Extraction Method D (PRP and RM)

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

This protocol allows for adequate DNA extraction from archival samples.

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 D (PRP and RM). **protocols.io**
dx.doi.org/10.17504/protocols.io.indcda6
Published: 05 Jul 2017

Before start

Separate PCR-free facility

Materials

- ✓ Buffer AL 19075 by Contributed by users
- Buffer AW1 19081 by Qiagen
- Buffer AW2 19072 by Qiagen
- Buffer AE 19077 by Qiagen
- Buffer ATL 19076 by Qiagen
- ✓ Ethanol by Contributed by users

Protocol

Extraction

Step 1.

Digestion of ca. 50 mg sample with Buffer ATL (Qiagen, Hilden, DE) and Proteinase K overnight at 56 °C.

AMOUNT
50 mg Additional info: Buffer ATL

NOTES

GigaScience Database 26 Jun 2017

Incubation was performed with rotation of the samples in an oven.

Extraction

Step 2.

After pelleting remaining material, remove the supernatant and add to Buffer AL and ethanol.

Extraction

Step 3.

Transfer the solution to DNeasy Mini spin column (Qiagen, Hilden, DE)

Extraction

Step 4.

Centrifuge the solution at 8000 rpm.

Extraction

Step 5.

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

Extraction

Step 6.

Prior to elution, incubate DNA in the membrane with elution Buffer AE for 20 min at 37 °C.

Extraction

Step 7.

Proceed elution by centrifugation at 8000 rpm for 1 min.

Extraction

Step 8.

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