DNA Extraction of Fresh and frozen blood samples

Workflow

DNA Extraction → PCR

Specimen

Type: Fresh and frozen blood samples in EDTA-containing vacutainer tubes.

Materials

Reagents:

RLB (Red blood cell lysing buffer): 0.155 mol /LNH4CL,10mmol/L KHCO3 AND 0.1 mol/L EDTA(Na2) in 1000 ml of distilled water. The PH adjust to 7.6.

Extraction buffer: 1.5 mol/L Tris pH 7.6, 0.4 mol/L disodium salt of ethylenediamine tetra acetic acid (Na2EDTA), pH 8, 2.5mol/L NaCl, 2%, cetyl trimethyl ammonium bromide (CTAB; Merck, Germany) 850ml H2O.Adjust the PH to 8.0 and make the final volume to 1 L.

10% SDS (sodium dodecyl sulfate)

B-Mercaptoethanol

Chloroform: isoamyl alcohol 24:1

Isopropanol

70% and 90% Ethanol (-20°C)

Equipment and supplies:

Microcentrifuge tubes

centrifuge

Pipette tips

Nanodrop

Vortex Mixer

Water/Dry Bath

Safety (Warning and Biohazard consideration):

Prior to commencing, read and understand the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (for example, isopropanol)

Protective clothing, including laboratory coat, gloves and protective glasses, must be worn at all times when performing this procedure.

Procedure:

Step 1:

Transfer 500 µL of fresh blood sample to an eppendorf tube. In case of frozen blood, thaw the sample at room temperature for 20-30 minutes before transferring the blood to the eppendorf tube.

Step 2:

Centrifuge the sample at 6000 rpm at 4°C for 10 min. Aspirate the plasma without touching the leukocyte layer.

Step 3:

Add 1 mL RLB and mix gently, Centrifuge the sample for 5 min at 3000 rpm. Remove the supernatant. Repeat this step 1-2 times until a white colored pellet is obtained.

Step 4:

Add 500 µL prewarmed DNA extraction buffer to the pellet followed by 30 µL of 10% SDS and 2 µL of B-Mercaptoethanol respectively and mixed gently, Incubate the tube at 56-60 °C for 60 min.

Step 5:

Add 500 µL of Chloroform-Isoamilalcohol solution (24:1) to the mixture after incubation and shaken well, then centrifuge the tube at 12000 rpm for 8 min at 4°C

Step 6:

Transfer the supernatant to a new tube, add an equal volume of chilled ethanol, shake the tube until fine white threads appear in the solution. Keep the sample in -20 °C for 30 min.

Step7:

Centrifuge the tube at 4 °C, 12000 rpm for 10 min. Discard the supernatant and add 500 µL ethanol 90%

Step8:

Centrifuge the tube at 4 °C, 12000 rpm for 5 min.

Step9:

Repeat Step 8 and 7 with 500 µL ethanol 70%

Step 10:

Discard the supernatant and let pellet to be dried at room temperature.

Step 11:

Dissolve the pellet in 100 µL of TE buffer or dH2O and store DNA solution at -20 °C

References:

A rapid and efficient DNA extraction protocol from fresh and frozen human blood samples

Pokhraj Guha ,Avishek Das ,Somit Dutta ,Tapas Kumar Chaudhuri

شناسنامه سند: CoreLab.106

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شرح سند	روش انجام استاندارد استخراج DNA از نمونه خون تازه و فریز شده را شرح می‌دهد.
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