A Recommended Procedure for DNA Extraction from Plant Tissues

Monsanto Biotechnology Regulatory Sciences

Overview

Purpose & Scope

This procedure describes a method to extract high quality genomic DNA from processed plant tissues (e.g., leaf, grain, or seed).

Monsanto has optimized and performed internal validation on these test methods using the protocols, procedures, conditions, equipment, reagents and test matrix described in the method protocols. While this method protocol has been verified to a high standard in Monsanto's laboratories, variations in laboratory conditions and capabilities require that this protocol must be considered only as a guideline for other users of this method. Each laboratory and user must validate each method protocol in their individual applications and in their laboratory, and conduct appropriate proficiency testing to establish the reliability, accuracy and reproducibility of the method for that use in that laboratory.

Important Precautions

- Follow all appropriate safety procedures. Safety glasses should be worn at **all times** in the laboratory. It is recommended to wear protective gloves during the entire procedure to protect the DNA from **contamination** with DNase from the skin.
- Chloroform is **dangerous** if inhaled and should be used under a fume hood.
- Gloves should be worn throughout this entire procedure to protect oneself from **irritant chemicals** and the DNA from **contamination** with DNases from the skin.

Abbreviations

The following abbreviations are used in this procedure:

Abbreviation	Definition
CTAB	hexadecyltrimethylammonium bromide
EDTA	ethylenediaminetetraacetic acid
H_2O	purified water appropriate for laboratory use (e.g., milli-Q, US filter)
RT	room temperature
Tris	tris(hydroxymethyl)aminomethane
TE	Tris EDTA

Contents

A Recommended Procedure for DNA Extraction from Plant Tissues

Monsanto Biotechnology Regulatory Sciences

DNA Extraction

Procedure Following are the steps to extract the DNA.

Note: Exact amounts and parameters listed (excluding incubation temperatures) are suggested. Volumes may be adjusted for the amount of tissue used. Tissue should be processed prior to extraction procedure. Possible methods of processing include a mortar and pestle with liquid nitrogen (leaf) or a commercial blender (grain or seed).

Step	Action
1	Weigh out 6 g of processed tissue into a 50 ml conical tube appropriate for centrifugation. **Note:** For unprocessed tissue, weighing may occur prior to processing as long as entire processed sample is transferred to the conical tube.
2	For each 6 g sample add 25ml of a solution consisting of 24.25 ml, pre-warmed (55°C) CTAB extraction buffer, 0.5 ml 2-mercaptoethanol (2-ME), and 0.25 ml of 10 mg/ml proteinase K for a final concentration of 2% (2-ME) and 100 ug/ml (proteinase K).
3	Incubate the tube for 60 minutes at 55°C. Cool the tube briefly on bench (10 minutes)
4	Add 20 ml of phenol:chloroform:isoamyl alcohol (PCI, 25:24:1). Cap the tube and mix vigorously by vortex or inversion.
5	Centrifuge for 10 minutes at 13,000 *x g* and 20-25°C to separate the aqueous and organic phases. Transfer the upper aqueous phase to a clean 50 ml conical tube.
6	**Repeat extraction two times for a total of three extractions (steps 4-5).**
7	Transfer upper aqueous phase to a new tube and add approximately 2/3 volume of -20°C isopropanol and gently invert the tube several times to mix.
8	To precipitate the DNA place the tubes at -20°C for at least 30 minutes and up to three days.
9	To pellet the DNA centrifuge the tubes at approximately 13,000 *x g* for 20 minutes at 4°C.
10	Redissolve the pellet in 4 ml of TE pH 8.0. Transfer to a 13-ml Sarstedt tube and add approximately 40 ul of 10 mg/ml RNase, then incubate at 37°C for 30 minutes.
11	To extract the DNA add 4 ml of chloroform:isoamyl alcohol (CIA, 24:1). Centrifuge for 10 minutes at approximately 13,000 *x g* at room temperature. Transfer the upper aqueous phase to a clean Sarstedt tube.
12	Repeat step 11 then add half volume of 7.5M ammonium acetate, gently mix by inversion/ pipette and add 2 volumes of 100% ethanol. Mix by inversion/ pipette and place at -20°C for 30 minutes to overnight.
13	Centrifuge at 13,000 *x g* for 20 minutes at 4°C to pellet the DNA.

