Cat. # 9763

For Research Use

TaKaRa

TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver.3.0

Product Manual

v201309Da

Table of Contents

I. Description ... 3
II. Components ... 3
III. Storage and shipping ... 3
IV. Preparation before experiment ... 4
V. Protocol ... 4
VI. Experimental examples ... 6
VII. Cautions ... 7
VIII. DNA extraction from different bacteria ... 8
IX. Troubleshooting ... 8

I. Description

TaKaRa MiniBEST Bacterial Genomic DNA Extraction Kit Ver. 3.0 is designed to purify genomic DNA from a variety of bacteria including gram-positive/negative bacteria. This system apply a special lysis buffer in combination with DNA preparation membrane to efficiently purify genomic DNA from bacteria. The protocol provides a simple method to achieve the rapid isolation of highly purified genomic DNA and the entire procedure can be accomplished within 20 minutes after cell lysis. Using the kit 1 - 20 μg of highly purified genomic DNA can be extracted from 1.0 - 5.0 x 10^9 bacteria (OD_{600} = 1, equivalent to 1 ml broth containing 1.0 x 10^9 cells). Genomic DNA prepared by this kit is suitable for a variety of applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other molecular biology experiments.

II. Components (50 reactions)

The kit contains Box I and Box II.

Box I (stored at -20℃)

Component	Amount
Proteinase K (20 mg/ml)	1 ml
Lysozyme (20 mg/ml)*1	1.25 ml x 2
RNase A (10 mg/ml)	500 μl

*1: Avoid repeated freezing and thawing. Once thawed, store at 4℃ for a long time.

Box II (stored at room temperature (15 - 25℃))

Component	Amount
Buffer BS	28 ml
Buffer GL*1	12 ml
Buffer GB*1	12 ml
Buffer WA*1	28 ml
Buffer WB*2	24 ml
Elution Buffer	14 ml
Spin Column	50
Collection tube	50

*1: Contains strong denaturant. Be careful to avoid contacting with skin and eyes. In the case of such contact, wash immediately with plenty water and seek medical advice.
*2: Before using the kit, add 56 ml of 100% ethanol. Mix well.

Reagents not supplied in this kit

1. 100% ethanol
2. Sterilized water

III. Storage and shipping

1. Box I can be stored at -20℃, once thawed Lysozyme, store it at 4℃ for a long time, and Box II can be stored at room temperature (15 - 25℃).
2. Box I can be shipped at -20℃, and Box II can be shipped at room temperature (15 - 25℃).

IV. Preparation before experiment

1. Adjust a water bath to 56℃ and 37℃ (when extracting the gram-positive bacteria).
2. If precipitation are present in Buffer GL, warm it at 65℃ and use after returning to room temperature.
3. Add 56 ml of 100% ethanol to Buffer WB and mix well before using it.
4. Pre-heat the Elution Buffer or sterile distilled water to 65℃ will improve elution efficiency.

