Complete automated DNA procedure to facilitate DNA database collection

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Abstract. To address the requirements of DNA database workflow, a complete automated procedure is necessary. Analysis by direct PCR on FTA® punches is possible. However, since DNA quantity is highly variable from one sample to another, genotyping STR profiling leads to a very high rate of invalidated genetic profiles (20-25%). In order to be in the best amplification conditions, it is important to isolate good quality DNA in a normalized concentration. To meet this requirement in one step, we used Smart D-N-Adem-kit for profiling (ADEMETCH) containing calibrated magnetic nanoparticles suitable with a fast and completely automated procedure, from DNA extraction to injection plate setup. The DNA extraction and normalization procedure was successfully validated on 2047 FTA® cards, leading to 95.5% success rate with av. PHR of 86.5%. DNA quantification is no longer necessary prior to STR typing. Similar results have been shown with buccal sample swabs**.**

Keywords: DNA database ; Magnetic nanoparticles ; DNA normalisation ; Automation ; STR profiling

1. Introduction

DNA Database is used in police investigations to search a DNA profile against all other international profiles that have been submitted for unsolved crime stains, convicted offenders, suspects, unidentified bodies and missing persons. Blood and buccal samples are often collected on paper cards as well as on forensic swabs. In this paper, we described a new automated procedure to address the requirement of DNA database workflow, approximately 200 000 FTA® cards per year in duplicate. DNA purification and normalization allow obtaining genetic profiles of homogenous intensity and of good quality. Contrary to this new automated procedure, direct PCR process does not allow normalizing DNA quantity by PCR reaction and therefore, generates a higher rate of artefacts. It is thus necessary to check on a larger number of profiles to clean them and, in that case, there is an increased risk of error. With the new automated procedure we describe in this article, GeneMapper software validates in a reliable way a large number of genetic profiles without human intervention (80.9%). This automated process is a real alternative to the direct PCR on FTA® punches in order to obtain, in first analysis, the best success rate of good quality genetic profiles. Therefore, genetic profiles analysis is faster than direct PCR and strongly reduces risk of error.

2. Materials and methods

2.1 Samples

2047 saliva and 36 bloods on Whatman® FTA® card were analysed. Double analysis was performed on saliva samples and single analysis on blood samples. Buccal cells on Swabs (cotton, nylon…etc) from different suppliers (Puritan, GE) have been analysed.

2.2 DNA extractions and normalisation

DNA extraction and normalisation procedure was performed from three punches of 3.2 mm of diameter for saliva samples and 1 punch for blood samples. FTA® cards were punched with BSD 600-Duet semi-automated punch instrument in a polypropylene microplate. Automatic DNA extraction was performed with Hamilton Microlab® STAR and STARlet workstations using Smart D-N-Adem-kit for Profiling from Ademtech, according to manufacturer instructions. For swabs, cells were lysed in 500µl and DNA extraction and normalisation were performed from 50µl of lysate.

2.3 PCR amplification and detection

PCR amplification plates were automatically prepared on Hamilton Microlab® STAR workstation in 96-well PCR plates. Before DNA pipetting, DNA microplate was placed on an Agencourt SPRIPlate super magnet plate in order to fix smart-Adembeads at the bottom wells of the DNA plate. PCR amplifications were performed using the Applied Biosystems AmpFl STR Identifiler Plus Kit using 2 µL of DNA template for a total reaction volume of 12.5 µL. Following the PCR amplification, 1 µL of each sample was diluted in 8.7 µL Hi-Di™ Formamide (Applied Biosystems) and 0.3 µL GeneScan™ 500 LIZ® size standard (Applied Biosystems). PCR products detection was performed in a 384-wells reaction plate automatically prepared on an Eppendorf epMotion 5070®. All DNA fragments were separated and detected by capillary electrophoresis on a 24-capillary 3500xL Genetic Analyzer (Applied Biosystems) or on a 3130xL Genetic Analyzer (Applied Biosystems)

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2.4 Data Analysis

PCR products injected on 3500xL Genetic Analyzer were analyzed with GeneMapper® ID-X v1.2 software (Applied Biosystems) and PCR products injected on 3130xL Genetic Analyzer with GeneMapper® ID v3.2 software (Applied Biosystems). Analyses were performed using manufacturer provided allelic ladders, bins and panels.

