Extraction Method E (PRP)

Faezah Mohd Salleh,Jazmin Ramos-Madrigal,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 fresh sample (tissue material)

Citation: Faezah Mohd Salleh,Jazmin Ramos-Madrigal,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 E (PRP). **protocols.io**

dx.doi.org/10.17504/protocols.io.inecdbe

Published: 05 Jul 2017

Before start

Clean

Materials

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

Protocol

Extraction

Step 1.

Cut tissue into small pieces.

Extraction

Step 2.

Add 500 µl Lyse 1, 50 µl Lyse 2 and 10 µl Proteinase K.

AMOUNT

50 µl Additional info: Lyse 2

AMOUNT

10 µl Additional info: Proteinase K

Extraction

Step 3.

Incubation in a shaker at 65°C for 12 hours.

Extraction

Step 4.

Centrifuge for 10 min. 13000rpm.

Extraction

Step 5.

Discard supernatant without disturbing the pellet.

Extraction

Step 6.

Add 375 µl Lyse 3

AMOUNT

375 µl Additional info: Lyse 3

Extraction

Step 7.

Vortex for 20 sec.

Extraction

Step 8.

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

Extraction

Step 9.

Centrifuge 20 min. 13000rpm.

Extraction

Step 10.

Transfer the supernatant to a new tube.

Extraction

Step 11.

Add 640 µl Isopropanol.

AMOUNT

640 µl Additional info: Isopropanol

Extraction

Step 12.

Mix by inversion.

Extraction

Step 13.

Centrifuge for 15 min 13000 rpm.

Extraction

Step 14.

Remove the supernatant (don´t touch the pellet).

Extraction

Step 15.

Add 150 µl Ethanol.

AMOUNT

150 µl Additional info: Ethanol

Extraction

Step 16.

Centrifuge for 5 min 13000 rpm.

Extraction

Step 17.

Remove the Ethanol with a pipette (don´t touch the pellet).

Extraction

Step 18.

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

Extraction

Step 19.

Re-suspend the pellet in 100 µl ddH2O water.

AMOUNT

100 µl Additional info: ddH2O

Extraction

Step 20.

Prior to library construction, analyze small aliquots of each extract on Nanodrop (Thermo Fischer Scientific, Darmstadt, DE) for estimation of DNA concentration.