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Multiplex STRs amplification from hair shaft: Validation study

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Abstract. Mt-DNA analysis which is widely used in forensic genetics in case where the amount of DNA is very small or degraded, is unfortunately a complex and time-consuming procedure; hence, in the present study, we have evaluated the ability to perform multiplex STRs amplification and the reproducibility of results obtained using different extraction procedures. We verified that, in some cases where there is a sufficient quantity and a good quality of medulla cells inside the hair stem, a multiplex amplification can be performed and this is very useful for obtaining in a single step the typing of many loci avoiding the loss of DNA.

Keywords: Hair shaft; STRs; Multiplex

1. Introduction

Mt-DNA analysis that is widely used in forensic genetics in case where the amount of DNA is very small or degraded, is unfortunately a complex and time-consuming procedure; hence, since several years in other of our previous papers, we have demonstrate that it is possible to amplify in single-plex DNA extracted from hair shaft. In the present study, we have evaluated the ability to perform multiplex STRs amplification and the reproducibility of results obtained [1,2].

2. Materials and methods

In order to minimize the possibility of contamination, all extractions were set up in a Gelman laminar flow cabinet in a dedicated laboratory.

In particular, we analysed 20 hair shafts (from different body region) belonging to known donors (2 male and 2 female): all hairs were stored at room temperature after their collection and analysed in different slot (till to 12 months) to simulate evidences that are commonly found in a crime scene.

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For the analysis, we used different DNA extraction procedures such as paramagnetic silica particles, silica membrane column, and phenol–chloroform [3–7].

As reference, we used saliva samples and hair roots coming from the same donors.

All extracts were purified by Microcon 100 treatment and then the extracted DNA has been quantified in Real-Time PCR by Quantifiler Human DNA Quantification kit (Applied Biosystems) using a 7300 Real-Time PCR System.

DNA amplification was carried out in a laboratory different from the one dedicated to the extraction so that amplified products never entered the extraction laboratory.

DNA was extracted from saliva samples by Instant Gene Matrix (Biorad) treatment while hairs root were extracted by IQ Tissue and Hair Extraction kit (Promega). Amplifications were performed using the GeneAmp PCR System 9700, 2400, 2720 (Applied Biosystems) and the following kits:

a) AmpF/STR Identifiler kit (Applied Biosystems) that simultaneously amplifies 15 STRs loci: D19S433, D3S1358, D5S8118, D8S1179, vWA, TH01, D13S317, D21S11, TPOX, FGA, D7S820, D16S539, D18S51, CSF1PO, D2S1338 and Amelogenin [8].
b) Mentype Argus X-UL (Biotype) that is a new kit for fast and reliable profiling of the following 5 unlinked X-chromosomal STRs markers DXS8378, DXS7132, HPRTB, DXS7423 and Amelogenin [9].
c) AmpF/STR Y-Filer (Applied Biosystems) that coamplifies the following 16 Y-STRs loci: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, YGATAH4 DYS437, DYS438, DYS448 [10].

The cycling reactions were started with an initial 94 s delay for 12 min and the number of PCR cycles increased in the following way using a reduced reaction volume: for AmpF/STR Identifiler until 42 cycles, for Argus X-UL until 35 cycles and for Power Y System until 38 cycles. Different positive and negative controls were enclosed during the amplification steps so to verify the possibility of any contamination from a laboratory source, since the high sensibility of the procedure. In particular we used as positive controls two different samples of human DNA with a known profiles: one from a man at the concentration of 0.1 ng/μl and the other one from a woman at the same concentration. All PCR tests were performed twice.

PCR products were analysed by capillary electrophoresis using the automatic sequencer ABI PRISM 3130 and its software (Data Collection, GeneMapper v3.2). For fragment length determination of the products, were used for the calibration, the allelic ladder from each kit as external standard and the internal size standards LIZ 500,ROX550.

3. Results and discussion

We have evaluated the ability of each method to extract DNA, the quantity of human DNA extracted with each procedure, the ability to perform multiplex STRs amplification, the presence of partial profile or unbalanced peaks or allelic dropout, the reproducibility of results obtained.

We found that the DNA extraction procedure employing silica membrane column gave good results only if the extraction lysis is modified by adding some DTT, for increasing the extraction power. This procedure seems to be useful since it gives DNA quantity similar to the phenol–chloroform treatment in less time avoiding the employment of hazard reagents, while the method with paramagnetic silica particles showed a minor extraction efficiency in absence of root. Microcon purification is a fundamental step for removing any inhibitors (hair dye) that could be coextracted with DNA; in fact, in the absence of this step, we often observed the inhibition of internal control (IPC) during the amplification for quantitation by real-time. The quality of DNA profiles obtained depends on the quality and quantity of medulla cells inside the stem. These last ones are influenced by different factors: the age of hair (aging pigment granulations and medulla

become rarefied; the air balls are more frequent and they seep into the medulla cells and interrupt the continuity of the medulla, causing the presence of some stem traits without medulla), treatment with cosmetics (in fact, strong decolouration treatments often damage hair and they may destroy medulla cells), the body region (pubic hairs are the best candidate since they are generally coarse and wiry in appearance and have a continuous medulla or interrupted by small gaps, while head hairs generally show thick and discontinuous medulla). All the above factors together with the sizes of STRs analysed and the sensibility of the kit used influence amplification results; in fact, we generally observed partial DNA profiles and, in a few cases, only with fresh pubic hair samples, full profiles were obtained. In general, AmpF/STR Identifiler kit gave the best results compared with the other kits showing good balance between alleles and less allelic dropout and less background noise. In each case, profiles obtained were reproducible in the different analysis performed and were compatible with the ones of control samples.

4. Conclusions

Low copy number (LCN) DNA profiling is a technique sensitive enough to analyze just a few cells. The extreme sensitivity of the method requires many precautions either during the analysis to reduce the risk of contamination from personnel within the laboratory itself and between samples than during the interpretation of data since the possibility to observe artefacts or high stutters (due to the increased number of cycles), allelic dropout (preferential amplification). Our findings showed that, in cases where there is a sufficient quantity and a good quality of medulla cells inside the hair stem, a multiplex amplification can be performed and this is very useful for obtaining in a single step the typing of many loci avoiding the loss of DNA. The ability to identify STRs markers in difficult samples as hair shafts gives a great opportunity to obtain DNA profiles useful for any further comparison or searching in DNA database.

References

[1] A. Barbaro, G. Falcone, A. Barbaro, DNA typing from hair shaft: a case report, European Symposium on Human Identification–Innsbruck (1998 June).
[2] A. Barbaro, G. Falcone, A. Barbaro, DNA typing from hair shaft, in: Progress in Forensic Genetics, vol. 8, Elsevier, 2000.
[3] P.S. Walsh, D.A. Metzger, R. Higuchi, Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, BioTecniques 10 (1991) 506.
[4] InstaGene Matrix Protocol, User's Manual, Biorad, 2002.
[5] DNA IQ™ System–Small Sample Casework Extraction kit, User Manual, Promega, 2002.
[6] NucleoSpin Tissue User's Manual, Macherey Nagel, 2004.
[7] J. Sambrook, E.F. Fritsch, T. Maniatis, 2nd edition. Molecular Cloning: A Laboratory Manual, vol. 2, Cold Spring Harbor Laboratory Press, New York, 1989.
[8] AmpF/STR Identifiler PCR amplification kit, User 's Manual, Applied Biosystems, 2001.
[9] Mentype Argus X-UL kit, User 's Manual, Biotype, 2004.
[10] AmpF/STR Y-Filer PCR amplification kit, User 's Manual, Applied Biosystems, 2005.