

Isolation of Genomic DNA from bacteria with the JETQUICK System

The JETQUICK 'Tissue' kit can be adapted to the isolation of genomic DNA from different bacterial species. Genomic DNA from either gram-positive or gram-negative bacteria can be isolated according to the following protocol modifications. Related products are reported to be successfully used with species like *Escherichia coli*, *Bacillus subtilis*, *Bordetella pertussis*, *Borrelia burgdorferi* and *Legionella pneumophila*.

Depending on the source of the bacterial cells please choose one of the following protocol adaptations. All of these adaptations are going over into the JETQUICK 'Tissue' protocol at some point. This point of transition is exactly described for each of the bacterial protocols.

For the isolation of DNA from swabs (Protocol B) or gram-positive cells (Protocol D) there are some extra buffers and enzymes that are not supplied with the kit and have to be provided by the user.

Protocol A (Isolation of bacterial DNA from biological fluids)

- 1.) Pellet bacterial cells by centrifugation for 3 min at 13,000 rpm (~15,000 x g). Remove the clarified supernatant completely with a pipette.
- 2.) Resuspend the bacterial pellet in **200 µl of buffer T1** (supplied with the JETQUICK Tissue kit).
- 3.) Follow the JETQUICK Tissue protocol from step 2 of part A.

Protocol B (Isolation of bacterial DNA from eye, nasal, pharyngeal, or other swabs)

- 1.) Collect samples and place in 2 ml PBS containing a common fungicide. Incubate for several hours at room temperature.
- 2.) Pellet bacterial cells by centrifugation for 3 min at 13,000 rpm (~15,000 x g). Remove the clarified supernatant completely with a pipette.
- 3.) Resuspend the bacterial pellet in **200 µl of buffer T1** (supplied with the JETQUICK Tissue kit).
- 4.) Follow the JETQUICK Tissue protocol from step 2 of part A.

Protocol C (Isolation of genomic DNA from gram-negative bacterial cultures)

a) Plate cultures

- 1.) Remove bacterial cells from culture plate with an inoculation loop and suspend in 200 µl of buffer T1 (supplied in the JETQUICK Tissue kit) by vigorous stirring.

2.) Follow the standard JETQUICK Tissue protocol beginning with step 2 of part A.

b) Suspension cultures

- 1.) Transfer 1 ml of bacterial culture into a microfuge tube and centrifuge for 3 min at 13,000 rpm (~15,000 x g) to pellet the bacterial cells. Remove the clarified supernatant completely with a pipette.
- 2.) To the cell pellet add **200 µl of buffer T1** (supplied with the JETQUICK Tissue kit) and resuspend the cells completely by vortexing vigorously or pipetting up and down.
- 3.) Follow the standard JETQUICK Tissue protocol beginning with step 2 of part A.

Protocol D (Isolation of genomic DNA from gram-positive bacterial cells)

Some hard-to-lyse bacterial species (especially gram-positive bacteria) require a pre-incubation with specific enzymes such as lysozyme or lysostaphin (i.e. *Staphylococcus spec.*) to disrupt the rigid multilayered murein cell wall. For these species please use the following protocol:

- 1.) Pellet bacterial cells by centrifugation for 3 min at 13,000 rpm (~15,000 x g). Remove the clarified supernatant completely with a pipette.
- 2.) Resuspend the bacterial cells in 200 µl of the following buffer containing the described amount of the appropriate enzyme.

**20 mM Tris-HCl (pH 8.0),
2 mM EDTA,
1.2% Triton-X-100**

**20 mg/ml lysozyme OR
200 µg/ml lysostaphin**

The appropriate enzyme should be added fresh to the buffer immediately before use.

- 3.) Incubate for at least 30 min at 37°C.
- 4.) Continue with step 3 (part A) of the standard JETQUICK Tissue protocol (addition of proteinase K), but reduce the incubation time at 56°C from several hours to 30 min. This should be enough time to disrupt the bacterial cells completely.
Follow the standard JETQUICK Tissue protocol from this point.