Forensic Science International: Genetics 3 (2008) 1–6

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Typing of 48 autosomal SNPs and amelogenin with GenPlex SNP genotyping system in forensic genetics

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ARTICLE INFO

Article history:
Received 11 April 2008
Received in revised form 19 June 2008
Accepted 30 June 2008

Keywords:
GenPlex
Autosomal SNPs
Amelogenin
Forensic genetics

ABSTRACT

GenPlex (Applied Biosystems) is a new SNP genotyping system based on an initial PCR amplification followed by an oligo ligation assay (OLA). The OLA consists of the hybridization of allele and locus specific oligonucleotides (ASOs and LSOs) to PCR products and posterior ligation of ASOs and LSOs. The ligation products are immobilized to microtitre plates and reporter oligonucleotides (ZipChute® probes) are hybridized to the ligation products. ZipChute® probes are subsequently eluted and detected using capillary electrophoresis. Applied Biosystems developed the GenPlex SNP genotyping system with amelogenin and 48 of the 52 SNPs used in the 52 SNP-plex assay developed by the SNP*for*ID consortium. The system requires equipment that is usually found in forensic genetic laboratories. The use of a robot for performance of the pipetting steps is highly recommendable.

A total of 286 individuals from Denmark, Somalia and Greenland were investigated with GenPlex using a Biomek® 3000 (Beckman Coulter) robot. The results were compared to results obtained with an ISO 17025 accredited SNP typing assay based on single base extension (SBE). With the GenPlex SNP genotyping system, full SNP profiles were obtained in 97.6% of the investigations. Perfect concordance was obtained in duplicate investigations and the SNP genotypes obtained with the GenPlex system were concordant with those of the accredited SBE based SNP typing system except for one result in rs901398 in one of 286 individuals most likely due to a mutation 6 bp downstream of the SNP. Reproducible SNP genotypes were obtained from as little as 250 pg of DNA.

1. Introduction

The SNP*for*ID consortium [1] developed a PCR multiplex for amplification of 52 autosomal SNPs [2]. The SNPs were detected in a single base extension (SBE) assay using the SNaPshot® kit (AB: Applied Biosystems, Foster City, CA, USA) together with capillary electrophoresis (CE) of the SBE products and multicolour fluorescence detection of the extended oligonucleotides. The SBE assay was chosen as the first platform for analysis of the SNP multiplex because most forensic genetic laboratories have the equipment that is needed for the analysis of SBE products. It has been demonstrated that the 52 SNP*for*ID SNPs are well suited for forensic genetic investigations in crime [2], paternity and immigration cases [3]. An SBE based SNP typing assay with 49 of the 52 SNP*for*ID SNPs [3] was recently accredited in our laboratory according to the ISO 17025 standard for forensic genetic investigations in relationship testing.

The SNaPshot® kit has some drawbacks that make it a challenge to analyze the CE data files. The signal strengths of the four colours in the SNaPshot® kit are not well balanced, which can make it difficult to distinguish between homozygotes and heterozygotes. In addition, the Taq polymerase used in the kit extend primers and PCR products unspecifically with an A nucleotide [4,5], which results in a relatively high background in the green colour. Therefore, we are trying to find more suitable platforms that (1) allow more precise and accurate results to be obtained, (2) are well suited for small and medium scale productions, and (3) can be used with the usual equipment in a modern, forensic genetic laboratory. As part of the projects of the SNP*for*ID consortium, other platforms than the SBE based one were investigated [1,6–10], but none of them have been implemented in routine work. Applied Biosystems used the information offered by the SNP*for*ID consortium to develop a SNP multiplex typing system for 48 autosomal SNPs and amelogenin based on the GenPlex system, which is a modification of the SNPlex™ genotyping system (AB) [11]. The SNPlex™ system

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doi:10.1016/j.fsigen.2008.06.007

begins with a multiplex OLA reaction that is followed by PCR amplification of the ligation products. The initial OLA is not sensitive and 30–40 ng DNA are generally needed to obtain a reliable reaction [11]. The GenPlex system begins with PCR amplification of the template DNA followed by an OLA reaction. In this way, the GenPlex system becomes more sensitive. In both systems, allele and locus specific oligonucleotides (ASOs and LSOs) and reporter oligonucleotides (ZipChute™ probes) are used for the analysis [11,12].

