High yield DNA extraction from bones using a full demineralization approach

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Introduction

Since the end of 2001, the International Commission on Missing Persons (ICMP) has used a silica-based DNA extraction protocol with a 76% success rate [1] for autosomal STR typing on over 34.000 skeletal remains submitted for testing in the former Yugoslavia. During the last seven years over 15.000 persons have been successfully DNA matched to families of the missing. Given the small proportion of cases where ICMP's standard extraction method has failed, and an increased proportion of more highly degraded samples in case work performed today, ICMP has developed and validated an alternative DNA extraction procedure to increase the success rate of the identification of challenging samples. A full demineralization extraction protocol was optimized on 70 samples, demonstrating a considerable improvement of the DNA yields and performances with inhibited samples.

In addition, the protocol has been adapted to the QIAcube robotic platform to have the possibility of automating a part of the process for DVI operations requiring a very high throughput.

Materials and Methods

Material: 70 samples previously extracted using the standard silica based approach were used for this study. These samples were divided into subsets to evaluate the DNA yield (40), to assess the performances against inhibitors (32) or to test the post extraction clean-up protocol (30).

Digestion setup: 1 gram of bone powder was digested overnight at 56°C in 15ml of lysis buffer composed by EDTA 0.5M (Gibco) and sodium-N-laurylsarcosinate (0.01g.ml^{-1}) (Fluka).

Extraction setup: the lysates were first concentrated on Amicon ultra-15 (Millipore) and transferred into 5 volumes of PBI buffer (QIAGEN). The DNA was extracted using QIAquick columns with 2 extra washes with PE buffer. The regular ICMP protocol [2] starts from 3g to 5g of bone powder and DNA is extracted using a silica based method.

Post extraction clean-up : in some instances inhibitors were carried over during the extraction. A purification procedure was developed using the QIAquick columns and additional washes with PE buffer.

Automation setup: a custom protocol has been developed in partnership with QIAGEN. The procedure is identical to the manual extraction in terms of the reagents and volumes used and takes place after the addition of PBI buffer.

DNA quantification and amplification: samples were quantified using the Quantifiler Human DNA Quantification kit (Applied Biosystems) and amplified with the PowerPlex 16 kit (Promega) on a 3100 automated sequencer.

Results

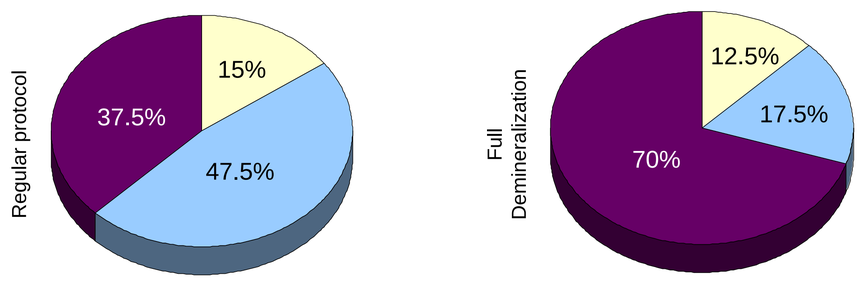
DNA yields: Unlike the standard method used previously, the new demineralization method results in the nearly complete digestion of solid bone material. DNA yields per gram of bone powder varied from 53pg to 68.89ng. Out of the 40 samples tested, 35 (87.5%) showed higher DNA yields per gram with the full demineralization approach.

STR profiles quality: the profiles generated using the regular protocol were compared to the those obtained using the full demineralization approach by counting the number of reportable alleles. ICMP's threshold for submission is 11 loci with reportable alleles.

70% of the full demineralization samples would have been submitted vs. 37.5% for the regular protocol and 30% of the samples gave a full profiles vs. 2.5%.

Quality of the profiles obtained for 40 samples extracted using regular protocol and full demineralization approach.

These charts do not take into account re-amplifications or re-extractions.



Performances with inhibited samples: 32 samples with various inhibition levels were tested with the full demineralization approach. The threshold cycle (Ct) value of the Quantifiler Internal PCR Control (IPC) was compared to the value previously obtained with the regular protocol.

The full demineralization approach was efficient in removing the inhibitors in 29 out of the 32 inhibited samples tested (90.6%).

