

Genomic DNA Miniprep Method

note: (o.n. 4°C) = steps indicated like this can be extended overnight without negative impact on the results

- 1 place 300 mg of fresh leaf material into plastic bag, add 1.2 ml of **2*CTAB-buffer** add 2% of β -Mercaptoethanol just before use, seal the bag and macerate.

(o.n. 4°C)
- 2 incubate bags containing macerated leafs for at least 60 min at 65°C (can be extended for several hours)
- 3 cut the edge of the bag and empty the solution (~900 μ l) into a 2.2 ml microfuge tube
- 4 add 800 μ l **Chloroform:Isoamylalcohol** (24:1)

(o.n. 4°C)
- 5 place the tubes into rack and then into overhead-shaker (half-speed) for 15 min
(after shaking, o.n. 4°C)
- 6 centrifuge for 15 min at 10'000 x g (13'000 rpm, Heraeus Biofuge pico)
- 7 take 800 μ l of the supernatant and transfer into 1.5 ml microfuge tube
- 8 add 5 μ l **RNase A solution**, mix by inverting the tubes, incubate for 15 min at 37°C
- 9 precipitate the DNA by adding 560 μ l **Isopropanol** (0.7 Vol !) and inverting the tube until well mixed (do this at room temperature to prevent extensive precipitation of carbohydrates)
- 10 to pellet the DNA centrifuge for 10 min at 10'000 x g (13'000 rpm, Heraeus Biofuge pico) at 4°C (a white pellet should be clearly visible at this stage)
- 11 remove the supernatant by aspiration (use disposable yellow tip attached to a vacuum line; be careful, the pellet is only loosely attached to the wall of the tube!)
- 12 add 1 ml of **Wash solution I** and incubate for 15 min

(o.n. 4°C)
- 13 aspirate the supernatant carefully (see step 11) and replace by 1 ml of **Wash solution II**
- 14 short incubation (~ 5 min, if pellet remains for a too long period of time in wash II it becomes translucent and is difficult to see while aspirating wash II) and aspiration of **Wash solution II**
- 15 short spin and aspiration of the remaining traces of **Wash solution II**
- 16 dry the pellet 5-10 min at room temperature (or 1 min in speedvac). If pellet is overdried dissolving of the DNA is difficult and takes some time.
- 17 dissolve the pellet in 80-100 μ l **TE-buffer** (pH 8.0), measure DNA concentration by UV-absorption at $A=260$ nm. (If many samples are going to be processed estimate DNA conc. of some samples and dissolve the pellets in the following in an appropriate volume to end up with a working-DNA-solution and to avoid further estimation of DNA conc and dilution steps)

Solutions:

2*CTAB

2% CTAB (Hexadecyltrimethylammoniumbromid, F 52369, FLUKA AG, Switzerland)	20 g
200 mM Tris/HCl (pH 8.0)	200 ml of 1 M stock
20 mM EDTA	40 ml of 0.5 M stock
1.4 M NaCl	81 g
1.0% PVP (Polyvinylpyrrolidone K30)	10 g

adjust to 1 l with sterile ddH₂O, sterile filtrate solution,

add 20 ml of BME just before use 0.28 M β-Mercaptoethanol (BME) (Mr=78.13; d=1.115)

RNase A [10 mg/ml]

RNase A (Fluka 83832)	200 mg
10 mM sodium acetate pH 5.2	60 µl 3 M stock
100 mM Tris/HCl pH 7.4	2 ml of 1 M stock

dissolve RNase A in 18 ml 0.01 mM NaAc and boil in water bath for 30 min, cool down slowly to room temperature, add 0.1 vol 1M Tris/HCL, check remaining DNase activity and store in aliquots at -20°C

Wash solution I

76% Ethanol	760 ml of 100% Ethanol
200 mM Sodium acetate (anhydrous)	66.7 ml of 3 M NaAc
	adjust to 1 l with sterile ddH ₂ O

Wash solution II

76% Ethanol	760 ml of 100% Ethanol
10 mM Ammonium acetate	771 mg NH ₄ Ac
	adjust to 1 l with sterile ddH ₂ O

TE-buffer

10 mM Tris/HCl pH 8.0	10 ml of 1 M stock
1 mM EDTA	2 ml of 0.5 M stock
	adjust to 1 l with sterile ddH ₂ O

Literature:

Stein N., G. Herren and B. Keller (2001) A new DNA extraction method for high-throughput marker analysis in a large genome species like *Triticum aestivum*/ L. Plant Breeding 120, 354-356.