The GenPlex SNP genotyping system was evaluated for forensic genetic applications by the SNP*for*ID consortium [10]. The investigations suggested that GenPlex is likely to offer a comparable, if not improved, alternative to SBE for forensic SNP genotyping. Philips et al. [12] calculated the match probability of the 48 SNP GenPlex assay to be 9.6×10^{-18} in Europeans and 6.9×10^{-16} in Africans, and calculated the average PI to 4.2×10^{4} in three CEPH families and 1.0×10^{6} in 24 German trios.

The large number of pipetting steps makes the GenPlex system vulnerable to pipetting errors if performed manually, and if a large number of samples are processed, the technical staff may be exposed to injuries in their arms.

We have conducted a preliminary study in order to evaluate the results and the challenges of the GenPlex SNP genotyping system. The GenPlex protocol was performed using a simple robot (Biomek® 3000, BC: Beckman Coulter, Fullerton, CA, USA) for the majority of the pipetting steps.

2. Materials and methods

2.1. Samples and DNA preparation

A total of 286 samples from 111 Danes, 88 Greenlanders and 87 Somalis from paternity and immigration cases were typed. DNA of approximately two thirds of the samples was extracted from 200 μl blood using the QIAamp DNA blood mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations. Approximately one third of the samples were buccal cells collected on FTA® cards (Whatman Inc., Clifton, NJ). We obtained 1.2 mm diameter punches of each sample using a BSD600-Duet semi-automated puncher (BSD Robotics, Queensland, Australia). The punches were washed with Milli-Q water and dried for 45 min at 60 °C using a Biomek® NX Span-8 (BC) together with a heater-shaker (Variomag®, Daytona Beach, FL).

2.2. SNP typing with GenPlex

The SNP markers used in the GenPlex system are shown in Table 1. The GenPlex protocol was described in detail elsewhere [12].

Briefly, the protocol included:

(1) *PCR reaction*: A multiplex PCR reaction with 98 primers that amplified 48 autosomal SNP loci and amelogenin (Table 1) was performed in a GeneAmp® PCR system 9700 thermo cycler (AB) according to the manufacturer's recommendations. The lengths of the amplicons with the autosomal SNP loci were between 59 bp and 115 bp, and the lengths of the amplicons with amelogenin were 111 bp for the Y chromosome and 105 bp for the X chromosome. The sequences of the oligonucleotides were based on the SNP*for*ID 52 SNP-plex assay. Two microliters of a 10 times diluted DNA extract (containing between 1 ng and 5 ng DNA) or a 1.2 mm diameter punch were mixed with 8 μl or 10 μl of PCR master mix, respectively. The PCR master mix contained multiplex PCR mix (AB) and primer mix (AB).

Table 1
The detected alleles of the 48 SNPs and amelogenin in the GenPlex system and the SNP*for*ID 52 SNP-plex assay

Markers rs numbers	GenPlex		SNP*for*ID 52 SNP-plex assay
	Detected nucleotides	Fluochrome	Detected nucleotides [2]
rs740910	C T	FAM™	A G
rs1490413	A G	FAM™	C T
rs1335873	A T	FAM™	A T
rs1979255	C G	FAM™	C G
rs1493232	G T	FAM™	G T
rs2040411	C T	FAM™	A G
Amelogenin	–	FAM™	Not included
rs1528460	A G	FAM™	A G
rs717302	A G	FAM™	A G
rs251934	C T	FAM™	A G
rs8037429	C T	FAM™	A G
rs891700	A G	FAM™	A G
rs901398	A G	FAM™	C T
rs873196	C T	FAM™	A G
rs964681	A G	FAM™	C T
rs737681	A G	FAM™	C T
rs1463729[a]	A G	FAM™	A G
rs1360288	C T	FAM™	A G
rs1382387	A C	FAM™	G T
rs1413212	A C	FAM™	C T
rs2056277	C T	FAM™	A G
rs2107612	C T	FAM™	A G
rs1015250	C G	FAM™	C G
rs1005533	C T	FAM™	A G
rs729172	A C	FAM™	G T
rs10495407	A G	FAM™	C T
rs1357617	A T	FAM™	A T
rs719366	A G	FAM™	C T
rs1031825	A C	dR6G	G T
rs733164	A G	dR6G	A G
rs938283	A G	dR6G	C T
rs2111980	C T	dR6G	A G
rs1886510[a]	A G	dR6G	A G
rs914165	A G	dR6G	C T
rs354439	A T	dR6G	A T
rs763869	A G	dR6G	C T
rs2076848	A T	dR6G	A T
rs1024116	A G	dR6G	A G
rs1355366	A G	dR6G	C T
rs735155	A G	dR6G	C T
rs1454361	A T	dR6G	A T
rs727811	A C	dR6G	A C
rs917118	C T	dR6G	A G
rs2831700	A G	dR6G	C T
rs907100	C G	dR6G	C G
rs1029047	A T	dR6G	A T
rs2046361	A T	dR6G	A T
rs722098	C T	dR6G	A G
rs876724	C T	dR6G	C T

