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Short Communication

Optimal amplification conditions for D16S3399 polymorphic STS *axin-1* gene marker

ANJA KAFKA[1,2], VESNA MUSANI[3], NIVES PEĆINA-ŠLAUS[1,2]

[1] Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine University of Zagreb, Šalata 12, HR-10000 Zagreb, Croatia.

[2] Department of Biology, School of Medicine, University of Zagreb, Šalata 3, HR-10000 Zagreb, Croatia.

[3] Division of Molecular Medicine, Laboratory for Hereditary Cancer, Ruđer Bošković Institute, Bijenička cesta, HR-10000 Zagreb, Croatia.

Correspondence
Nives Peićina-Šlaus
Laboratory of Neurooncology,
Croatian Institute for Brain Research
School of Medicine University of Zagreb,
Šalata 12, HR-10000 Zagreb
E-mail: nina@mef.hr

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Abstract

We investigated D16S3399 marker and affirmed it as a highly polymorphic marker useful for the analysis of the human axin-1 gene. Axin-1 acts as a tumor suppressor gene and its protein is an inhibitor of the Wnt signaling pathway. We report on heterozygosity status, alleles frequency observed in a preliminary group of Croatian subjects and the optimal amplification conditions for D16S3399 marker. The amplified CA repeat was confirmed by direct sequencing.

FINDINGS

In this report we would like to present data on the optimal amplification conditions and observed percentage of heterozygosity of the D16S3399 (also known as 16PTEL03, TEL-16p03 and Tel-16p03) polymorphic STS marker for the *axin-1* gene. The literature and gene databases lack this data without any specific characterization and required experimental conditions. The marker is reported as STS (sequence tagged site) and found by e-PCR *(1)* in *Homo sapiens* sequences. We investigated D16S3399 marker and affirmed it as a highly polymorphic marker very useful for microsatellite instability (MSI) and loss of heterozygosity (LOH) analysis of the *axin-1* gene. *Axin-1* (aliases: AXIN, hAXIN, MGC52315, Axis inhibitor 1, NCBI Locus ID 8312) acts as a tumor suppressor gene and its protein is an inhibitor of the Wnt signaling pathway *(2-4)*. As a scaffold protein, its main role is binding multiple members of Wnt signaling and formation of the β-catenin destruction complex *(5)*. *Axin-1* gene is located on the short arm of the chromosome 16 at position 16p13.3, extending from 337 440 to 402 676. *Axin-1* has two predominat isoforms, isoform a consists of 11 exons and has full gene transcript product length of 3675 bp. Isoform b lacks an in-frame exon in the 3' coding region and is shorter with sequence length of 3567 bp *(6)*.

D16S3399 polymorphism is 183 bp long dinucleotide CA repeat that extends from 145 245 bp telomeric region to position 145 427 bp of the chromosome 16p. The primers used were found on National Center for Biotechnology Information site, UniSTS Integrating Markers and Maps http://www.ncbi.nlm.nih.gov/genome/sts/sts.cgi?uid=9302&MAXHITS=6

Experimental Procedures

The D16S3399 polymorphic region linked to the *axin-1* gene was amplified in a volume of 25 μl. Different conditions were tested includ-

