High efficiency DNA extraction from bone by total demineralization

การสกัดสารพันธุกรรมจากกระดูกให้มีประสิทธิภาพสูงโดยการขจัดแร่ธาตุออกทั้งหมด

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นำเสนอโดย

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INTRODUCTION

Identification

- Missing person
- Mass disasters
- Ancient DNA

The only and almost always the best

Environmental conditions

- Microorganism
- Heat
- Water / humidity
- soil condition
- Duration

Bone Structure

Bone is a growing tissue made up mainly of collagen, a protein that provides

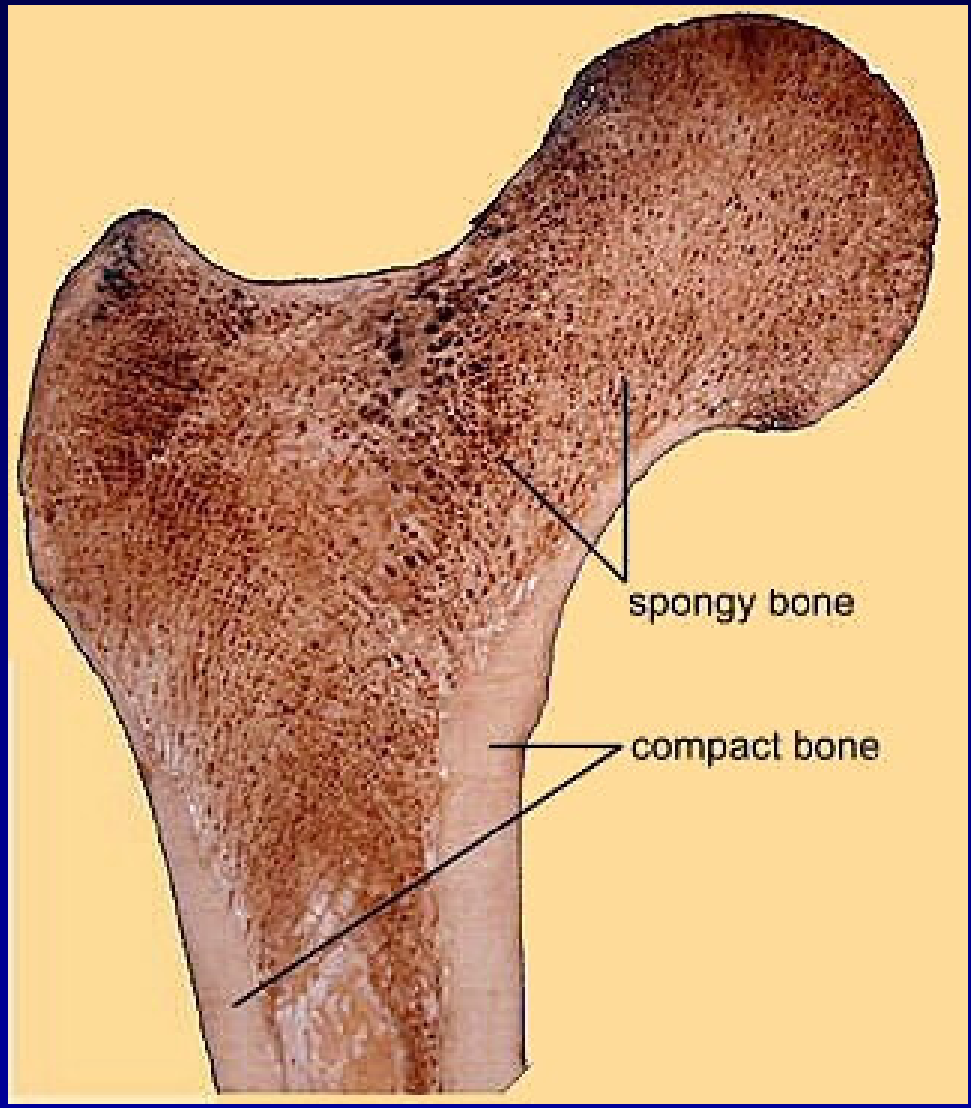
a soft framework, and minerals that add strength and harden the framework

