Development of a forensic DNA phenotyping panel using massive parallel sequencing

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ABSTRACT

The HIrisPlex-S system, targeting a total of 41 SNPs, allows the simultaneous eye, hair and skin color prediction from DNA. In the present study, we developed a massive parallel sequencing (MPS) multiplex assay in order to genotype all the HIrisPlex-S markers in degraded casework samples. PCR amplicons sizes of target regions were kept below 180 bp, in order to allow analysis of degraded DNA samples. Individuals with known phenotype, artificially degraded DNA samples and a set of 2800M control DNA dilutions were sequenced on a Ion PGM System, in order to evaluate the concordance testing results and the forensic suitability of this 41-plex MPS assay. Full and reliable profiles could be obtained with 0.1 ng of input DNA. The increment of the number of PCR cycles results in improvement of sensitivity or in typing results but an increase of artifacts were also observed.

1. Introduction

Forensic DNA Phenotyping (FDP) provides the ability to predict the human externally traits from unknown sample donors, directly from biological materials found at crime scene. This new branch of forensic genetics obtained a great importance for its potential use as a supplementary investigative tool whenever conventional DNA profiling fails to provide a match with any reference profiles. DNA phenotyping is useful to infer appearance predictions from bone remains, for missing person cases and mass disaster victim identification (DVI) scenarios, mostly in case where known relatives are unavailable. In the last years, Chaitanya et al. [1–3] introduced the HIrisPlex-S System (https://hirisplex.erasmusmc.nl/), based on 41 DNA variants generated from two forensically validated SNaPshot multiplex assay by using capillary electrophoresis (CE), for the simultaneous prediction of eye, hair and skin of an individual. However, one of the most limitation for SNaPshot genotyping assay is the limited number of variants per single assay. This limit can be overcome using massively parallel sequencing (MPS) technology, which provides new opportunities to obtain genetic data for hundreds of loci in a single assay. Recently, several studies [4,5] provided preliminary results for the forensic validation of MPS designed assay for the HIrisPlex-S System.

In the present study, the samples were genotyped by sequencing using a MPS multiplex assay targeting all the 41 SNPs included in HIrisPlex-S System. MPS panel was designed maintaining the amplicons size below 180 bp, in order to investigate the usefulness of the panel with degraded DNA samples.

2. Material & methods

MPS assay for the HIrisPlex-S 41 SNP loci [1] was designed by using the Ion AmpliSeq Designer tool (https://ampliseq.com/). Two primer pools for multiplex PCR reactions were designed, with amplicons range between 124 bp and 174 bp. To investigative the effectiveness of the panel in forensics, different types of samples were analyzed: buccal samples (reference samples, i.e. individuals with known phenotypes) together with artificially degraded DNAs. Sensitivity study was performed by using the 2800M DNA (Promega, WI, USA) as control, diluted to concentrations of 5 ng/μl, 1 ng/μl, 500 pg/μl, 100 pg/μl, 50 pg/μl and 25 pg/μl.

After assessing the DNA quantity and DNA degradation index (DI) by Quantifiler™ Trio DNA Quantification Kit, libraries for MPS analysis were prepared with Precision ID Library kit according to the user guide (MAN0015830). Amplifications were performed with DNA input ranging from 5 ng to 25 pg and testing different number of PCR cycles (21 and 25). After PCR, the two multiplex reactions were pooled and processed as a unique sample. Each library was submitted to emulsion PCR by using the Ion PGM™ Hi-Q™ View OT2 Kit. The template-positive Ion PGM™ Hi-Q™ View ISPs were enriched on Ion OneTouch™ ES Instrument and sequenced on Ion PGM™ System by using Ion PGM™ Hi-

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Q™ View sequencing Kit, 318 chip v2 types and 200 base read mode. 20 barcoded libraries were sequenced on one chip.

Raw data was processed by the Torrent Suite version 5.0.4 and the reads aligned against human reference genome (GRCh37/hg19). Variant calling of the 41 SNPs was performed using HID SNP Genotyper Plugin v.4.3.2. Genotype data of the 41 DNA variants can then be uploaded to the easy-to-use DNA Phenotyping web tool found at https://hirisplex.erasmusmc.nl/ to generate individual prediction probabilities for 3 eye colors, 4 hair colors and 5 skin color categories.

3. Results

The average coverage of sequenced libraries by MPS was 3168.7 and the uniformity of coverage of 95% (mean). The relative Depth of Coverage (rDoC) across all loci sequence was calculated as the ratio of Depth of Coverage (DoC) at single locus to total DoC. Results shown a uniform rDoC distribution across all 41 loci both in reference samples than in degraded DNA samples, regardless of the amplicons' size.

