

# Extraction method F (CR)

Faezah Mohd Salleh, Jazmin Ramos-Madriral, Fernando Penaloza, Shanlin Liu, Mikkel-Holger S Sinding, Riddhi P Patel, Renata Martins, Dorina Lenz, Jorns Fickel, Christian Roos, Mohd Shahir Shamsir, Mohammad Shahfiz Azman, Burton K Lim, Stephen J Rossiter, Andreas Wilting, M Thomas P Gilbert

## Abstract

Gen-IALFirst All-tissue DNA extraction kit -This protocol provides an efficient DNA extraction and purification of historic sample (tissue material)

**Citation:** Faezah Mohd Salleh, Jazmin Ramos-Madriral, Fernando Penaloza, Shanlin Liu, Mikkel-Holger S Sinding, Riddhi P Patel, Renata Martins, Dorina Lenz, Jorns Fickel, Christian Roos, Mohd Shahir Shamsir, Mohammad Shahfiz Azman, Burton K Lim, Stephen J Rossiter, Andreas Wilting, M Thomas P Gilbert Extraction method F (CR). **protocols.io**  
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## Before start

Ancient DNA lab

## Materials

- ✓ Proteinase K by Contributed by users
- ✓ Isopropanol by Contributed by users
- ✓ Ethanol by Contributed by users

## Protocol

### Extraction

#### Step 1.

Cut tissue (25-50 mg) into small pieces.

### Extraction

#### Step 2.

Add 500 µl Lyse 1.

 **AMOUNT**

500 µl Additional info: Lyse 1

### Extraction

#### Step 3.

Add 50 µl Lyse 2.

☐ AMOUNT

50 µl Additional info: Lyse 2

Extraction

**Step 4.**

Add 10 µl Proteinase K.

☐ AMOUNT

10 µl Additional info: Proteinase K

Extraction

**Step 5.**

Incubate in a thermomixer at 65°C for 12 hours.

Extraction

**Step 6.**

Centrifuge for 10 min at 13000rpm.

Extraction

**Step 7.**

Discard supernatant without disturbing the pellet.

Extraction

**Step 8.**

Add 375 µl Lyse 3

☐ AMOUNT

375 µl Additional info: Lyse 3

Extraction

**Step 9.**

Vortex for 20 sec.

Extraction

**Step 10.**

Leave it for 5 min in the freezer (-20°C)

Extraction

**Step 11.**

Centrifuge 20 min at 13000rpm.

Extraction

**Step 12.**

Transfer the supernatant in a new tube.

Extraction

**Step 13.**

Add 640 µl isopropanol.

☐ AMOUNT

640 µl Additional info: Isopropanol

Extraction

#### Step 14.

Mix by inversion.

Extraction

#### Step 15.

Centrifuge for 15 min at 13000 rpm.

Extraction

#### Step 16.

Remove the supernatant (don't touch the pellet).

Extraction

#### Step 17.

Add 150 µl ethanol.

 AMOUNT

150 µl Additional info: Ethanol

Extraction

#### Step 18.

Centrifuge for 5 min at 13000 rpm.

Extraction

#### Step 19.

Remove the ethanol with a pipette (don't touch the pellet).

Extraction

#### Step 20.

Dry the pellet (37°C open lid max 5 min).

Extraction

#### Step 21.

Re-suspend the pellet in 100 µl Buffer AE.

 AMOUNT

100 µl Additional info: Buffee AE

Extraction

#### Step 22.

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