[a] SNPs not included in the accredited version of the SNP*for*ID 52 SNP-plex assay.

(2) *PCR reaction clean-up*: Unincorporated DNA bases and primers were removed by adding 2 μl ExoSAP-IT® (USB Corp., Cleveland, OH, USA) directly to the PCR reaction. The incubation was carried out in a GeneAmp® PCR system 9700 thermo cycler (AB) according to the manufacturer's recommendations.

(3) *OLA reaction*: The PCR products were used as templates for hybridization and ligation of two kinds of oligos: (i) biotinylated locus specific oligos (LSOs) hybridized to sequences immediately downstream of the SNP positions (17–30 bp); and (ii) allele specific oligos (ASOs) bound immediately upstream of the LSOs. The 3′ end of the ASOs hybridized to the SNP position. There were two different ASOs for each SNP locus. Each ASO was identified by a reporter sequence complimentary to a ZipChute® detection probe (see below) [11,12]. The PCR products were mixed with 10 μl of OLA reaction mix containing SNPlex™ OLA

Table 2
In house criteria for allele calling

SNP	Alleles		Heterozygote	Homozygote allele 1	Homozygote allele 2
	Allele 1	Allele 2	Allele1/allele2	Allele 1/allele 2 >than	Allele 1/allele 2 <than
rs1005533	C	T	0.7–1.6	29	0.038
rs1015250	G	C	0.4–3.5	56	0.029
rs1024116	G	A	0.3–5.9	46	0.048
rs1029047	T	A	0.4–3.5	18	0.071
rs1031825	C	A	0.5–2.6	37	0.040
rs10495407	G	A	0.7–1.7	47	0.038
rs1335873	A	T	0.7–1.6	42	0.016
rs1355366	G	A	0.6–1.7	78	0.027
rs1357617	A	T	1.0–2.0	33	0.030
rs1360288	C	T	0.2–6.7	23	0.063
rs1382387	C	A	0.7–1.4	68	0.036
rs1413212	A	C	0.5–2.1	67	0.077
rs1454361	A	T	0.6–1.9	64	0.019
rs1463729	A	G	1.2–3.0	55	0.026
rs1490413	G	A	0.4–3.4	48	0.056
rs1493232	T	G	0.5–2.2	70	0.011
rs1528460	G	A	0.5–2.8	66	0.020
rs1886510	G	A	0.5–2.8	18	0.083
rs1979255	G	C	0.5–2.7	35	0.014
rs2040411	T	C	0.5–2.3	43	0.019
rs2046361	A	T	0.7–1.6	16	0.050
rs2056277	C	T	0.5–2.3	82	0.053
rs2076848	A	T	0.5–3.0	39	0.056
rs2107612	T	C	0.6–1.8	30	0.091
rs2111980	T	C	0.5–3.0	35	0.036
rs251934	T	C	0.5–2.4	75	0.026
rs2831700	G	A	0.6–2.0	25	0.040
rs354439	T	A	0.7–1.6	48	0.029
rs717302	A	G	0.6–1.9	71	0.019
rs719366	A	G	1.0–2.1	62	0.067
rs722098	T	C	0.4–2.8	35	0.029
rs727811	C	A	0.5–2.1	45	0.015
rs729172	A	C	0.5–2.5	101	0.063
rs733164	G	A	0.4–3.5	24	0.045
rs735155	A	G	0.6–1.9	41	0.026
rs737681	G	A	0.6–2.1	101	0.024
rs740910	T	C	0.6–1.7	82	0.037
rs763869	G	A	1.3–3.0	47	0.077
rs8037429	C	T	0.5–2.3	27	0.022
rs873196	T	C	0.8–1.4	48	0.010
rs876724	T	C	0.2–5.2	15	0.067
rs891700	G	A	0.6–2.1	44	0.020
rs901398	A	G	0.7–1.6	59	0.036
rs907100	C	G	0.3–3.8	16	0.125
rs914165	G	A	0.5–2.8	50	0.036
rs917118	C	T	0.6–1.9	44	0.030
rs938283	G	A	0.7–1.6	30	0.019
rs964681	A	G	0.5–2.5	49	0.032

