Steps:

I. Nuclei Purification

1. Supplement Nuclear Purification Buffer (NPB) to 0.2 mM DTT, 0.5 mM PMSF, 0.5 mM spermadine, 0.25 mM spermine.

   * For 20 ml: 8 µl 0.5 M DTT, 100 µl 100 mM PMSF, 20 µl 0.5 M spermadine, 10 µl 0.5 M spermine.

2. Lightly thaw pellet(s) on ice until “slushy” consistency, and scoop into mortar.

3. Add liquid N2 to mortar, and grind worm pellet using pestle. Repeat 3-4 x until a fine powder.

4. Re-suspend ground-pellet in 10 ml supplemented NPB (sNPB) and transfer to dounce-homoginizer. Apply 50 strokes on ice.

5. Centrifuge 2 minutes, 100 x g, room temperature to pellet debris and carcasses.

6. Apply nucleosome supernatant over a 7.5 ml chilled sucrose cushion (10 mM Hepes, pH 7.5, 30 % w/v sucrose, 1.5 mM $MgCl_2$). Centrifuge 12 minutes, 4° C, 1,300 x g. Remove cushion with pipette, and re-suspend nuclei in 5 ml sNPB. Repeat sucrose cushion purification (2x total). Wash by re-suspending nuclei in 1 ml packed nuclear volume sNPB and re-centrifuging for 5 minutes, 4° C, 1,300 x g.

II. Mononucleosome Extraction and Purification

1. Re-suspend nuclei in 2x packed nuclear volume sNPB (typically 100-200 µl). Quantify chromatin by aliquoting 3 replicates of 2 µl to a 1.5 ml tube. Add 98 µl 2 M NaCl (1:50 dilution), and bath-sonicate 30 seconds, and vortex 1 minute. Then nanodrop relative to an appropriate blank. *Concentration of DNA = A_{260} x dilution factor x 50 ng/µl*

2. Add 1 M $CaCl_2$ to 5 mM final concentration, and equilibrate nuclei 37° C for 5 minutes.

3. Add Mnase (NEB) to fragment chromatin; 0.015 µl/µg chromatin, and incubate 5 minutes 37° C. Longer digestion will start to overdigest chromatin, losing yield, while shorter digestion will lead to more di- and tri-nucleosomes which will lead to avidity bias.

4. Add 100 mM EGTA to 20 mM final concentration, and add 5 M NaCl dropwise to 400 mM final concentration while vortexing on the lowest setting. Incubate 10-20 minutes 4° C with rotation to extract nucleosomes from nuclei. Centrifuge max speed, 4° C, 5 minutes to pellet nuclear debris.

5. Transfer mono/di-nucleosomes to new tubes. Aliquot 10% input to a new tube and keep on ice. Measure in triplicate by nanodrop, or Qubit* if you don't have a lot of material. Dilute with ChIP buffer to ~20 ng/µl to (1) reduce salt for IP and (2) stabilize nucleosomes. Don't dilute nucleosomes < 5 ng/µl, as they begin to fall apart at low concentrations.

*Note a 0.6 M solution will give a blunted signal (~10% drop) of actual chromatin concentration by Q-bit.