V. Protocol

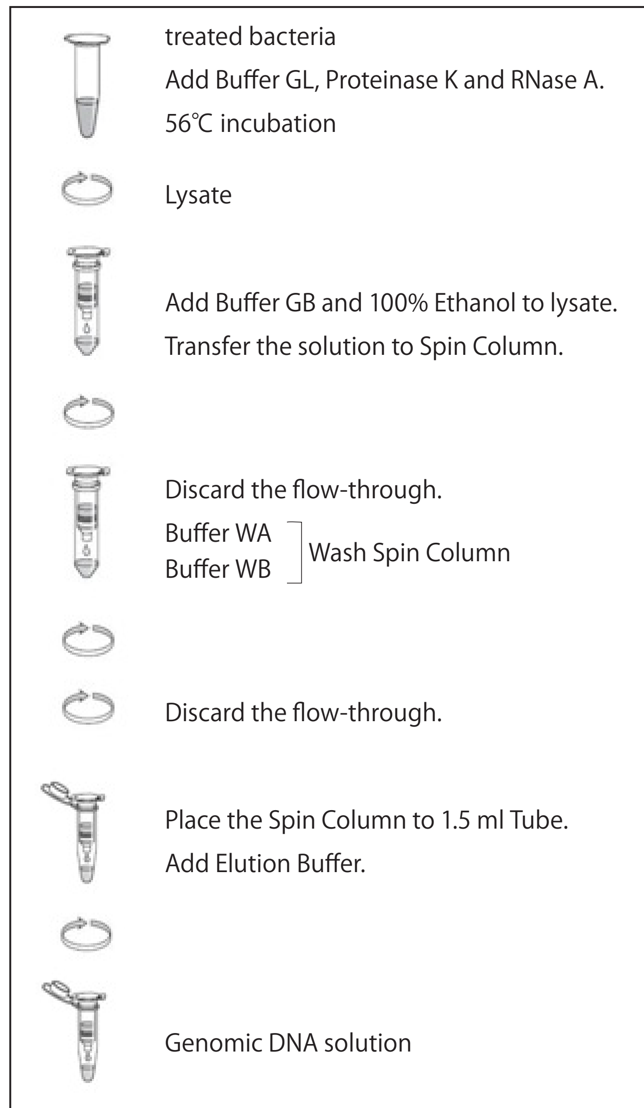

Figure 1. Flow chart

The flow chart of protocol is in Figure 1. The procedure after bacteria lysis can be accomplished in 20 minutes. The whole procedure includes bacteria lysis, DNA binding with column and DNA purification. Protocol in detail is as below.

● Lysis of gram-negative bacteria

(1) Collect 1.0 - 5.0 x 10^9 of bacteria into 1.5 ml tube.
Centrifuge at 12,000 rpm for 2 minutes.
Discard the supernatant.

(2) Add 180 μl of Buffer GL, 20 μl of Proteinase K (20 mg/ml) and 10 μl of RNase A (10 mg/ml). Mix well and incubate it into 56℃ water bath for 10 minutes. The solution should appear clear, and clarify.

(3) Add 200 μl of Buffer GB and 200 μl of 100% ethanol to the solution and mix well.

● Lysis of gram-positive bacteria

(1) Collect 0.5 - 2.0 x 10^9 of bacteria into 1.5 ml tube.
Centrifuge at 12,000 rpm for 2 minutes. (If the bacteria is overgrown or the cell wall of the bacteria has special structure, the amount of starting material should not exceed 1.0 x 10^9 cells.)
Discard the supernatant.

(2) Add 500 μl of Buffer BS, 50 μl of Lysozyme (20 mg/ml). Mix well and incubate it into 37℃ water bath for 60 minutes. (Mix by inversion once every 20 minutes.)

(3) Centrifuge at 12,000 rpm for 5 minutes.
Discard the supernatant.

(4) Add 180 μl of Buffer GL, 20 μl of Proteinase K (20 mg/ml) and 10 μl of RNase A (10 mg/ml).
Mix well and incubate it into 56℃ water bath for 10 minutes. The solution should appear clear, and clarify.
If the solution is not clear after 10 minutes incubation, extend the lysis time to 30 minutes and mix the solution every 5 minutes by pipetting.

(5) Add 200 μl of Buffer GB and 200 μl of 100% ethanol to the solution and mix well.

1. Place Spin Column into Collection Tube. Transfer the solution to Spin Column. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.
2. Add 500 μl of Buffer WA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
3. Add 700 μl of Buffer WB into Spin Column. Take care to add Buffer WB along the tube wall of Spin Column to wash off any residual salt.
Centrifuge at 12,000 rpm for 1 minute.
Discard the flow-through.
Note: Make sure the amount of 100% ethanol specified on the bottle label has been added to the Buffer WB.
4. Repeat Step 3.
5. Place Spin Column into Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
6. Place Spin Column into a new 1.5 ml tube. Add 50 - 200 μl of Elution Buffer or sterile distilled water to the center of the membrane. Let it stand for 5 minutes at room temperature.
Note: Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.
7. Centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
If more yield is needed, the flow-through can be re-added into the center of the membrane or add 50 - 200 μl of Elution Buffer or sterile distilled water and let it stand for 5 minutes at room temperature and centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
8. Quantification of the genomic DNA.
The extracted genomic DNA can be quantitatively determined by electrophoresis or absorbance measuring.

VI. Experimental examples

1. Purification of Genomic DNA from Gram-positive Bacteria
   Approximate 10 μg, 6 μg, 6 μg, or 8 μg of highly-purified genomic DNA has been extracted from 2.0 x 10^9 of *Bacillus subtilis*, *Lactobacillus plantarum*, *Acidiphilium facilis*, or *Kocuria kristinae*, respectively. The result of electrophoresis is in Figure 2.

