File S1 Supporting Methods and Materials: NIB-and-Grab DNA isolation protocol

1. Pellet cells (~6 x 10^8), rinse in distilled water, and transfer to a 15 ml conical tube. (Cells may be frozen at - 20˚C at this point.). Add 0.2 ml NIB to the cell pellet and vortex briefly. (NIB = Nuclear Isolation Buffer: 17% glycerol, 50 mM MOPS buffer, 150 mM potassium acetate, 2 mM magnesium chloride, 0.5 mM spermidine, and 0.15 mM spermine; pH is adjusted to 7.2 after all ingredients are dissolved.) Add 0.9 ml acid-washed glass beads. (There will be no obvious liquid above the level of the beads.)

2. Vortex at the highest rate with a vigorous vortexer, 5 to 10 times for 30 seconds, returning the cells to ice for 30 seconds between bursts of vortexing. Check for cell lysis by microscopy. Continue vortexing until the desired percentage of broken cells is achieved (~90%). Add 0.6 ml NIB to the broken cells, vortex briefly and transfer the solution to a fresh microfuge tube. Repeat with another 0.6 ml of NIB. (There should now be ~1.3 ml transferred to the microfuge tube.) Spin down nuclei, cell walls and unbroken cells in a microfuge for 20-30 minutes at top speed at 4°C. Pipette off the supernatant. Keep the pellet on ice.

3. Resuspend the nuclei in 0.3 ml TEN (50 mM Tris, 50 mM EDTA, 100 mM NaCl) to which 1 ml of RNAse A (10 mg/ml) has been added. Keep on ice 5 minutes. Add 12 ml of 25% Sarkosyl. Invert tube gently a few times to mix. Add 1 ml proteinase K (20 mg/ml). Invert tube again a few times to mix and transfer to 37°C for 30 minutes.

4. Spin the lysed nuclei for 5 minutes at 4°C at top speed in a microfuge. Remove the supernatant with a pipette tip to a screw-cap microfuge tube. Add 0.3 ml phenol:chloroform:isoamyl alcohol (24:24:1). Invert a dozen times to mix the phases. Let it rest and repeat another dozen inversions. Spin the samples at top speed in a microfuge to separate the phases (the top aqueous phase should be clear).

5. Use a wide-bore tip or cut off the tip of a 200 µl pipette tip to increase the diameter of the opening and carefully remove the aqueous phase to a clean microfuge tube. It will take several attempts to recover all of the aqueous phase. Avoid taking any of the white interface. (There should be ~0.25 ml of the aqueous phase.)

6. Add 2 volumes (~0.5 ml) of absolute ethanol that contains 0.5 M potassium acetate. Slowly and gently mix the phases by inverting the tube. The DNA should spool. When all of the aqueous phase has been incorporated into the alcohol phase, the DNA spool should collapse and fall to the bottom of the tube. Pull off the liquid with a pipette tip and discard. To the DNA pellet, add 0.3 ml 70% ethanol. Spin 5 minutes at top speed. Aspirate the ethanol, invert the tube and let it air dry for 30 minutes.

7. Resuspend the DNA in 75 µl (or so) of TE (Tris pH 8.0; 1 mM EDTA) by gently flicking the tube a few times. Incubate the tube overnight (or longer) at 4˚C. It will probably take the DNA overnight or longer to completely go into

solution. Store the DNA at 4˚C. Be aware that there may be RNA nucleotides/fragments in the solution so an OD_{260} reading is likely to give an overestimate of the DNA concentration. Run a microliter on a gel to confirm the DNA concentration. The average size of the DNA is greater than 50 kb.