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# Nonisotopic SSCP and competitive PCR for DNA quantification: p53 in breast cancer cells

Eric P.H.Yap and James O'D.McGee

University of Oxford, Nuffield Department of Pathology and Bacteriology, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK

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PCR product yield does not quantitatively reflect the original concentration of template DNA due to variations in amplification efficiency (1, 2). This necessitates the coamplification of known amounts of a control standard. The control template may be of different size (1, 2) but this assumes that length difference does not affect amplification efficiencies. Alternatively, an internal restriction site may be created or deleted in the mutant competitor (2); however digestion-resistant heteroduplexes must either be minimised by limiting the reaction to the linear phase or maximised by a denaturation-reannealing step that results in a binomial distribution of duplexes. These difficulties may be circumvented by the use of single-strand confomation polymorphism (SSCP) (3) to detect single base-pair differences. We describe here the quantification of the p53 gene and alleles in genomic DNA extracted from MDA cells and human breast cancer biopsies respectively, using a rapid nonisotopic SSCP method.

The p53 gene in the breast cancer cell line, MDA-MB-231 (ATCC), can be distinguished from the cDNA plasmid by nonisotopic SSCP, based on a single base polymorphism ('G'vs 'C') in codon 72 (4). Serial 1:2 dilutions of plasmid DNA were added to fixed amounts of MDA DNA. A 185-bp fragment spanning the polymorphic site was amplified in 20 μl reactions by PCR. p53 was also amplified from paired blood and tumour DNA samples from breast cancer patients. Non-isotopic SSCP was then performed (4).

Three bands representing a DNA duplex and two single strands were seen with the slower migrating single-stranded band showing sequence-dependent mobility (Fig. 1a, b). The relative amount of allelic products correlated with the initial concentrations of templates in the dilution series visually (Fig. 1a, b) and by densitometry (Fig. 1c). Increasing the amount of target DNA gave the expected shift in cross-over of relative band intensities. Furthermore, increasing the yield to saturation limits by lowering the denaturation temperature (5) did not affect the band pattern and only increased the brightness of both bands (Fig. 1a). The initial amount of DNA template could be quantitated in absolute terms within a factor of 2. Relative loss of a p53 allele in human tumour DNA of constitutionally heterozygous individuals could also be demonstrated (Fig. 1d).

Ethidium bromide stained SSCP gel electrophoresis is rapid and may be routinely used in screening PCR products for mutations or polymorphisms. When combined with competitive PCR, it could be applied to measurement of mRNA or gene dosage. SSCP of isotope-labelled PCR products followed by autoradiography or scintillation may provide greater quantitative precision. It should be emphasized that competitive PCR does not compensate for variability in amplification efficiency due to different physical and biochemical states of the DNA templates (e.g., linearized and supercoiled, aldehyde cross-linked and native).

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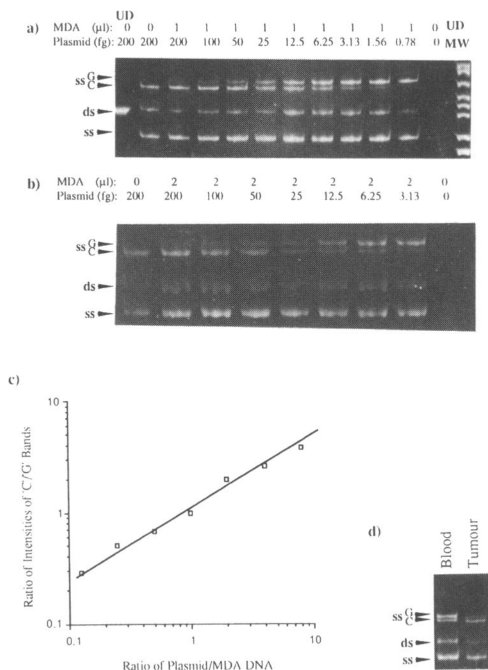


**Figure 1.** 5 μl of alkali-denatured p53 competitive PCR product was electrophoresed on 0.4 mm thick non-denaturing 10% polyacrylamide + 5% glycerol gels in 0.5×TBE buffer, at 15–30 V/cm for 2 h at 22°C. Double-stranded (ds) DNA, single-stranded (ss) bands of 'G' and 'C' alleles, undenatured (UD) controls and MspI-digested pBR322 marker (MW) are indicated. **a)** 1 μl/tube of MDA DNA equivalent to 12.5 fg p53, amplified through 50 cycles with 90°C denaturation in latter 40. **b)** 2 μl/tube of MDA DNA corresponding to 25 fg p53, amplified through 40 standard cycles. **c)** Densitometric readings of photographic negative of gel (b) showing linearity of relative band intensities with quantity of DNA. **d)** p53 amplified from 1 μg of blood and tumour DNA from a breast cancer patient.