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Population genetic study of 15 STRs loci using AmpF/STR Identifiler™ kit

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Abstract

Identifiler™ is the most recent kit from Applied Biosystems. It uses a five-dye fluorescent system and so coamplifies the repeat regions of 15 STR loci and a segment of the X–Y homologous gene Amelogenin. In the present study, we analyzed allelic and genotypic distribution of STRs loci in three populations from Southern Italy (Calabria): Reggio Calabria, Catanzaro, and Cosenza.
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Keywords: STRs alleles frequencies; Identifiler™ loci; Italian cities

1. Introduction

Identifiler™ is the most recent kit from Applied Biosystems. It uses a five-dye fluorescent system for automated DNA fragment analysis. By adding an additional dye, more loci can be multiplexed in a single PCR amplification compared to some previous four-dye systems. Identifiler™ kit coamplifies the repeat regions of 15 STRs repeat loci (D19S433, D3S1358, D5S8118, D8S1179, vWA,TH01 ,D13S317,D21S11, TPOX, FGA, D7S820, D16S539, D18S51, CSF1PO, D2S1338. A segment of the X–Y homologous gene Amelogenin is also amplified, for gender diagnosis. Using capillary electrophoresis, it takes about 30 min to type 1 sample and up to 48 samples a day can be typed automatically. The purpose of this study is to calculate in three Calabrian (Southern Italy) cities (Reggio Calabria, Catanzaro, Cosenza) the allelic frequencies of AmpFlSTR Identifiler™ loci.

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2. Materials and methods

2.1. DNA extraction

In order to minimize the possibility of contamination, all extractions were set up in a Gelman laminar flow cabinet in a dedicated laboratory. DNA was extracted, by Instant Gene Matrix (Biorad) treatment, from blood samples of unrelated healthy donors (100 per each city), belonging to the population examined since three generations at least. Samples were boiled for 8 min in a 6% Chelex solution and then amplified directly [1,2]. Extracts were quantified by the Quantiblot—Human DNA Quantitation kit [3].

2.2. DNA amplification

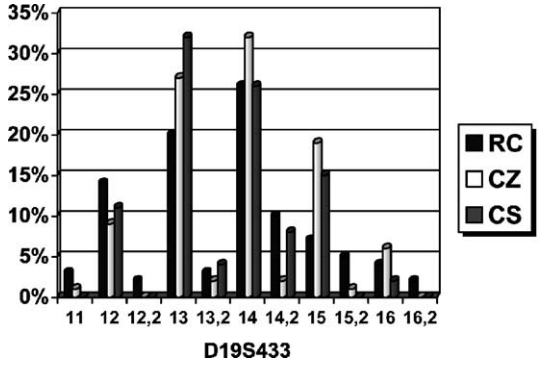
Amplification was carried out in a laboratory different from the one dedicated to the extraction, so that amplified products never entered the extraction laboratory. STR amplification was carried out according to the Identifiler™ kit protocol using GeneAmp 9600, 9700 and 2400 thermal cyclers (Perkin Elmer) with positive and negative controls [4].

2.3. Electrophoretic analysis

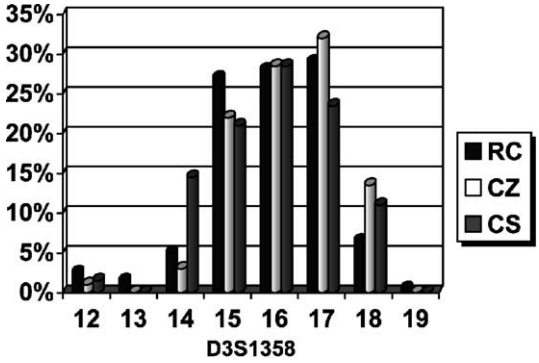
The analysis of 0.5 ul of the amplification products was performed by capillary electrophoresis on two ABI PRISM 310 Genetic Analyzers employing ABI softwares (DATA Collection, GeneScan Analysis, Genotyper Fragment Analysis). For fragment length determination of products, the internal lane DNA standard LIZ 500 (Orange dye) was used for calibration [5,6].

3. Results

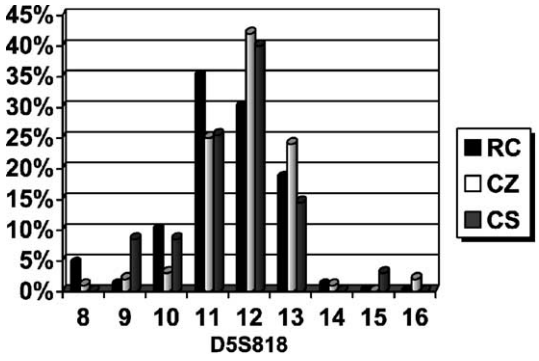
The distribution of allelic frequencies in the three populations above mentioned is showed for each locus in the following graphics (see Graphs. 1–15).



