Genomic Mini AX BACTERIA

DNA isolation from bacteria

cat # 060-60 **Protocol**

Notes:
- Store lysozyme at -20 °C
- Store Proteinase K solution, BS suspension buffer and Columns Mini AX at temperture range from 4 to 8 °C

1. Centrifuge **0.2-1.0 ml** overnight bacterial culture, discard supernatant and suspend the bacterial pellet in **100 µl** of **BS** suspension buffer.
2. Add **10 µl** of **Lysozyme** and incubate for **15 min** at **37 °C**.
3. Subsequently add **900 µl** of **LS** lysis suspension (Note: Mix lysis suspention thoroughly by inverting the bottle several times before use) and **20 µl** of **Proteinase K** solution.
4. Mix the whole contents and incubate for **30 min** at **50 °C**. Mix from time to time by inverting the tube.
   RNA digestion (optional): If sample is to be RNA-free, RNA may be removed by adding 5 µl of RNAse (10 mg/ml) and incubating for 5 min at room temp.
5. Vortex vigorously sample for **15 s** and spin for **5 min** at **10 000-14 000 RPM**.
6. During the sample centrifugation, equilibrate the column by adding **800 µl** of **K1** equilibrating solution. Allow to pass the whole volume of solution by gravity.
7. After sample centifugation, transfer supernatant to an equilibrated column and allow it to flow by gravity.

   Note: At the bottom of the tube there should be visible solid, compact pellet. It is a mixture of non-lysed fragments of sample material and particles from lysis suspension.
8. Wash the column by adding **1.5 ml** of **K2** wash solution. Allow to pass the whole volume of washing solution by gravity.
9. Repeat washing step by adding **1.5 ml** of **K2** wash solution. Allow to pass the whole volume of washing solution by gravity.
10. Add to the column **250 µl** of **K3** elution solution and allow it to pass the column.

    Note: The aim of this step is ti reduce the total volume of eluate since the dead volume of the column is 0.3 ml
11. Transfer the column to a new 2 ml precipitation tube (included) and elute DNA by adding **1 ml** of **K3** elution solution to the column.

    Note: The column drop director possesses proper fitting that allows easy attachment to the precipitation tube.

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12. Add to the eluted DNA **800 µl** of **PM** precipitation mix. Mix the whole sample by inverting the tubes a few times and spin for **10 min** at **10 000 RPM**.

    Note: PM solution contains precipitation enhancer and it should be intensively mixed before use by few times vigorous hand shaking.

13. Carefully discard supernatant and add **500 µl** of **70% ethanol** (not included) to the precipitation tube. (Note: At the bottom of the precipitation tube there should be visible the light-blue DNA pellet.) Mix the sample and spin for **3 min** at **10 000 RPM**.

14. Carefully discard supernatant and air dry the DNA pellet for **5 min** at **room temp.** in the up-side-down position of the precipitation tube.

    Note: If there are any leftovers (small droplets) of alcohol on the tube walls they should be removed with a sterile cotton buds.

15. Dried DNA pellet can be dissolved in desired volume of Tris buffer (10 mM, pH 8.5) (included) or sterile nuclease-free water or TE buffer (not included)

    Note: The blue color of DNA precipitate enables visual control of DNA dissolution process.

additional notes

1. The purifiction column flow rate depends directly on quantity and size of DNA molecules. High content of high molecular weight DNA decreases the flow rate. The DNA amount exceeding 20 µg loaded onto column may even lead to flow stoppage. In such cases the column should be placed in the 15 ml collection tube and centrifuged in a swing-out rotor at **3 000 - 4 000 RPM** for **1 min**. The centrifugation can be performed both, after the loading step and durind the washing step with K2 solution and K3 solution.

   Subsequently, the DNA elution step should be performed as follows: Transfer the column to 15 ml tube (not included) and add **1 ml** of **K3** elution solution. Wait 2 min.Spin for **1 min** at **3000 RPM**. Transfer the eluate to 2 ml precipitation tube (included). Then follow the standard procedure from point 12.

2. The chromatography purification of DNA can be paused at any time while sample is loaded onto column. The purification process can be continued after up to 15-hours-long pause with no influence on quality or quantity of purified DNA.

   During the pause of the DNA purification the 15 ml tube with the column inside has to be closed with the screw cap to avoid membrane desiccation and subsequent DNA lost.

   The volume of the solution in the 15 ml tube enables the easy location of the procedure step after which the DNA purification process was paused:

   ~ **0.5 ml** – after the column equilibration,
   ~ **1.5 ml** – after the sample loading step,
   ~ **3 ml** – after the first washing step with K2 solution,
   ~ **4.5 ml** – after the second washing step with K2 solution

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