**CTAB DNA Extraction Protocol (for 2 x 96 well plates)**

* Add 1 tungsten carbide bead (Qiagen: 69997) to each well of a 1mL 96-well extraction plate (Axygen plate: AX-MTS-11-8-C, lids: AX-MTS-8CP-C-S)
* Place 1-2 small leaves in each well (store plates at -80°C)
* Preheat 110mL CTAB buffer in 65°C water bath (add a bit extra)
* Grind frozen plant tissue in Quiagen shredder. Repeat (refreeze and grind) until leaf material is a fine dust. Can freeze either with liquid nitrogen, or with storage at -80°C
* Centrifuge for 1-2mins so crushed material settles down
* Add 220uL RNaseA to CTAB buffer and vortex
* Add 500uL CTAB buffer to each well (put new lids for this step or else they will leak, and ensure they are on tight) and vortex
* Put plates in 60°C incubator overnight
* Centrifuge at 3000rpm for 1.5mins
* Put in freezer (-20°C) for 20mins (or until tubes are not warm)
* Add 500uL of Chloroform: Isoamylalcohol (24:1) to each well of plant material plate, invert until well dissolved (when it turns white in colour). Again, put new lids on for this step or else they will leak. Before inversion, ensure lids are on very tight
* Centrifuge at 5000rpm for 20mins (can be longer)
* Add 300uL Binding Buffer and 200uL ethanol to each well of a new 1mL 96-well plate
* Transfer 300uL of supernatant into the new plate
* Transfer all 800uL of sample (set pipette volume to 850ul so all is collected) to a 96-well Epoch membrane plate (sitting on top of a 2mL deepwell plate)
* Spin on high speed for 4.5 minutes
* Discard flow through and add 500uL of Wash Buffer
* Spin on high speed for 2 minutes
* Discard flow through (or move to another deepwell plate) and add another 500uL of Wash Buffer
* Spin on high speed for 15 mins to make sure membrane is dry
* Place the Epoch membrane plate (Epoch: 2020-001) on a Quiagen barcode plate (Qiagen: 19588) or standard PCR plate for elution
* Add 100uL of elution buffer (Tris-HCl pH 8.0) to each well and incubate at room temperature for 2 mins
* Spin on high speed for 1 min

**CTAB solution**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **100mL** | **500mL** | **1L** |
| **CTAB** | 2g | 10g | 20g |
| **Tris HCL (1M, pH8)** | 10mL | 50mL | 100mL |
| **NaCl (1.42M)** | 8.29g | 41.49g | 82.98g |
| **EDTA (500mM)** | 4mL | 20mL | 40mL |

- Add water to make the solution to the total volume

- Put solution on magnetic mixer to dissolve

**Binding Buffer: 3M GuHCl 3.75M NH4Ac pH 6**

|  |  |
| --- | --- |
|  | **1L** |
| **Guanidine Hydrochloride** | 573.18g |
| **H2O** | 500mL |
| **7.5M Ammonium Acetate** | 500mL |

- Adjust pH to 6 using glacial Acetic acid

**Wash Buffer: 10 mM Tris-HCl pH 7.5, 80% ethanol**

|  |  |
| --- | --- |
|  | **1L** |
| **1M Tris-HCl pH 7.5** | 10mL |
| **96% ethanol** | 800mL |
| **H2O** | 190mL |