High peak ratio ranges for hetero- and homozygotes calculated for each SNP.

master mix (AB), OLA oligos (AB), gender oligos (AB) and dATPs, and incubated in a GeneAmp® PCR system 9700 thermo cycler (AB) according to the manufacturer's recommendations.

(4) *Binding of biotinylated OLA products to a solid phase for the ZipChute® hybridization*: Ten microliters of biotinylated OLA product were added to a streptavidin coated capture microtitre plate (AB). The capture of the biotinylated OLA product was performed in an Eppendorf Thermomixer comfort (Eppendorf) at 700 rpm and room temperature for 1 h.

(5) *ZipChute® probe hybridization*: A total of 25 μl of a mix containing 98 ZipChute® identifying probes were hybridized to the captured OLA products. The hybridization was carried out at 37 °C and 700 rpm for 1 h in an Eppendorf Thermomixer Comfort (Eppendorf).

(6) *Elution of the ZipChute® probes*: After removing the non-hybridized products by washing the microtitre plate four times with 1× Hybridization Wash Buffer (SNPlex™ system, AB), the ZipChute® probes were eluted by incubating the samples at 37 °C for 5 min with sample loading reagent (AB).

(7) *Capillary electrophoresis*: The eluted ZipChute® probes were mixed with LIZ™ 48-plex size standard (SNPlex™ system). Samples were transferred to the electrophoresis plates with one negative control and multiple wells containing allelic ladders. Products were separated by capillary electrophoresis in an ABI3130*xl* Genetic Analyzer (AB) using POP-7™ polymer (AB). The injection voltage was 1 kV for 10 s. The run voltage was 15 kV for 400 s. The results were recorded as fragment sizes and relative fluorescence units (RFUs).

With the exception of the PCR reaction, all the post-PCR steps included in the GenPlex protocol were performed using a Biomek® 3000 (BC). Details regarding the automation of the GenPlex protocol using Biomek® 3000 (BC) are described elsewhere [13].

2.3. SNP typing with SBE with SNaPshot®

All the samples had previously been SNP genotyped using a modified version of the SNP*for*ID 52 SNP-plex assay, where 49 of the 52 SNPs are included [3]. Two of the 48 SNPs typed with the GenPlex system (rs1463729 and rs1886510), were not typed with the SBE assay (Table 1).

2.4. Sensitivity study

DNA from two individuals was purified from 200 μl blood using the QIAamp DNA blood mini kit (Qiagen GmbH, Hilden, Germany) and quantified with the Quantifiler® Human DNA Quantitation kit (AB) on an AB 7900 (AB) according to the manufacturer's recommendations.

Five concentrations of DNA from two individuals were prepared for the sensitivity experiment. The PCR was carried out using 2 μl DNA extract with the following amounts of DNA: 500 pg, 250 pg, 100 pg, 50 pg and 20 pg. The sensitivity experiments were performed in triplicate.

2.5. Data interpretation

All the results were analyzed using the cluster analysis programme implemented in the GeneMapper™ 4.0 software. All the tools needed to analyze GenPlex with GeneMapper™ were facilitated by Applied Biosystems.

