3% CTAB DNA extraction protocol

(modified from Zeng et al. 2002, Acta Botanica Sinica 44; 694-697)

- grind frozen tissue
- add 1 ml of CTAB-free buffer (cold) + 6 μl β-mercaptoethanol
- mix by inversion, keep 10 minutes on ice
- spin at 10.000 g for 10 minutes
- discard the supernatant
- (if you use particularly nasty tissue, you can repeat this step)

- add 500 μl of pre-warmed 3% CTAB + 5 μl of β-mercaptoethanol
- incubate at 65°C for 30-60 minutes in a water bath
- let the tubes cool down a little bit
- add 500 μl of chloroform-isoamylalcohol 24:1, vortex
- spin at full speed for 5 minutes
- move the aqueous phase to a new tube

- add 0.1 volumes of NaCl 5M, mix
- add 0.7 volumes of cold isopropanol, mix
- leave 20 minutes at -20°C
- spin at max speed for 15 minutes
- wash pellet with 1 ml of cold 75% ethanol
- spin at max speed for 5 minutes
- repeat wash
- dry pellet

- resuspend in 400 μl of high salt TE + 2 μl of RNAseA (20 mg/ml)
- incubate at 37°C for one hour, mixing from time to time until the pellet dissolves
- add 800 μl of cold 100% ethanol
- (optional: put at -20°C for 20 minutes)
- spin at max speed for 15 minutes
- wash pellet with 1 ml of cold 75% ethanol
- spin at max speed for 5 minutes
- repeat wash
- dry pellet and resuspend in 100 μl of TE (or more, as you prefer)

CTAB-free buffer

Tris-HCl pH 8.0	200 mM
EDTA	50 mM
NaCl	250 mM

3% CTAB buffer

Tris-HCl pH 8.0	100 mM
EDTA	25 mM
NaCl	1.5 M
CTAB	3% (w/v)
PVP	1% (w/v)

High salt TE

1 M NaCl in TE buffer