SNP Analysis 1: DNA Extraction

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Abstract

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Before start

This protocol is derived from

[Reference: GeneMATRIX Quick Blood DNA Purification Kit, version 1.2, EURx company]

Protocol

Activation of column

Step 1.

Apply 40µl of activation Buffer QB onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

***Warning #1**

Addition of Buffer QB onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.

***Warning #2**

The memrane activation should be done before starting isolation procedure.

Sample preparation

Step 2.

Add 200µl blood/body fluid into a new 1.5-2 mL tube.

Add 10µl Proteinase K and next 200µl Sol QB buffer.

Vortex the mixture thoroughly and incubate for 20 min at 60°C inside an incubator.

Add 200µl 96**%** ethanol in the same tube of DNA sample.

Vortex the mixture throughly and centrifuge for 1 min at 12000 rpm.

***Warning #3**

For sample volumes less than 200µl, add PBS to adjust the volume to 200µl.

***Warning #4**

If RNA-free DNA is crucial for downstream applications, add 2µl RNase A**.** Mix by vortexing and incubate 5 min at room temperature.

***Warning #5**

If purifying DNA viruses, it is recommended to start with 200µl serum or plazma to prepare pure viral DNA (cellular DNA-free)

Washing of lysate DNA

Step 3.

Transfer the lysate sample into the spin-column and place in a new clean collection tube.

Centrifuge for 2 min at 12000 rpm.

Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

Add 500µl Wash QBX1 buffer to the spin-column and centrifuge for 1 min at 12000 rpm.

Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

Add 500µl Wash QBX2 buffer to the spin-column and centrifuge for 2 min at 12000 rpm.

*** Warning #6**

Continue centrifugation at all steps, if not all of the sample passed through the column.

Collection of DNA

Step 4.

Place the spin-column in a new collection tube (1.5-2 mL)

Add 50-200 µl of Elution buffer (10 mM Tris-HCl, pH 8.5) heated to 60°C to elute bound DNA in column.

Incubate the spin-column/collection tube assembly for 5 min at room temperature with the cap is closed.

Centrifuge the spin-column for 1 min at 12000 rpm.

*** Warning #7**

Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-column do not touch the spin column walls with the micropippete.

*** Warning #8**

The researcher is free to use other types of Elution solutions regarding further use of DNA sample.

*** Warning #9**

The elution can be repeated once again as described in steps 15-17**.** This step improves DNA recovery from the column. A new collection tube can be used

to prevent dilution of the first eluate or collection tube from step 15 can be reused to combine the eluates.

*** Warning #10**

More than 200µl should not be used to elute into a single 1.5 ml microcentrifuge tube, as the spin-column will come into contract with the eluate, causing DNA contamination.

Storage of DNA sample

Step 5.

Discard the spin-column, cap the collection tube, label as appropriate.

DNA isready for further experiment

It can be stored either at 2÷8^{o}C or at -20^{o}C.

Warnings

* All the related steps must be done in a biotech lab using appropriate clothing and equipment.

* All centrifuging steps must be performed by an expert.

* Please learn well about the hazards of all chemical used in this protocol.