USEFULNESS OF POWERPLEX® FUSION SYSTEM AS TYPING METHODOLOGY OF CODIS AND ESS GENETIC MARKERS IN A IMMIGRANT POPULATION FROM CABO VERDE IN LISBOA

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Introduction

Cabo Verde is an archipelago located on the West African coast. It was discovered in 1460 by Portuguese explorers and it was colonized by them and by Africans. Culturally, Cabo Verde is characterized by an admixture of European and African origin elements, being one of the countries with the highest number of emigrants in Portugal [1].
PowerPlex® Fusion System kit (Promega Corporation) consists of a multiplex that amplifies 24 loci (D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, VWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA, D22S1045, DYS391 and Amelogenin) in a single PCR reaction. These genetic markers include all Short Tandem Repeats (STRs) encompassed in the Combined DNA Index System (CODIS) and in the European Standard Set (ESS) [2,3].
In order to implement or modify technologies for forensic DNA analysis there are two types of validation required: developmental and internal. The developmental validation studies accuracy, reproducibility and precision of the procedure by the manufacturer, and it should precede the use of this novel methodology for forensic DNA analysis. Internal validation is performed by each laboratory of forensic DNA analysis to demonstrate that established method and procedure perform as expected in the laboratory [4-7].
Parameters such as final volume of PCR reaction, DNA concentration range, stutter peaks ratio and heterozygote peak height ratios, were determined. Beyond the method implementation, one of the aims of this work is sample costs-saving by the laboratory maintaining the quality of the obtained results.

Materials and Methods

Buccal swab samples from 500 unrelated individuals form Cabo Verde, currently living in Lisboa were studied. DNA was extracted using Chelex® 100 method [8], quantified by real-time PCR using Quantifiler® Human DNA Quantification Kit (Applied Biosystems) in a 7500 Real-Time PCR System (Applied Biosystems) and analyzed with HID Analysis software v1.2 (Applied Biosystems). Amplification was performed with PowerPlex® Fusion System kit (Promega Corporation) in a GeneAmp® 9700 Gold Plate (Applied Biosystems), DNA fragment detection and identification was achieved with capillary electrophoresis in an ABI PRISM® Genetic Analyser 3130xl sequencer (Applied Biosystems) and using GeneMapper® ID-X v1.4 (Applied Biosystems).
Some changes in the kit manual protocol were carried out, during the implementation of PowerPlex® Fusion System kit, as regards the final volume of PCR reaction used in amplification and the number of cycles, in order to obtain a complete genetic profile (Table 1).

Table 1 – Final volume of PCR reaction and number of PCR cycles for PowerPlex® Fusion System kit Manual (Promega Corporation) and for PowerPlex® Fusion System kit implemented protocol in SGBF.

	Final Volume of PCR reaction	Number of PCR cycles
PowerPlex® Fusion System (Promega) kit Manual	25µL	30 Cycles
PowerPlex® Fusion System kit implemented protocol in SGBF	6,25µL	28 Cycles

Results and Discussion

During the implementation of PowerPlex® Fusion System kit (Promega Corporation) in the Serviço de Genética e Biologia Forenses (SGBF), it was first determined that the minimum final volume of PCR reaction to obtain a complete genetic profile is 6.25µL. For a final amplification volume of 6.25µL it was observed that the minimum concentration of required DNA in a sample, amplified with 28 cycles is 0.04ng/µL. Regarding the minimum concentration of DNA required to obtain a full genetic profile, it shows that the PowerPlex® Fusion System kit (Promega Corporation) is a sensitive kit that allows the analysis of samples with low DNA concentration.
The genetic profiles obtained with this amplification conditions were compared with genetic profiles obtained by the kits already in place in lab routine, the PowerPlex® 16 HS (Promega Corporation) and the AmpFlSTR® Identifiler® Plus (Applied Biosystems), between common loci, verifying coincidence between them.
Stutter peak and heterozygote peak height ratios were identified and its proportion determined for all markers in the 500 obtained genetic profiles (Table 2). Results show that the average stutter peaks products do not exceed 10% of the full-length allele product in any of the genetic markers. The average balance between peaks, in heterozygote markers, was above 80% in all *loci*.
Through the analysis of stutter peak and heterozygote peak height ratios, PowerPlex® Fusion kit (Promega Corporation) reveals a great capacity in the characterization of CODIS and ESS markers, laying the observed results within the expected values.
In order to evaluate the capacity of distinguishing two contributors in the same sample, a mixture was prepared in different proportions (1:10; 1:1 and 10:1) and analyzed through the same protocol as the reference samples. The obtained electropherograms were studied, using the stutter peak and heterozygote peak height ratios assignment for each locus previously determined, revealing that in case of forensic casework samples containing mixtures (Figure 1), this information is a valuable tool to distinguish true alleles and artefacts; and also to identify the number of contributors in a sample.
There were no off-ladder alleles detected and it was possible to type microvariants which were not present at the allelic ladder of the kits already in place in lab routine.

