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Protocol: fast and high-performance Eucalyptus DNA extraction in 96-well plate method

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Forestry genetics and genomics require a great number of samples to produce accurate results. This is a simple method with high-throughput DNA extraction that combined CTAB protocol with fast rupture tissue and centrifuge steps in plates to obtain good yield, quality and low price. Approximately 576 samples can be processed for day. The obtained DNA is suitable for use in PCR-based study like Microsatellites. This method employs 96 Racked Collection Microtubes (Qiagen). *Eucalyptus* tissue 15mg was placed in each well together with a tungsten bead and 420ul of CTAB2X buffer distributed with a multichannel pipette, the samples were disrupted to full power in a TissueLyser (Qiagen) for 3min. Then we added 360ul of CIA24:1 to each tube and both racks were centrifuged at 4000 RPM for 20min (ThermoScientific SorvallST16). Afterwards the upper phases were transferred carefully to clean rack-wells and 150ul of isopropanol were added to each well and centrifuged at 4000RPM for 20min. The liquid phases were removed and the pellets were washed twice with ethanol 70% and centrifuged at 4000RPM for 5min. Finally the pellets were resuspended in 100ul of RNAse and incubated at 37°C for 60min. To evaluate the yield and purity, this prococol was compared against results obtained with four commercial extraction kits: DNeasy Plant Handbook® (Qiagen), Power Plant DNA® Isolation Kit (MOBIO), Wizard® Genomic DNA Purification Kit (Promega), ZR Plant / Seed DNA MiniPrep® (Zymo). The procedure of rupture was the same for all protocols and in all cases racks of 96 wells were used. The data of absorption spectrum indicate an average concentration of 400ng/ul for CTAB protocol, Qiagen 150ng/ul, Wizard 165ng/ul MOBIO 85ng/ul for Zymo. Comparing this method to the commercial is demostrated that the final concentration superior, the time os processing is lower, also it is considerable cheaper.