70% of bone

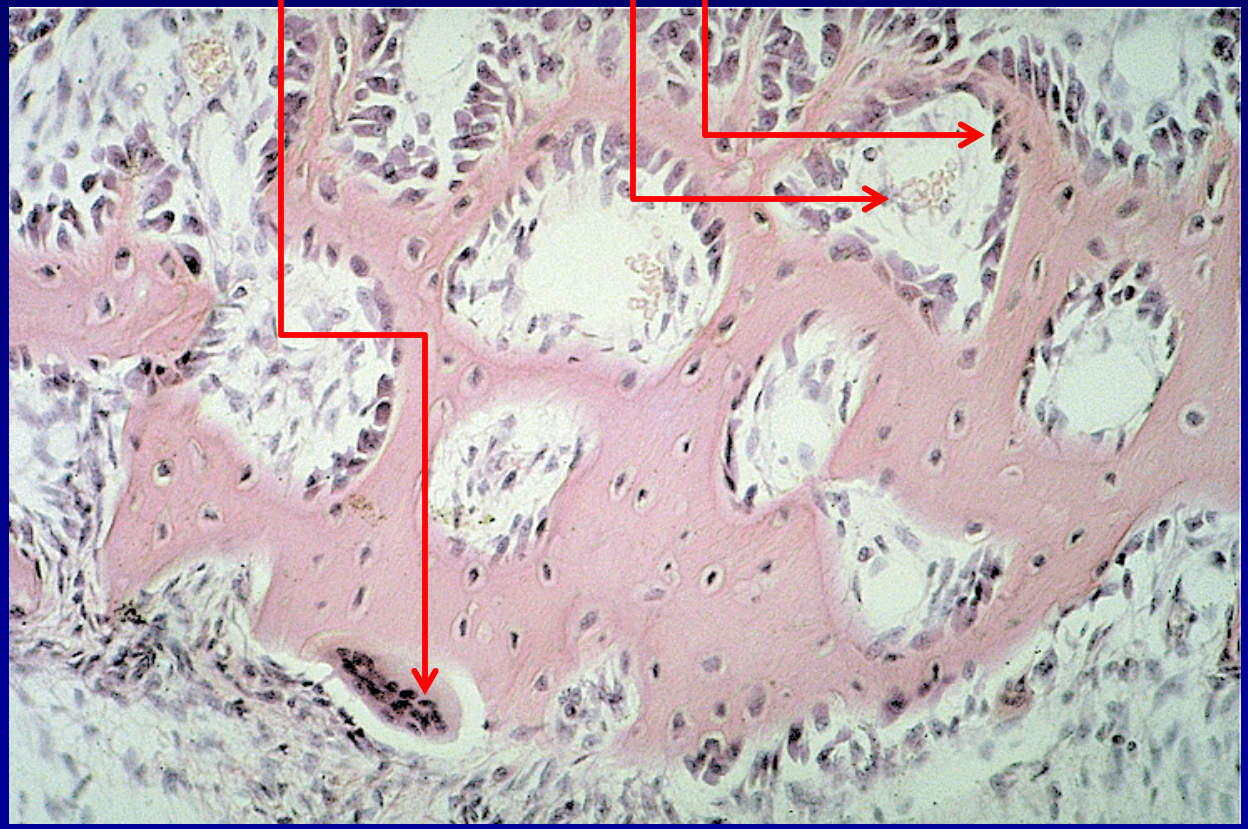
- inorganic mineral
- calcium phosphate, calcium carbonate, calcium fluoride, calcium hydroxide

- Compact bone

- Spongy bone



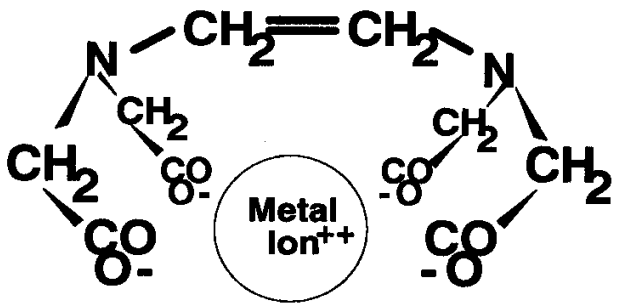
- **Cellular structure**
  - Osteoblast
  - Osteocyte
  - Osteoclast



Most of the current DNA extraction protocols

Ethylene diamine tetra-acetic acid (EDTA)

- Demineralization
- Inactivates DNAses by chelating bivalent cations such as Mg++ or Ca++



Ethylenediaminetetraacetic acid (EDTA) chelates a metal ion

Bone extraction protocols

- Incubated bone powder in a lysis buffer
- Collected the supernatant
- Discarded undissolved powder

Alternative extraction protocols

- Use demineralization steps
- wash/soak bone powder in large volumes of EDTA
- Extraction of bone powder
- DNA is discarded in the EDTA wash solutions