3. Results and discussion

The DNA extraction and normalization procedure was successfully validated on 2047 buccal FTA® cards (Table 1) leading to a 95.5% success rate with av. PHR of 86.5%. More than 80% of the genetic profiles were automatically validated by GeneMapper® ID-X v1.2 software without human checking. With this new DNA extraction process, PCR products can be injected as well on 3500xL as on 3130xL sequencers, however artefacts are very low with the 3500xL sequencer. The difference of peaks intensity of PCR products injected on 3130xL and 3500xL sequencers is due to the performances of the 3500xL device which is more sensitive than the 3130xL one. Rate of genetic profiles with artefacts is higher on the 3130xL sequencer than on the 3500xL one because the threshold of saturation on 3500xL device is higher. Our results show that the DNA process could be also performed on blood FTA® cards.

Table 1: Genetic profiles results injected on 3500xL Genetic Analyzer according to analysis conditions and samples origin

	Saliva		**Blood**
	Single Analysis	Double Analysis	Single Analysis
Number of samples	2047	2047	36
Success rate	100.0%	95.5%	100.0%
Full profile for both duplicates	ND	95.5%	100.0%
Automatic validation by software	84.1%	80.9%	98.8%
Profiles with artefact	0.6%	0.6%	1.2%
Average PHR	86.5%	86.5%	84.7%
Average Peak Height	3767 RFU	3659 RFU	5084 RFU

Validation criteria of genetic profiles were the following ones for PCR products injected on 3500xL Genetic Analyzer : Peak height of homozygous alleles 800 RFU minimum; Peak height of heterozygous alleles 400 RFU minimum; PHR must be equal or greater than 30% in double analysis and 50% in single analysis. Validation criteria were the same for PCR products injected on 31030xL Genetic Analyzer except peak heights which were 400 RFU and 100 RFU minimum for homozygous and heterozygous alleles respectively.

Buccal swabs used in this study were cotton and nylon swabs with wood or plastic shaft. Sufficient DNA quantity was obtained for generate full STR profiles (Table 2). DNA extraction process eliminates potential PCR inhibitors. These results suggest that, even without DNA quantification step, 100% of profiles were successful with av. PHR of 86.6%.

Table 2: DNA extraction from swab using Smart D-N-Adem kit for profiling

Collection Product	**qPCR data (Quantifiler Human)**		**STR profiling (Identifiler Plus)**	
	Av. Conc. (pg/µl)	Av. Total yiel (ng)	Success Rate	Av. PHR
Cotton (wood shaft) / 25-806 1WC (Puritan)	3460	173	6/6	85.9%
Cotton (plastic shaft) / 25-805 1PC (Puritan)	1309	65	3/3	84.3%
Nylon (plastic shaft) / 25-3306 1PN (Puritan)	5025	251	3/3	92.0%
Omniswab / WB10-0004 (GE)	2788	139	9/9	87.1%
Total average	**3088**	**154**	**100%**	**86.6%**

IPC Quantifiler Human : Ct = 26.51 (cv = 0.6%)

4. Conclusions

This automated process is a real alternative to the direct PCR on FTA® punches in order to obtain, in a first analysis, the best success rate of good quality genetic profiles (PHR of 86,5%). The classical direct PCR process does not allow normalizing DNA quantity by PCR reaction and therefore, generates a higher rate of artefacts. It is thus necessary to check on a largest number of profiles, to clean them and such a process increase the risk of error and is time consuming. Furthermore, DNA quantification is no longer necessary prior to STR typing. This new procedure is now accredited IS0 17025 and currently used in routine for high-throughput STR profiling. Furthermore, Smart D-N-Adem-kit for profiling can also be successfully used for efficient buccal samples swabs processing leading to high quality genetic profiles.