



B29 Performance Comparison of the Penta D and Penta E Loci With the D2S1338 and D19S433 Loci in the Massive DNA Identification Effort in Former Yugoslavia

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The decisions involved in the selection of materials needed for the operation of a large-scale DNA testing effort involving skeletal remains and the results of those studies will be discussed.

The primary mission of the International Commission on Missing Persons (ICMP) is to help resolve the fate to the tens of thousands who are still missing in the former Yugoslavia from the conflicts in the 1990s. Paramount to the realization of success in this mission has been the development of a state-of-the-art DNA testing system which possesses a capacity of processing up to ten thousand skeletal cases per year. In order to implement such a DNA testing system, the selection of appropriate equipment, methodologies and systems is critical. One of these critical components is the choice of an appropriate STR multiplexing. This is especially true today since faced with numerous STR multiplex kits from various providers, in addition to cost, also differ in the loci included in the analysis.

One of the first experiences in the ICMP's DNA department was that certain STR loci in closed enclaves such as Srebrenica offer relatively low levels of diversity inside the associated reference population. In the case of Srebrenica, where the number of missing are in thousands, and number of family reference blood samples are in tens of thousands, half-band sharing between the DNA profiles of bone samples and an unrelated family reference sample is a frequency occurrence, even when 15 loci have been successfully profiled. Because of this, either multiple family members must be profiled for each missing person or extended DNA testing involving additional STR loci, mtDNA or Y-chromosome testing must be performed on both the bone sample in question and the corresponding blood family references. Still, for a large-scale DNA identification effort such as this one, it is important that bulk processing, and therefore the bulk of identifications, is performed as often as possible by a single multiplex reaction, or in other words, one kit. This approach saves two very important elements for operation of this scale: time and money.

Among the kits that have been validated for ICMP casework are two 16 multiplexing kits, Promega's PowerPlex® 16 system and ABI's AmpFLSTR® Identifier™ system. With the exception of Penta D, Penta E, D2S1338, and D19S433, these two kits amplify the same loci, including all of the CODIS 13 loci. The former two are incorporated in the PowerPlex 16 system, while the latter two are incorporated inside ABI's Identifier™ five-dye kit.

The Pentas are 5-base repeats, ranging from in size 375bp to 471bp (for Penta E), and from 368 bp to 438 bp (for Penta D). In the PowerPlex 16 kit they are labeled with blue (Fluorescein) and green (JOE) dyes, respectively. Naturally, due to their size, the Pentas are on the far right of the allelic ladder, appearing as the fifth loci in blue, or sixth loci in green.

D19S433 and D2S1338 loci are four-base repeats. D19S433 is significantly shorter than both Pentas with a size range of 106 bp to 144 bp and appears as the first loci in yellow of the Identifier's allelic ladder. D2S1338 ranges from 300 bp to 365 bp and, due to its greater size, is the last loci to appear in green.

The statistical significance of these four loci have been investigated within the appropriate reference population of the families of the missing, as well as the results obtained from both blood and bone samples. In addition, the amplification response to the reduced DNA quantities as recovered from skeletal remains, as well as the appearance of artifact peaks from bacteria, can represent a problem in allele calling. For these purposes, 500 blood and 50 bone samples were analyzed using both kits. Statistical analysis indicates that Penta E and Penta D have the higher power of discrimination and heterozygosity percentage. Paternity statistics favor the Pentas, especially Penta E, which scored highest Typical Paternity Index, and also had the highest observed power of exclusion among these four loci. Differences in the sensitivity and bacterial peak emergence were observed as well.

STR, DNA, ICMP