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Genomic DNA Preparation from RNAlater™ Preserved Tissues Protocol

*Copied from http://www.ambion.com/techlib/misc/genomicDNA_rnalater.html

Required Materials

Razor blade or scissors
55ºC incubator
Nuclease-free 2.0 ml microfuge tubes
Nuclease-free water
1 M Tris, pH 8.0
Buffer saturated phenol
0.5 M EDTA
Chloroform
10% SDS
3 M Sodium acetate, pH 5.2
Proteinase K
95% Ethanol

Protocol

Prepare digestion buffer:
60 mM Tris, pH 8.0
100 mM EDTA
0.5% SDS

Remove tissue from RNAlater and mince well. Cutting the tissue into very small pieces is absolutely essential to achieving adequate digestion. Place in a 2.0 ml eppendorf tube with 1.5 ml digestion buffer.

Add Proteinase K to a final concentration of 500 µg/ml. Mix by inversion. Incubate the sample at 55ÆC for approximately 4 hours with rotation. A standard hybridization oven and bottle can be used for the digestion. Simply tape the tubes to the bottle allowing enough room for clearance and set the oven temperature to 55ÆC. Samples should be allowed to digest until little or no cellular matter is visible.

Remove the tubes from the oven and divide each sample into 2 even aliquots (approximately 750 µl each). Add 750 µl 50:50 phenol:chloroform and invert rapidly (do not vortex) for 2 minutes in an eppendorf rack. Spin samples on high in a microcentrifuge for 10 minutes at room temperature. Remove aqueous (upper) phase to a new 2 ml tube using a 1 ml pipet tip with the end cut off. The wide bore will prevent shearing of the DNA. Be careful not to disturb the interface (it may be gooey). Repeat phenol:chloroform extraction 2 more times. If interface is still dirty, additional extractions are recommended.

Extract the sample once with chloroform. Remove aqueous (upper) phase to a fresh 2 ml tube and measure volume. Add 1/10 volume 3M sodium acetate, pH 5.2, and 1

volume 95% ethanol at room temperature. Mix by inversion. DNA should spool. Remove DNA with a pipet tip to a fresh tube. Wash DNA in 70% ethanol by inversion, do not vortex. After washing for 5 minutes, pull off 70% ethanol and let pellet air dry on bench overnight.

Add an appropriate volume TE, pH 7.4, to each pellet. For approximately 100 mg original tissue, pellet can be resuspended in 100 to 200 µl TE. Let sit in refrigerator overnight to resuspend. Alternatively, pellet and TE can be GENTLY shaken at room temperature for a few hours (for example on a rocking platform). Time for resuspension is dependent on the pellet size. DNA SHOULD NOT BE RESUSPENDED BY PIPET OR VORTEX, AS IT WILL SHEAR!!!

DNA can be quantitated by spectrophotometry. Read a 1/20 dilution of the sample in TE at A260 and A280. Remember to use TE as a blank to zero the spectrophotometer. Use the following equation to calculate µg/ml DNA:
A260 x dilution factor x 50 = µg/ml