| WCSB, Wageningen University | IBISBA-SOP-WU15 |
|---|---|
| | Version 1.0 |

# EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Genomic DNA isolation

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# Instruction

## *Genomic DNA isolation*

### *1. Introduction / Purpose*

This protocol describes the isolation of bacterial genomic DNA, using the kit GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich®). The protocol focusses on Gram-negative bacteria. The kit can also be used for Gram-positive bacteria, which is not described in this protocol. The information for Gram-positive bacteria can be found in the manual provided with the kit. The manual can also be found here:

https://www.sigmaaldrich.com/technical-documents/protocols/biology/genelute-bacterial-genomic-dna-kit.html

**Keywords:** Genomic DNA - isolation

### *2. Equipment and chemicals*

#### *2.1. Equipment*

- 37 °C water bath or heating block
- 55 °C water bath or heating block
- Microcentrifuge (2 mL tube, rotor equipped)

#### *2.2. Chemicals*

- Ethanol (95%–100%)
- Molecular Biology Reagent Water

### *3. Media and Buffers*

- GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich®)


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## 4. Procedures

Preparation instructions

- Preheat a water bath or heating block to 55°C.

- Thoroughly mix reagents: Examine reagents for precipitation. If any reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves and cool to room temperature before use.

- Dilute wash solution concentrate: Dilute the concentrate with 10 mL (10 prep package), 80 mL (70 prep package), or 360 mL (350 prep package) of 95–100% ethanol. After each use, tightly cap the diluted Wash Solution to prevent the evaporation of the ethanol.

- Reconstitute proteinase K: Dissolve the powder in one bottle of Proteinase K in water to obtain a 20 mg/mL stock solution, according to Table 1. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed. This product as supplied is stable at room temperature.

- Method

- **Harvest cells:** Pellet 1.5 ml of an overnight bacterial broth culture (See Protocol: Standard cultivation of *P. putida* and *E. coli* in LB medium) by centrifuging for 2 min at >12000 g. Remove the culture medium completely and discard. If the bacteria are propagated in rich media such as Terrific broth, it will be necessary to reduce the volume of starting material to 0.5 ml of an overnight bacterial broth culture to avoid overloading the GenElute columns.

- **Resuspend cells:** Resuspend the pellet thoroughly in 180 [Symbol]l of Lysis Solution T/Buffer STL for Gen Elute Mammalian Genomic DNA Kit. If residual RNA is a concern perform the Optional RNase A treatment.

- **Optional RNase A treatment:** If RNA-free genomic DNA is required, add 20 [Symbol]l of RNase A solution, mix, and incubate for 2 min at room temperature.

- **Prepare for Cell Lysis:** Add 20 [Symbol]l of the Proteinase K solution to the sample. Mix and incubate for 30 minutes at 55[Symbol]C.

- **Lyse cells:** Add 200 [Symbol]l of Lysis solution, vortex thoroughly for about 15 seconds and incubate at 55[Symbol]C for 10 minutes. A homogeneous mixture is essential for efficient lysis.

- **Column preparation:** Add 500 [Symbol]l of the Column Preparation solution to each pre-assembled GenElute Miniprep Binding Column (with a red o-ring, not to be confused with other GenElute kits) seated in a 2 ml collection tube. Centrifuge at >12000 g for 1 minute. Discard the eluate.

- **Prepare for binding:** Add 200 [Symbol]l of ethanol (95-100%) to the lysate and mix thoroughly by vortexing for 5-10 seconds. A homogeneous mixture is essential.

- **Load Lysate:** Transfer the entire contents of the tube into the binding column. Use a wide bore pipette tip to reduce shearing the DNA when transferring the contents into the column. Centrifuge at ≥ 6500 × g for 1 minute. Discard the collection tube containing the eluate and place the column in a new 2 mL collection tube.

- **First Wash:** Add 500 µL of Wash Solution 1 (W0263) to the column and centrifuge for 1 minute at ≥ 6500 × g. Discard the collection tube containing the eluate and place the column in a new 2 mL collection tube.

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- **Second Wash:** (Important Reminder: *Verify that ethanol has been added to the bottle of Wash Solution Concentrate*) Add 500 µL of Wash Solution to the column and centrifuge for 3 minutes at maximum speed (12,000–16,000 × g) to dry the column. The column must be free of ethanol before eluting the DNA.
  Centrifuge the column for an additional 1 minute at maximum speed if residual ethanol is seen. You may empty and re-use the collection tube if you need this additional centrifugation step. Finally, discard the collection tube containing the eluate and place the column in a new 2 mL collection tube.

- **Elute DNA:** Pipette 200 µL of the Elution Solution (B6803) directly onto the center of the column; centrifuge for 1 minute at ≥ 6500 × g to elute the DNA. To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Solution, then centrifuge.
  **Optional:** A second elution can be collected by repeating step 10 with an additional 200 µL of Elution Solution and eluting into a new 2 mL collection tube or into the same 2 mL collection tube as used for the first eluate. The yield can be improved by 20–50% when performing a second elution.

- The eluate contains pure genomic DNA. For short-term storage of the DNA, 2–8 °C is recommended.

- For longer-term storage, –20 °C is recommended. Avoid freezing and thawing, which causes breaks in the DNA strand. The Elution Solution will help stabilize the DNA at these temperatures.

## *5. Remarks / troubleshooting*

- The protocol focuses on Gram-negative bacteria. The kit can also be used for Gram-positive bacteria, which is not described in this protocol. The information for Gram-positive bacteria can be found in the manual provided with the kit.

## *6. Biosafety*

No biosafety issues are associated with this protocol.

## *7. Acknowledgements*

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