Isolation of Purified DNA Using a Bone DNA Extraction Kit

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1. Introduction

Bones constitute one of the most challenging sample types in forensic casework, and the extracted DNA is often low in quantity and degraded reducing the chances of a usable STR profile. Also, old and poor conditions of bones present additional challenges for processing. We assessed a cellulose based resin in comparison with DNA IQ™ resin and bench-marked against a modified organic method for processing contemporary and old bones. Additionally, preprocessing buffers – Bone Incubation Buffer and Demineralization Buffer (Lorielle et al., 2007) were compared for DNA extraction efficiency using the DNA IQ™ resin chemistry and further compared to a competitor workflow. The results indicate that preprocessing of bone samples using demineralization buffer, followed by DNA purification using the DNA IQ™ chemistry offers an easy, automatable workflow for obtaining DNA that is pure and free of inhibitors. The extracted DNA was compatible with downstream applications such as PowerQuant® System and PowerPlex® Systems, thus enabling accurate human DNA quantification and STR profile generation.

2. Initial evaluation of bone DNA purification

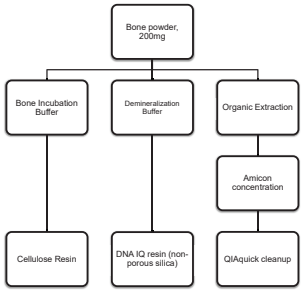
Material

Period	Bone Type	Number of bones
From 1939-1945	World War II Femur	5
From 1870-1880	Deadwood Femur	4
Contemporary	Femur	6

Method

Three workflows were evaluated:

a) Preprocessing with Bone Incubation Buffer (BIB) and Purification using cellulose binding chemistry
b) Preprocessing with Demineralization Buffer and purification using Maxwell® DNA IQ™ Casework Pro Kit
c) Organic extraction followed by QIAquick cleanup



3. Quantification of human DNA

	POWERQUANT CONCENTRATION VALUES											
	AUTO CONC (ng/ul)			DEG CONC (ng/ul)			Y CONC (ng/ul)			AUTO/DEG Ratio		
Sample	BIB/Cellulose	Demin/IQ	Mod Organic	BIB/Cellulose	Demin/IQ	Mod Organic	BIB/Cellulose	Demin/IQ	Mod Organic	BIB/Cellulose	Demin/IQ	Mod Organic
1 - World War II femur 2011-296-1246(3)	0.2462	0.9027	No Cq	0.0156	0.0064	No Cq	0.0094	0.0321	No Cq	15.79	9.48	No Cq
2 - World War II femur 2010-484-1570(3)	0.0884	0.0078	No Cq	0.0042	0.0008	No Cq	0.0253	0.0024	No Cq	21.05	9.75	No Cq
3 - World War II femur 2009-268-743	0.0208	0.0445	No Cq	0.0008	0.0024	No Cq	0.0055	0.0109	No Cq	26.00	18.54	No Cq
4 - World War II femur 2011-226-625(3)	0.0034	0.0005	No Cq	No Cq	No Cq	No Cq	0.0014	0.0002	No Cq	No Cq	No Cq	No Cq
5 - World War II femur 243-658	0.0314	0.0157	No Cq	0.0008	0.0004	No Cq	0.0087	0.0036	No Cq	39.25	39.25	No Cq
6 - Deadwood femur 007.002	0.0235	0.0013	No Cq	0.0014	No Cq	No Cq	0.0072	0.0006	No Cq	16.79	No Cq	No Cq
7 - Deadwood femur 001.002	0.0117	0.0208	No Cq	0.0004	No Cq	No Cq	0.0030	0.0003	No Cq	29.25	No Cq	No Cq
8 - Deadwood femur 010.001	0.0052	0.0011	No Cq	0.0003	No Cq	No Cq	0.0017	0.0004	No Cq	17.33	No Cq	No Cq
9 - Deadwood femur 008.001	0.0423	0.0065	No Cq	0.0052	0.0019	No Cq	0.0206	0.0043	No Cq	8.13	3.42	No Cq
10 - Contemporary femur 0017.12	0.2354	0.0386	0.0597	0.0627	0.0090	0.0116	0.1059	0.0171	0.0286	3.75	4.29	5.15
11 - Contemporary femur 0054.12	0.0016	0.0014	No Cq	No Cq	No Cq	No Cq	0.0011	0.0005	No Cq	No Cq	No Cq	No Cq
13 - Contemporary femur 063	12.4220	3.9132	0.0364	1.1054	0.3278	0.0036	3.1728	1.5418	0.0111	11.24	5.81	10.11
13 - Contemporary femur 0063.12	0.0168	0.0163	No Cq	0.0009	0.0052	No Cq	0.0072	0.0045	No Cq	22.00	13.58	No Cq
14 - Contemporary femur 0021.12	1.1619	3.3526	0.0239	0.0126	0.0787	No Cq	0.2381	0.9960	0.0042	92.21	42.60	No Cq
15 - Cyprus femur CHE-8	0.4342	0.2132	No Cq	0.0234	0.0159	No Cq	0.1295	0.0563	No Cq	15.29	13.41	No Cq

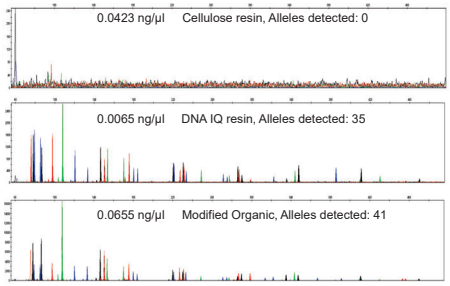
All eluates were quantified by the PowerQuant® System. The modified organic samples failed to amplify for all but two contemporary bone samples. The two Maxwell® chemistries had amplification of the autosomal target for all samples, with the cellulose chemistry having higher yields overall. The [Auto]/[Deg] ratio indicated a high level of degradation in all samples tested.

