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Extraction method A (FMS and CR)

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Abstract

This protocol allows for adequate DNA extraction from fresh tissue samples.

Materials

MATERIALS

- Buffer AW1 **Qiagen Catalog #**19081
- Buffer AW2 **Qiagen Catalog #**19072
- Buffer AE **Qiagen Catalog #**19077
- Buffer ATL **Qiagen Catalog #**19076
- Ethanol

Before start

Separate PCR-free facility

Extraction

1 Cut tissue into small pieces.

2 Digestion of ca. 25 mg sample with Buffer ATL (Qiagen, Hilden, DE) and Proteinase K overnight at 56 °C.

25 mg Sample

Note

Incubation was performed on a thermomixer with 800 rpm

3 After pelleting remaining material, remove supernatant and added to Buffer AL and ethanol.

4 Transfer the solution to DNeasy Mini spin column (Qiagen, Hilden, DE) and centrifuge at 8000 rpm.

5 DNA purification following manufacturer's protocol with Buffer AW1 and Buffer AW2 (Qiagen, Hilden, DE).

6 Prior to elution, incubate DNA in the membrane with elution Buffer AE for 5 min at room temperature.

00:05:00 Incubation at RT

7 Elution proceeds by centrifugation at 8000 rpm for 1 min.

00:01:00 Centrifugation

8 Measure DNA concentration on Nanodrop (Thermo Fischer Scientific, Darmstadt, DE).