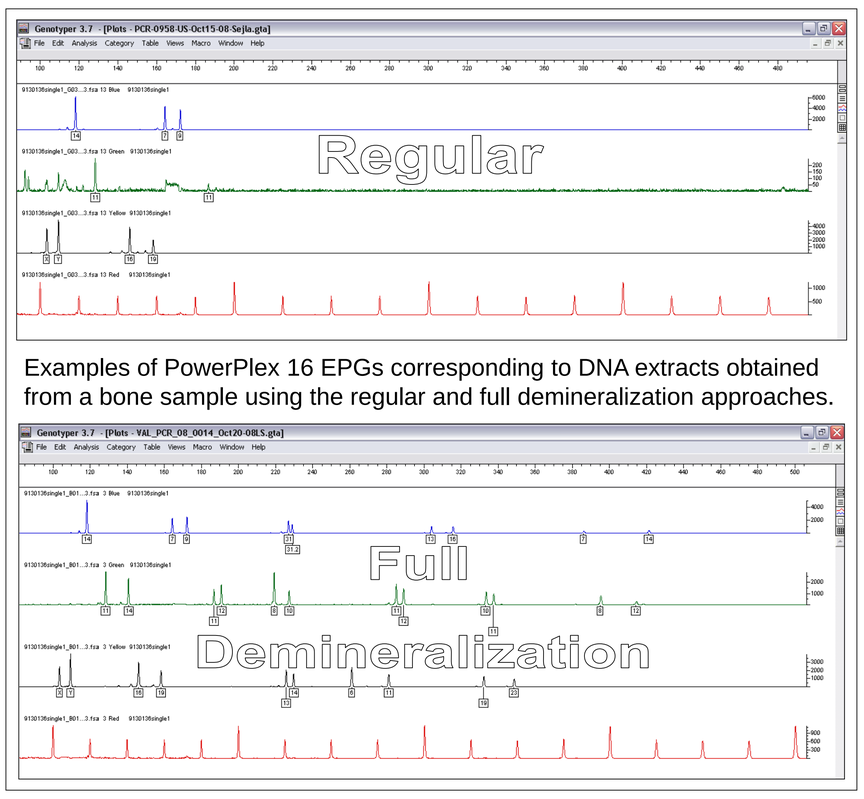
For 13 samples (40.6%), including 7 completely inhibited, the Ct IPC values were reduced below 29 cycles (moderate concentration of inhibitors).

Samples presenting Ct IPC values > 30 cycles were re-purified using the QIAquick columns

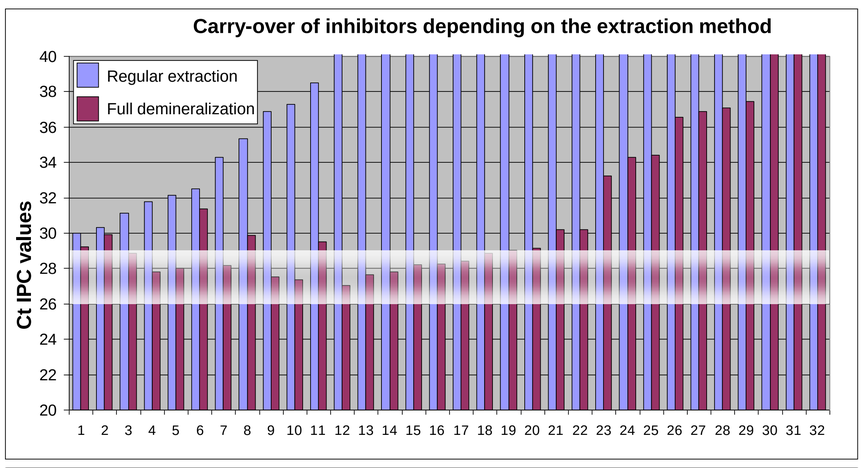
Post extraction clean-up: 30 DNA extracts with a wide range of Ct IPC values were selected to test the purification protocol.

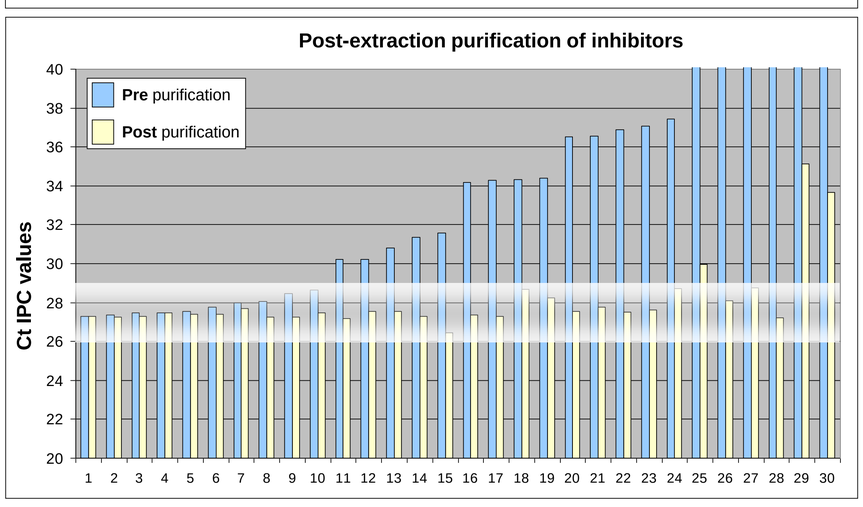
Before the post extraction clean-up, 20 extracts yielded Ct IPC values > 30 cycles. The purification process led to a decrease of the Ct IPC < 29 cycles for 17 samples (85%). For the three remaining samples a second round of purification was successfully applied.

The DNA loss during the process appeared to be negligible.



Examples of PowerPlex 16 EPGs corresponding to DNA extracts obtained from a bone sample using the regular and full demineralization approaches.





Conclusions

The full demineralization protocol generates higher DNA yields per gram of bone powder and improves significantly the typing results.

The use of smaller bone powder quantities (1g vs. ~4g) combined with the increased number of washes of the QIAquick membrane leads to a substantial reduction of the PCR inhibitors carried over during the extraction.

For highly inhibited samples, the post extraction clean-up procedure is efficient in decreasing the inhibition to an acceptable level.

In addition to the reduction of sample re-processing, the full demineralization protocol is 20% cheaper than the previous silica based procedure.

References

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[2] Davoren et al. Highly Effective DNA Extraction Method for Nuclear Short Tandem Repeat Testing of Skeletal Remains from Mass Graves. *Croat Med J.* 2007;48:478-85

icmp International Commission on Missing Persons