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Autosomal SNP typing of forensic samples with the GenPlex™ HID System: Results of a collaborative study

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ABSTRACT

The GenPlex™ HID System (Applied Biosystems – AB) offers typing of 48 of the 52 SNP*for*ID SNPs and amelogenin. Previous studies have shown a high reproducibility of the GenPlex™ HID System using 250–500 pg DNA of good quality. An international exercise was performed by 14 laboratories (9 in Europe and 5 in the US) in order to test the robustness and reliability of the GenPlex™ HID System on forensic samples. Three samples with partly degraded DNA and 10 samples with low amounts of DNA were analyzed in duplicates using various amounts of DNA. In order to compare the performance of the GenPlex™ HID System with the most commonly used STR kits, 500 pg of partly degraded DNA from three samples was typed by the laboratories using one or more STR kits. The median SNP typing success rate was 92.3% with 500 pg of partly degraded DNA. Three of the fourteen laboratories counted for more than two thirds of the locus dropouts. The median percentage of discrepant results was 0.2% with 500 pg degraded DNA. An increasing percentage of locus dropouts and discrepant results were observed when lower amounts of DNA were used. Different success rates were observed for the various SNPs. The rs763869 SNP was the least successful. With the exception of the MiniFiler™ kit (AB), GenPlex™ HID performed better than five other tested STR kits. When partly degraded DNA was analyzed, GenPlex™ HID showed a very low mean mach probability, while all STR kits except MiniFiler™ had very limited discriminatory power.

1. Introduction

The usefulness of SNP typing in forensic genetics has previously been pointed out in several publications [1–3]. Short DNA fragments with SNPs can be amplified, which makes it possible to analyze SNPs in partly degraded DNA samples [1,2,4]. Moreover, the low mutation rate of SNPs is an important advantage in kinship analysis [3]. The SNP*for*ID consortium [5] selected 52 biallelic SNP markers with high levels of polymorphism in the major population groups [1]. Short amplicons (up to 115 bp) were used to analyze the 52 SNPs and the mean match probability was at least 5.0×10^{-19}. Several platforms can be used to analyze SNPs [6–10]. The most widely used assay is the single base extension (SBE) assay using the SNaPshot® kit

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(AB: Applied Biosystems, Foster City, CA, USA) because it is easy to implement in forensic genetic laboratories. The SBE-based SNP typing assay of the SNP*for*ID 52-plex was validated for forensic genetic investigations [4] and it performed better than the most commonly used STR kits when partly degraded DNA samples were investigated [2,4]. Nevertheless, the unspecific addition of dA nucleotides at the end of the PCR products by the Taq polymerase [11] and the fact that the signal strength of the four colours in the SNaPshot® kit (AB) is unbalanced makes the interpretation of the results challenging [12].

Applied Biosystems developed the GenPlex™ HID System using 48 of the 52 SNPs in the SNP*for*ID 52-plex and amelogenin. The GenPlex™ HID protocol is based on the SNPplex™ assay [13] but in this case, the protocol starts with a PCR reaction, which makes the method more sensitive and suitable for forensic applications. The GenPlex™ HID protocol contains a large number of pipetting steps that makes it laborious and time consuming. However, the use of a simple robot reduces the laboriousness of the protocol [14]. A limited number of laboratories have successfully tested the GenPlex™ HID System [15,16] and demonstrated that it is a sensitive and reproducible SNP typing method when good quality samples are analyzed.

In order to test the performance of the GenPlex™ HID System in challenging forensic genetic samples, as well as the handling of a complex typing protocol in routine casework laboratories, an international exercise was organized within the framework of the EDNAP and ENFSI groups. Fourteen laboratories (9 in Europe and 5 in the USA) participated in the exercise and 13 DNA samples (three naturally degraded DNA samples from case work and 10 DNA samples from blood from healthy donors) were SNP typed using the GenPlex™ HID System at various DNA concentrations. The results of the GenPlex™ HID System were compared to the results obtained by typing 500 pg of partly degraded DNA with six commonly used STR kits.