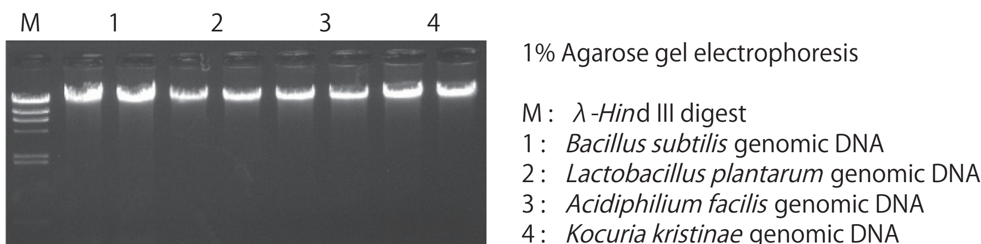

Figure 2. Electrophoresis of Genomic DNA from Gram-positive Bacteria

2. Purification of Genomic DNA from Gram-negative Bacteria
   Approximate 6 μg, 8 μg, 8 μg, or 10 μg of highly purified genomic DNA has been extracted from 2.0 x 10^9 of *Acetobacter aceti*, *Enterobacter aerogenes*, *Serratia marcescens* Sb, or *Escherichia coli* JM109, respectively. The result of electrophoresis is in Figure 3.

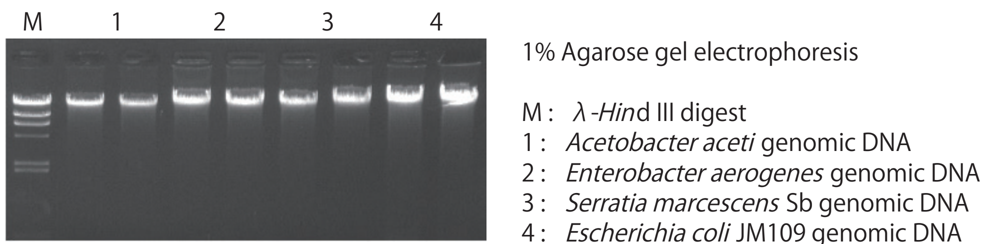

Figure 3. Electrophoresis of Genomic DNA from Gram-negative Bacteria

3. Purification of Genomic DNA from *Staphylococcus aureus* and PCR amplification of 3.4 kb fragment
   Approximate 6 μg of highly purified genomic DNA has been extracted from 2.0 x 10^9 of *Staphylococcus aureus*. Using the genomic DNA as template, 3.4 kb DNA fragment of R. *Sau*3A I gene was amplified by PCR. The results of electrophoresis are in Figure 4.

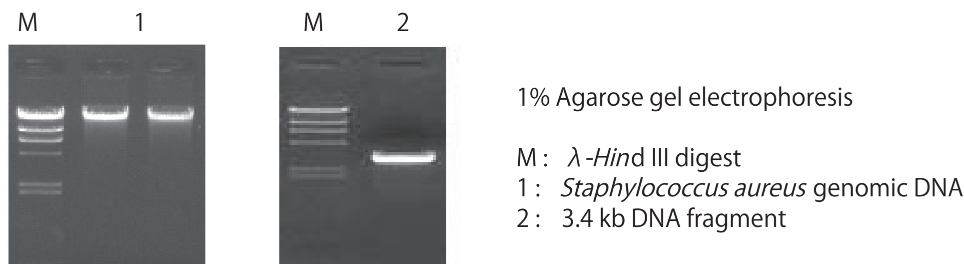

Figure 4. Electrophoresis of Genomic DNA from *Staphylococcus aureus* and fragment amplified by PCR

4. DNA yield from different starting amount of bacteria
   Using the kit, genomic DNA were extracted from 1.0 x 10^9, 2.0 x 10^9 and 5.0 x 10^9 of *Microbacterium oxydans* and eluted with 200 μl Elution Buffer.

