

ISOLATION OF DNA FROM PLANT TISSUES CONTAINING POLYPHENOLICS AND POLYSACCHARIDES

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High quality DNA can be isolated directly from most plants with DNAzol®ES (Cat. No. DN 128). However, some plant tissues contain high concentrations of reactive polyphenolic and polysaccharide contaminants that co-isolate with DNA.

I. REMOVAL OF POLYPHENOLICS

The disruption of some plant cells may release reactive phenolic compounds which oxidize rapidly to form phenoxides that undergo irreversible electrophilic substitution reactions with proteins and nucleic acids. DNA extracts that are contaminated with polyphenolics may contain a sticky, dense, colored gelatinous residue that cannot be separated from the DNA. In order to extract higher quality DNA from these plant tissues, we recommend supplementing DNAzol ES with polyvinylpyrrolidone(PVP).

Required Reagents: 2-Mercaptoethanol (2-ME). Polyvinylpyrrolidone (soluble 10,000 Mw). Prepare a 10 % stock solution (w:v) by adding 1.0 g of PVP to 9.0 ml of DNAzol ES. This 10% PVP-DNAzol ES stock solution is stable for at least 1 month.

PROTOCOL

1. Dilute the 10% PVP-DNAzol ES stock solution with DNAzol ES to obtain a 2% PVP-DNAzol ES solution.
2. Immediately before use, add 2-mercaptoethanol to the 2% PVP-DNAzol ES solution to achieve a 2-mercaptoethanol final concentration of 1% (V:V).
3. Pulverize 1 g of the plant tissue according to the DNAzol ES protocol. Mix the plant tissue with 3.0 ml of the 1% 2-ME, 2% PVP-DNAzol ES working solution. Perform DNA isolation according to the DNAzol ES protocol.

NOTE: Plant tissues are very heterogeneous and the optimal concentration of PVP may vary for different plant tissues. A 2% PVP-DNAzol ES solution is most versatile but experiments may be performed using a concentration range of 1 - 5 % PVP to identify the optimal quantity of PVP for a specific plant tissue. Please note that increasing PVP concentration may increase DNA purity, but excessive amounts of PVP will significantly decrease DNA yield.

II. REMOVAL OF POLYSACCHARIDES

Some plants contain large quantities of polysaccharide that may co-isolate with DNA. Extracts that contain polysaccharides are usually very viscous and these contaminants can be removed during DNA isolation by sedimentation at low temperature (See Note 4 of the DNAzol ES protocol). If the sedimentation at low temperature step was not employed, polysaccharide contaminants can be removed from the solubilized DNA / NaOH extracts by low salt-ethanol precipitation (Michaels, S.D., M.C. John and R. M. Amasino. *BioTechniques* 17:274,1994).

Required Reagents: Prepare a 10X salt solution containing 2.5 M NaCl, 20 mM EDTA and 0.5 M Tris-HCl, pH 8.0.

PROTOCOL

1. Perform DNA isolation according to the DNAzol ES protocol and solubilize DNA in 8 mM NaOH.
2. Mix 1 volume of the 10X salt solution with 9 volumes of DNA solubilized in 8 mM NaOH.
3. Precipitate polysaccharides while maintaining the DNA in solution. With continuous mixing, slowly add 0.35 volumes of ethanol to the DNA-salt solution. Ethanol must be added slowly and with mixing to avoid local elevations in ethanol concentration that can precipitate DNA. Incubate the mixture for 15 minutes on ice and centrifuge at 10,000 g for 10 minutes at 4 C.
4. Transfer the supernatant containing the DNA to a clean tube and add an equal volume of isopropanol to precipitate the DNA. Mix the samples by inversion, incubate for 5 minutes at room temperature and pellet the DNA by centrifugation at 6,000 g for 4 minutes at 4 - 25 C.
5. Decant the supernatant and wash the DNA pellet for 5 minutes with 1 ml of 70% ethanol.
6. Sediment the DNA for 4 minutes at 6,000 g, decant the supernatant and solubilize the DNA in 8 mM NaOH as outlined in the DNAzol ES protocol.