Appendix 1.

Protocol with minor modifications for Isolation of Genomic DNA from Gram Positive and Gram Negative Bacteria with Wizard® Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA)

1. Centrifuge 1 ml of overnight culture for 2 minutes at 13 000 rpm and remove the supernatant. For gram positive, proceed to next step. For gram negative, go to step 5.
2. Resuspend the pellet in 480 µl of 50 mM EDTA.
3. Add 120 µl of 10mg/ml lysozyme.
4. Incubate at 37°C for 40 minutes and centrifuge for 2 minutes at 13 000 rpm.
5. Add 600 µl of Nuclei Lysis Solution.
6. Incubate at 80°C for 5 minutes.
7. Add 3 µl of RNase Solution and invert the tube a few times.
8. Incubate at 37°C for 40 minutes.
9. Add 200 µl of Protein Precipitation Solution, vortex for 20 seconds.
10. Incubate on ice for 5 minutes and centrifuge for 3 minutes at 13 000 rpm.
11. Transfer the supernatant to a microcentrifuge tube containing 600 µl isopropanol. Mix by inversion.
12. Centrifuge for 3 minutes at 13 000 rpm.
13. Pour off the supernatant and add 600 µl of 70% EtOH and invert the tube several times.
14. Centrifuge for 3 minutes at 13 000 rpm. Aspirate the EtOH.
15. Air-dry the pellet for 10 - 15 minutes.
16. Add 100 µl of DNA Rehydration Solution and incubate the tube for 1 hour at 65°C.
17. Store the DNA at 4°C.