SNP Analysis 2: PCR product Clean–Up

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Abstract

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Before start

This protocol is derived from
[Reference: GeneMATRIX Basi DNA Purification Kit, version 1.0, EURx company]

Protocol

Activation of column

Step 1.

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

*** Warning #1**

Addition of Buffer Uni onto the center of the resin enables complete wetting of memranes and maximal binding of DNA.

*** Warning #2**

The membrane activation should be done before starting isolation procedure.

Sample preparation

Step 2.

This protocol is suggested for the purification of PCR product if necessary.

Add 400µl of Basic buffer to DNA sample and mix.

Pour the mixture into spin-column/receiver tube assembly.

Spin down in a centrifuge at 11 000 x g for 1 minute.

*** Warning #3**

Maximum volume of an initial DNA sample can not exceed 200µl.

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Washing of sample DNA

Step 3.

Remove spin column, pour the supernatant off, replace back the spin-column and place into centrifuge.

Add 500 µl of Wash UX1 buffer and spin down at 11 000 x g for 1 minute.

Remove spin column, pour the supernatant off, replace back the spin-clumn.

Add 650 µl of Wash UX2 buffer and spin down at 11 000 x g for 1 minute.

Remove spin column, pour the supernatant off, replace back the spin-column.

Spin down at 11 000 x g for 2 minutes to remove traces of Wash UX2 buffer.

Collection of sample DNA

Step 4.

Place spin-column into a new clean receiver tube (1.5-2 mL). Add 50-150 µL of Elution buffer to elute bound DNA from column.

Incubate spin-column/receiver tube assembly for 2 minutes at room temperature.

Spin down at 11 000 g x for 1 minute.

*** Warning #4**

Addition of eluting buffer directly onto the center of the membrane improves DNA yield.

To improve recovery of larger DNA fragments (above 5 kb) it is recommended to elute with buffer heated to 80ºC.

*** Warning #5**

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

*** Warning #6**

It is possible to reduce the volume of eluting buffer below 50 µl (no less than 20µl). However, recovery of DNA will gradually decrease.

Storage of DNA sample

Step 5.

Remove spin column, cap the receiver tube, and label as appropriate.

Isolated DNA is ready for further experiments.

It can be stored at 2-8°C or (preferred) at -20°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.