Laboratory Strategy of the Austrian DNA Intelligence Database

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INTRODUCTION

The Austrian DNA Intelligence Database project was installed October 1st, 1997. It consists of two components, the executive branch which is located at the ministry of the Interior in Vienna, and the laboratory unit situated at the Institute of Legal Medicine, University of Innsbruck. Reference material of suspected persons is collected by means of buccal scrapes by police officers and sent to the laboratory anonymously. The personal data of the suspected person is administrated at the Ministry of the In-terior exclusively. Each reaction tube containing a buccal scrape is unambiguously characterized by its barcode, which is the only link between the personal data and the obtained DNA profile of the suspected person. Parallel, unidentified casework samples are collected at crime scenes and processed at the laboratory again taking advantage of the barcode technology.

LOGISTICS

The laboratory produces sets, containing sterile buccal scrapes, barcoded reaction tubes and a blank ID-form, all identified by the same barcode. Throughout the entire laboratory operation, a sample is addressed by its barcode. This is achieved by an MS-Access based application, which was set up for DNA databasing purposes. This software provides a workflow design. A sample is directed through extraction, amplification, detection and analysis, reporting the current position and status of the sample. Thus, extraction lists, parameters for PCR set-up, sample sheets for electrophoresis and injection lists are provided by the program never allowing for manual input or change of sample information. Samples failing to amplify or producing unexpected results are designated to separate lists according to the problem which occurred during the process and wait to be repeated. The program also takes care of quality control issues, transport of the results to the Ministry of the Interior via a high-security data solution and storage of the extracted DNA after the typing process.

METHODOLOGY

When a buccal scrape sample is returned to the laboratory, its barcode undergoes validation. Since the barcode itself has been produced at the institute, a sample can be recognized as valid. Remarks to the sample can be added interactively using dialog windows, again being linked to the barcode information. Subsequently, the sample is forwarded into the extraction list. DNA from the epithelial buccal cells from the scrapes is isolated performing the Chelex extraction method (Walsh *et al.*, 1991). The reference samples are typed on the basis of highly polymorphic Short Tandem Repeat (STR) loci using the Second Generation Multiplex (SGM, Forensic Science Service, Sparkes *et al.*, 1996 a,b). Amplification products are detected fluorescently on automated capillary electrophoresis devices (ABD Genetic Analyzer CE 310) and analyzed using GeneScan™ software (PE/ABD). Analyzed data are then imported into Genotyper™ software (PE/ABD) for macro-automated allele designation. Analysis and manual inspection of the data is performed independently by two experienced analysts. The obtained results are then compared by the Access-based software and forwarded to a transfer list to be sent onto the DNA database in the Ministry of the Interior.

AUTOMATED FEATURES

The Austrian National DNA Database project was implemented into an existing forensic DNA laboratory performing routine casework analysis. Thus, a new sample management has been developed to increase the sample through-put, not only by expansion of manpower, but also by simplifying the typing process of reference samples. The relatively simple and reproducible nature of buccal scrape samples compared to more complex casework stains offered the application of standardized protocols and the implementation of appropriate robotic devices. The processing of the reference samples involves four generations of reaction tubes, which are all — except the first - loaded by robots to avoid mixing-up of samples.

First generation. Buccal swabs are returned to the laboratory in 1.5 ml reaction tubes, which are barcode labelled. In these Chelex extraction is performed after decantation of the transport medium.

Second generation. An aliquot of the extracted DNA is transferred into another 1.5 ml vial. This is performed by a 4 channel robotic microplate processor (3002 Rosys/Anthos, Switzerland). These vials serve as both source tube of DNA for amplification set-up as well as storage medium for the extracted DNA after analysis.

Third generation. The PCR is set up in 0.2 ml reaction tubes changing into the 96 well microtiterplate format on the same robotic device. Amplification is performed in 9600 GeneAmp™ Thermocyclers (PE), located in the *Amplified DNA* work area.

Fourth generation. Aliquots of the amplification products are transferred into a 96 well format tray, combined with deionized formamide and internal lane standard (GeneScan™ 500 Tamra, PE/ABD) using an ASYS HiTech™ (Asys) robotic device, which is again located in the amplification area. Subsequently the amplification products are denatured, cool chilled on ice and loaded on the capillary electrophoresis device. After analysis, the amplification products are discarded, and the extracted DNA is stored at —20°C.

ANALYSIS OF THE STR PROFILES

Analysis and allele calling of the STR fragments is a process which involves both automated routines applying macros on commercially available software (Genotyper™ 2.0, ABD) and subsequent manual inspection of the results independently by two analysts. Analyzed data from GeneScan™ 2.1 software are imported into an empty Genotyper™ file including categories for allele size ranges and macros to perform the peak labelling. Prior to the determination of the fragment sizes of the peaks, the internal lane standard of each injection (up to 105 per run) is controlled by inspection in the plot window. Subsequent runs on a capillary may probably deviate from each other due to variations of the electrophoretic conditions. To eliminate run-to-run variability of fragment size values in categories, the offset for each ladder fragment is calculated according to the actual size. Allelic ladders are injected every 20 to 25 samples. The offset values determined by one of the ladder injections are subsequently compared to all other ladder injections to control the performance of the entire run.

Fragment lengths are labelled according to the recommended nomenclature, with respect to a ±0.5 bp floating bin window. A filtering option deletes labels from peaks known to contain no valid information for the profile (e.g. stutter bands, background noise, and peaks missing the non-template base addition). After manual inspection of the electropherograms, the peak labels are imported into the Access-based application, where the two independent analyses are compared automatically. Differences between the independent analyses are reported as well as rare alleles and failed STR loci. Affected samples can be directed to lists for repeated processing. Finally, the results identified by the corresponding barcode are sent onto the DNA database for matching purposes.

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