Insertion/Delection Polymorphism and forensic aplications: A preliminary Study

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INTRODUCTION

Human genetic identification is usually based on the study of STR markers, robust and reliable for samples containing relatively small quantities of DNA [1]. Recent advances in forensic genetics have focused on the development of genotyping assays using shorter amplicons, in order to improve the successful amplification of degraded samples. Single Nucleotide Polymorphisms (SNP) and Insertion/Deletion polymorphisms (INDEL), length polymorphisms created by insertions or deletions of one ore more nucleotides in the genome [2], have considerable potential in this kind of forensic samples, usually present in identification caseworks, since they can combine desirable characteristics of both, STR and SNP [3].

MATERIAL and METHODS

In this study, a set of 30 biallelic Delection/Insertion polymorphisms (DIP or INDEL) distributed over 19 autosomes plus Amelogenin in a single multiplex PCR reaction was applied to 100 healthy and unrelated caucasian individuals (50 males and 50 females) selected from casework samples undergoing forensic investigations.

DNA was isolated from blood stain cells by chelex ® [4] method and DNA concentrations were estimated by Real Time PCR using the Quantifiler™ Human DNA quantification kit on an ABI Prism 7500 (Applied Biosystems). INDEL's amplification was performed with DIPplex® kit (Qiagen) in an ABI Prism 3130*xl* (Applied Biosystems), according to manufacturer's instructions.

Allele distribution, Observed Heterozigosity(OH) and Expected Heterozigosity (EH), and Hardy Weinberg (HWE) departure were estimated by Arlequin 3.5.1.2. [5].Statistical parameters to evaluate forensic efficiency, such as Discrimination Power (DP) and Power of Exclusion (PE) for each locus were calculated using PowerStatsv12 (Promega).

RESULTS

Table 1-Allele distribution, observed heterozygosity(OH), Hardy Weinberg equilibrium(HWE), Discrimination Power (PD) and Power of Exclusion (PE)

Marker	Freq. of Allele Deletion	HWE	OH	EH	DP	EP
HLD77	0.5689	0.5115	0.4482	0.4933	0.6377	0.1501
HLD45	0.4712	0.8300	0.4827	0.5012	0.6264	0.1715
HLD131	0.4425	1.0000	0.4942	0.4962	0.6232	0.1794
HLD70	0.3850	1.0000	0.4712	0.4763	0.6125	0.1571
HLD6	0.5689	0.5113	0.4482	0.4933	0.6488	0.1295
HLD111	0.4827	0.5196	0.4597	0.5022	0.6488	0.1402
HLD58	0.5574	0.3903	0.4482	0.4962	0.6382	0.1456
HLD56	0.2988	0.6089	0.3908	0.4215	0.5701	0,1115
HLD118	0.5689	0.3901	0.5402	0.4933	0.6043	0.1875
HLD92	0.5517	1.0000	0.5057	0.4975	0.6043	0,1875
HLD93	0.5574	0.0855	0.4023	0.4962	0.6305	0.1643
HLD99	0.4252	0.8288	0.5057	0.4916	0.6415	0.1501
HLD88	0.5114	0.8311	0.5172	0.5026	0.6398	0.1595
HLD101	0.5057	0.0516	0.3908	0.5028	0.6536	0.1305
HLD67	0.3735	0.6548	0.4482	0.4707	0.6270	0.1393
HLD83	0.4425	0.6675	0.4712	0.4962	0.6322	0.1718
HLD114	0.5689	0.5117	0.4483	0.4933	0.6264	0.1755
HLD48	0.3563	0.4806	0.5057	0.4613	0.5894	0.1919
HLD124	0.4482	0.1913	0.5747	0.4975	0.5983	0.2220
HLD122	0.6609	0.8110	0.4713	0.4508	0.5862	0.1501
HLD125	0.4770	0.3908	0.4483	0.5018	0.6284	0.1795
HLD64	0.3965	0.0708	0.3793	0.4814	0.6392	0.1176
HLD81	0.5805	0.5093	0.4483	0.4898	0.6258	0.1646
HLD136	0.4885	0.5228	0.5402	0.5026	0.5852	0.2508
HLD133	0.4942	1.0000	0.5056	0.5028	0.6248	0.1875
HLD97	0.5632	0.5243	0.4597	0.4948	0.6397	0.1501
HLD40	0.5000	0.6718	0.4713	0.5029	0.6377	0.1643
HLD128	0.5747	1.0000	0.4827	0.4916	0.6166	0.1718
HLD39	0.5632	0.8301	0.4827	0.4948	0.6322	0.1368
HLD84	0.3620	0.6431	0.4943	0.4646	0.5952	0.1706

Each one of caucasian individuals was typed for the 30 indels. Allele frequencies of the alleles deletion, Observed and Expected Heterozigosity, Hardy Weinberg departure p-values, Discrimination Power and Power of Exclusion for each locus are shown in table 1.

Most of the allele frequencies are around 0.500, with the exceptions of HLD56 and HLD122, 0.2988 (under 0.3000) and 0.6609 (above 0.6500), respectively.

All studied markers followed Hardy Weinberg expectations ($\alpha < 0.05$).

All markers showed heterozigosity values higher than 0.30.

The cumulative power of discrimination is 0,9999999999998.

An electrophorogram of the 30 indel plex obtained from one male caucasian individual, analysed using Genemapper ID v3.2 software is represented in Figure 1.

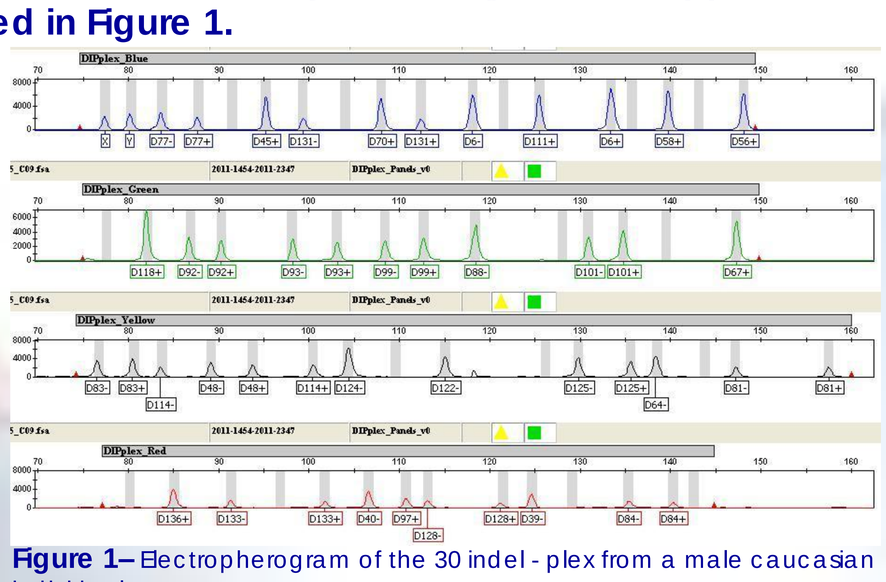

Figure 1– Electropherogram of the 30 indel - plex from a male caucasian individual.

CONCLUSIONS

The DIPplex ® kit enables the simultanous amplification and analysis of 30 small indels in a very easy way. This kit proved to be a very sensitive and robust assay, since it was possible to obtain good results even samples with small amounts of DNA (50pg/µl).

Statistical analysis revealed that the 30 biallelic markers can provide satisfactory levels of informativeness for forensic demands.

In the near future, this assay will be a valuable routine tool in combination with STR typing, specially in degraded DNA samples.

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