Bacterial Genomic DNA Extraction Kit

Project number: B669951

Storage conditions: room temperature.

Products

individual parts making up a compound	50T
Buffer GTL	15ml
Buffer GL	15ml
Buffer GW1 (concentrate)	13ml
Buffer GW2 (concentrate)	15ml
Buffer GE	15ml
Proteinase K	25mg
Proteinase K Storage Buffer	1.25ml
Spin Columns DM with Collection Tubes	50

Products

This kit is suitable for extracting high purity total DNA from Gram-negative and Gram-positive bacteria. 106-108 cells can be processed at a time, and up to 20 μg of total DNA can be obtained within one hour without the need for toxic solvents such as phenol or chloroform, and without the need for ethanol precipitation. The optimized buffer system enables the DNA in the lysate to be efficiently and specifically bound to the silica matrix centrifugal adsorption column, while other contaminants can flow through the membrane, and the inhibitors of PCR and other enzymatic reactions can be effectively removed through a two-step washing step, and finally washed off with low-salt buffer or water, so that high-purity DNA can be obtained. The purified DNA can be used for downstream experiments such as digestion, PCR, Real-Time PCR, library construction, Southern Blot and molecular labeling, molecular labeling and other downstream experiments.

Self-contained reagents: anhydrous ethanol; Enzymatic Lysis Buffer is required for extraction of Gram-positive bacteria.

Enzymatic Lysis Buffer was prepared by 20 mM Tris, pH 8.0; 2 mM Na2-EDTA, pH 8.0; and 1.2% Triton X-100. 121° C sterilization for 20 minutes, and the appropriate amount of Lysozyme was added at a final concentration of 20 mg/ml.

Pre-experiment Preparation and Important Notes

1. Add 1.25ml Proteinase K Storage Buffer to Proteinase K to dissolve it and store it at -20℃. Do not leave the prepared Proteinase K at room temperature for a long time, and avoid repeated freezing and thawing to avoid affecting its activity.
2. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller DNA fragments and a decrease in the amount of extracted DNA.
3. If extracting genomes from bacterial cultures with high accumulation of secondary metabolites or thick cell walls, it is recommended that samples be collected early in the logarithmic phase.
4. Anhydrous ethanol should be added to Buffer GW1 and Buffer GW2 according to the instructions on the label of the reagent bottle before first use.
5. Before use, please check Buffer GTL and Buffer GL for crystallization or precipitation. If crystallization or precipitation occurs, please re-dissolve Buffer GL and Buffer GTL in a 56℃ water bath.
6. If the downstream experiments are sensitive to RNA contamination, 4μl of DNase-Free RNase A (100mg/ml) can be added before adding Buffer GL. RNase A is not provided in this kit.

If the extracted samples are Gram-positive bacteria, customers need to prepare their own Enzymatic Lysis Buffer to treat the bacteria, which requires the use of Lysozyme (lysozyme) at a concentration of 20 mg/ml, which is not provided in this kit.

procedure

i Extraction of genomic DNA from Gram-negative bacteria

1. Take 1-5 ml of bacterial culture (106-108 cells, maximum 2×109 cells) and put it into a centrifuge tube (provided), centrifuge it at 12,000 rpm (~13,400×g) for 1 minute, and aspirate the supernatant as much as possible.
2. Add 180 μl Buffer GTL to the precipitate and shake to resuspend the bacteria.
3. Add 20 μl of Proteinase K, vortex and mix well, incubate at 56° C until the solution becomes clear, and invert or shake the centrifuge tube at intervals during the incubation to disperse the sample.

Note: If RNA removal is required, add 4 μl of RNase A solution at a concentration of 100 mg/ml after the above steps are completed, shake to mix, and leave for 5-10 minutes at room temperature.

4. Add 200μl Buffer GL and mix well with vortexing and shaking. Add 200μl of anhydrous ethanol and mix well with vortexing and shaking.

Centrifuge briefly so that the solution on the walls of the tube collects at the bottom.

Note: 1) If multiple samples are manipulated together, Buffer GL and anhydrous ethanol can be mixed in equal proportions and then added together, shaking to mix.

2) The addition of Buffer GL and anhydrous ethanol may produce a white precipitate that will not affect subsequent experiments.

5. Add all of the solution obtained in step 4 (including the precipitate formed) to the Spin Columns DM in the collection tube, or if the solution cannot be added all at once, transfer it several times. centrifuge at 12,000 rpm for 1 minute, discard the waste solution, and return the column to the collection tube.

6. Add 500 μl of Buffer GW1 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and return the adsorption column to the collection tube.

