On DNA typing of hard tissues

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INTRODUCTION

DNA preparation from tissues is necessary for forensic and pathological case work. Various methods for handling tissues and samples have been described. Mostly it's possible to receive muscles, lymph nodes or other organs from autopsy material in sufficient quantities and good quality. The amount of degraded DNA correlates directly with the duration of the postmortem period (Bär 1988). Tissues with a high content of fibers and minerals such as skin, tendon, also prostata and uterus, cartilage, bone and tooth (root) are more resistant to environmental factors and can protect cells and their DNA longer than other parenchymatous tissues as brain, muscle or liver.
In this paper are shown first experiences in DNA preparation of hard an solid tissues, the yield and quality of DNA in dependence of storage time and give some methodological advices.

MATERIAL

Specimens of all tissues from the same 40 years old male accident victim who died immediatly. Cadaver was kept at 4° C.
Prepared tissues samples:
- skin with complete lamination (without subcutis)
- tendon (Achilles tendon)
- cartilage (hyalin from joints, elastic from epiglottis, fibrocartilage from intervertebral disk)
- bone from the skull
- teeth from the dentist

METHODS

Storage conditions:
Fresh cutted tissue pieces from autopsy material, frozen at -80° C immediatly, after one, after two and after three weeks storage time in tubes at room temperature.
- The pieces were rasped in frozen conditions to aliquots of appr. 500 mg and stored again in the freezer till steps of cell lysis. We used a rasp with a very deep and rough profile for getting chips of bone or teeth or a pappy mass of skin, tendons and cartilages. All kinds of tissues had to rasp quickly because the material thaws within one minute and you will get only smear.
  For rasping the small pieces of hyalin cartilage they should be embedded in frozen water because of their very smooth surface.

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- Before lysis with Proteinase it is useful to decalcify the rasped bones and teeth:
  For one aliquot of 500 mg take 40 ml of high molar EDTA.
  Gently shaking overnight at 50° C (look at established methods in histological labs).
  After decalcification wash two times with isotonic sodiumchloride.
- Lysis with various concentrations and combinations of Proteinase K, Collagenase A (Boehringer) and Hyaluronidase (Dessau).
  For one aliquote mix gently with 10 ml of 0,01 M Tris-HCl (pH 7,6), 0,01 M EDTA, 0,1 M NaCl (pH 8,0) and 2 % SDS.
  Before addition of SDS it is useful to shake vigorously the mixture for getting a homogenious distribution of all tissue particles.
- Deproteinization two times with phenol-chloroform (1:1) and once with chloroform-isoamylalcohol (24:1).
- At least remove the upper phase because of impurities at the wall of the tubes such as insoluble minerals, filling substances of teeth, rests of tissue after incomplete lysis.
- DNA precipitation, digestions with restriction enzyme (Hinf I) and electrophoresis with known and established procedures.
- Hybridisation and visualization with the Digoxigenin labelled B.E.S.T.-Probe MZ 1.3.

RESULTS

1. Total yield of DNA from fresh prepared tissues (Table 1, Fig. 1).
2. We found clear less postmortal degradation of DNA prepared from hard and solid tissues compared with parenchymatous tissues (Fig. 2).
3. DNA from bone can be obtained with and without decalcification. After decalcification we got a higher yield of DNA (appr. 100 %). DNA in a tooth can only be recovered from the pulp and the directly surrounded parts of the dentin.
4. For all kinds of tissues the amount of Proteinase could be limited to 2 mg/500 mg rasped material. Less than 1 mg gave no sufficient desintegration and lysis. After testing Collagenase and Hylase alone with different concentrations we did not find lysis of the samples.
5. Only in combination of Proteinase and 300 units of Hyaluronidase it was possible to reduce the amount of Proteinase to less than 1 mg/500 mg rasped material. Other combinations of Proteinase, Collagenase and Hyaluronidase gave no additional effect.
6. There were no fundamental differences in reaction of the various kinds of tissue to the enzymes.
7. All kinds of tissues of the same body showed identical band patterns.

Table 1.

Total yield of DNA from fresh tissues.

Yield in ug/g tissue			
skin	325	bone	300
tendon	187	tooth	5
cartilage	612	prostata	580

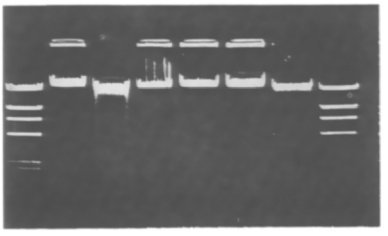

Fig. 1.

High molecular weight control of DNA from fresh autopsy material: lambda, skin, tendon, cartilage, bone, tooth, prostata (from left to right)

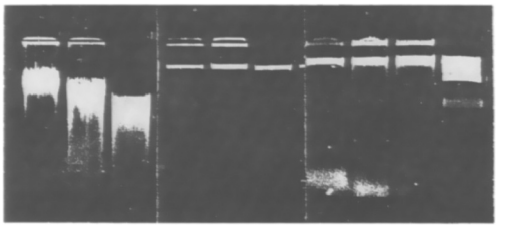

Fig. 2.

High molecular weight control of DNA prepared from muscle, tendon and bone after storage time of one, two and three weeks at room temperature (from left to right)

DISCUSSION

The described mechanic destruction of tissues by rasping is simple but good for efficiency of digestion with Proteinase. Furthermore there is no danger of contagiousness by microdrops during homogenization with a conventionel apparatus.
Commonly digestion with Proteinase will be sufficient. Using of Hyaluronidase and Proteinase together could be important only for analysis of tissues which are very rich in cells and fibers. The influence of the enzymes used for cell lysis to activity of restriction enzymes should be investigated in a further study. The comparison of enzyme activity between Proteinase, Collagenase and Hyaluronidase is difficult (definition of units). Thatswhy the enzyme dosis choosed in our lab should go in control. Using of Collagenase type "A" was decided after recommendations of Boehringer. Main contents of the phiols are lyophilized Collagenase and some proteolytic activities.
For DNA analysis after longer postmortem period we recommend preparation of bone or prostata and uterus as showed in this paper. It is not necessary to take bone marrow; also compact structures of pelvis or skull are suitable.

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