2. Material and methods

2.1. DNA samples

Three DNA extracts from cadaveric remains (paraffin-embedded tissue, nails and blood) with partly degraded DNA that gave partial STR profiles with the AmpFℓSTR® SGM Plus® PCR amplification kit (AB) were selected. None of the partly degraded DNA samples showed STR results corresponding to amplicons longer than 185 bp, weak results were obtained with amplicons between 135 bp and 185 bp and strong results were observed with amplicons lower than 135 bp. In addition, DNA was extracted from blood collected from 10 healthy volunteers.

All samples were investigated in quadruplicate by the organizing laboratory using the GenPlex™ HID System (AB) and the SBE-based 49plex SNP assay [12]. Concordant results were obtained from at least two of the investigations. The results obtained by the organizing laboratory were used as reference.

The protocols were approved by the Danish ethical committee (KF-01-037/03).

2.2. DNA extraction and quantification

DNA from the three samples with partly degraded DNA was extracted using a standard phenol–chloroform procedure. DNA from blood samples from 10 healthy donors was extracted from 15 μL of blood using the MagAttract DNA blood midi M48 kit (Qiagen GmbH, Hilden, Germany) and a Tecan Freedom EVO® robot (Tecan trading AG, Switzerland) [17]. The DNA samples were quantified with the Quantifiler® Human DNA Quantitation kit (AB) using an AB 7900 (AB) real time PCR analyzer according to the manufacturer's recommendations.

2.3. Shipment of samples and reagents

DNA extracts and the critical reagents needed for SNP typing with the GenPlex™ HID System (AB) were sent on dry ice by courier to the participating laboratories.

2.4. GenPlex™ HID SNP typing

The participating laboratories typed the degraded DNA samples in duplicate with the GenPlex™ HID System using 500 pg, 250 pg, 100 pg, 50 pg and 20 pg of DNA in the PCR reaction. Ten blood DNA extracts were SNP typed in duplicate by the participating laboratories with the GenPlex™ HID System adding 50 pg, 25 pg, 10 pg, 5 pg and 2 pg of DNA to the PCR reaction. The DNA titrations were prepared by each participating laboratory.

Details on the GenPlex™ HID procedure are published elsewhere [15]. Briefly, a multiplex PCR reaction was performed using 2 μL of DNA with amounts indicated above. All laboratories performed the PCR reaction in a GeneAmp® PCR System 9700 thermal cycler (AB) according to the manufacturer's recommendations. Leftover primers and unused dNTPs were removed from the PCR products using 2 μL of ExoSAP-IT® (USB Corp., Cleveland, OH, USA). An oligo ligation assay (OLA) was performed using the PCR products as templates. The OLA assay required the phosphorylation of the oligos. This may be performed at the same time as the OLA assay or as a step preceding the OLA assay. Twelve of the fourteen participating laboratories phosphorylated the oligos prior to the OLA assay, while two of them performed the phosphorylation reaction and the OLA assay in a single step (Table 1). All the

Table 1
Details of the GenPlex™ HID protocol.

Lab #	Previous experience with GenPlex	ABI sequencer	No. of steps in the OLA reaction	OLA product (μL)	Sample loading reagent (μL)
1	Yes	3130xl	2	10	17.5
2	Yes	3130	2	5	10
3	Yes	3730xl	2	10	17.5
4	No	3130xl	2	10	10
5	No	3130xl	2	10	10
6	No	3130xl	2	5	10
7	Yes	3130xl	1	5	10
8	Yes	3130xl	1	10	10
9	No	3130xl	2	10	17.5
10	No	3130xl	2	10	17.5
11	Yes	3130xl	2	5–10	10–17.5
12	Yes	3130xl	2	5	10
13	No	3130xl	2	10	10
14	Yes	3130xl	2	10	17.5