gDNA Extraction

Genomic DNA extraction was performed using the Genomic DNA Kit from Qiagen. The list and preparation of the buffers used is at the end of the protocol.

1. Bacteria was grown to a total number of 4.5 x 10^9 cells.
2. Prepare Buffers B1, B2, QBT, QC, and QF according to the tables below.
3. For each prep, add 2 µL of RNase A solution (100 mg/mL) to a 1 mL aliquot of Buffer B1.
4. Dissolve lysozyme in distilled water to a concentration of 100 mg/mL.
5. Prepare QIAGEN Protease stock solution in distilled water or use QIAGEN Proteinase K stock solution.
6. Pellet bacteria from an appropriate volume of culture by centrifugation at 3000 5000 x g for 5 10 min.
7. Discard the supernatant, ensuring that all liquid is completely removed.
8. Resuspend the bacteria pellet from step 3 in 1 mL of Buffer B1 (with RNase A) by vortexing at top speed.
9. Add 20 µL of lysozyme stock solution (100 mg/mL), and 45 µ of QIAGEN Protease or QIAGEN Proteinase K stock solution.
10. Incubate at 37°C for at least 30 min.
11. Add 0.35 mL of Buffer B2 and mix by inverting the tube several times or by vortexing for a few seconds.
12. Incubate at 50°C for 30 min.
13. Equilibrate a QIAGEN Genomic-tip 20/G with 1 mL of Buffer QBT and allow the QIAGEN Genomic-tip to empty by gravity flow.
14. Vortex the sample for 10 s at maximum speed and apply it to the equilibrated QIAGEN Genomic tip. Allow it to enter the resin by gravity flow.
15. Wash the QIAGEN Genomic-tip with 3 x 1 mL of Buffer QC.
16. Elute the genomic DNA with 2 x 1 mL of Buffer QF.
17. Precipitate the DNA by adding 1.4 mL (0.7 volumes) room-temperature (15 25°C) isopropanol to the eluted DNA.
18. Mix and centrifuge immediately at >5000 x g for at least 15 min at 4°C.
19. Carefully remove the supernatant.
20. Wash the centrifuged DNA pellet with 1 mL of cold 70% ethanol.
21. Vortex briefly and centrifuge at >5000 x g for 10 min at 4°C.
22. Carefully remove the supernatant without disturbing the pellet.
23. Air-dry for 5 10 min and resuspend the DNA in 0.1-2 mL of a suitable buffer (e.g. TE buffer)
24. Dissolve the DNA overnight on a shaker or at 55°C for 1-2 h.

Table 1: Buffer composition and storage

Buffer	Composition	Storage
Buffer B1	50 mM Tris·Cl, pH 8.0 50 mM EDTA, pH 8.0 0.5% Tween® -20 0.5% Triton X100	2-8°C or RT
Buffer B2	3 M guanidine HCl 20% Tween-20	2-8°C or RT
Buffer QC (wash buffer)	1.0 M NaCl 50 mM MOPS, pH 7.0 15% isopropanol	2 8°C or RT
Buffer QF (elution buffer)	1.25 M NaCl 50 mM Tris·Cl, pH 8.5 15% isopropanol	2-8°C or RT
TE	10 mM Tris·Cl, pH 8.0 1 mM EDTA, pH 8.0	RT

Preparation of buffers

B1: Dissolve 18.61 g $Na_2EDTA \cdot 2H_2O$ and 6.06 g Tris base in 800 mL distilled water. Add 50 mL 10% Tween-20 solution and 50 mL 10% Triton X-100 solution. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water.

B2: Dissolve 286.59 g guanidine HCl in 700 mL distilled water. Add 200 mL of 100% Tween-20. Adjust the volume to 1 liter with distilled water. pH does not need to be adjusted.

QC: Dissolve 58.44 g NaCl, 10.46 g MOPS (free acid) in 800 mL distilled water. Adjust the pH to 7.0 with NaOH. Add 150 mL pure isopropanol. Adjust the volume to 1 liter with distilled water.

QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 mL distilled water. Adjust the pH to 8.5 with HCl. Add 150 mL pure isopropanol. Adjust the volume to 1 liter with distilled water.