Jul 15, 2024

High Molecular Weight (HMW) DNA Extraction Protocol for Tissue Sample

DOI
dx.doi.org/10.17504/protocols.io.kqdg3xdrpg25/v1

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Protocol Citation: Aswini Leela Loganathan 2024. High Molecular Weight (HMW) DNA Extraction Protocol for Tissue Sample. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg3xdrpg25/v1

Protocol status: Working
We use this protocol and it's working

Created: January 22, 2024

Last Modified: July 15, 2024

Protocol Integer ID: 93868

Abstract

This is an organic extraction protocol used for high molecular DNA extraction for tissue samples. This protocol is suitable for obtaining gDNA for PacBio Sequel IIe library preparation and sequencing.

HMW DNA Extraction Method

9h 12m 10s

1 **Lysis the tissue sample.**
Add 500 µL of lysis buffer to 50 - 150 mg of Sample of sample. Make sure the tissue sample has been finely cut.

2 **Denatures and digest proteins that are subsequently hydrolyzed with Proteinase K.** 10s
Add 10 µL of Proteinase K and vortex for 00:00:10 .

3 Incubate on a shaking incubator at 55 °C for 02:00:00 (or till dissolved). 2h

4 **Remove RNA with RNAse .**
Add 5 µL of RNAse and vortex briefly.

5 Incubate in a shaking incubator for at 37 °C .

6 **Partitioning of lipids and debris into an organic phase using P:C:IA.**
Add 500 µL of P:C:IA (25:24:1). Vortex until an emulsion is formed.

7 Centrifuge at Room temperature for 00:10:00 at 10000 x g . 10m

8 Pipette 200 µL of the aqueous layer into a new tube.

9 **Neutralize the charges on the sugar-phosphate backbone of the DNA with Sodium Acetate.**
Add 1/10th : 20 µL 3M Sodium Acetate. Vortex gently (avoid creating bubbles).

10 **Precipitation Step.**
Add 2 Vol : 440 µL of ice-cold 100% Ethanol. Mix gently and slowly by inverting the tube.

11 Incubate at -20 °C for 02:00:00 . 2h

12 Centrifuge at 10000 x g, Room temperature, 00:02:00 . 2m

13 **Wash Step.**
Wash with ice-cold 70% ethanol.

14 Centrifuge for 00:02:00 at 10000 x g . 2m

15 Repeat **Wash Step** (Step 13 and 14) **2** more times.

16 Air dry for at least 00:30:00 . 30m

17 **Final elution.**
Add 35 µL of elution buffer (EB) and mix gently using a wide bore tips or by tapping.

18 **Storage.**
Store the gDNA at 4 °C