Dialysis vs. Qiagen M48

Two Alternative Procedures for Purifying Ancient DNA from Bone and Teeth Extracts

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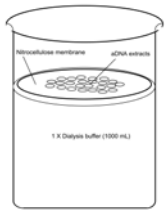
Abstract:
Aside of extensive requirements for sample pretreatment in order to avoid and remove contaminations, the retrieval of ancient DNA from historical and archaeological samples is a challenging task: Naturally, hard tissue remains are the predominant source materials for molecular archaeologists. Due to diagenetic effects, only minute amounts of DNA are preserved in ancient bones and teeth; sample composition and extraction procedure(s) result in a variety of impurities (accessory compounds), that have to be removed prior to PCR analysis.
Here we present two alternative procedures for the purification of ancient DNA from hard tissue raw extracts. Dialytic DNA purification (**manual**) is contrasted to **semiautomated** processing (Qiagen M48 Robot) focussing on temporal and logistic factors as well as on DNA yield and quality.

Dialysis:
experimental set-up



After decalcification and ProtK-digestion raw extracts are transferred to a floating filter as shown above. Transient gain of volume (osmotic effect) requires spatial distribution to ensure controlled confluence during incubation.

Dialysis (see timeline, column left) against hypotonic buffer solution removes impurities (salts, small organic molecules, EDTA, etc.).

Subsequent ethanolic precipitation results in purified, concentrated DNA, suitable for PCR. analysis.

Basic principle:
"Molecular Sieving", dilution of objectionable compounds, precipitation;

laboratory work flow




drying, cleaning, surface decontamination,...
powdering: grinder, mortar, drill,...
bone/tooth powder 1-2 g total
transfer to a 1,5 ml screw tube
powder: 60-80 mg / extraction | **powder:** 220-250 mg / extraction
add 625 µl 0,5 M EDTA, pH=8,0
Dialysis hands on procedure | incubate @ 56°C for 36-48 hours for decalcification ensure permanent agitation! | **QIAGEN M48** semiautomated
prepare dialytic set up | add 25 µl Proteinase K (20 mg/ml) & 25 µl DTT (0,39 M) incubate @ 56°C for 3-5 hours for Protein digestion optional: boost the reaction with 10 µl ProtK ensure permanent agitation | **prepare M48 robot**
spin down 1 min., 14.000 rpm, 30-well-rotor
load entire clear supernatant on dialytic filter (approx 550-600 µl)
incubate over night, max. 14 hours
recover purified DNA: 450 µl portions of purified DNA perform precipitation: 3-5 corresponding tubes dissolve pellets in 20-33 µl each
transfer clear supernatant to 2ml sample tubes (2 portions / extraction, 200 - 250 µl max. each)
start M48 robot extraction protocol TraceSample v3.1 choose 12 samples / 50 µl elution volume
recover purified DNA (2 times 50 µl per sample),
pool corresponding samples yield 100 µl of purified DNA each

documentation & sample pretreatment
physical break up (powdering)
chemical break up (DNA extraction)
DNA **purification**
DNA **amplification** (PCR - polymerase chain reaction)
Post-PCR analyses: **Genotyping** & Sequencing

Qiagen M48:
BioRobot

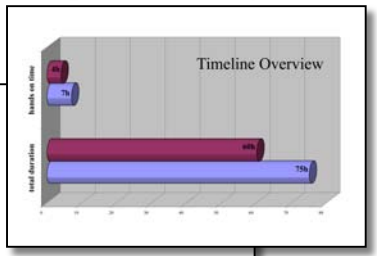
After decalcification and ProtK-digestion raw extracts are transferred to the Qiagen M48 BioRobot.

