STS for minisatellite 33.1 (D9S49): direct typing by PCR

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The minisatellite locus 33.1 was initially isolated from a human genomic library by hybridization screening with a tandem repeat probe (1). This locus has since been localised by somatic cell hybrid analysis and linkage mapping to the long arm of chromosome 9 (data not shown). PCR typing at this minisatellite locus has already been described (2) in which PCR products were detected by hybridization. Here we describe the direct typing of this locus by PCR, a process which may also be used to generate a probe for use in Southern blot analysis of genomic DNA.

200 ng genomic DNA was amplified using primers 33.1A [5' CTTTCTCCACGGATGGGATGCCAC 3'] and 33.1B [5' GCCGTGTCACCCACAAGCTTCTGG 3'] in a 20 μ l reaction (3) with 2.5 units of Taq polymerase; after 24 cycles (95°C, 1 minute; 67°C 1 minute; 70°C 10 minutes) and a single 'chase' consisting of the last two segments only, PCR products were analysed on a 1.5% agarose gel. Figure 1 shows the results of an analysis of a family group (F = father, M = mother, D = daughter) and an additional, unrelated individual (U). As an alternative to direct analysis by PCR, the PCR product from lane M was recovered from the gel, and 2 ng subjected to reamplification for 16 cycles (+ chase) under the same conditions as for amplification from genomic DNA. This resulted in a high yield (about 500 ng) but also in the degeneration of the product into a smear of different sized products (2). From this smear, a 0.5–1.5 kb fraction was recovered, of which 10 ng was oligo-labelled and used to probe a Southern blot of *Dde*I-digested genomic DNA. Filters were washed at 65°C in 0.1 $\times$ SSC, 0.01% SDS. Figure 2 shows the result of this method of analysis on DNA from the same individuals as shown in Figure 1.

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Figure 1.

Figure 2.