PURO Bacteria DNA

For isolation of genomic DNA from bacteria

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Innovación y Desarrollo en Biotecnología

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Contents

Contents	50 preps
Buffer GA	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TE	15 ml
Proteinase K	1 ml
Spin Columns CB3	50
Collection Tubes 2 ml	50
Handbook	1

Storage

PURO Bacteria DNA can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the can be stored at 2-8°C.

Introduction

PURO Bacteria DNA is based on silica membrane technology and special buffer system for many kinds of sample's gDNA extraction. The spin column made of new type of silica membrane which can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA purified by PURO Genomic DNA is highly suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Yield of Genomic DNA with PURO Genomic DNA

Sample	Quantity	DNA yield
Bacterial culture	10^6-10^8 cells	5-20 µg

Important Notes

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to DNA size reduction.
2. If precipitates formed in Buffer GA or Buffer GB, warm the buffer to 56°C until the precipitates fully dissolve.
3. All centrifugation steps should be carried out in a conventional table-top centrifuge at room temperature (15-25°C).

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Pipet 1-5ml bacterial culture suspension in a centrifuge tube by centrifuging for 1 min at 10,000 rpm (~11,500 × g). Discard supernatant.
2. Add 200 µl Buffer GA. Mix thoroughly by vortex.

   Note: For difficult-broken Gram-positive bacteria, you can skip Step 2, add lysozyme, the specific methods are: add 180 µl enzymatic lysis buffer (20 mM Tris·Cl, pH 8.0; 2 mM sodium EDTA; 1.2% Triton® X-100; add lysozyme to 20 mg/ml immediately before use). Incubate for at least 30 min at 37°C.

   If RNA-Free genomic DNA is required, add 4 µl RNase A (100 mg/ml, should be prepared by user, Cat. no. RT405-11), mix by vortex for 15s, and incubate for 5 min at room temperature (15-25°C).

3. Add 20 µl Proteinase K. Mix thoroughly by vortex.
4. Add 220 µl Buffer GB to the sample, mix thoroughly by vortex, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
5. Add 220 µl ethanol (96-100%) to the sample, and mix thoroughly by vortex for 15 s. A white precipitate may form on addition of ethanol. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
6. Pipet the mixture from step 5 into the Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the spin column into the collection tube.
7. Add 500 µl Buffer GD to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
8. Add 700 µl Buffer PW to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
9. Add 500 µl Buffer PW to \Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
10. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely.

    Note: The residual ethanol of buffer PW may affect downstream application.
11. Place the Spin Column CB3 in a new clean 1.5 ml centrifuge tube, and pipet 50-200 µl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

Note: If the volume of eluted buffer is less than 50 µl, or it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest chose buffer TE or distilled water (pH 7.0-8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.