Table 2 - Stutter peak and heterozygote peak ratios for PowerPlex®Fusion System. Values obtained from 500 genomic samples amplified using GeneAmp® 9700 Gold Plate. One microliter of each sample was electrophoresed in ABI PRISM® Genetic Analyzer 3130xl sequencer and the results analyzed by GeneMapper® ID-X v1.4.

Marker	Stutter peak ratio (minus stutter)	Stutter peak ratio (plus stutter)	Heterozygote peak height ratio
D3S1358	5.39%	0.18%	85.93%
D1S1656	5.84%	0.19%	86.17%
D2S441	2.64%	0.13%	85.62%
D10S1248	7.61%	0.05%	85.20%
D13S317	2.66%	0.16%	84.13%
Penta E	1.51%	0.07%	83.09%
D16S539	4.39%	0.42%	80.98%
D18S51	6.72%	0.39%	86.42%
D2S1338	9.39%	0.16%	86.55%
CSF1P0	3.36%	0.15%	82.94%
Penta D	0.78%	0.05%	82.76%
TH01	0.96%	0.21%	80.76%
vWA	4.45%	0.13%	86.36%
D21S11	5.16%	0.30%	84.88%
D7S820	2.40%	0.10%	86.36%
D5S818	3.19%	0.30%	83.31%
TPOX	0.66%	0.20%	83.55%
DYS391	2.81%	0.04%	80.30%
D8S1179	5.61%	0.40%	88.03%
D12S391	6.45%	0.26%	85.87%
D19S433	4.01%	0.06%	85.07%
FGA	5.09%	0.15%	84.06%
D22S1045	5.06%	1.14%	80.51%

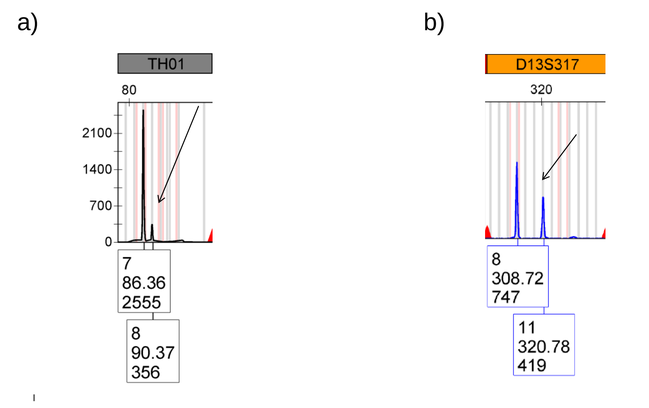

Figure 1 – Representative electopherograms of two markers resulting from the prepared mixture used to evaluate the capacity to distinguish true alleles/artefacts and to identify the number of contributors in a sample, where we can observe that we are in the presence of more than one contributor, because:
a) the difference between allele 7 and allele 8 is 13.93%, being the average stutter value for TH01 marker 0.21%;
b) the difference between alelle 8 and alelle 11 is 56.09%, being the average balance between heterozygous peaks height at D13S317 marker 84.13%.

Conclusions

By analysing the 500 samples with the PowerPlex® Fusion kit (Promega Corporation) we found that it is very useful in CODIS and ESS markers characterization. The implemented method exhibits conclusive results and complete genetic profiles. With the implementation of this method by reducing the final volume of amplification it was possible to reduce laboratory costs. This kit has revealed, in comparison with kits used in SGBF routine, a better capacity on microvariants detection which were not present at the allelic ladder of the other PCR kits.

References

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