Rapid microsatellite analysis of paraffin embedded tumour specimens from patients with hereditary non-polyposis colorectal cancer

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Abstract
In screening for hereditary non-polyposis colorectal cancer (HNPCC)—an autosomal dominant disorder characterised by mutations in mismatch repair genes—detection of microsatellite instability is an important diagnostic criterion. The mono- or dinucleotide repeat DNA sequences are usually amplified from formalin fixed, paraffin embedded tissue by polymerase chain reaction after numerous time consuming steps including deparaffinisation, DNA extraction, and purification. A rapid single step method for direct DNA analysis is described, based on preincubation of paraffin embedded tissue with Triton X-100 followed by DNA amplification with fluorescence labelled primers and electrophoresis in an automated sequencer. This procedure allows precise allele sizing and analysis of genetic instability, is more efficient and time saving, reduces the risk of contamination, and is therefore of particular interest in screening for HNPCC.

Keywords: hereditary non-polyposis colorectal cancer; rapid microsatellite analysis; paraffin embedded tissue

Microsatellites represent DNA loci with simple sequence repeats that are frequently and widely dispersed throughout the human genome. Owing to allelic polymorphisms they represent a valuable source for human genetic linkage studies with applications in forensic medicine, population genetics, paternity affiliation, and molecular cancer research. In hereditary non-polyposis colorectal cancer (HNPCC)—an autosomal dominant disorder accounting for two to six per cent of all colorectal cancers in the Western world—length alterations in two to six per cent of all colorectal cancers in autosomal dominant disorder accounting for polyposis colorectal cancer (HNPCC)—an important diagnostic criterion. The mono- or dinucleotide repeat DNA sequences are usually amplified from formalin fixed, paraffin embedded tissue by polymerase chain reaction after numerous time consuming steps including deparaffinisation, DNA extraction, and purification. A rapid single step method for direct DNA analysis is described, based on preincubation of paraffin embedded tissue with Triton X-100 followed by DNA amplification with fluorescence labelled primers and electrophoresis in an automated sequencer. This procedure allows precise allele sizing and analysis of genetic instability, is more efficient and time saving, reduces the risk of contamination, and is therefore of particular interest in screening for HNPCC.

Here we describe a modified protocol for convenient direct PCR amplification of microsatellite markers from paraffin embedded tissue. For HNPCC screening, representative 5 µm sections of paraffin embedded tumour and peritumour tissue were mounted onto slides and dried for 60 minutes at 50°C. Areas of 2–3 mm² in size were microdissected and the tissue transferred into PCR tubes containing 20 µl of 1% Triton X-100 (Sigma, Deisenhofen, Germany). After 10 minutes’ incubation at 95°C, the PCR mixture was added and the amplification was started. We successfully amplified several mono- (BAT26, BAT40) and dinucleotide repeat loci (D2S119, D2S123, D2S136, D3S1266, D3S1298, D5S346, D15S120, D17S250, and D18S58) on chromosomes 2, 3, 5, 15, 17, and 18, respectively, using fluorescence labelled 5’ primers. The products of the examined microsatellite loci ranged in size from 80 to 300 bp and were electrophoresed using an automated ABI 310 DNA sequencer. Using primers labelled with HEX (4,7,2’-4’,5’,7’-hexachloro-6-carboxy-fluorescein), TET (4,7,2’,7’-tetrachloro-6-carboxy-fluorescein), and 6-FAM (6-carboxy-fluorescein), we simultaneously analysed up to seven amplified microsatellite loci in one electrophoresis lane. The accuracy and efficiency of MIN assessment of tumour DNA from HNPCC patients were compared with the conventional extraction of genomic DNA using proteinase K digestion and phenol-chloroform standard techniques. As shown in fig 1, no difference in the electrophoresis patterns between the two methods was observed. We were able to assess MIN and loss of
heterozygosity equally in all loci examined. Accuracy of allele sizing, which is important for linkage studies, was identical between the two techniques.

The amplification rate of paraffin embedded tissue specimens using the above described technique was significantly higher (130/140 tested loci) compared with the conventional extraction method (114/140 tested loci; p < 0.01). Using conventional protocols, MIN analysis of paraffin embedded tumour specimens requires three to four days. Application of the method described here reduces the duration for MIN analysis to 12 hours. Although lysis with Triton X-100 has recently been suggested for direct PCR from paraffin embedded tissue, no applications for microsatellite amplification with fluorescence labelled primers followed by automated DNA electrophoresis have been reported. Since the technique we describe is less time consuming, more efficient, and reduces the risk of contamination by limiting the number of steps required it might replace current DNA extraction and purification steps, especially for clinical screening purposes. In addition it is also applicable to genetic linkage studies.