Hi-Flow® – Novel Large Volume Columns for DNA Extraction

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Fig. 1 Protein Ark Hi-Flow Column

ABSTRACT

The ideal DNA isolation protocol should provide a maximum yield of DNA free from inhibitory compounds that can affect downstream applications. It also is important to minimize sample handling steps, as every manipulation provides an opportunity for contamination. Reduced sample manipulation also limits the loss of DNA through repeated extractions and transfers. The latter benefit becomes even more critical in cases where the quantity of sample (e.g., bone) available for DNA isolation is minimal.

Larger volumes of digestion buffer are being used to more fully demineralize pulverized bone samples. One way to handle the increased volume of crude extract is to employ ultrafiltration for sample concentration, buffer exchange, and removal of contaminants smaller than the nominal molecular weight cutoff of the device's filtration membrane. These devices must be used in conjunction with another purification method such as organic extraction or silica column purification in order to sufficiently reduce co-purifying inhibitory compounds. Silica based columns, slurries, and resins have long been available for DNA isolation, but the current methodology is geared toward extraction of DNA from small volumes. Limited volume has proven to be a substantial disadvantage of applying silica-based extraction methods to bone-derived samples. This study describes a silica-based device that is large enough to process the entire volume of crude extract in a single step, which greatly reduces time and manipulation necessary to perform purification. Silica columns (Hi-Flow columns) with a larger volume capacity have been produced (Protein Ark Ltd, Sheffield, UK) to effectively handle commonly encountered working volumes of crude DNA extract. This simplification of the protocol substantially reduces the number of handling steps and sample transfers required, as well as eliminates the use of hazardous compounds such as phenol and chloroform.

MATERIALS AND METHODS

Bone section preparation
- Surface decontamination – 5 min. in 3% NaOCl, 4 rinses in ddH_2O, 1 rinse 100% ethanol, air dry.
- Pulverization – Freezer mill 15 impacts/sec., 5 min. grind cycle

Demineralization and protein digestion
- All extractions performed with 0.5 ± 0.03 g bone powder and 3 ml of digestion buffer
- Hi-Flow Digestion Buffer - 0.5 M EDTA, 1% sodium lauroyl sarcosinate (sarkosyl), 100 ug/ml ProK
- Ultra4 + MinElute Digestion Buffer – 0.5 M EDTA, 0.5% sodium lauroyl sarcosinate, 666.67 ug/ml ProK (Loreille *et al.*2010)
- 56° C overnight on rocking platform

Recovery/purification of DNA from crude extract

<u>UltraFiltration</u>

Membrane with defined pore size used to retain larger molecules; followed by use of MinElute® columns (Qiagen Inc., Valencia CA) for final cleanup.
- Amicon® Ultra4 (Millipore, Inc., Billerica, Massachusetts) – 4 ml capacity, NMWL 30,000

<u>Silica</u>

DNA adsorbed to silica in high salt solution, eluted in low salt solution: Bind → Wash → Elute
- MinElute® columns – 0.75 ml capacity
- Hi-Flow columns (Fig. 1) – 20 ml capacity

DNA Quantification
- Human DNA quantification (in ng/µl) was multiplied by the volume of extract recovered to give total DNA recovery in nanograms (ng).
- Three sequential elutions were performed on Hi-Flow columns (shown as stacked columns in Figs. 2 and 4)
- Two sequential elutions were performed on MinElute columns (shown as stacked columns in Figs. 2 and 4)
- Quantifiler ™ (Life Technologies Corp., Carlsbad CA) results are shown for Bone 23, a cortical section from a humerus that had been infiltrated by fungal growth (Fig. 2)
- Quantifiler results for Bone 25, a section form the far distal end of a femur that had very little cortical bone, (Fig. 4)

STR Peak Heights
- Identifiler Plus® (Life Technologies Corp., Carlsbad CA) was used for DNA profiling
- Representative peak heights are shown from the green channel of Identifiler Plus, which includes D3S1358, TH01, D13S317, D16S539, and D2S1338
- The loci/alleles in each graph are ordered from smallest amplicon on the left of the graph to largest amplicon on the right

RESULTS AND DISCUSSION

DNA Recovery

• The Hi-Flow columns produced DNA yields comparable to those obtained with the Amicon Ultra4 30K columns+ the MinElute column, but showed less inhibition as ascertained by the performance of the Quantifiler internal positive control (data not shown).

• Bones that required longer centrifugation times in the Ultra4 device gave poorer results overall with regard to yield and profile quality. For example, extract from Bone 25 had to be centrifuged for 90 minutes to reduce the volume sufficiently (Figs. 4, 5).

• A single 15 ml wash step (vs. 3 x 5 ml washes) for the Hi-Flow columns yielded better profiling results as well as a reduction in processing time and handling steps.

