High Molecular Weight DNA extraction protocol

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This protocol takes advantage of the modified nuclei enrichment method from Zhang et al (1995), CTAB DNA extraction, and the Short Read Eliminator (SRE) from Circulomics.

**Reagents:**

Nuclei enrichment:

1. Homogenization buffer (HB) 10X stock: 0.1M Tris, 0.5M KCl, 0.1M EDTA, 10 mM spermidine, 10 mM spermine, final pH ~9.5 adjusted with NaOH. Store at 4°C.
2. HB 1X: HB 1X, 0.5M sucrose, 0.2% Triton X-100, 4% PVP-40. Adjust pH to ~9.5. Can be stored at 4°C for up to 3 months. Add beta-mercaptoethanol to 0.3% before use.
3. Liquid nitrogen
4. A filtering system: filter paper with pores >30 micron or cheese cloth; 50ml tube.

CTAB:

1. CTAB: 2% CTAB, 100 mM Tris (pH 8.0), 1.4 M NaCl, 20 mM EDTA, add 0.1% beta-mercaptoethanol before use.
2. TE: 10mM Tris, 1mM EDTA
3. 24:1 Chloroform:Iso Amyl Alcohol
4. Isopropanol

Short Read Eliminator:

Can be purchased here (<https://www.circulomics.com/store/Short-Read-Eliminator-Kit-p131401036>)

Optional beads cleaning:

KAPA Purebeads or AMPure beads

**Protocol**

1. **Nuclei enrichment**
2. Add 0.3% beta-mercaptoethanol to 1X HB (4°C) and put on ice.
3. Grind tissue in liquid nitrogen with mortar and pestle to fine powder. Transfer the powder to 25-50ml 1X HB. Mix thoroughly with pipetting and then mix gently on ice (manually or with magnetic stir bar) for 15 min.
4. Make a funnel with 4 layers of filter paper and put it on a 50ml tube. Wet the funnel with 1X HB to reduce absorption. Filter the homogenized mix from step 2 through this funnel.
5. Distribute the resulting filtrate to 2 ml tubes and centrifuge at 1200 g, 4°C, 20 min. Discard aqueous layer, resuspend the nuclei in 1000 ul HB.
6. Make a new funnel with 4 layers of filter paper and filter all of the homogenized mix through it. Centrifuge the filtrate at 1200 g, 4°C, 20 min.
7. Discard aqueous layer, resuspend the nuclei in 750 ul HB in each tube. Centrifuge the filtrate at 1200 g, 4°C, 20 min. Discard aqueous layer, resuspend the nuclei in 750 ul HB. Repeat 2 to 3 times.

[Safe stopping point, the resulting nuclei is stable in 4°C HB]

1. **CTAB**
2. Add 750 ul 2% CTAB in each 2 ml tube. Preheat at 55°C.
3. Suspend the nuclei pellets from last step in 750 ul CTAB. The pellets may be distributed into multiple tubes. Mix thoroughly with pipetting.
4. Incubate samples at 55˚C for 1hr. Mix gently every 15min by reversing the tube up and down.

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From now on the DNA is in the solution. Avoid vigorous pipetting or vertexing.

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1. Add equal volume of 24:1 Chloroform:Iso Amyl Alcohol and mix well by gently shaking tubes.
2. Centrifuge for 5-10 minutes at 5000 g.
3. Pipette off the aqueous phase without sucking up any of the middle or chloroform phases. Pipetting slowly helps with this.
4. Place the aqueous phase into a new tube. Estimate the volume of the aqueous phase. Add 0.54 volumes of isopropanol.
5. Mix well by reversing the tube up and down. Let sit in room temperature for 15 min to 1 hour. (Longer times will tend to yield more DNA, but also more contaminants). Do not chill in fridge unless no pellet is observed.
6. Centrifuge for 5 min at maximum speed. Carefully pipette off the liquid.
7. Add 700µl of cold 70% Ethanol to wash the pellet. Pour or pipette off the liquid, being careful not to lose the pellet with your DNA. Add 700µl of cold 95% Ethanol to wash the pellet. Pour or pipette off the liquid, being careful not to lose the pellet.
8. Dry the pellet at room temperature.
9. Resuspend samples with 80µl TE buffer. Allow to resuspend for 1hr at 55˚C. HMW DNA can be difficult to dissolve. You may see jelly-like things in the solution. This is the non-homogenized DNA.

[Safe stopping point]

To further clean your DNA and to remove these non-homogenized DNA, you can apply an optional beads cleaning.

[Safe stopping point]

1. **SRE**

See protocol from Circulomics. Measure the concentration of the DNA and make sure it is at the recommended range (>50 ng/ul). Higher concentration (>150 ng/ul) is usually fine. After adding SRE and centrifuge, the DNA pellet is transparent and very difficult to see. Dissolve the pellet in TE 55˚C 1h or 4˚C over night.