PROTOCOL 2.

DNA isolation from 124 yers old Balkan chamois bone remains using QIAquick® PCR purification kit and QIAcube® robotic workstation

Things to be done before the start:

- **All surfaces used in the laboratory should be wiped down with a 10% of sodium hypochlorite and 70% ethanol solution before and after each use.**
- **Equipment (e.g. stainless steel grinding jars and balls, pipettes, tips) should be irradiated under the UV light for 30 minutes, before and after use**
- **Add the appropriate amount of ethanol (96–100%) to Buffer PE before use to obtain working solutions.**
- **The QIAcube performs fully automated processing of up to 12 samples.**
- **Pour PE and EB buffers into two 30 ml QIAcube reagent bottles.**
- **Place 30 ml QIAcube reagent bottles into the position 5 (PE) and 6 (EB) of the QIAcube reagent bottles rack.**
- **Place QIAquick column and 1,5 ml microcentrifuge tubes into the position 1 and 3 of the QIAcube® Rotor Adapters.**
- **Place the loaded QIAcube Rotor Adapters into the QIAcube centrifuge buckets.**
- **Place two prefilled tip racks (1000 µl) onto the worktable of the QIAcube.**

1) Cut 0.5 to 1 g of molar and/or maxilla bone using Dremel® machine.

2) Wash bone cut twice with 10% of sodium hypochlorite for 30 s, soak in water for 5 min, rinse using 96% ethanol and then dry at room temperature.

3) Ground bone cut for 20 min at 30 Hz in the TissueLyser (Qiagen®) using two stainless steel grinding jars and 10 mm stainless steel grinding balls.

4) Incubate 0,5 g of bone powder in 10 ml of lysis buffer (0,5 M EDTA pH 8, 1 % N-laurylsarcosinate and 6,7 mg of proteinase K) overnight in an incubator at 56 °C on a rocking or rotated platform.

5) Centrifuge lysates with completely digested bone powder for 5 min at 1800 x *g* to pellet any particulates.

6) Concentrate lysates to approximately 300 µl by centrifugation for 50 to 60 min at 5000 x g in Amicon® Ultra-15 100K Centrifugal Filter Devices.

7) Transfer each 300 µl of concentrated lysates to a new 2 ml safe lock (Eppendorf®) microcentrifuge tubes.

8) Add 1500 µl Buffer PB to each concentrated lysate and mix.

9) Open the QIAcube door and place 2 ml tubes into the QIAcube shaker adapter. Place tube lids into the slot at the edge of the shaker adapter.

   - Remove caps from 30 ml QIAcube reagent bottles

10) Close the QIAcube door and launch QIAcube robotic workstation choosing: QIAcube® Customized Protocol Sheet IDCR1179 (see QIAcube Protocol Sheet).

- In these steps DNA molecules are bounded, washed, dried and finally eluted from DNeasy Mini spin column as a pure DNA isolate.

10) When the protocol run has finished, a message is displayed in the touchscreen confirming that the samples have been processed.

11) Remove the 1,5 ml microcentrifuge tubes containing purified DNAs from the rotor adapters.

- Discard sample tubes, used rotor adapters, and reagent according to safety regulations.
- Replace the lids of the reagent bottles and close tightly
- Empty the waste drawer

12) Isolated DNA solution should be kept at -20 °C for longer period of time or at 4-8 ° C for several days

References:

1) Amicon® Ultra15 Centrifugal Filter Unit - User guid
2) Amory et al. 2012. Automatable full demineralization DNA extraction procedure from degraded skeletal remains. Forensic Science International: Genetics 6: 398–406.
3) QIAcube® - User Manual
4) QIACube and QIAquick PCR Purification Kit - QIAcube® Protocol Sheet
5) QIAquick® - PCR Purification Kit

6) Laboratory equipment and accessories used for DNA isolation

Dremel 3000-15 Multitool

Tissue Lyser Qiagen®

Grinding jars and balls for Tissue Lyser

Stuart® Hybridisation oven SI30H

Amicon® Ultra 15 mL centrifugal filter devices

Sigma 4-16 Centrifuges

QIAquick® PCR purification kit

QIAcube®

QIAcube® Filter-Tips 1000 µl

QIAcube® 30 ml Reagent Bottles

QIAcube® Rotor Adapter