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D2S1338 RARE ALLELE IDENTIFICATION AND CONFIRMATION BY DIRECT SEQUENCING

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An out of locus allele was observed while analyzing the sample data generated using the AmpFISTR® Identifiler™ PCR amplification kit. Since both loci adjacent to the observed allele, D16S539 and D2S1338, showed a single-peak homozygous pattern, it was necessary to confirm the origin of the allele by direct sequencing. Locus D2S1338 was amplified using the specific primers, the PCR products were purified and cloned into pSC-B-amp/kan plasmid vectors. The clones with an insert of suitable length were identified and sequenced using the M13 universal primers. The sequence analysis and comparison with the reference sequence obtained from the NCBI GenBank revealed that the allele indeed belongs to the D2S1338 locus and that it is consistent with (TGCC)₆ (TTCC)₇ repeat pattern. In this way a possible misinterpretation was avoided and it is a recommended procedure when a rare allele like this one is encountered.

Keywords: DNA typing, STR loci, Allele variant, DNA sequencing, Croatian population