Bead-Beater DNA Preparation Protocol

1. Pellet cells/material for 5 minutes at 10,000g
2. Resuspend pellet in 800µl lysis solution
3. Add 0.8g of 0.1mm glass beads (or equal volume)
4. Disrupt for 30 seconds at maximum speed.
    a. Time many vary depending upon starting material/organisms present
5. Centrifuge at 10,000g for 3 minutes
6. Transfer supernatant to new 1.5ml eppendorf tube.
7. Add equal volume phenol/chloroform.
8. Incubate at 68°C for 5 minutes with 2-3 mixes.
9. Centrifuge at 10,000g for 3 minutes.
10. Transfer aqueous phase to new 1.5ml eppendorf.
11. (OPTIONAL) Do another phenol/chloroform extraction.
    a. Dependent upon previous 260/280 readings from similar sample types.
12. Add 2 volumes 95% EtOH and 1/10th volume NaAcetate (3M)
13. Precipitate at -20°C O/N.
14. Centrifuge at 10,000g for 45-60 minutes at 4°C.
15. Wash with 70% EtOH.
16. Centrifuge 10,000g for 30 minutes at 4°C.
17. Air dry pellet
18. Resuspend in DNase/RNase-free TE (pH 7.0 or 8.0)

Lysis Solution
2.7g NaAcetate (33mM)
5.0g SDS (17.5mM)
0.34g EDTA (~3mM)
pH 5.5
q.s. to 1 Liter total volume