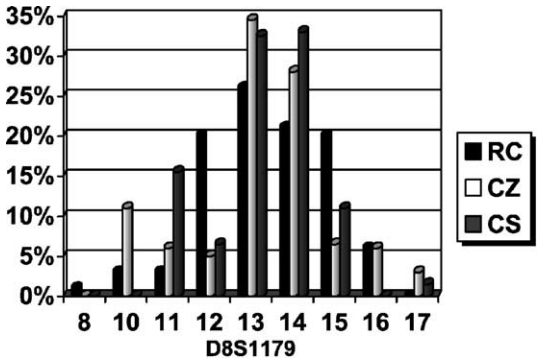
Graph. 1. Locus D19S433: Distribution of allelic frequencies.



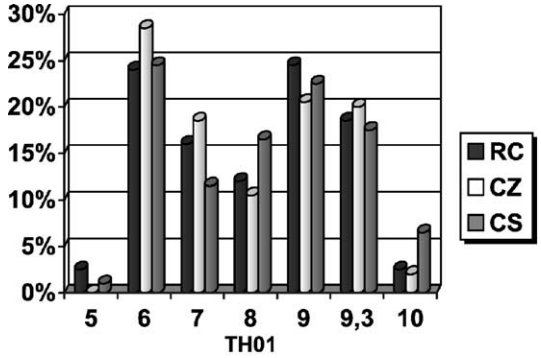
Graph. 2. Locus D3S1358: Distribution of allelic frequencies.



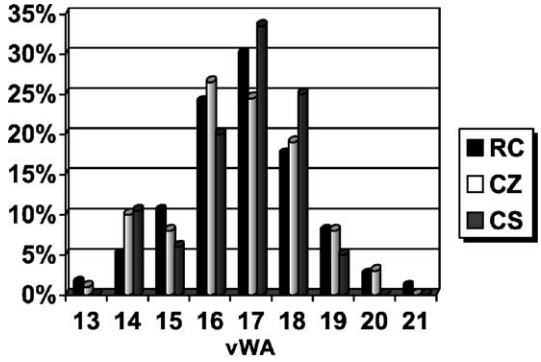
Graph. 3. Locus D5S818: Distribution of allelic frequencies.



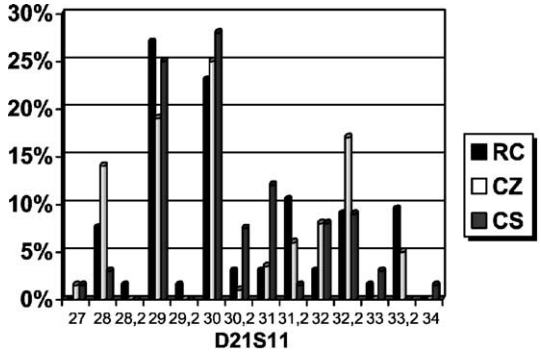
Graph. 4. Locus D8S1179: Distribution of allelic frequencies.



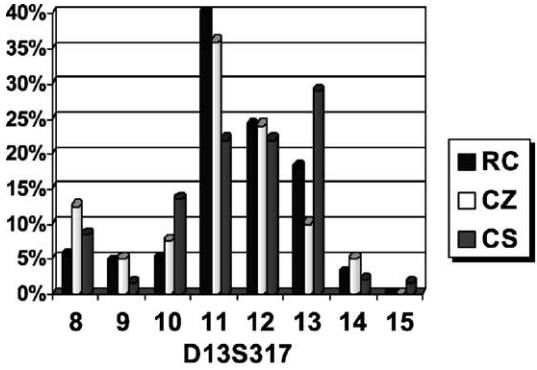
Graph. 5. Locus TH01: Distribution of allelic frequencies.



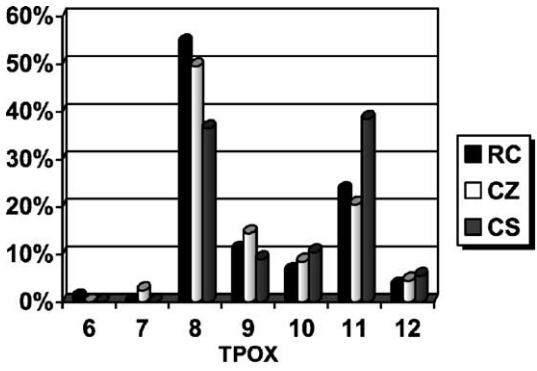
Graph. 6. Locus vWA: Distribution of allelic frequencies.



