HLA-DQα TYPING BY DNA AMPLIFICATION OF SINGLE HUMAN HAIR

Caenazzo L., Crestani C., Ponzano E., Bonan G., Cortivo P.

Institute of Legal Medicine,University of Padua, Via Falloppio 50, 35121 Padova, Italy

INTRODUCTION

One of the best characterized marker systems in the forensic identification with PCR is the HLA-DQα with the Amplitype™ kit.
The possibility of detecting this system in a single human hair was first described by Higuchi (1). Threafter, a number of Authors have discussed the system's power and methods of extraction (2-6). In fact, the choice of extraction method represents the crucial step in the determination of the efficacy of the amplification, particularly when consisering a single human hair without bulb.
This paper describes the results of a simple and pratical method for the extraction of PCR-amplifiable DNA from human hair for the HLA-DQα gene.
DNA from single human hair, with or without bulb, was extracted by incubation with 0.85% NaCl and Proteinase K and then amplified and typed with the Amplitype™ HLA-DQα kit.

MATERIALS AND METHODS

Sample preperation

The samples were obtained from hairs which had been pulled out directly from the scalp, or had fallen spontaneously. In either case, hairs with and without bulb were present. Samples were kept in nylon bags for up to six months. The amount of sample extracted ranged from a single, 1 cm-long hair to 4 hairs with or without bulb.

Extraction procedure

Each hair sample was first washed in water with soap, rinsed in dH_2O, then cut in small pieces and introduced into an eppendorf tube. Subsequent incubation was with the following buffer:
1 ml 0.85% NaCl +
100 μl buffer K (containing 25 mM $MgCl_2$ 20 mM Tris pH 8.5,0.5% Tween 20)+
10 μl Proteinase K (10mg/ml).
The tubes were then shaken vigorously and allowed to stand in a water bath at 65°C for 3 h.
Following incubation, the supernatant was removed and dispensed into another eppendorf tube, while the pieces of hair were rinsed with 500 μl of 0.85% NaCl before being added to the eppendorf tube containing the supernatant. The solution was centrifuged at 13000 rpm for 15 min., the supernatant discarded, and the eppendorf tube inverted to allow the pellet to dry. 40 μl of dH_2O were subsequently added to the tube.
The contents of the eppendorf tube were then boiled for 10 min. and kept at -20°C until use.

Amplification and typing

30 μl of extracted DNA were amplified by PCR using the Amplitype™ DQα kit (CETUS) according to the recommended protocol. Positive and negative controls were used.
Before typing with reverse dot blot each amplification was checked by employing 10% polyacrylamide gel electrophoresis in 1x TBE buffer.
The amount loaded for each sample was 10 μl of amplified material.
The band of 239/242 amplified fragment was visualized with ethidium bromide.
It was noted that if the band was visible, 35 μl of amplified material, were sufficient to achieve dot blot typing.

RESULTS AND DISCUSSION

DNA was extracted from locks containing up to 4 strands (from 1 to 6 cm), as well as from single shafts with or without roots.
Best results were achieved using by incubating the sample with 0.85% NaCl, buffer K and Proteinase K at 65°C for 3 h.
Each extraction required the final addition of 40 μl of dH_2O. Obviously, under the same conditions more DNA is extracted when the hair root is also avaible, but even a single 1 cm-long hair shaft will provide enough extract for one amplification.

Advances in Forensic Haemogenetics 5 (c) Springer-Verlag Berlin Heidelberg

It is notheworthy that the use of the conditions here described are sufficient not only to extract DNA form a single hair shaft (without bulb), but also to permit the extraction process to be repeated from the same sample (Fig.1).

REFERENCES

1) Higuchi R., von Beroldingen C.H., Sensabaugh G.F., Erlich H.A. (1988). DNA typing form single hairs. Nature 332: 543-546.
2) Singer-Sam J., Tanguay R.L., Riggs A. (1989). Use of Chelex to improve the PCR signal from a small number of cells.
Amplification 3:11
3) Chelex protocols (1990) in Amplitype™ User Guide.Cetus Corporation, Emerville CA.
4) Jung J.M., Comey C.T., Baer D.B., Budowle B. (1991). Extraction strategy for obtaining DNA from bloodstains for PCR amplification and typing of the HLA DQα gene.
Int.J.Leg.Med. 104: 145-148
5) Comey C.T., Budowle B.,Adams D.E., Baumstark A.L., Lindsey J.A., Presely L.A. (1993). PCR amplification and typing of the HLA DQα gene in forensic samples.
J.Forens.Sci. 38:239-249
6) Okajima H., Yamamoto T., Kojima T., Uchichi R., Katsumata Y.(1993) Amplification of HLA-DQA1 gene from bloodstains by polimerase chain reaction.
Jpn.J.Legal Med. 47:6-12

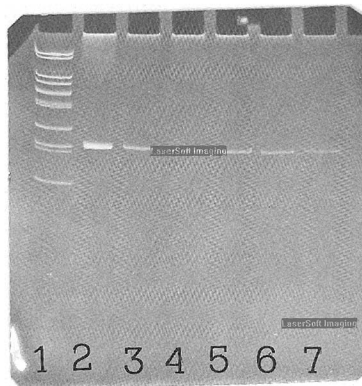

Fig.1 Amplification of HLA DQα system.
Lane 1: Marker VI Boehringer; lane 2: control DNA; lanes 3, 4 and 5: hairs with bulbs: 2, 3 and 4 hairs respectively; lane 6: hair without bulb; lane 7: repetetive extraction of sample in lane 6.

Advances in Forensic Haemogenetics 5 (c) Springer-Verlag Berlin Heidelberg