A Recommended Procedure for DNA Extraction from Plant Tissues

Monsanto Biotechnology Regulatory Sciences

Procedure (Continued)

14	Rinse the DNA pellet twice with 70% ethanol and remove residual ethanol by vacuum.
15	Resuspend DNA in 1 ml TE, pH 8.0 and incubate at 65°C for 1 hour with periodic gentle mixing.
16	Centrifuge the DNA solution at 16,000 *x g* for 10 minutes at 4°C. Transfer the aqueous portion to a clean tube without disturbing the pellet and store at 4°C.

Attachments

Materials

Equipment The following equipment are used in this procedure (**equivalents may be substituted)**

Equipment	Number/Specification
Centrifuge	Beckman Coulter Avanti J-251
Centrifuge tubes: • 50 ml conical • 13 ml Sarstedt	 • Corning Cat. No. 430290 • Sarstedt Cat. No. 60.540
Shaker	LabLine Enviro 3527
Thermometer	VWR Cat. No. 61222-504
Vacufuge	Eppendorf 5301 22 82 010-9
Water bath	Precision Cat. No. 51220046

Reagents The following reagents are used in this procedure (**equivalents may be substituted**)

Reagent	Number/Specification
24:1 chloroform:isoamyl alcohol	Sigma Cat. No. C-0549
25:24:1 phenol:chloroform:isoamyl alcohol	Sigma Cat. No. P-3803
Ammonium acetate 7.5M	Sigma Cat. No. A-2706
CTAB	Sigma Cat. No. H-6269
0.5 M EDTA, pH 8.0	GibcoBRL Cat. No. 15575-038
100% ethanol	AAPER
NaCl	Sigma Cat. No. S-5150
2-mercaptoethanol	Bio-Rad Cat. No. 161-0710
10 mg/ml RNase A	Roche Cat. No. 109 169
Isopropanol	EM Science Cat. No. PX1835-9
1 M Tris HCl pH 8.0	Sigma Cat. No. T-3038
Proteinase K	Roche Cat. No. 3 115 836
RNase	Roche Cat. No. 1 579 681

Buffers and Solutions

Overview	The following describes the preparation, storage, and stability of the buffers and solutions used in this procedure. **Note:** Volumes may be scaled as needed. Equivalent reagents may be substituted. To filter sterilize, vacuum filter through a maximum 0.45 micron filter into a sterile receptacle.
CTAB Extraction Buffer	**CTAB Extraction Buffer (2%) -** For 500 ml: • 10 g CTAB (1.5% w/v) • 50 ml 1 M Tris HCl, pH 8.0 (75 mM) • 20 ml 0.05 M EDTA, pH 8.0 (100 mM) • 140 ml NaCl (5M) • 290 ml H_2O Store at RT for up to 5 years.
TE Buffer, pH 8.0	**TE Buffer, pH 8.0** - For 250 ml: • 2.5 ml 1 M Tris HCl, (10 mM) • 0.5 ml 0.5 M EDTA, (1 mM) • Add H_2O to 250 ml • Filter sterilize Store at RT for up to 5 years.
70% (v/v) Ethanol	**70% (v/v) Ethanol** - For 200 ml: • 140 ml100% ethanol • 60 ml H_2O Store at RT for up to 5 years.
Proteinase K	**Proteinase K 10 mg/ml** - For 5 ml: • 5 ml H_2O • 0.05g proteinase K Store at -20°C for up to 2 years.
RNase	**RNase 10mg/ml-** For 5 ml: • 5 ml H_2O • 0.05 g • Boil for 10 minutes Store at -20°C for up to 2 years.