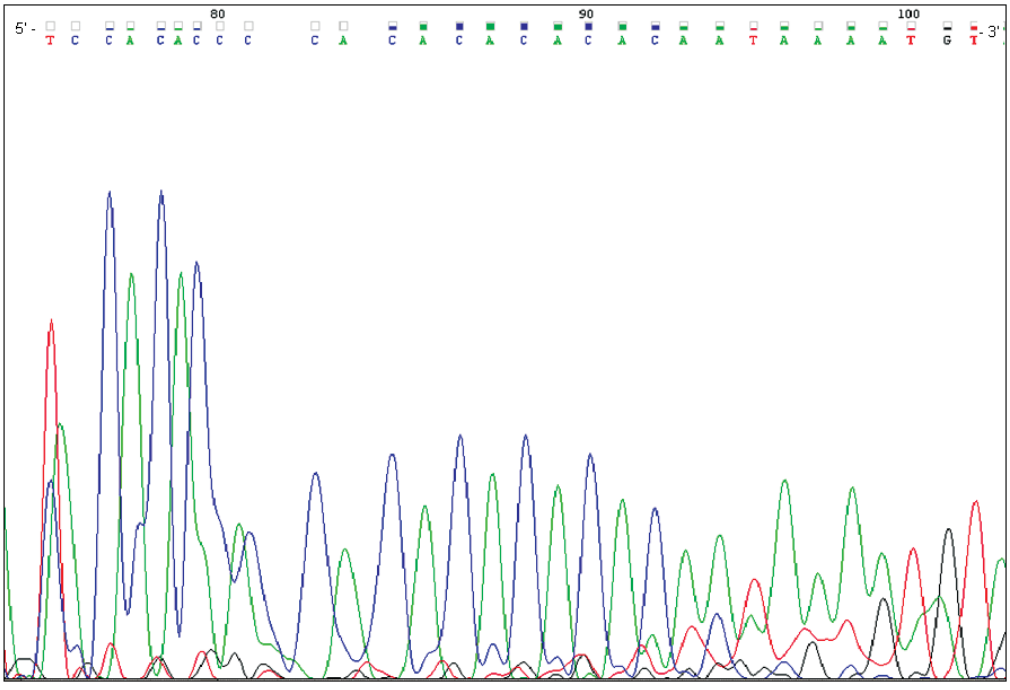

Figure 1. *Sequence for the D16S3399 polymorphic STS marker. 183 bp fragment was sequenced with appropriate primers, and analyzed on ABI PRISM 310.*

ing $MgCl_2$ concentrations, template DNA concentrations, primer and dNTP concentrations and Taq units. The optimal conditions were as follows: 5 pmol of each primer (5'- GGGCCATTATTCAGCCAATC -3' and 5'-ACCTAGATCCCTCCAGGTTT -3'), 200-400 ng DNA, 2.5 µl PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3), 2 mM $MgCl_2$, 2.5 mM of each dNTP, 5 U/µl Taq polymerase (Promega, USA).

Optimal polymerase chain reaction (PCR) conditions for D16S3399 marker were as follows. Reaction was preceded by 3-minute initial denaturation at 95°C. Samples were subjected to 35 cycles of three-step amplification consisting of 30 seconds of denaturation at 96°C, 30 seconds of annealing at 56°C and 30 seconds of extension at 72°C adding 1 extra second after every cycle. A final extension at 72°C was lengthened to 10 minutes. PCR products were analyzed on 2% agarose gels.

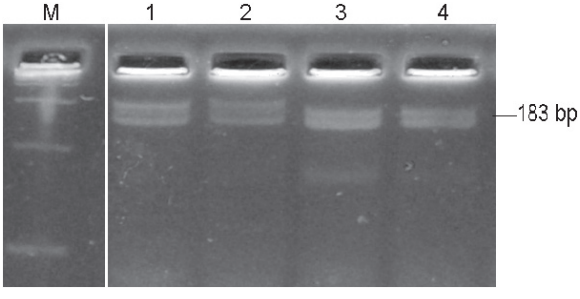

Figure 2. *Alleles of the D16S3399 visualized on Spreadex EL 400 Mini gels (Elchrom Scientific, Switzerland) stained with SyberGold (Molecular Probes, Netherlands). Lane M- molecular DNA standard; lanes 1, 2, 3, 4 heterozygous samples from blood and human tissue.*

In order to verify that our PCR products are compatible with the reported sequence on the web we sequenced our 183 bp product. We confirmed D16S3399 by direct sequencing.

PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. DNA fragments were cut out and isolated from agarose gel (QIAquick gel extraction Kit; QIAGEN, Hilden, Germany). Sequencing reactions were performed with appropriate primers in a BigDye Terminator v1.1; Applied Biosystems, Foster City, USA, and analyzed on ABI PRISM 310. All sequences were confirmed by bidirectional sequencing of PCR products generated by at least two independent reactions (Figure 1).

Allelic heterozygosity of the D16S3399 was visualized on Spreadex EL 400 Mini gels (Elchrom Scientific, Switzerland) stained with SyberGold (Molecular Probes, Netherlands). The percent of heterozygous samples was 83%. (Figure 2).

Our results of the experimental PCR were compatible to the e-PCR of this STS. The reported optimal conditions for D16S3399 polymorphic STS marker would be useful for further analysis of the *axin-1* gene. Specific ge-

netic variations represent important tools for human genetics *(7, 8)* and contribute to the many analyses of diseases including chromosomal abnormalities in tumors.

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