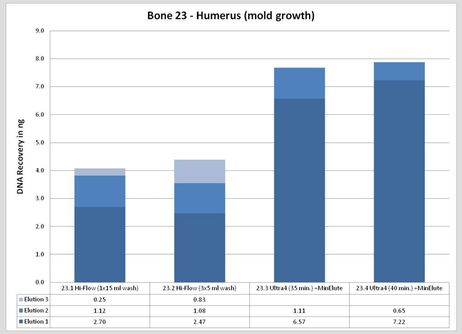

Fig. 2 – Representative quantification results. Ex: Bone 23

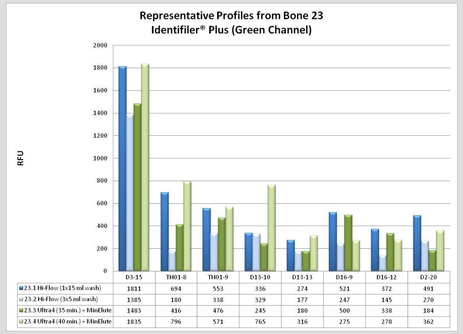

Fig. 3 – Representative DNA profiling results. Ex: Bone 23

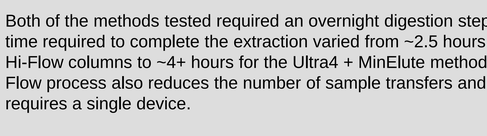

Fig. 4 – Representative quantification results. Ex: Bone 25

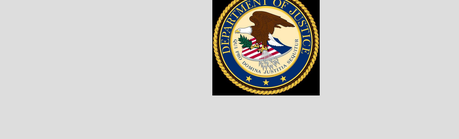

Fig. 5 – Representative DNA profiling results. Ex: Bone 25

	Bone 8		Bone 23		Bone 24		Bone 25	
Extraction Method	28 Cycles	29 Cycles	28 Cycles	29 Cycles	28 Cycles	29 Cycles	28 Cycles	29 Cycles
Hi-Flow (1x15 ml wash)	10	12	26		28	28	22	
Hi-Flow (3x5 ml wash)			24	26	28	28	22	25
Ultra4 + MinElute	13	13	26	25	28	27	11	20
Ultra4 + MinElute	11	15	26	26	28	28	14	18

Table 1 – Total number of alleles amplified from extracts obtained using Identifiler Plus at 28 or 29 cycles

CONCLUSIONS

DNA Profile

Both methods yielded comparable number of alleles per profile (Table 1), except for Bone 25, which required extended centrifugation in the Ultra4 device.

Ease of Use

Both of the methods tested required an overnight digestion step, but the time required to complete the extraction varied from ~2.5 hours for the Hi-Flow columns to ~4+ hours for the Ultra4 + MinElute method. The Hi-Flow process also reduces the number of sample transfers and only requires a single device.

References

•Amicon Bioseparations, Amicon® Ultra-4 centrifugal filter devices, User guide, 2007.

•M. Hoss, P. Jaruga, T.H. Zastawny, M. Dizdaroglu, and S. Paabo, DNA damage and DNA sequence retrieval from ancient tissues. Nucleic Acids Res 24 (1996) 1304-7

•B.M. Kemp, and D.G. Smith, Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int 154 (2005) 53-61.

•C. Keyser-Tracqui, E. Crubezy, and B. Ludes, Nuclear and mitochondrial DNA analysis of a 2,000-year-old necropolis in the Egyin Gol Valley of Mongolia. Am J Hum Genet 73 (2003) 247-60.

•O.M. Loreille, T.M. Diegoli, J.A. Irwin, M.D. Coble, and T.J. Parsons, High efficiency DNA extraction from bone by total demineralization. Forensic Sci Intl: Genetics 1 (2007) 191-195.

•O.M. Loreille *et al.*, Integrated DNA and Fingerprint Analyses in the Identification of 60-Year-Old Mummified Human Remains Discovered in an Alaskan Glacier. J Forensic Sci 55(3):813-8.

•Qiagen, MinElute® spin handbook, MinElute® PCR purification kit, 2004.

•N. Tuross, The biochemistry of ancient DNA in bone. Experientia 50 (1994) 530-5.

•D.Y. Yang, B. Eng, J.S. Waye, J.C. Dudar, and S.R. Saunders, Technical note: improved DNA extraction from ancient bones using silica-based spin columns. Am J Phys Anthropol 105 (1998) 539-43.

Acknowledgements

Funding for this project provided by the National Institute of Justice (NIJ) Grants 2009-DN-BX-K188 and 2009-DN-BX-K058

Protein Ark Hi-Flow columns generously provided by Mike Brownleader, whose collaboration has been essential to this research