DNA extraction for rainforest plant species

modified from Scott & Playford 1996
Jer-Ming Hu Aug 1st, 1996

Grind 0.2g plant material using liquid N2 or using Ottawa sand (.2g)
+ 4ml Extraction Buffer at RT
Ú
filter w/ Miracloth into eppendorf tube, spin 14000X rpm for 5 min
Ú
remove supernatant, add remain filtrate and repeat the last step
Ú
resuspend pellet w/ 400ml Wash Buffer
Ú
+ 100ml 5% Sarkosyl, incubate at RT for 15 min
Ú
(add b-mercaptoethanol to CTAB Buffer, preheat at 55ºC)
+ 1 ml CTAB Buffer, incubate at 55ºC for at least 30 min
Ú
centrifuge 14000X rpm for 5 min, take supernatant and separate to two tubes
Ú
+ 700ml CI (chloroform/isopropanol)
Ú
centrifuge 10000X for 8 min, tranfer supernatant to new tube
Ú
+ 10ml RNase, incubate at 37ºC, 15 min
Ú
+ 65ml 7.5M NH4OAc
Ú
+ 700ml ice-cold 95% ethanol, put into -80ºC for 30 min (or -20ºC overnight)
Ú
4ºC centrifuge for 15 min, air dry, resuspend pellet in 50ml TE

Reference:

Scott, K.D. and J. Playford 1996. DNA extraction technique for PCR in rain forest plant species. BioTechniques 20(6): 974-978.