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Experiences from DNA analysis in Sweden for the identification of tsunami victims

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Abstract. After the tsunami 453 Swedish citizens were reported missing. The first reference samples ante mortem (AM samples) were received on January 4. A total of 757 samples including 124 from the PKU bio-bank were collected within a few weeks. The DNA analysis started on January 12. The genetically best references, 566 samples, were selected and 86% were analyzed by the end of February. At March 7 several laboratories got a request to take part in the analysis of post mortem (PM) samples. After quality test we received 600 samples on April 5 to be analyzed within 6 months. Of these 133 samples were withdrawn. By July 22 the remaining 467 were analyzed. The success rate was 90% with 421 good profiles and a request for 46 new samples.

Keywords: DNA extraction; Bone; PCR; STR; Capillary electrophoresis

1. Introduction

The complexity of the disaster was a new challenge for the Swedish authorities. In many cases all family members comprising several generations were reported missing. Also, DNA analysis for the identification of small children was imperative. On January 10 the National Police Board requested the National Board of Forensic Medicine to assist in DNA analysis. The legislation for the use of medical bio-banks in Sweden (2002: 297, 5th chap., 2nd §) was temporarily changed. This made PKU-samples (phenylketonuria) available between February 8, 2005 and June 30, 2006 for the identification of human remains in mass disasters. Only the National Police Board and The National Board of Forensic Medicine could require these samples.

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2. Material and methods

2.1. AM samples and DNA extraction

A total of 757 AM–DNA samples were collected, of these 566 were chosen for analysis. 442 were buccal swabs from close relatives and 124 were samples from the PKU bio-bank. Two samples from each person were analyzed.

DNA was extracted using the Qia-amp® MiniKit (Qiagen) protocol.

2.2. PM samples and DNA extraction

PM samples consisted of various pieces of mostly femur, but also ribs and a few teeth. The bone samples contained remains of soft tissue. Diaphyseal bones were first cleaved with an oscillating bone saw, flushed with plenty of hot tap water and cleaned manually. Thereafter soaked in 95% ethanol for a few minutes, rinsed with 0.5% Na-hypochlorite, wiped with a paper towel and dried overnight at 50 °C in open autoclave bags.

Next day, the dried bones were smashed to pieces, frozen in liquid N_2 and homogenized in polycarbonate vials using a Freezer/Mill 6850-115 (SPEX CertiPrep). The grinding cycles used were: T1 for 1 min, T2 for 2.5 min and T3 for 5 min. For larger bones, in large vials, 4–5 cycles and for smaller bones, in small vials, 7–15 cycles.

Between samples the vials were rinsed with tap water, soaked for a few minutes in 0.5% Na-hypochlorite, rinsed with tap water and wiped dry with Kleenex or left to air dry.

Approximately 2×1 g bone powder per sample was used for DNA extraction. The bone powder was mixed with buffer and digested with Proteinase K at 56 °C overnight. The method was mainly according to Rainio et al. [1]. In short: two phenol–chloroform and one chloroform extractions using 15 mL Phase Lock Gel Light tubes (Eppendorf); concentration on Centricon® 30 columns (Millipore); purification with Qiaquick® PCR Purification Kit (Qiagen), eluted in 45 μL. Series of 18 samples with extraction controls included for every new batch of reagents were processed.

2.3. PCR amplification, electrophoresis and analysis

Of both AM and PM extractions 1.2 μL of DNA was used for PCR amplification. The setup was as follows: each of the two AM–DNA extracts was amplified according to the Identifiler™ protocol (reaction volume 10 μL), giving two parallel results.

For each of the two PM–DNA extracts two separate amplification protocols were used, thus giving four parallel results. One amplification was according to the Identifiler protocol, the other: 94 °C denaturation for the first 10 cycles, lowering to 90 °C for the following 20 cycles according to the "forensic" protocol by Biotype®. DNAs with difficult profiles were also amplified using either the Identifiler or the Biotype protocol extended by two cycles.

DNA profiles were determined using capillary electrophoresis, ABI3100. The electropherograms were evaluated using GeneMapper ID 3.1 (Applied Biosystems™).

3. Results

The PCR workload from the AM and PM tsunami samples was close to 1/4 of a year's production in the laboratory.

Only AM samples from one generation relatives, and only one for each missing person were analyzed. The 566 samples generated over 1100 PCR reactions. Most were done within a few days after

Table 1
The number of extractions and amplifications needed to give full profiles

	Theoretical	In fact	Difference	Difference in %
Samples	467			
Extractions	934	1 036	+102	+10.92
PCR amplifications	1 868	2 699	+831	+44.48

registration. All laboratory methods were manual. Chain of custody was assisted using our paternity program FABIAN, adjusted for this purpose. The bulk of samples was completed by mid February.

The PM bone samples generated 2699 PCR reactions (Table 1). Of the 467 samples analyzed 421 resulted in good profiles. New samples had to be requested in 46 cases. The overall success rate was 90%.

In general 2/3 of the samples gave full profiles after the first set of four PCR reactions. Most of the extra PCRs needed were therefore generated by 1/3 of the samples. An average of 2.2 extractions and 5.8 PCR analyses per bone sample were done. The procedure described proved to be robust. We found no contamination in the extraction or PCR controls.

4. Conclusion and lessons learned

The analysis of the AM samples was very straightforward. The Police collected the samples and pedigrees were submitted later on. After selection of one best reference only 75% of the samples collected were analyzed. A pedigree analysis before sample collection could have reduced the workload both for the Police and the laboratory.

Major difficulties arouse when submitting AM profiles to the TTVI IMC (Thailand Tsunami Victim Identification, Information Management Center). File format and profiles were accepted by mid April.

The analysis of the PM samples proved to be much more laborious than anticipated. However, the strategy with two extractions and four PCRs, with two different protocols, proved to be fruitful. This strategy was a guarantee for good quality profiles and most certainly also reduced the processing time. The unsuccessful cases consisted mostly of too small samples for a complete procedure.

PM profiles were converted from the text format generated in GeneMapper ID to an Excel format accepted by PlassData at TTVI in Phuket. This “format converter” also checked for inconsistencies between parallel profiles.

To this date (September 5, 2005) DNA analysis has been the basis for the identification of more than 200 deceased. Of these, 45 were Swedish citizens for which 31 of the AM–DNA profiles were derived from PKU samples. More are to be expected.

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References

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