

QUICK METHOD OF DNA EXTRACTION IN 96-WELL FORMAT

1. Use the special 96 well 2mL deep plates (Phenix-MPI-009) (always use the plate with one cut corner) for DNA extraction using quick method.
2. Cut about one inch long fresh leaf into 2-3 pieces and place into plate well and cover the plate with kimwipe, then put the plate into lyophilizer for 3-4 days.
3. Using the bead tray add medium beads into the plates and using qiagen clamp, place the plates into qiagen mixer mill for grinding the tissue for 5-10min at 25 cycles/sec speed.
4. Using 8 channel pipette add 750 μ L of CTAB or SDS buffer solution (60°C) and place the plate into levitation machine (preheated to 60°C) and run at 60°C for 1-1.5 hrs.
5. Let the plate cool down to room temperature and add 750 μ L (or equal volume) of Chloroform:Octanol (24:1) for CTAB method or Chloroform:Isoamyl alcohol (24:1) for SDS method. Mix thoroughly using levitation machine for about 30 min at room temperature (heater off). Centrifuge plate at room temperature at 4000 rpm for 15 minutes.
6. Transfer the supernatant (using 8 channel pipette with wide bore tips) to a new two cut corner plate and add 2/3 volume of isopropanol (room temp) for CTAB method or 2 volumes of chilled absolute ethanol (for SDS method). Mix the samples by pipetting in and out gently using wide bore tips for precipitation.
7. Centrifuge plate at room temperature at 4000 rpm for 10 minutes and drain the solution by retaining the DNA pellet in the plate. Wash DNA with 1mL of 70% ethanol. Leave at room temperature for 30 minutes, plate can be left overnight in 4°C for washing.
8. Quick spin for 4 min and drain the ethanol, air dry the DNA pellet, and add 200-300 μ L of 1mM Tris and 1 μ L of RNase (10mg/mL). Leave at 4°C overnight before use. This method would yield about 30-50 μ g of DNA that can be used for PCR analysis for more than one year.

CTAB EXTRACTION BUFFER

Stock	10 mL	50 mL	200 mL	750 mL	1500 mL
dd H ₂ O	7.8 ml	39	146	585	1170
2M Tris pH 7.5	0.5 ml	2.5	20.0	37.5	75
5M NaCl	1.4 ml	7.0	28.0	105	210
0.5 M EDTA pH 8.0	0.2 ml	1.0	4.0	15	30
CTAB	0.1 g	0.5	2.0	7.5	15
14M BME	0.1 ml	0.5	2.0	7.5	15

CTAB = Cetyl Trimethyl Ammonium Bromide

BME = 2- mercaptoethanol: Add to warmed buffer just prior to use in the hood.

SDS EXTRACTION BUFFER

Stock	75mL	150mL	1000mL
1 M Tris, pH=8.0	7.5	15	100
5M NaCl	7.5	15	100
0.5M EDTA pH=8.0	7.5	15	100
20% SDS	3.15	6.3	42

Add 0.38 g sodium bisulfite/100 mL of buffer before use and adjust pH 7.8-8.0 with NaOH.