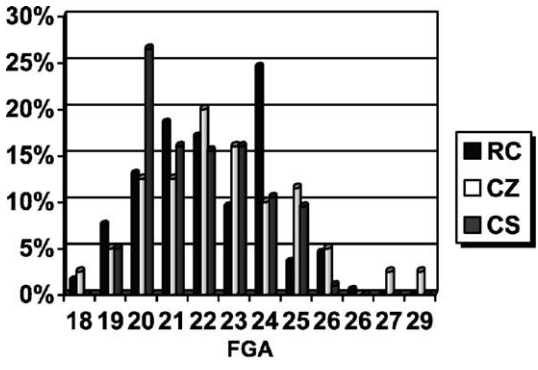
Graph. 7. Locus D21S11: Distribution of allelic frequencies.



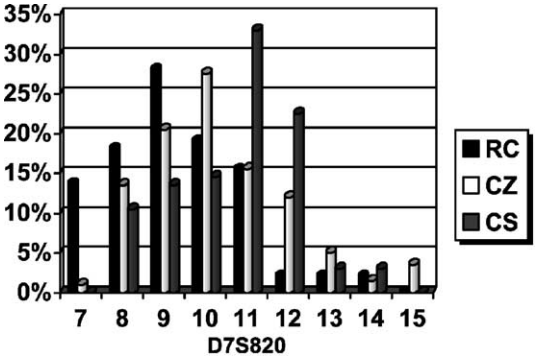
Graph. 8. Locus D13S317: Distribution of allelic frequencies.



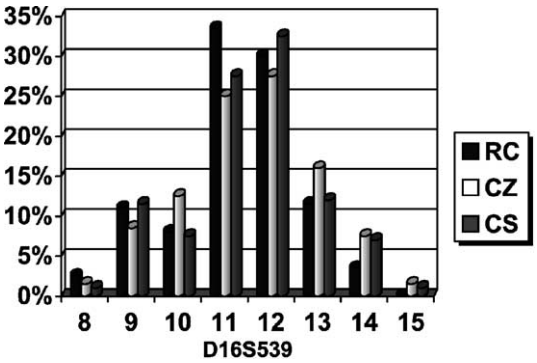
Graph. 9. Locus TPOX: Distribution of allelic frequencies.



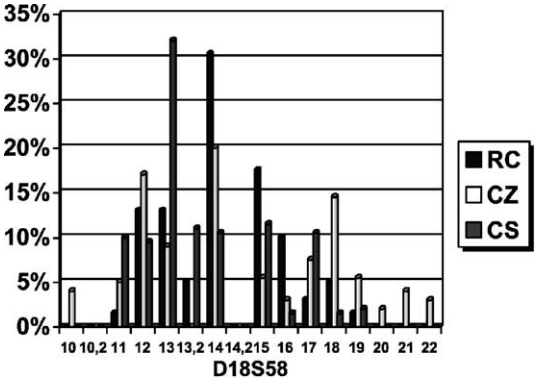
Graph. 10. Locus FGA: Distribution of allelic frequencies.



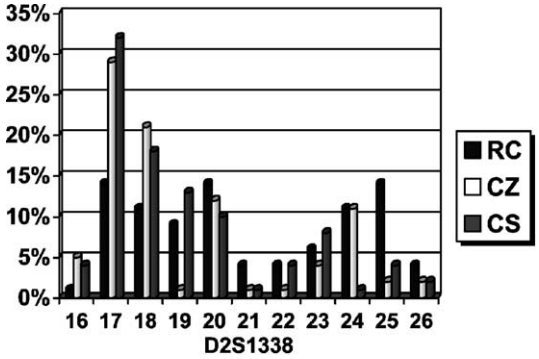
Graph. 11. Locus D7S820: Distribution of allelic frequencies.



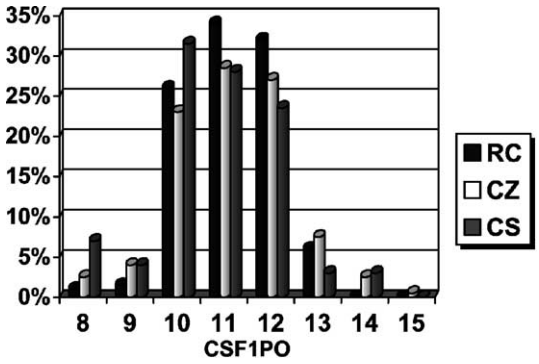
Graph. 12. Locus D16S539: Distribution of allelic frequencies.



Graph. 13. Locus D18S58: Distribution of allelic frequencies.



Graph. 14. Locus D2S1338: Distribution of allelic frequencies.



Graph. 15. Locus CSF1PO: Distribution of allelic frequencies.

4. Conclusion

With the high levels of polymorphism of AmpFlSTR Identifiler™ loci, our results confirm that the kit is suitable for identification and underscores the importance of the generation of local databases for STRs when these markers are used in forensic casework.

Acknowledgements

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