7. Add 500 μl of Buffer GW2 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note: Step 7 can be repeated if further DNA purity is required.

8. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorbent column at room temperature for several minutes to dry thoroughly. Note: The purpose of this step is to remove residual ethanol from the adsorbent column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

9. Place the adsorption column in a new centrifuge tube, add 50-200 μl Buffer GE to the middle part of the adsorption column overhanging the center of the adsorption column, leave it at room temperature for 2-5 minutes, centrifuge it at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20 ℃. note: 1) If the downstream experiments are sensitive to the pH or EDTA, the elution can be done with sterilized water. The pH of the elution solution has a great influence on the elution efficiency. If water is used as the elution solution it should be ensured that its pH is 7.0-8.5 (the pH of water can be adjusted to this range with NaOH), and the elution efficiency is not high when the pH is lower than 7.0.

2) Incubation at room temperature for 5 minutes prior to centrifugation increases yield.

3) Re-elution with an additional 50-200 μl Buffer GE or sterilized water can increase the yield.

4) If the final concentration of DNA is to be increased, the DNA eluate obtained in step 9 can be re-spiked onto the adsorbent membrane and step 9 repeated; if the elution volume is less than 200 μl, the final concentration of DNA can be increased, but the total yield may be reduced. If the amount of DNA is less than 1 μg, elution with 50 μl Buffer GE or sterilized water is recommended.

(5) DNA stored in water will be affected by acidic hydrolysis. For long-term storage, it is recommended to elute with Buffer GE and store at -20℃.

i. Extraction of genomic DNA from Gram-positive bacteria

1. Take 1-5 ml of bacterial culture (106-108 cells, maximum 2×109 cells) and put it into a centrifuge tube (provided), centrifuge it at 12,000 rpm (~13,400×g) for 1 minute, and aspirate the supernatant as much as possible.

2. Add 180μl Enzymatic Lysis Buffer (self-provided) to resuspend the bacteria. Enzymatic Lysis Buffer is prepared as described in the Self-Prepared Reagents section in the front of the manual.
3. Incubate at 37° C for 30 minutes.
4. Add 20μl Proteinase K and mix well. Add 200μl of Buffer GL and mix well with vortexing and shaking.
Note: Do not add Proteinase K directly to Buffer GL.
Incubate at 5.56° C for 30 minutes.
Note: 1) If desired, incubation at 95° C for 15 minutes will inactivate the pathogen, but 95° C incubation will cause some DNA degradation.
(2) If RNA removal is required, add 4μl of RNase A solution at a concentration of 100mg/ml after the above steps are completed, shake and mix well, and leave for 5-10 minutes at room temperature.
6. Add 200μl of anhydrous ethanol and mix well with vortex shaking.
Note: The addition of anhydrous ethanol may produce a white precipitate that will not affect subsequent experiments.
7. Add all of the solution obtained in step 6 (including the precipitate formed) to the Spin Columns DM that have been loaded into the collection tube, and if the solution cannot be added all at once, it can be transferred in several times. centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and put the column back into the collection tube.
8. Add 500 μl of Buffer GW1 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
9. Add 500 μl Buffer GW2 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge the column at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the column back into the collection tube.
Note: Step 9 can be repeated if further DNA purity is required.
10. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.
Note: The purpose of this step is to remove residual ethanol from the adsorption column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
11. Place the adsorption column in a new centrifuge tube (self-provided), add 50-200 μl of Buffer GE to the center of the adsorption column overhanging the center of the adsorption column, let it stand at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20℃.
Note: 1) If the downstream experiment is sensitive to pH or EDTA, you can use sterilized water for elution. The pH of the eluent has a great influence on the elution efficiency, if water is used as the eluent should ensure that its pH is

7.0-8.5 (you can use NaOH to adjust the pH of the water to this range), and the elution efficiency is not high when the pH is lower than 7.0.

2) Incubation at room temperature for 5 minutes prior to centrifugation increases yield.

3) Re-elution with an additional 50-200 μl Buffer GE or sterilized water can increase the yield.

4) If the final concentration of DNA is to be increased, the DNA eluate obtained in step 11 can be re-spiked onto the adsorbent membrane and step 11 repeated; if the elution volume is less than 200 μl, the final concentration of DNA can be increased, but the total yield may be reduced. If the amount of DNA is less than 1 μg, elution with 50 μl Buffer GE or sterilized water is recommended.

(5) DNA stored in water will be affected by acidic hydrolysis. For long-term storage, it is recommended to elute with Buffer GE and store at -20℃.