In house criteria for allele calling were established by calculating independent cut off values for homo- and heterozygotes for each SNP. We collected the peak heights that were higher than 10 relative fluorescent units from 521 samples in an Excel (Microsoft©) spread sheet. The results of the first 51 samples used to optimize the GenPlex reaction in our laboratory were not used to establish rules for allele calling because the results were considered special due to the training situation. The ratios between the two peak heights obtained for each SNP in each sample were calculated in heterozygotes (peak ratio between two alleles) and homozygotes (peak ratio between the real allele and any spurious peak, if present). Taking into account that every SNP behaved differently, the peak height ratios were analyzed independently for each SNP, and different cut off values for hetero- and homozygote calls were defined for each SNP (Table 2). In order to calculate the peak height ratio cut off values for homozygotes, the peak ratios were analyzed using the discriminant analysis tool implemented in the SPSS statistical package (SPSS Inc., Chicago, IL, USA). A SNP type was considered to be "homozygotic" if the peak height ratio was closer to the centroid value of the homozygotes than to that of the heterozygotes. The cut off values were calculated independently for the two possible alleles of each SNP. The "heterozygotic" cut off values were calculated as the arithmetic mean values of all peak height ratios in heterozygotes plus/minus 4 times the standard deviations, which is expected to include 99.9% of the observed values.

Peak heights that were lower than 100 RFUs in a presumed homozygote or lower than 50 RFUs in a presumed heterozygote were not automatically called and the results were scrutinized manually.

SNP typing results that did not fall into the clusters of homo- or heterozygotes were classified as indeterminable by the automated scoring system. These results were scrutinized manually.

3. Results and discussion

3.1. SNP typing results with the GenPlex SNP genotyping system

A total of 286 samples were typed in duplicate with the GenPlex SNP genotyping system. Of the 572 SNP types, 97.6% showed a complete SNP profile (Table 3) and all the SNP profiles of the duplicate investigations were concordant (Table 3). Of the 13,156 SNP genotypes that were typed with the GenPlex system and the SBE assay, only one discrepancy was detected (99.99% concordant results) (Table 3). The discrepancy was found in rs901398 in one individual. We detected two alleles using the SBE reaction but only one allele using the GenPlex assay. Sequencing of the region around the SNP rs901398 in the individual showed that the individual had a punctual mutation [C/T] 6 bp downstream of rs901398. The mutation most likely explains the 'null' allele when using the GenPlex system through a mechanism that decreases the hybridization between the ASO/SNP/OLA complex and the template. The mutation did not affect the efficiency of the SBE reaction because the SBE primer binds immediately upstream of rs901398.

3.2. No or inconclusive SNP typing results with the GenPlex SNP genotyping system

Of the 572 SNP typings, 521 were collected in an Excel (Microsoft©) spread sheet and were analyzed using in house criteria (Table 4). Of the 25,529 genotypes, 0.3% were locus dropouts (peak heights lower than 10 RFUs), 0.6% of the results were below 50 RFUs in heterozygotes or 100 RFUs in homozygotes, and 1.8% of the results could not be clearly assigned as homo- or heterozygotic.

3.3. Performance of individual SNPs in the GenPlex SNP genotyping system

Fig. 1 shows the signal (measured as RFUs) obtained for each SNP when 250–500 pg of DNA template was used (mean ± standard deviation).

According to our results, a few SNPs – especially rs2831700 and rs907100 – generally gave lower signals than the remaining SNPs and only one SNP (rs907100) showed a remarkably high fraction of

Table 3
Results of the GenPlex SNP genotyping system

	Total	*N*	%
Complete profiles[a]	572	558	97.6
Concordant profiles[a]	286	286	100
Correct SNP genotypes[b]	13,156	13,155	99.99[c]

[a] Duplicate investigations of 286 individuals.
[b] GenPlex SNP typing compared with accredited SBE based SNP typing. The number of genotypes is 286 (samples) times 46 (SNPs shared by GenPlex and the SBE based method). Amelogenin results were concordant with the gender of the individuals typed in all cases.
[c] One individual was called as G in rs901398 with GenPlex and as AG with SBE.

Table 4
Inconclusive results of 25,529 genotypes (49 markers × 521 samples) analyzed with in house criteria

	N	%
No result (<10 RFUs)	86	0.3
Weak result[a]	143	0.6
Possible 'heterozygotes'[b]	55	0.2
Possible 'homozygotes'[c]	407	1.6
Total number of inconclusive SNP genotypes	691	2.7

[a] Weak results defined as—heterozygotes: <50 RFUs; homozygotes <100 RFUs.
[b] The results could not be clearly assigned as heterozygotes.
[c] The results could not be clearly assigned as homozygotes.

