1. Resuspend beads in 112.5 µL extraction buffer (including 1% SDS).
2. Add 12.5 µL of 20 mg/ml Proteinase K (for a final concentration of 2X) and incubate overnight at 65 °C with low shaking (300-600 rpm).

Day II. DNA purification and assessment of digestion efficiency
DNA purification

1. **Phenol:Chloroform:Isoamylacohol (PCI) extraction:**
    a. Add 1X volume (125 µl) of PCIa
    b. Shake or vortex for 20 sec
    c. Spin for 10min at 19800xg at room temperature
    d. Keep upper layer and transfer to a new tube.
2. **Purify DNA by ethanol precipitation:**
    a. Add 0.1X (12.5 µl) volume of sodium acetate and 2.5X volume (312.5 µl) of cold 100% ethanol
    b. Incubate for >15 min at -80°C
    c. Spin for >15 min at max speed at 4°C (longer is better, we routinely spin for >30 min)
    d. Wash pellet with cold 75% ethanol and spin for 5 min at max speed at 4°C
    e. Air dry pellet for max 10 min at room temperature
    f. Resuspend pellet in 25 µL TE or 10mM Tris
    g. Add 1µl RNase A (20 mg/ml) and incubate for 15 min at 37°C to digest RNA

1. Quantify DNA concentration by Qubit. You can also save some sample for bioanalyzer.
2. Run DNA on a 3.5 % TAE / 3% TBE agarose gel. You should see mono-nucleosomal (~150 bp) and di-nucleosomal (~300 bp) bands. MNase concentrations that yielded approximately 70% mono-nucleosomal and 30% di-nucleosomal DNA were selected d for subsequent experiments.

Here is one of our early gels comparing MNase digestion with and without beads. We ultimately chose to use 2.5 Units of MNase for 10 NC14 embryos, as we found that samples that were slightly under-digested produced better libraries.

- Qubit Concentrations:
    - ***With (+) beads***
        - 2.5 U – 1.48 ng/uL
        - 5 U – 1.3 ng/uL
        - 10 U – 0.774 ng/uL
        - 15 U – 0.710 ng/uL
        - 20 U – 0.488 ng/uL
    - ***Without (-) beads***
        - 5 U – 0.69 ng/uL
        - 10 U – 0.36 ng/uL
        - 15 U – 0.39 ng/uL
        - 20 U – 0.146 ng/uL