DNA Extraction protocol (modification of https://www.biomedcentral.com/content/supplementary/1471-2164-12-553-S3.PDF)

100X TE (Tris-EDTA) stock buffer: (1.0 M Tris base, 0.1 M EDTA)
To make 1 L

- Place 121.14 g Tris-base and 29.22 g of EDTA (or 37.22 g of EDTA disodium salt: dihydrate) into a 1.5 L beaker.
- Add distilled water to a volume of 750 mL.
- Adjust pH with concentrated HCl to pH 7.0.
- Add distilled water to a final volume of 1 L.
- Place the 100X TE in glass bottles and store at room temperature

1X TE
To make 1L

- Place 10 mL 100X TE stock solution in a beaker
- Bring up to 1L with distilled water and ice

3M sodium acetate (MW=82.03)
To make 100 mL

- Place 24.61 g of sodium acetate in a beaker and bring up to ~75 mL with distilled water
- Adjust pH to 5.2
- Bring up to 100 mL with distilled water
- Store in aliquots of 2 mL at -20˚C

20% w/v SDS
To make 100 mL

- Place 20 g SDS in a beaker and bring up to 100 mL with distilled water
- Store in a glass bottle at room temp

20% v/v Triton x-100
To make 100 mL

- Place 20 mL of Triton x-100 into a volumetric flask.
- Bring up to 100 mL with distilled water.
- Stir using a magnetic stirrer until the liquid is homogeneous.

MEB (MPD-Based Extraction Buffer)
(To make 300 / 600 / 1200 mL)
H = higher concentrations to deal with plants with large amounts of secondary metabolites

-35.45g	70.91g	141.82g	2-methyl-2,4-pentanediol (Hexylene glycol)
-0.91g	1.81g	3.63g	Pipes
-0.61g	1.21g	2.42g	MgCl2.6H2O
-0.57g	1.14g	2.28g	sodium metabisulfite
-1.50g	3.00g	6.00g	sodium diethyldithiocarbamate

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-200ml	400ml	800ml	distilled water
-8.77g	17.54g	35.08g	L-lysine
-0.68g	1.36g	2.72g	EGTA

- Add all chemicals to a (500/1000/1500 mL) beaker together with the water
- Mix using a magnetic stirrer.
- Add 6g / 12g / 24g or 12g (H) / 24g (H) / 48g (H) of PVP-10 a little at a time to prevent clumping
- Add highly conc HCL to the medium until the pH is between 3 and 4 and is not drifting
- Add 1M NaOH until pH is 6.0
- Add crushed ice until total vol is 300ml / 600ml / 1200ml
- cover and place at 4°C (if possible, gently stirring)

- Add 2-mercaptoethanol (in fume cupboards) just before use

-120µl	240µl	480µl	2-mercaptoethanol
-0.6ml (H)	1.2ml (H)	2.4ml (H)	

MPDB (2-methyl-2,4 pentanediol buffer)
(To make 50 / 100 / 200ml)
H = higher concentrations to deal with plants with large amounts of secondary metabolites

-2.95g	5.90g	11.80g	2-methyl-2,4 pentanediol
-5.90g (H)	11.80g (H)	23.60g (H)	
-0.15g	0.30g	0.60g	Pipes
-0.10g	0.20g	0.40g	MgCl2.6H20
-0.10g	0.20g	0.40g	sodium metabisulfite
-0.25ml	0.50ml	1.00ml	Triton x-100
-1.50g	3.00g	6.00g	L-lysine
-0.11g	0.22g	0.44g	EGTA

- Add all chemicals to a 100/200/250 mL beaker
- Add distilled water to a volume of ~45/75/150 mL
- Add NaOH until pH is 7.0
- Bring total vol to 50ml / 100ml / 200ml with distilled water.
- Cover and place at 4°C (if possible, gently stirring)

- Add 2-mercaptoethanol (in fume cupboards) just before use

-19.5µl	39µl	78µl	2-mercaptoethanol
-0.25ml(H)	0.5ml (H)	1ml (H)	

Other Chemicals
- Phenol/chloroform/isoamyl alcohol (24:24:1)
- 100% Ethanol
- 70% (v/v) Ethanol

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Preparation

1. Prepare MEB and MPDB as previously described. Ideally, these buffers are prepared before any tissue is harvested (i.e., the fresher the tissue, the better the result). **NOTE: Do not yet add the 2-mercaptoethanol.**
2. Prepare 1 L of 1x TE as described above (make sure to keep TE ice cold).
3. Pre cool the blender by placing it in a cold room or filling it with ice.
4. Pre cool the centrifuges to 4°C.
5. Prepare a water bath at 60°C.

