Genomic DNA Isolation Protocol - MiniBeadbeater

1. Label 1 screw-capped tube and 4 microfuge tubes (1.5 ml) for each sample to be used for cell lysis, extractions and precipitation of genomic DNA.

2. Centrifuge culture in a clinical centrifuge (5 min at max. speed) and carefully pour off media. Resuspend cell pellet by vortexing and place cell suspension in a yellow screw-capped tube. Use a microfuge to pellet cells, then remove remaining liquid media. Add TE (10 mM Tris, 1 mM EDTA) to a total volume of 0.75 ml. (If needed, wash pellet with 5 M NaCl to remove polysaccharides before resuspension.)

3. Add the following to the screw-capped tube (wear gloves when using phenol and chloroform):

0.5 ml glass beads (0.425-0.6mm Sigma #G9268)
18 µl 10% SDS
0.75 ml Tris-buffered phenol/chloroform (1:1 mix) in hood

Shake at high speed, 100 sec. on MiniBead Beater. Remove to ice.

4. Spin 10', 13,000 X g at 4˚C (in microcentrifuge).

5. Remove top aqueous layer, avoiding the thin white layer of denatured proteins (however, take the white precipitate at interface if it is very thick and fluffy, meaning it contains genomic DNA). Transfer to new microfuge tube and extract with an equal volume phenol/chloroform (an extraction means vortexing for 10 seconds followed by a 2 min centrifuge at max. to separate phases; the aqueous phase containing DNA will be on top).

6. a. Extract with an equal volume of chloroform to remove phenol and remove top aqueous phase to a fresh tube.
b. Then add 2 µl RNase (10 mg/ml) and incubate 10' at 37°C to digest RNA in the sample.

7. Extract RNased sample with chloroform to remove degraded RNA and protein. Remove 400 µl of the top aqueous phase to a fresh tube.

8. To precipitate genomic DNA, add:

1/10th volume 3M sodium acetate (40 µl)
2-2.5 volumes 95% ethanol (1 ml)

mix by rocking microfuge tube back and forth; if genomic DNA is at a high concentration you will observe it come out of solution as white threads; low concentration will appear uniform whitish color.

9. Centrifuge 10 min. 4° in microcentrifuge, discard supernatant. Wash pellet with 70% cold ethanol up to 3 times until no phenol smell remains. Vacuum dry. Resuspend pellet in 50 µl TE.

10. Assay by A_{260} and using 50 µg/ml conversion factor for double stranded DNA.
i.e.) A_{260} X dil. factor X 50 µg /ml · A^{260} X 0.001 ml/µl = DNA conc. in µg/µl
(try diluting 2 µl of your DNA in 98 µl water for a dilution factor of 50; be sure to use a quartz spectrophotometer cuvette since glass will not let UV light pass)

11. Store genomic DNA at -20˚C. Place phenol and chloroform wastes in proper container and allow the extraction tubes to dry in the fume hood.