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MutS binding protects heteroduplex DNA from exonuclease digestion *in vitro*: a simple method for detecting mutations

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MutS and its homologues are highly conserved proteins which, with *Mut*H and *Mut*L repair DNA mismatches (1). The *Mut*S protein binds to heteroduplex DNA at the site of mismatch and can recognise single base mismatches as well as deletions or insertions of up to four bases (2). A purification protocol for the purification of *E.coli MutS* has been described previously (3) and the protein is now available commercially (United States Biochemicals Ltd). Here we describe a system to detect *MutS* binding which is sensitive and specific. After *MutS* binding to heteroduplex DNA, the heteroduplexes are exposed to exonuclease digestion. Bound *MutS* protects mismatch containing sites from the exonuclease, leaving a partially degraded molecule. Homoduplexes are digested to completion (Figure 5). This *MutS*-exonuclease protection (MutEx) assay detects and localises heteroduplex sites to within 20 bases. The method should detect any point mutation and small insertions or deletions in heteroduplex DNA. The largest fragment so far analysed is 493 bases but the upper size limit of the assay is likely to be limited only by the processivity of the exonuclease. We found that T7 polymerase had exonuclease activity which was well suited to this method. The exonuclease protection method could also be applied to the study of other DNA binding proteins as an alternative to gel retardation or footprinting.

Fluorescent primers were synthesised to direct the PCR amplification of a 493 bp fragment containing human cystic fibrosis transmembrane regulator (CFTR) exon 11. Four previously sequenced CFTR exon 11 mutations plus wild type homozygotes were amplified using the fluorescent primers FAM 5′ AGC AAT GTT GTT TTT GAC CAA CTA 3′ (blue) and JOE 5′ GCA CAG ATT CTG AGT AAC CAT AAT 3′ (green). PCR products were purified by centrifugal dialysis using Centricon 100 tubes (Amicon). In each case PCR product (50 μl) was diluted to 2 ml in TE buffer, pH 8 and concentrated to a final volume of 25–30 μl. The final wash used exonuclease buffer (50 mM Tris (pH 7.5), 7 mM $MgCl_2$, 5 mM DTT). The OD260 of the product was determined after which heteroduplexes were formed by heating to 95°C for 5 minutes followed by cooling to 65°C for 1 hour. For *MutS* binding, 5 pmole of *MutS* in 5 μl dilution buffer (50 mM Hepes (pH 7.2), 100 mM KCl, 1 mM EDTA, 1 mM DTT) was incubated with 2 pmole of DNA on wet ice. After 1 hour 10 units of T7 polymerase (New England Biolabs) were added and the tube was transferred to a 37°C waterbath. The reaction was stopped after digestion for 3–5 minutes by the addition of 10 μl gel loading buffer (deionised formamide containing 10 mM EDTA). The samples were heated to 90°C and then loaded with size standards (Applied Biosystems Rox 2500) onto a polyacrylamide sequencing gel (6%, containing 7 M Urea) for analysis on an Applied Biosystems 373 automated DNA sequencer using Applied Biosystems 672 Genescanner software. Fragment sizes were estimated using a third order least squares approximation. Figures 1–4 illustrate the detection of four different mutations in CFTR exon 11 (1717-1 G→A, R553X, G551D, S549N) by this method. Distinct peaks were seen clearly on both strands, which were absent in control samples. The signal to noise ratio of the assay was high, although small background peaks could be seen. The position of the major

Table 1.

Mutation	Observed fragment size (blue)	Distance from primer to mutation (blue)	Observed fragment size (green)	Distance from primer to mutation (green)
wild type	none	NA	none	NA
1717-1 (G→A)	189	184	326	308
R553X (C→T)	269	256	246	236
G551D (G→A)	259	250	256	242
S549N (G→A)	253	245	263	247

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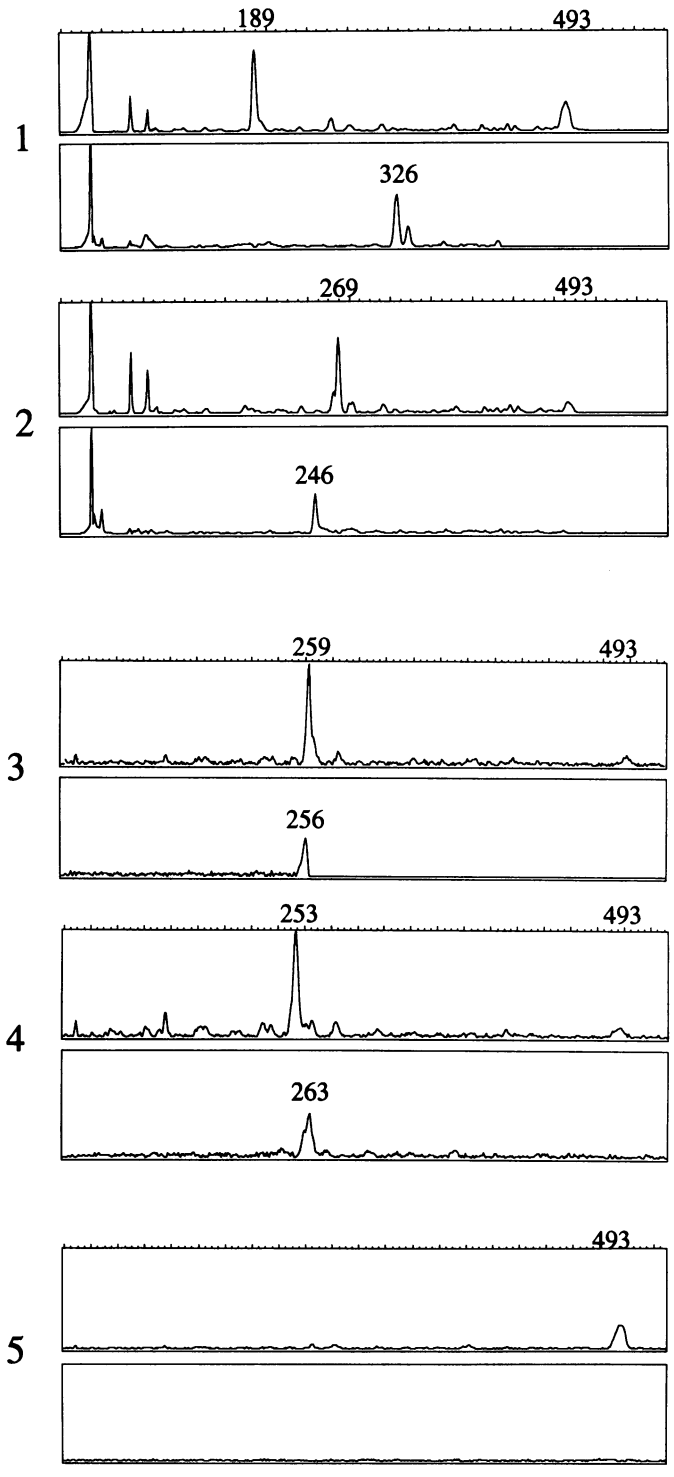

Figures 1–5. MutEx assay results for CFTR Exon 11 mutations: **1**, 1717-1 G→A; **2**, R553X; **3**, G551D; **4**, S549N; **5**, Wild Type. See text for further details.

peak shifted according to the mutation being tested and were seen at the same position reproducibly between and within assays on both strands. (Table 1). Peaks were not seen in the absence of *MutS*. Although the results reported here were obtained using fluorescence labelling and automated fragment analysis, radioactively labelled heteroduplex molecules detected autoradiographically should work equally well.

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