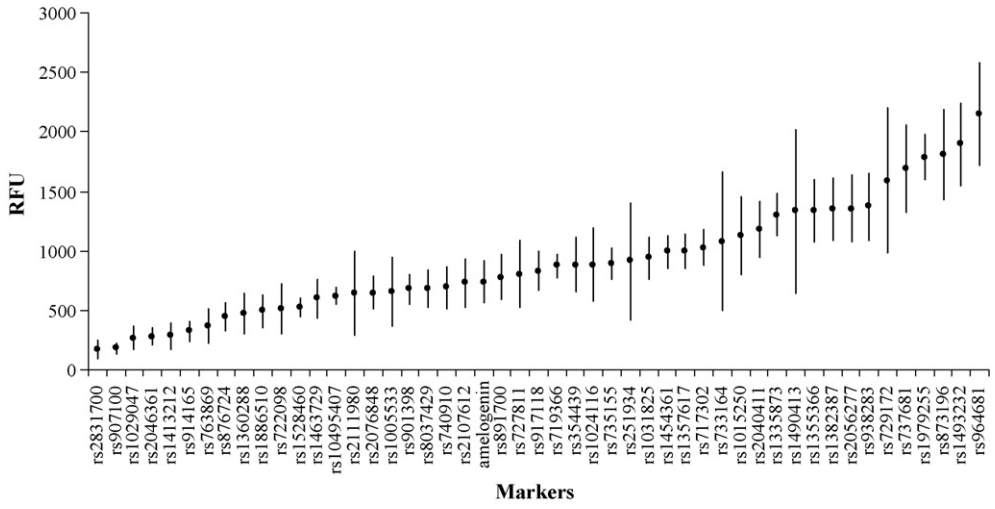

Fig. 1. Signals obtained with the GenPlex system. The mean peak height value ± the standard deviation (measured as RFUs) is shown for each SNP and amelogenin. DNA from two individuals was analyzed in triplicates using 250 pg and 500 pg for each sample. The average of the results obtained with both 250 pg and 500 pg DNA was used because the responses were almost identical (see Fig. 2).

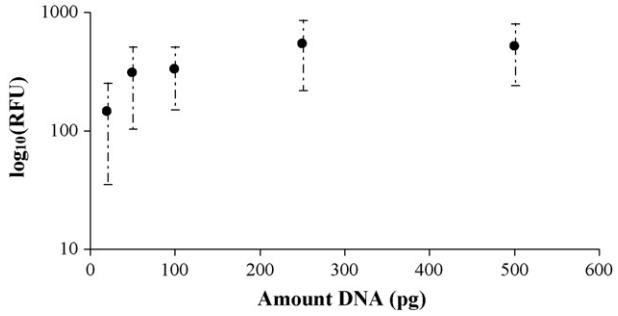

Fig. 2. Sensitivity of the GenPlex SNP genotyping system. DNA from two individuals was used in the following amounts: 500 pg, 250 pg, 100 pg, 50 pg and 20 pg. RFU values from homozygotes were normalized by division by two. Abscissa: amount of DNA (pg) used in the PCR; ordinate: $\log_{10}$(RFU).

SNP typing results that did not lead to clear SNP types (9.2%) using in house criteria. Moreover, the fact that this SNP showed one of the lowest signal strengths suggests that the chemistry for this SNP should be optimized. Problems with typing of rs907100 have also been noticed by others (D. Ballard, personal communication).

In addition to the performance of individual markers, the variation of the signal strengths could be explained by differences in the fluorescence between the two dyes used. Eight out of the ten SNPs that showed the lowest peak heights were labelled with dR6G (green), and eight out of the ten SNPs that showed the highest peak heights were labelled with FAM™ (blue).

The mean peak height ratio between the two alleles of presumed heterozygotes calculated for each of the 48 SNPs varied between 1 and 3.

3.4. Sensitivity of the GenPlex SNP genotyping system

Fig. 2 shows the results of the GenPlex SNP genotyping system performed with 500 pg, 250 pg, 100 pg, 50 pg and 20 pg DNA from two individuals.