Genotyping results of sensitivity study shown that complete and reliable genotypes could be obtained when the amount of DNA range from 5 ng to 0.1 ng. Occurrence of one drop-out event was observed with 50 pg of input DNA and 76% of correct genotypes were observed by using 21 PCR cycles (Fig. 1). The same quantity of input DNA but amplified with 25 PCR cycles results in two drop-out events and 88% of correct genotypes. Finally, when 25 pg of DNA was amplified, the number of no calls increased, with 63% of correct genotypes observed by using 21 PCR cycles, while with 25 PCR cycles we observed 78% of correct genotypes and 12% drop-out events (Fig. 1).

The two artificially degraded DNA samples gave different genotyping results. One sample with high degraded DNA (DI = nc, not calculable) were amplified with approximately 1 ng of input DNA using 21 PCR cycles. Full genotype concordant with the reference sample profile was obtained. The other artificially degraded DNA sample showed DI not calculable and low amount of DNA (not quantifiable as showed DNA quantity below the limit of quantification, loq) was amplified using 21 PCR cycles. Partial profile was obtained with only few loci correctly genotyped, but with very low coverage.

The five individuals with known phenotype were amplified with approximately 1 ng of input DNA using 21 PCR cycles and gave complete profiles for all SNPs. The prediction probabilities for eye, hair and skin colors were inferred through the DNA Phenotyping web tool and concordant results between predicted and effective phenotypes were obtained. In one case, an expected very pale skin failed to be predicted as the results shown a p-value of 0.393 for Pale Skin and for 0.544 for Intermediate Skin.

4. Discussion

This study proposes to develop MPS multiplex assay in order to genotype all the 41 SNPs, included in the HIrisPlex-S system and to investigate its usefulness in challenging forensic samples.

We genotyped a set of phenotype known reference samples together with a set of artificially degraded DNA samples. A sensitivity test was evaluated to determine the minimum input DNA need to obtained a complete 41 SNPS HIrisPlex-S System profile. To evaluate the forensic suitability of this MPS-based assay, DNA Phenotyping web tool were used to perform a statistical prediction of phenotypes of tested samples.

The MPS results showed a good performance of the designed panel. The 41 loci have been uniformly amplified and sequenced in all different types of samples, without difference between reference and degraded DNAs and regardless of the amplicons' size.

As assessed by sensitivity test, full and reliable profiles could be obtained with 0.1 ng of input DNA. It is interesting to note that the increment of the number of PCR cycles results in improvement of sensitivity or in typing results but an increase of artifacts were also observed. Concordant results observed for artificially degraded samples confirm that the high degradation index did not influence the correct genotyping and that the critical parameter that affect the result is the quantity of input DNA. Considering the area under the receiver characteristic operating curves (AUC) as an overall measure for prevalence adjusted prediction accuracy, we have found correct prediction even with 100 pg.

5. Conclusion

The advantage of MPS to target several SNP loci in a single sequencing run allows to better exploring the suitability of DNA phenotyping in forensic caseworks.

The performance of this MPS multiplex assay panel was evaluated dealing with low quantity and quality DNA. The results show that full profiles can be obtained with 0.1 ng–5 ng, even if DNA presented a highly degradation index. MPS assay were able to generate complete and accurate phenotypic prediction, even with 100 pg of input DNA. The increment of the number of PCR cycles results in an improvement of correctly genotyping and phenotyping for samples with low amounts of degraded sample but higher frequencies of artefacts were found. Further validation studies with additional samples and testing intermediate number of PCR cycles are needed for better assess its effectiveness and usefulness in forensic caseworks.

In conclusion, the study performed until now has demonstrated that the designed MPS assay is sensitive and allow reliable genotypes, supporting further investigations.

Declaration of Competing Interest

None.

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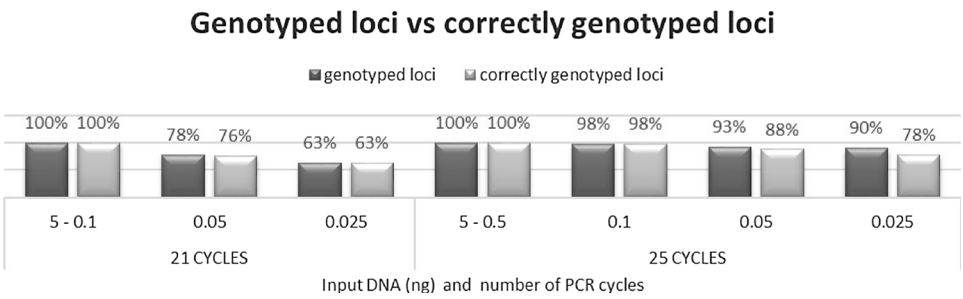

Fig. 1. An assessment of the 41-SNPs panel's sensitivity on control DNA samples. Full profiles were observed down to 100 pg DNA input and 21 PCR cycles. With 25 PCR cycles, an increase of discrepancies between called loci versus correctly genotyped loci were observed.

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