbach J, Gyapay G, Dib C, et al. A second generation linkage map of the human genome. *Nature* 1992;359:794-801.
- 5 Ziegler JS, Su Y, Corcoran KP, et al. Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics* 1992;14:1026-31.
- 6 Cawkwell L, Bell SM, Lewis FA, Dixon MF, Taylor GR, Quirko P. Rapid detection of allele loss in colorectal tumours using microsatellites and fluorescence DNA technology. *Br J Cancer* 1993;67:1262-7.
- 7 Riordan JB, Rommens JM, Kerem BS, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066-72.
- 8 Estivill X, Morral N, Casals T, Nunes V. Prenatal diagnosis of cystic fibrosis by multiplex PCR of mutation and microsatellites. *Lancet* 1991;338:458.
- 9 Zielenski J, Markiewicz D, Rinisland F, Rommens JM, Tsui LC. A cluster of highly polymorphic dinucleotide repeats in intron 17b of the CFTR gene. *Am J Hum Genet* 1991;49:1256-61.
- 10 Lindeman R, Kouts S, Woodage T, Smith A, Trent RJ. A dinucleotide repeat polymorphism of D15S10 in the Prader-Willi chromosome region (PWCR). *Nucleic Acids Res* 1991;19:5449.
- 11 Mutirangura A, Ledbetter SA, Kuwano A, Chinault AC, Ledbetter DH. Dinucleotide repeat polymorphism at the GABA-a receptor beta3 (GABRB3) locus in the Angelman/Prader-Willi region (AS/PWS) of chromosome 15. *Hum Mol Genet* 1992;1:67.
- 12 Mutirangura A, Kuwano A, Ledbetter SA, Chinault AC, Ledbetter DH. Dinucleotide repeat polymorphism at the D15S11 locus in the Angelman/Prader-Willi region (AS/PWS) of chromosome 15. *Hum Mol Genet* 1992;1:139.
- 13 Mutirangura A, Greenberg F, Butler MG, et al. Multiplex PCR of three dinucleotide repeats in the Prader-Willi/Angelman critical region (15q11-q13): molecular diagnosis and mechanism of uniparental disomy. *Hum Mol Genet* 1993;2:143-51.
- 14 Feener CA, Boyce FM, Kunkel LM. Rapid detection of

- CA polymorphisms in cloned DNA: application to the 5' region of the dystrophin gene. *Am J Hum Genet* 1991;48:621-7.
- 15 Clemens PR, Fenwick RG, Chamberlain JS, *et al.* Carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophy families using dinucleotide repeat polymorphisms. *Am J Hum Genet* 1991;49:951-60.
 - 16 Beggs AH, Kunkel LM. A polymorphic CACA repeat in the 3' untranslated region of dystrophin. *Nucleic Acids Res* 1990;18:1931.
 - 17 Noble JS, Taylor GR, Stewart AD, Mueller RF, Murday VA. A rapid PCR-based method to distinguish between fetal and maternal cells in chorionic biopsies using microsatellite polymorphisms. *Disease Markers* 1992;9:301-6.
 - 18 Oudet C, Hanauer A, Clemens P, Caskey T, Mandel JL. Two hot spots of recombination in the DMD gene correlate with the deletion prone regions. *Hum Mol Genet* 1992;1:599-603.
 - 19 Odleberg SJ, White R. A method for accurate amplification of polymorphic CA-repeat sequences. *PCR Methods and Applications* 1993;5:7-12.
 - 20 Ellis LA, Taylor GR, Banks R, Baumberg S. MutS binding protects heteroduplex DNA from exonuclease digestion in vitro: a simple method for detecting mutations. *Nucleic Acids Res* 1994;22:2710-1.