4. Alleles detected using PowerPlex® Fusion System

	PowerPlex® Fusion Alleles detected		
Sample	BIB/Cellulose	Demin/IQ	Mod Organic
1 - World War II femur 2011-296-1246(3)	0	38	40
2 - World War II femur 2010-484-1570(3)	0	20	41
3 - World War II femur 2009-268-743	0	30	38
4 - World War II femur 2011-226-625(3)	0	0	7
5 - World War II femur 243-658	0	18	23
6 - Deadwood femur 007.002	0	4	23
7 - Deadwood femur 001.002	0	0	17
8 - Deadwood femur 010.001	0	5	12
9 - Deadwood femur 008.001	0	35	41
10 - Contemporary femur 0017.12	27	42	42
11 - Contemporary femur 0054.12	0	6	14
13 - Contemporary femur 0063.12	0	24	20
14 - Contemporary femur 0021.12	1	20	43
15 - Cyprus femur CHE-8	0	28	36

Following PowerQuant® amplification, STR analysis by PowerPlex® Fusion was performed on all samples. Despite having the highest yield by PowerQuant® System, the Maxwell® BIB/cellulose samples performed poorly in STR analysis, with no alleles detected in all but two contemporary samples. The modified organic method had the best performance in STR, with alleles detected in all samples tested, followed by the Maxwell Demin/ DNA IQ™ samples, with alleles detected in all but two samples.

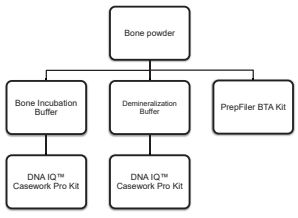
5. PowerPlex® Fusion Profile



Normalized DNA template was amplified using PowerPlex® Fusion System and electrophoresed on Applied Biosystems 3500 Genetic Analyzer using manufacturer's recommendation for injection and run conditions. This STR for one of the Deadwood femur samples is indicative of most of the samples tested, with no detection of alleles in the BIB/cellulose samples and similar STR profiles between Demin/DNA IQ™ and modified organic samples.

Based on the results obtained we proceeded to compare BIB and Demin buffer as preprocessing for DNA IQ™ purification

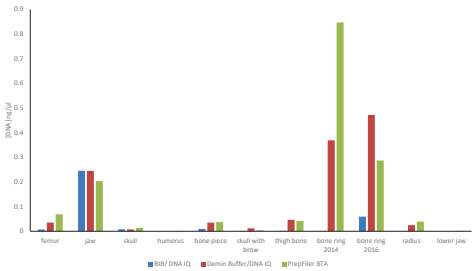
6. Comparison of preprocessing buffers



Three workflows were evaluated:

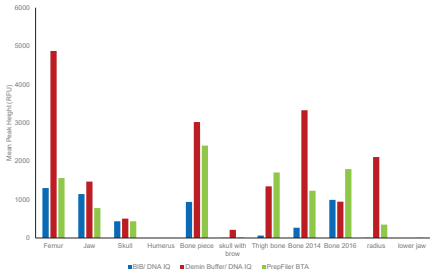
a) Preprocessing with Bone Incubation Buffer (BIB) and Purification using Maxwell® DNA IQ™ Casework Pro Kit
b) Preprocessing with Demineralization Buffer and purification using Maxwell® DNA IQ™ Casework Pro Kit
c) Purification using PrepFiler BTA kit following manufacturer's recommendations

7. Quantification of human DNA



Ten bone samples of various ages were preprocessed using Bone Incubation Buffer (BIB) or Demineralization Buffer (DB) and purified using DNA IQ™ chemistry on the Maxwell® RSC instrument. Bone samples were also processed in parallel with PrepFiler BTA Kit. The purified DNA was quantified using Quantifiler Trio and DNA concentration of the autosomal target is shown in the figure above.

8. Mean Peak heights of alleles detected



DNA purified using the three workflows were normalized and amplified using PowerPlex® Fusion 6C System and electrophoresed on a Applied Biosystems 3500 instrument with recommended injection and run conditions. Mean Peak Height (RFU) of the alleles obtained in PowerPlex® Fusion 6C profiles are shown in the figure above.

9. Conclusions

Initial testing at the University of North Texas

- The cellulose chemistry was unsuitable for STR analysis, despite having the highest yields by quant. This may be due to carryover of inhibitors.
- The modified organic method had the best performance in STR, followed closely by Demin Buffer / DNA IQ™ chemistry.
- The Maxwell® methods were much easier and quicker to execute compared to the modified organic method.

Comparison of Preprocessing Buffers

- The demineralization preprocessing had better performance in STR, in terms of mean peak height and alleles detected, compared to the bone incubation buffer preprocessing workflow.
- The Demin Buffer / DNA IQ™ method and PrepFiler BTA methods performed comparably in generating STR profiles from bone samples.

Reference: Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Sci Int Genet. 2007 Jun;1(2):191-5.

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