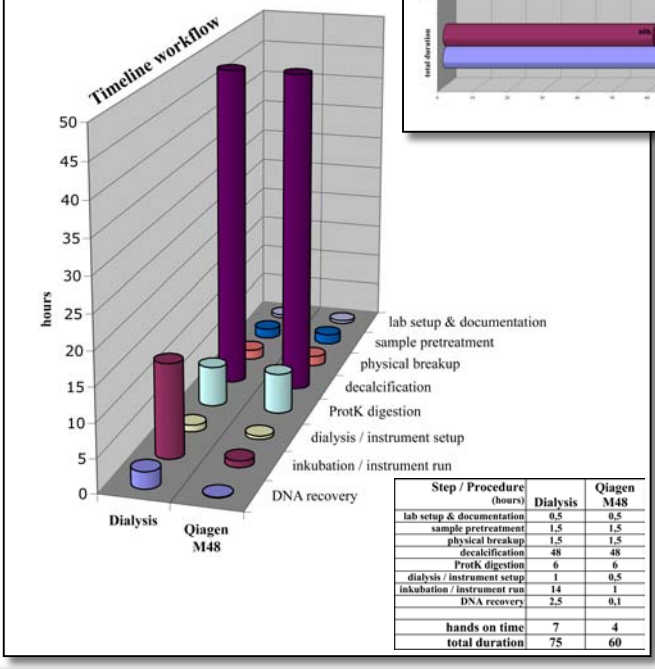
DNA is attached to glass beads with a ferromagnetic core.

Operating a magnetic device allow separation of DNA/glass-bead-aggregates from residual raw extracts.

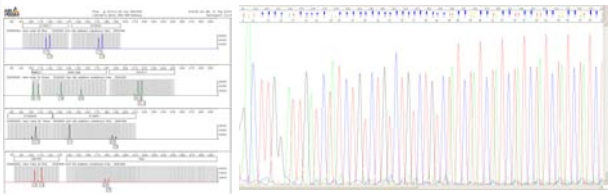
Wash steps and final recovery (DNA release from glass beads) results in purified, concentrated DNA, suitable for PCR. analysis.

Basic principle:
DNA-isolation by attachment to glass-beads enabled by electromagnetic separation;





Step / Procedure (hours)	Dialysis	Qiagen M48
lab setup & documentation	0,5	0,5
sample pretreatment	1,5	1,5
physical breakup	1,5	1,5
decalcification	48	48
ProtK digestion	6	6
dialysis / instrument setup	1	0,5
inkubation / instrument run	14	1
DNA recovery	2,5	0,1
hands on time	7	4
total duration	75	60



Conclusions / Recommendations:

- total time: **M48 saves** about **15 hours** compared to dialysis (6 samples / batch).
- hands on time: **M48 saves** about **3 hours** compared to dialysis (6 samples / batch).
- contamination: semiautomatic **M48** has a **significantly reduced risk** of foreign DNA introduction (closed working platform, UV-decontamination procedure, automated sample handling).
- source material: Dialysis requires approx. **60 mg bone/tooth powder** for yielding 100 µl of purified DNA **versus 250 mg** for M48.
- logistics: **Dialysis** has shown to be suitable for **small scale** flow capacities; **M48** extracts multiples of 6 samples (**medium to large scale** flow capacity); alternatively, the Qiagen EZ1 robot is capable of single extractions. **Additionally, Dialysis** requires extensive preparations (assembling the beakers, dialytic buffer and filters) and **handling** when harvesting and precipitating the purified DNA.
- costs: **both** systems require approx. **10 €/extraction** for reagents and lab consumables, instrument and other lab equipment not included.
- DNA yield and data quality: remarkably the **total DNA yield** (ng DNA per g bone powder) employing **dialysis** is more than **10 times higher than M48**! However, the **DNA-quality** of both procedures is **comparable** referring to successful DNA typing. **None** of the extracts from both procedures showed **inhibition**.

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Acknowledgements to:
Monika Radacher, Christian Starke & Edith Tutsch-Bauer

Poster presented at SECVNDUS CONVENTUS ARCHÆOMETRIÆ - **MMX**
Zweiter Österreichischer Archäometriekongress - 13. & 14. Mai 2010, Salzburg

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