Preparation of Bacterial Genomic DNA

1) Inoculate a 5 ml culture and incubate overnight
2) Transfer in hemolysis tube and spin the cells down until a compact pellet forms. Discard the supernatant.
3) Resuspend the pellet by repeating pipetting in a mix of 567µl TE buffer and 5µl RNAse A. Add 15 µl 10% SDS and 4 µl proteinase K (18 $mg.mL^{-1}$). Mix thoroughly and incubate 1h at 37°C or a few minutes at 65°C until all the cells are lysed.

   The solution should become viscous as the detergent lyses the bacterial cell walls.
4) Add 100µl of 5M NaCl and mix thoroughly.

   This step is very important since a CTAB-nucleic acid precipitate will form if salt concentration drops below 0.5M at room temperature (Murray and Thompson, 1980). The aim here is to remove cell wall debris, denaturated protein and polysaccharides complexed with CTAB, while the nucleic acids in solution.
5) Add 80µl of CTAB/NaCl (10% w/v; 0.7M) solution. Mix thoroughly and incubate 10min at 65°C.
6) Add approximately equal volume of chloroform/isoamyl alcohol (0.7-0.8ml), mix thoroughly and spin 4-5 min in a centrifuge.

   The extraction removes CTAB-protein/polysaccharides complexes. A white interface should be visible after centrifugation.
7) Remove aqueous, viscous supernatant to a fresh Falcon tube, leaving the interface behind. Add an equal volume of phenol/chloroform/isoamyl alcohol, extract thoroughly and spin for 5 min.

   With some bacterial strains the interface formed after chloroform extraction is not compact enough to allow easy removal of the supernatant. In such cases, most of the interface cans be fished with a sterile toothpick before removal of the supernatant. Remaining CTA precipitates is then removed in a phenol/chloroform extraction.
8) Add 1 volume of isopropanol, shake and spin. Wash with 70% ethanol, dry and dissolve the pellet in 50µl Tris pH = 7 or water.