Extraction of DNA from bacterial

1- **Pellet** bacteria by centrifugation for 10 min at (7500 rpm).

2- **Resuspend** bacterial pellet in 180 µl Buffer ATL (supplied in the QIAamp DNA Mini Kit) for lysis.

3- **Add** 20 µl proteinase K, mix by vortexing, and incubate at 56°C until the complete lysis.Vortex occasionally during incubation to disperse the sample.

4- **Briefly** centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

5- **Add** 200 µl Buffer AL to the sample, mix by pulse-vortexing for 15 s, and incubate at 70°C for 10 min., Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

6- **Add** 200µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

7- **Apply** the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.

8- **Add** 500 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.

9- **Add** 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed 14,000 rpm for 3 min.

10- **Place** the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

11- **Place** the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE or distilled water. Incubate at room temperature for 1 min, and then centrifuge at (8000 rpm) for 1 min.

PCR Procedures:

Set up PCR reaction Reagents in a tube:

Volume (µl)	Reagent
5 µl	• Sample (DNA)
1 µl F and 1 µl R	• Primers (forward and reverse)
12.5 µl	• Enzyme (DNA polymerase, *Taq)* • dNTPs (A, T, G, C) • Buffer(50 mM KCl; 10 mM Tris-HCl; 1.5 mM MgCl2) } 2X PCR Master Mix
5.5 µl	• Water DNAase free water
25 µl	Total reaction volume