The signal strengths were reproducible with 500 pg and 250 pg DNA. The quality of the results decreased with lower amounts of DNA. When the amount of DNA used was 50 pg or 20 pg, the peak height ratios in both homo- and heterozygotes showed unexpected values and some alleles were miscalled. In most cases, a heterozygote was called as a homozygote because one of the alleles was not called (Fig. 3a). We also observed that homozygotes were called as heterozygotes because a background peak was called as a real allele (Fig. 3b). To obtain reliable results, the amount of DNA must be higher than 100 pg. Highly reproducible results are obtained with 250–500 pg DNA.

3.5. The GenPlex SNP genotyping system in forensic laboratories

The GenPlex SNP genotyping system involves a large number of pipetting steps that makes manual performance tiresome. However, it was relatively uncomplicated to set up the GenPlex method on a Biomek® 3000 (BC) robot. The time needed to complete the protocol (around 11–12 h) was not shortened, but the amount of

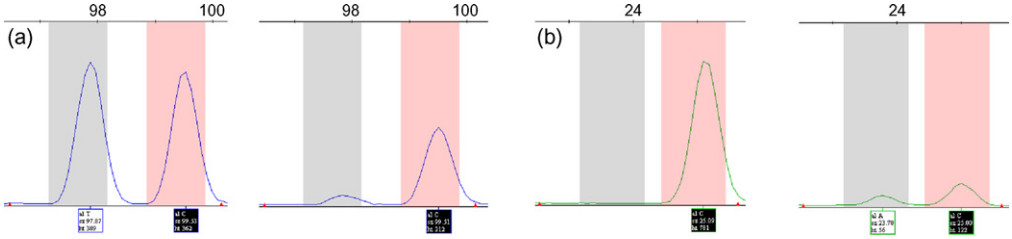

Fig. 3. Sensitivity study. (a) Allele dropout observed when the amount of template DNA used in the PCR was decreased from 500 pg (left) to 20 pg (right). (b) False heterozygote observed when the amount of template DNA was decreased from 500 pg (left) to 20 pg (right).

manual work was dramatically reduced. With the exception of the Biomek® 3000 (BC), the equipment required to perform the GenPlex protocol is found in the great majority of modern, forensic genetic laboratories.

AB recommends the use of POP-7™ for the GenPlex SNP genotyping system, while most forensic genetic laboratories use POP-4 for STR typing. In a production-scale forensic laboratory, a common polymer to run both applications (GenPlex and STRs) on the CE instrument would be ideal.

Only few SNPs (especially rs907100) showed relatively low quality results. However, the present version of the GenPlex assay is not developed and validated for forensic investigations. The 48 SNP GenPlex assay can most likely be improved by optimization of the PCR amplification and/or the OLA reaction.

GeneMapper™ 4.0 (AB) is a reasonable tool for experimental calling of SNP alleles detected by the GenePlex SNP genotyping system. However, the tools for discrimination between homo- and heterozygotes SNPs must be improved for practical forensic genetic case work. It might be worth introducing a 'learning algorithm' that can optimize the analyses compensating for systematic deviations in the results obtained in different laboratories.

3.6. Conclusions

In conclusion, we found that the GenPlex SNP genotyping system is a sensitive and reproducible SNP typing method. It was possible to automate the majority of the pipetting steps on a simple robot. Complete, reproducible SNP profiles were obtained from as little as 250 ng DNA. Full SNP profiles were obtained in the majority of the samples. The SNP profiles of duplicate testings were all concordant and the results were concordant with those of the accredited, SBE-based SNP typing system except for one genotype in rs901398 most likely due to a mutation in the oligo-binding region close to SNP.

Although the GenPlex SNP genotyping system is an experimental assay, the results obtained were at least as robust as those obtained with the accredited SBE based system. This is most likely due to a combination of a robust chemistry in the GenPlex SNP genotyping system and the possibility to automate the pipetting steps on a robot. The GenPlex SNP genotyping system is an attractive method for relationship testing. Further investigations are needed to evaluate the GenPlex method for SNP typing in crime case work.

Acknowledgments

We thank Applied Biosystems, Foster City, CA, California, for making the 49 SNP GenPlex assay reagents available for us. We also thank Beatriz Sobrino (Institute of Legal Medicine, University of Santiago de Compostela), David J. Ballard (Department of Haematology, Barts and The London, Queen Mary's School of Medicine and Dentistry), Yogesh R. Prasad (Applied Biosystems) and Rixun N. Fang (Applied Biosystems) for helpful discussions.

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