# ANNEX 1: Comprehensive List of Equipment, Disposables, Commercial Kits, Reagents, and Buffer Recipes for HMW DNA Extraction and Analysis

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## 1. Equipment:

- Precision digital balance
- Thermoblock
- Vortex-Genie™ 2 (Scientific Industries SI™)
- Refrigerated tabletop micro-centrifuge
- Refrigerated centrifuge (with JA-14 rotor for 50 mL tubes)
- Orbital Shaker
- Thermomixer
- HulaMixer™ Sample Mixer
- Magnetic tube rack (DynaMag™-2 Magnet)
- NanoDrop 1000 Spectrophotometer (Thermo Fisher)
- Qubit 3.0 Fluorometer (Thermo Fisher)
- Optical microscope
- BioRad Gel electrophoresis system
- Pulsed-field gel electrophoresis (PFGE) system: Gene Navigator™ System (Amersham Biosciences, USA).

## 2. Disposables:

- Scalpel
- Cutting board
- 50 mL conical tubes (ThermoFisher)
- Liquid Nitrogen LN2
- Porcelain mortar and pestle
- P10, P200, P1000 pipettes

- Pipette tips, 10 µl, 200 µl, 1000 µl
- Filter cloth (100 µm)
- Steriflip (20 µm pore size, 50 mL process volume) (Merck)
- Small nylon or synthetic paintbrush
- 1.5 mL Protein LoBind micro-centrifuge tubes (Eppendorf)
- Qubit™ Assay Tubes

**3. Commercial Kits:**

- DNAbsolute (Idylle)
- Nanobind plant nuclei big DNA kit (PacBio®)
- NANOBIND® PANDNA KIT (PacBio®)
- CelLytic™ PN Isolation/Extraction Kit (Sigma-Aldrich)
- Qubit™ dsDNA Quantification Assay Kit, Broad Range

**4. Reagents:**

- RNase A (10mg/mL, VWR)
- 2-mercaptoethanol, 14 M Sigma-Aldrich (M3148)
- TRITON ™ X-100 Sigma-Aldrich (X100)
- Trizma base Sigma-Aldrich (T4661)
- Potassium chloride (KCl) Sigma-Aldrich (P9541)
- Ethylenediaminetetraacetic acid (EDTA), 0.5 M, pH 8.0 ThermoFisher (15575020)
- Spermidine trihydrochloride Sigma-Aldrich (S2501)
- Spermine tetrahydrochloride Sigma-Aldrich (S1141)
- Sucrose Sigma-Aldrich (S0389)
- Sodium hydroxide (NaOH), 10 M Sigma-Aldrich (72068)
- Polyvinylpyrrolidone (MW ~360 kD) (PVP360) Sigma-Aldrich (PVP360)
- Ultra-pure water
- Isopropanol (100%)
- Ethanol (96–100%)
- TE buffer
- DNA Gel Loading Dye (6X) Thermo Scientific™
- GreenSafe Premium DNA gel stain (NZY tech)
- Agarose

**5. Buffer recipes:**

**5.1 SILEX protocol (Vilanova et al., 2021):**

- Extraction buffer: 2% (w/v) CTAB, 2% (w/v) PVP-40, 20 mM EDTA, 100 mM Tris HCl (pH 8.0) and 1.40 M NaCl.
- Protein precipitation buffer: 24:1 chloroform: isoamyl alcohol.
- Binding buffer: 2.5 M NaCl and 20% PEG 8000.
- Silica matrix buffer: Mix 5 g of silicon dioxide ($SiO_2$) with 50 ml of MilliQ water and let stand for 24 h. Discard the supernatant and resuspend the pellet in 50 ml of MilliQ water and wait for another 5 h. Discard the supernatant and resuspend the pellet in 1:1 (v/v) MilliQ water. Finally, add 10 µl of HCl 36% per ml of silica matrix solution obtained.
- Washing buffer: Fresh prepared 70% ethanol.
- Elution buffer: 10 mM Tris HCl (pH 8.0) and 1 mM EDTA (pH 8.0).

**5.2 Plant nuclei isolation (PacBio):**

- 10X HB Buffer - homogenization buffer (500 mL): Add the following reagents to a clean beaker and stir until dissolved: Trizma 0.1M, KCl 0.8M, EDTA 0.1M, Spermidine 17mM, Spermine 17mM. Bring to the final volume with ultra-pure water and adjust pH to 9 with NaOH. Store at 4ºC for up to 1 year.
- 1X HB Buffer – homogenization buffer (1L): Buffer 10X HB (10%), sucrose 0.5M (171.2g/L). Bring to the final volume with ultra-pure water. Store at 4ºC for up to 3 months.
- TSB Buffer– triton-sucrose buffer (100 mL): Triton X-100 (20%), Buffer 10X HB (10%), sucrose 0.05M. Bring to the final volume with ultra-pure water. Store at 4ºC up to 1 year.
- NIB Buffer – nuclei isolation buffer (500 mL): 97.5% Buffer HB 1X, 2.5% TSB, 1% PVP360. Store at 4ºC for up to one week.

**5.3 Electrophoresis buffers:**

- 50X TAE Buffer: 242g of Tris-base (MW = 121.14 g/mol) and dissolve in approximately 700 mL of deionized water. Carefully add 57.1 mL of 100 % glacial acid (or acetic acid) and 100 mL of 0.5 M EDTA (pH 8.0). Adjust the solution to a final volume of 1 L and adjust the pH to 8.5.
- 1X TAE working solution: 20 ml 50 x TAE in 1000 ml distilled water.
- 10X TBE Buffer: 108 g tris base, 55 g boric acid, 900 ml double-distilled H2O, 40 ml 0.5 M EDTA solution (pH 8.0)

- 1X TBE: Dilute 100 mL of TBE Buffer, 10x stock solution into 900 mL deionised water to make 1L of TBE Buffer. (Final concentrations are: 89 mM Tris base, 89 mM Borate and 2 mM Na2EDTA.) On dilution to 1X, check pH and adjust as required.