Molecular Biological System Transfer

Genomic DNA Isolation from Plant Material

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Introduction

The MBST DNA extraction kit provides a fast and simple method for the isolation of DNA from eukaryotic cells. Purification requires neither phenol and chloroform extraction nor ethanol precipitation.

DNA purification is grounded on a selective binding of nucleic acids to a silica-based membrane in the presence of chaotropic salts. Non-nucleic acids elements do not bind to the membrane and will be removed by centrifugation.

DNA is then purified of residual contaminants by passing the wash buffer twice through the membrane. DNA is then eluted in the elution buffer, and is ready for use for enzymatic reactions such as restriction analysis or PCR.

Warning: For research use only. The products listed in this manual are not for diagnostic or therapeutic use in humans or animals.

Protocol for DNA extraction from Plant material

- **Prepare a 55°C water bath**

- **Add 1.1 ml of distilled water to the proteinase K and mix well. The proteinase K solution should be stored at –20°C. It is recommended to store proteinase K in aliquotes of 120 – 220 µl at –20°C.**

If a precipitate has formed in lysis buffer, dissolve it by incubation at 50°C

- **Add 26 ml of ethanol (96-100%) to wash buffer (WB) before using the first time. The wash buffer (WB) is stable for several months at room temperature when properly stored (tightly closed).**

1- froze the fresh leaf material (up to 4 $cm^{2,}$ or up to 100 mg) in a liquid nitrogen bath until it is very brittle and is ground to a fine powder using a glass homogenizer **(If liquid nitrogen is not available, the leaf material can be homogenized using tweezer in the 300 µl lysis buffer and continue with the step 3)**.

(You can use instead leaf, up to 100 mg powdered seed or other plant material)

2- Transfer the powder into the clean eppendorf tube containing 300 µl lysis buffer.

3- add 20 µl proteinase K into the tube, mix by vortexing and incubate for 15 min at 55ºC

5- add 580 µl binding buffer to the tube mix by vortexing and incubate for 10 min at 70ºC

6- centrifuge for 1 min by 8000 x g and transfer the supernatant into the clean 1.5 ml eppendorf tube

7- place a spin column^{MBST} (A) in a 1.5 ml eppendorf tube. Apply the mixture from step 6 to the spin column^{MBST} (A), close the cap and centrifuge at 8.000 x g for 1 min. Remove the spin column^{MBST} (A)

(the filtration can be also performed in two steps)

and add 440 µl ethanol (100%) into the tube and mix thoroughly the tube containing the infiltrate.

8- apply the half of the mixture from step 7 into the new spin column^{MBST}(B) and centrifuge at 8.000 x g for 1 min. Remove the infiltrate and apply the other half of the mixture into the same column and centrifuge again for 1 min. at 8.000 x g. Place the spin column^{MBST}(B) in a new eppendorf tube

9- add 500 µl of wash buffer (WB) to the spin column^{MBST}(B) and centrifuge at 8.000 x g for 1 min. Place the spin column^{MBST}(B) in a clean eppendorf tube and discard the tube containing the infiltrate

10- place the spin column^{MBST}(B) in a clean eppendorf tube

11- again add 500 µl of wash buffer (WB) to the spin column^{MBST}(B). Centrifuge at 8.000 x g for 1 min. and discard the tube containing the infiltrate

12- place the spin column^{MBST} (B) in a clean eppendorf tube and centrifuge at full speed for a further 2 min to remove the ethanol completely. Place the spin column^{MBST}(B) in a clean eppendorf tube and discard the tube containing the infiltrate

13- add 35- 50 µl of elution buffer preheated to 70°C to the spin column^{MBST}(B), incubate at room temperature for 3 min., then centrifuge at 8.000 x g for 1 min.

14- repeat step 13 with the same solution in the tube from step 13

Quantitation and determination of purity of DNA:

DNA concentration should be estimated by agarose gel electrophoresis or determined in eluates at 260nm in a spectrophotometer blanked against elution buffer using the following formula:

[DNA (µg/ml)] = A_{260} x D (dilution factor) x 50

Ratio of absorbance at 260nm to absorbance 280nm determines the purity of DNA. $A_{260/280}$ is for pure DNA between 1.7 to 1.9.

Cautions:

The researcher is requested to handle with the chemical reagents in this kit with absolute care.

Please wear gloves and avoid contact with eyes, skin, and clothing.

Components

The MBST DNA extraction kit contains sufficient reagents for 50 DNA preparations.

Components:

Lysis buffer	16 ml
(If a precipitate has formed, dissolve by incubating at 50°C)	
Binding buffer	29 ml
Proteinase K	10 mg
Wash buffer	26 ml
Elution buffer	20 ml
Column A	50
Column B	50

Elution buffer is:

10 mM Tris-Cl pH 7.4 and 1 mM EDTA pH 8.0

All buffers and columns^{MBST} should be stored at room temperature. Proteinase K should be stored dry at 4°C and dissolved at -20°C in small aliquots.

Additional material required:

Ethanol (96%-100%)

Microtubes