Materials and methods

Bone samples

- 14 human bones in various states of preservation
- Ranging in age from 5 to 100 years post-mortem

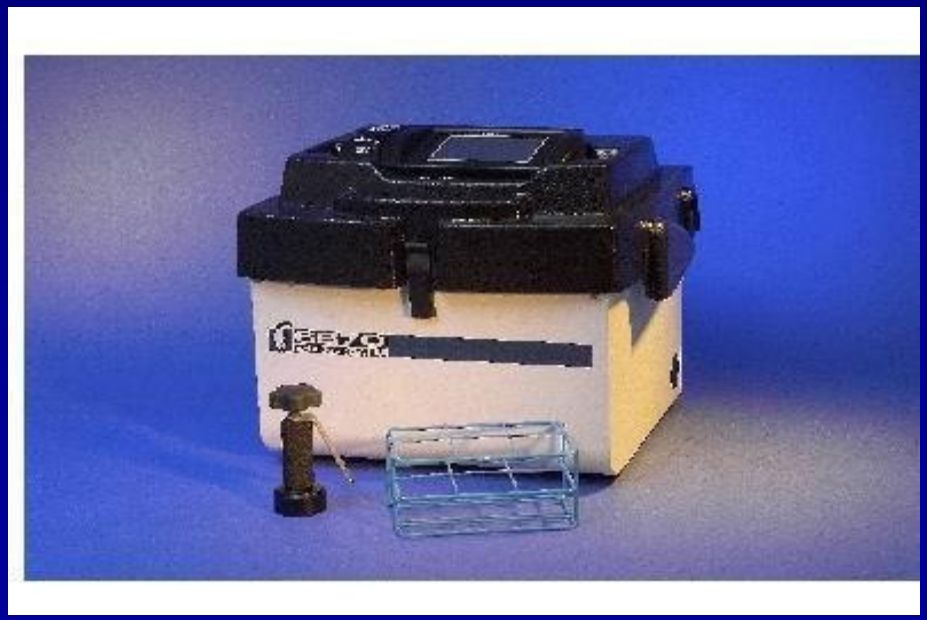
Pre-treatment of samples

- aluminum oxide sanding stone+ dremel tool (Dremel: Racine,WI)
- 20% bleach
- UV-irradiated water
- 100% ethanol
- air-dry overnight.

Physical breakup

2 different techniques

- 5 of the 10 samples were powdered in a cryogenic impact grinder (CertiPrep 6750 Freezer Mill, Spex/Mill, Spex, Metuchen,NJ)



- 1 sample was powdered in a sterilized Waring MC2 blender cup (Warring-Torrington, CT)

- 4 samples were powdered using both methods

Chemical breakup

Standard extraction protocol

Armed Forces DNA Identification Laboratory

(*AFDIL*)casework

- Bone powder 1–2 g
- 3 ml of an extraction buffer (10 mM Tris,pH 8; 100 mM NaCl; 50 mM EDTA, pH 8.0; 0.5% SDS) and 100 ul 20 mg/ml Proteinase K, 56 C (overnight)
- Phenol/chloroform/isoamyl alcohol
- Purification and concentration using TE buffer washes in a Centricon 100 centrifugal filter unit (Millipore)

Total demineralization protocol

- Bone powder incubated in 9–18 ml of the demineralization buffer(EDTA 0.5 M, 1%

  lauryl-sarcosinate) and 200 ul of 20 mg/ml Proteinase K, 56 C (overnight)
- Phenol/chlororm/isoamyl alcohol
- Concentrated to 2 ml using Centrifugal Filter Units(30 kDa, Amicon Ultra-15, Centricon+20, or Centriplus from Millipore)
- Transferred to centricon 30 centrifugal filter unit (Millipore) washed 3 times with irradiated water
- Eluted the final volume of all extracts was 100 ul

Real-time DNA quantification and Inhibition monitoring

- Each DNA extract was quantified using a real-time assay for relative quantification of a 143 bp fragment of mitochondrial
- Comparison to known quantities of 9947a DNA (Promega, Madison, WI)
- Internal positive controls (IPCs) were used for the detection of PCR inhibitors

mtDNA and STR typing

- mtDNA was sequenced
- STR amplifications using PowerPlex 16 system(Promega Corporation, Madison, WI) or the Yfiler system(Applied Biosystems, Foster City, CA)
- PCR products were separated on an Applied Biosystems 3100
- Analyzed using Genescan software version 3.7

Result

Total demineralization protocol

versus

standard protocol

Table 1

Treatments and relative quantities of mtDNA detected in samples extracted either by total demineralization or casework protocols

