The development of three SNP assays for forensic casework

T. Senge*, A. Junge, B. Madea

Institute for Legal Medicine, University of Bonn, Stiftsplatz 12, 53111 Bonn, Germany

Abstract. The present work introduces three novel validated SNP assays and describe their applicability in forensic casework. For this study three unlinked and non-coding SNPs named TSC0582423 (Chromosome 2), TSC0171847 (Chromosome 1) and TSC0741184 (Chromosome 3) with a balanced allele distribution were selected from the database of the SNP consortium. SNP detection was based on the 5′ nuclease system from Applied Biosystems. Before the use of this SNP-detection system in the laboratory routine, validation studies must be performed including determination of SNP genotypes by sequencing, sensitivity/reproducibility studies and population studies. Tests with artificial stains and with degraded DNA samples were also performed. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

SNPs (Single Nucleotide Polymorphisms) are the most abundant form of DNA polymorphisms in the human genome. They occur on average at every 1–2 kb and over 4 million SNPs have been identified yet [1–3]. The SNP consortium, a foundation organized for the purpose of providing public genomic data, had discovered and characterized nearly 1.8 million SNPs and published the corresponding sequences [4].

In this study three non-coding and unlinked SNPs (TSC0582423, TSC0171847, TSC0741184) were selected from the database of the SNP consortium and 5′ nuclease assays were developed and validated. The Validation Process includes the comparison of sequenced samples with the assays, sensitivity and reproducibility studies using samples with known genotype and population studies. To find out if these assays are suitable for forensic purposes tests with artificial stains and with degraded DNA samples were performed.

* Corresponding author. Tel.: +49 228 738 327; fax: +49 228 738 339.
E-mail address: serologie@uni-bonn.de (T. Senge).
2. Materials and methods

Candidate SNP sequences with an equated allelic distribution were taken from the database of the SNP consortium and 5’ nuclease assays were designed from these sequences using Primer Express Software (Applied Biosystems). DNA was extracted from saliva samples as well as from the stain-material using chelex 100 resin [5]. An alternative extraction method for stain-material was the First-DNA all-tissue Kit (Genial). Concentration was done with Microcon YM100 centrifugal filter devices (Amicon). Quantification of the extracted samples was done using Human Quantifier Quantification Kit (Applied Biosystems). Population studies were done with a group of 40 unrelated persons. The DNA of these persons was extracted from blood (according to [6]) and was photometrically quantified. STR analysis was done using the MPX2-Kit from Serac. Sequencing was done by Taq Cycle Sequencing (Applied Biosystems).

3. Results and discussion

For each SNP the genotype of each person determined by sequencing was identical with that determined by the assays. Sensitivity and reproducibility studies were carried out with template DNA amounts ranging from 5 ng to 50 pg (saliva samples). The test results show that an amount of at least 250 pg genomic DNA could be reproducibly typed with the developed SNP assays (TSC0171847, TSC0741184 = 250 pg and TSC0582423 = 100 pg). This is comparable to the sensitivity of the MPX2-Kit (data not shown), which is routinely used for typing stain material in our laboratory. The population studies show an equated allelic distribution of 58% T-Allele to 42% C-Allele for TSC0582423, 40% T-Allele to 60% C-Allele for TSC0171847 and 59% T-Allele to 41% C-Allele for TSC0741184 (n = 40 persons). These identified allele frequencies were comparable with those published by the SNP consortium. The tested artificial stains consisted of two cigarettes, two chewing gums, a bottle neck abrasion as well as a skin abrasion taken from under a fingernail. DNA extracted from the stain-material gave the expected genotypes as determined by the analysis of the corresponding saliva samples. This demonstrated that typical “fresh” stain-material can be typed effectively with the assays. To test if the developed SNP assays are also suited for typing degraded DNA-material environmental studies were performed. The samples consisted of blood on cotton, saliva on cotton, a chewed on chewing gum and three cigarettes from each person (n = 3 persons). The collected stains were placed outside and exposed to weather (like sunlight, rain, wind, etc.) to get aging conditions according to reality. Blood and saliva stains were also exposed to high humidity to get stains containing degraded DNA. In intervals of 14 days DNA was extracted from the stains using chelex 100 resin. The extracted DNA were typed with the developed assays (5’ nuclease SNP-typing) and in comparison with the MPX2-Kit (STR-typing Serac). For standard analysis, 600 pg and 1 ng of DNA were used in the 5’ nuclease assays and the MPX2-Kit, respectively. If the DNA concentration after extraction (and concentration) was too low to reach the standard mentioned above, maximal amounts of available DNA were used in the subsequent reactions. The DNA-typing functioned at an average of 28 days for blood on cotton and 14 days for saliva on cotton in the outside using the developed 5’ nuclease assays. DNA-typing using the MPX2-Kit (STR-typing Serac) is also possible but a drop out of the largest alleles occurred (Fig. 1). So the expectation is that the 5’ nuclease assays work better with degraded DNA than the MPX2-Kit (STR-typing). The DNA-typing of the cigarettes functioned for 14 days for both methods. The DNA-typing of the chewing gums using chelex 100 resin worked at an average of 42 days for the 5’ nuclease assays and 84 days for the MPX2-Kit.

Probably some inhibiting substances are coextracted from the chewing gum which affected the SNP assays more than the MPX2-Kit (STR-typing). To test this hypothesis an alternative method for
DNA extraction was used (First-DNA all-tissue Kit Genial). If the DNA was extracted from chewing gums using this alternative method the 5′ nuclease assays still functioned after 98 days with aged chewing gum. The MPX2-Kit showed allele drop outs at this timepoint. DNA-typing of all stains which were exposed to high humidity still worked after 98 days both for the 5′ nuclease assays and for the MPX2-Kit.

4. Conclusion

The results lead to the expectation that SNPs work better with higher degraded DNA than STRs. To get the same statistical power about 50 SNPs must be examined in contrast to about 8 STRs. So a possible application for SNPs could be the typing of stains with degraded DNA and a high DNA content. To type stains with a low DNA content an upstream multiplex PCR must be used before the 5′ nuclease assays.

References


Fig. 1. Electropherogram (MPX2-Kit; left) and X–Y diagram (5′ nuclease assays; right) from DNA extracted from 28 days aged blood on cotton. Electropherogram: upper row: fresh stain material; lower row: stain material 28 days later; * indicates allele drop out. X–Y diagram: I: TSC0582423, II: TSC0171847, III: TSC0741184; rhomb: TT-genotype; point: CC-genotype; triangle: TC-genotype; quadrate: NTC; each assay consisted of 1 control for each genotype and the samples from 28 days aged blood on cotton; the ellipses indicate the clusters of the blood samples.