Harvesting

1. Plants should be placed in the dark for 48hrs before harvesting (to reduce the starch content). Make sure that the plants are well watered during this period.
2. Collect young leaves and buds and submerge directly into the ice cold TE buffer.
3. Approximately 20-100g of fresh material is collected for one isolation.

Nuclei extraction

1. Add 2-mercaptoethanol to the MEB and MPDB (in fume cupboard).
2. Strain the TE buffer from the plant material (using sieve) and place plant material in the pre-cooled blender together with the MEB.
3. Homogenize using the highest available speed for 30 seconds.
4. Squeeze the homogenate through 4 layers of miracloth (all liquid should be removed).
5. Filter again through 4 fresh layers of miracloth, but allow this filtration to occur by gravity only (no squeezing) into an ice cold beaker (keep beaker on ice).
6. Add 20% Triton x-100 to the beaker to a final concentration of 0.5% while stirring (using a magnetic stirrer).
7. Leave the mixture to incubate for 30 minutes while gently stirring (in a cold room) or on ice.
8. Centrifuge the mixture at 800g for 20 minutes at 4°C.
9. Resuspend the pellet using a soft paint brush soaked in MPDB. Transfer the pellet to a 15 mL Falcon tube. Bring the volume up to 10 mL with MPDB.
10. Centrifuge at 650g for 20 minutes. Discard the supernatant.
11. Add a further 10 mL MPDB to the pellet and mix by gentle inversion. Centrifuge again at 650g for 20 minutes.
12. Discard the supernatant and if the pellet still contains green material, repeat step 11.
13. Resuspend the pellet (containing the nuclei) in the residual MPDB contained in the tube. Additional MPDB may be added if necessary, but keeping the volume low is recommended.

DNA extraction from Nuclei

1. Transfer the resuspended pellet to a 2 mL eppendorf tube (or to multiple tube if volume is greater than ~600µl).
2. Add 20% SDS (w/v) to a final concentration of 2% (w/v). Mix content of the tube by gentle inversion to lyse the nuclei.

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3. Heat the tube containing the nuclear lysate at 60°C for 10 minutes in a water bath. Cool to room temperature and add 5M sodium perchlorate to a final concentration of 1M.
4. Spin the lysate at max speed for 20 minutes. Transfer the supernatant to a new 2 mL tube using a 1 mL plastic pipette tip from which the bottom third has been removed. NOTE: perform all subsequent transfers of DNA containing solutions using such modified pipette tips to minimize shearing of DNA.

PCI extraction

1. Perform a DNA extraction by adding an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) to the nuclear lysate.
2. To minimize shearing, use a test tube rocker (18 cycles per minute for 30 minute) rather than a shaker to mix the organic and aqueous phases.
3. Centrifuge at 3000 g for 10 minutes. Transfer the upper aqueous phase into a new tube.
4. Perform a second PCI extraction (steps 1-3) followed by an extraction using only chloroform (instead of PCI).
5. Add one tenth the volume of Sodium Acetate to the supernatant. Mix by inverting (carefully) the tube.
6. Add two volumes of ethanol and mix thoroughly. The DNA should precipitate. DNA can be stored in this solution at -20° C indefinitely.
7. If 'high molecular weight' DNA is required, remove the precipitated DNA using a glass rod (or Shepherd's crook) into a clean tube. Add (as much as possible) 70% Ethanol to remove the salts from the precipitated DNA.
8. Remove the precipitated DNA to a new tube and allow to air dry (do not overdry the pellet or it will be difficult to dissolve).
9. Redissolve DNA in buffer of choice - either distilled water or 10 mM Tris-Cl, pH 8.5 are preferable.

   NOTE: Do not resuspend DNA in buffer containing EDTA if it is intended for sequencing.

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