Genomic Mini AX Staphylococcus

DNA isolation from Staphylococcus

cat # 067-60

Protocol

Notes:

- Store BS buffer suspension, Proteinase K solution and Mini AX columns at temp. from +4 to +8 °C.
- Store lysostaphin solution at -20 °C.
- DNA binding capacity of Mini AX column is 20 µg of DNA.

1. Centrifuge **0.5-1 ml** of overnight Staphylococcus culture, discard supernatants and suspend the pellets in **100 µl** of **BS** suspension buffer.
2. Add **20 µl** of **lysostaphin**. Mix whole contents and incubate for **20 min** at **37 °C**.

   Depending on the strain of Staphylococcus. enzymatic lysis efficiency may be significant differences. For strains particularly resistant to lysis. DNA must be isolated from the culture stopped in the logarithmic growth phase.
3. Subsequently add **900 µl** of **LS** lysis suspension and **20 µl** of **Proteinase K**.

   Note: Mix lysis suspension throughly by inverting the bottle several times before use.
4. Mix the whole contents and incubate for **10 min** at **50 °C**. Mix tube from time to time by vortexing.
   RNA digestion (optional): Add 5 µl of RNAse (10 mg/ml) (not included, # 1006-10, 1006-50). Mix sample by vigorous vortexing for 20 s and incubate for 5 min at room temp.
5. Vortex vigorously 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. Wait until the whole volume pass through the Mini AX columns by gravity.
7. Apply the supernatants onto pre-equilibrated Mini AX columns. Wait until the lysates pass through the Mini AX columns by gravity.
   Note: At the bottom of the Eppendorf 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. Add **1.5 ml** of **K2** wash solution. Wait until the K2 wash solution passes through the Mini AX columns by gravity.
9. Again add 1.5 ml of K2 wash solution. Wait until the K2 wash solution passes through the Mini AX columns by gravity.

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10. Add to the columns **250 µl** of **K3** elution solution and allow it to pass the columns.

    Note: The purpose of this step is to decrease the total volume of eluate, because the column void volume is about 250 µl.

11. Transfer the Mini AX columns to new precipitation tubes (included) and elute by adding 1 ml of K3 elution solution onto the Mini AX columns.

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

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

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

13. Carefully discard supernatants and add **500 µl** of **70% ethanol** to the precipitation tubes. (Note: At the bottom of the precipitation tube there should be visible the light blue DNA pellet.) Mix the samples and spin for **3 min** at **10 000 RPM**.

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

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

15. Dried DNA pellets 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 colour 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 for 1 min at **3000-4000 RPM**. The centrifugation can be performed both, after the loading step and durind the washing step with K2 solution.

   Subsequently, the DNA elution step (procedure point 8) sould be performed as follows: Transfer the columns to 15 ml tubes (not included) and add 1 ml of K3 elution solution, wait 2 min. Centrifuge for 1 min at 3000 RPM. Transfer the eluate to 2 ml precipitation tubes (included). Then follow the standard procedure from point 12.

2. The 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,
   ~ **2 ml** – after the sample loading step,
   ~ **3.5 ml** – after the first washing step with K2 solution,
   ~ **5 ml** – after the second washing step with K2 solution

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