Samples	Freezer mill method			Blender cup method		
	Bone powder (g)	A, demineralization	C, casework	Bone powder (g)	B, demineralization	D, casework
1	1.02	54	6	1.0	33	6
2	1.0	79	7	0.8	56	NR
3	1.0	42	2	1.02	43	6
4	0.6	2.5	0.8			
5	0.9	34	0.9			
6	1.0	10,668	2379			
7	1.0	393	158			
8	1.02	125	42	1.21	400	36
9	1.0	191	52			
10				1.01	20	0.07

Rows A and C were processed using the freezer mill, bone powder quantity listed (in g). Rows B and D were powdered with the blender cup method, bone powder quantity listed (in g). See Section 2 for detailed description of the real-time PCR assay results.

Result

Total demineralization protocol versus standard protocol

The total demineralization procedure yielded higher amounts of DNA than the standard protocol: on average 4.6

2.5 - 100+

Freezer mill versus blender cup

- DNA yields from freezer mill extractions did not yield more DNA

- The average DNA yields

  the blender cup (65 pg/ul)

  the freezer mill (44 pg/ul)

- 4 of the comparative extractions yielded more DNA with the freezer mill and 4 extractions yielded more with the blender cup

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Reduction of sample material

- The standard protocol, 1–2 g of bone powder
  Total demineralization protocol, 0.2 g of bone powder

- DNA yields from the total demineralization protocol (0.2 g of bone powder) were greater than the yields from 5 to 10 times more bone powder using the standard extraction

- DNA-yield per gram bone powder,
  the total demineralization protocol using 0.2 g of bone powder resulted in an average of 228 times more DNA than the standard protocol using 1–2 g

Table 2

Treatments and relative mtDNA quantities of samples extracted using the total demineralization protocol vs. the standard protocol

Sample	Extraction	Bone powder	Real-time data
A	Demin.	0.2	2.83
	Casework	1.96	0.16
		1.65	0.16
B	Demin.	0.2	28.56
	Casework	2.02	0.34
		1.29	1.33
C	Demin.	0.2	5.95
	Casework	1.93	0.18
		2.16	0.33
D	Demin.	0.2	31.42
	Casework	2.04	1.19

See Section 2 for detailed description of the real-time PCR assay results.

STR analysis

- Samples 6 and 7

total demineralization Protocol Full profile Partial profile

standard extraction protocol Full profile Partial profile

- Samples 8

total demineralization Protocol Partial profile

standard extraction protocol No proflle

- Samples 9

total demineralization Protocol Partial profile (13 loci)

standard extraction protocol Partial profile (4loci)

Table 3
STR profiles obtained from LCN STR analysis

Markers	Sample 3		Sample 8				Sample 9	
	Demineralization	Casework	Demineralization (FM)	Demineralization (BC)	Casework (FM)	Casework (BC)	Demineralization	Casework
D3S1358	16, 17	16, 17	15, 18	15, 18	–	–	14, 15	–
TH01	7, 8	8, –	9.3, –	9.3, –	–	–	9, 9.3	9, –
D21S11	24.2, 29		29, –	29, –	–	–	30, –	–
D18S51	–	–	–	–	–	–	–	–
Penta E	–	–	–	–	–	–	–	–
D5S818	11, 13	11, 13	11, 12	11, –	–	–	11, 12	12, –
D13S317	10, 12	10, 12	–	8, 13	–	–	10, 12	–
D7S820	8, 9	–	–	–	–	–	11, 12	–
D16S539	–	–	–	–	–	–	9, 12	–
CSF1PO	–	–	9, –	–	–	–	9, 11	–
Penta D	–	–	10, –	–	–	–	–	–
Amelo.	*X*, *Y*	*X*, *Y*	*X*, *Y*	*Y*, –	*X*, –	*X*, –	*X*, *Y*	*X*, –
vWA	16, 18	16, 18	17, –	17, –	–	–	18, –	18, –
D8S1179	15, –	15, –	14, –	–	–	–	13, –	–
TPOX	–	8, –	–	8, –	–	–	9, –	–
FGA	–	–	22, –	–	–	–	21, –	–

Sample 8 was extracted with both, total demineralization and casework protocol, using either the freezer mill (FM) and a blender cup (BC) method.

Conclusions

- Total demineralization of the bone powder significantly increases DNA yields
- DNA can be recovered from small quantities of starting material
- EDTA is a component of the lysis buffer and no DNA is lost
- mtDNA were only recovered when the total demineralization technique
- Increases the quality of STR profiles

THANK YOU