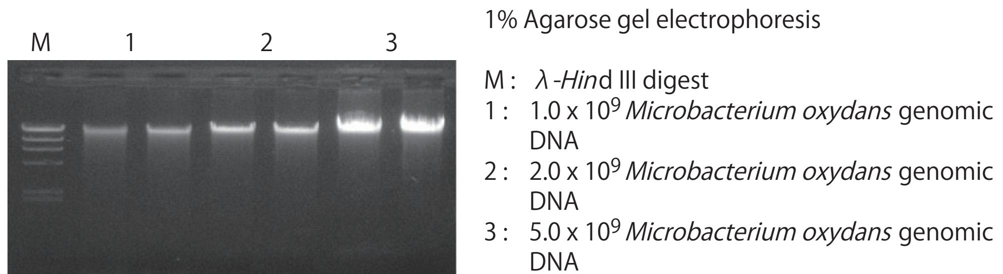

Figure 5. Genomic DNA extracted from different starting amount of *Microbacterium oxydans*

VII. Cautions

1. Use fresh experimental material to ensure genomic DNA extracted not to be degraded.
2. Some reagents contain chemical irritants. When working with these reagents, always wear suitable protection such as safety glasses, laboratory coat and gloves and operate at fume hood. Be careful to avoid contacting with eyes and skin. In the case of such contact, wash immediately with plenty water and seek medical advice.
3. If the genomic DNA is stored for a long time, it is recommended to save in the Elution Buffer.
4. The volume of bacteria sample cannot be too much. Usually 0.1 - 1 ml of overnight culture of bacteria can be used.

VIII. DNA extraction amount from different bacteria

The yields of DNA extracted from 2.0 x 10^9 of gram-positive bacteria are shown as follows.

Bacteria	DNA Yield
Microbacterium oxydans	6 - 10 μg
Bacillus subtilis	8 - 10 μg
Kocuria varians	6 - 10 μg
Staphylococcus aureus	3 - 8 μg
Enterococcus durans.	6 - 10 μg
Pediococcus pentosaceus	6 - 10 μg
Enterococcus gallinarum	5 - 8 μg
Anabaena flos-aquae	6 - 10 μg
Arthrobacter luteus	6 - 12 μg
Nocardia	5 - 8 μg
Brevibacterium linens	5 - 8 μg
Caryophanon latum L	5 - 8 μg
Caseobacter polymorphus	5 - 8 μg
Streptomyces	5 - 8 μg
Lactobacillus plantarum	5 - 8 μg

The yields of DNA extracted from 2.0 x 10^9 of gram-negative bacteria are shown as follows.

Bacteria	DNA Yield
Escherichia coli JM109	6 - 10 μg
Acetobacter aceti	6 - 10 μg
Acinetobacter calcoaceticus	10 - 15 μg
Acidiphilium organovorum 13H	10 - 15 μg
Enterobacter aerogenes	6 - 10 μg
Flavobacterium balustinum	10 - 15 μg
Plesiomonas shigelloides	6 - 10 μg
Serratia marcescens Sb	8 - 15 μg

X. Troubleshooting

Q1. How yield of genomic DNA is extracted?

A1. This kit is suitable for purification of genomic DNA from bacteria. The yield of genomic DNA differs by starting material. Generally, 5 - 15 μg of genomic DNA can be extracted from 2.0 x 10^9 of bacteria; 10 μg of genomic DNA can be extracted from 2.0 x 10^9 of *E. coli* JM109 ; 6 μg of genomic DNA can be extracted from 2.0 x 10^9 of *Staphylococcus aureus*.

Q2. The yield of genomic DNA is low or there is no yield, why?

A2. When the yield of genomic DNA is lower, the following aspects can be considered:

(1) The experimental sample is not enough, for instance, genomic DNA from 2.0 x 10^6 of bacteria can't be detected by electrophoresis.

(2) Incomplete lysis causes incomplete release of DNA. Since the bacterial cell wall structure is quite different at each species, so the lysis conditions depend on bacterial species. When bacteria solution is completely lysed, it should appear clear and clarification. When the bacteria content is higher, the solution is more viscous; Incomplete lysis causes the solution turbid. In that case it is recommended to extend the lysis time until overnight or to increase the amount of lysis buffer.

(3) Content of genomic DNA in sample is small, so increase the amount of starting material.
(4) Amount of starting material is too much so it's hard for lysis. Increase the amount of Lysozyme and Buffer GL appropriately and divide the sample into more than one to extract.
(5) Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.
(6) Strictly follow the protocol.

Q3. Genomic DNA extracted is degraded.
A3. (1) The bacteria are not fresh enough or not processed in time or not stored at low temperature after collecting. The sample should be stored at -80℃ and shipped in dry ice.
(2) There is residue DNase on experimental sample. Wash with Buffer WA once more.
(3) If the genomic DNA is stored for a long time, it is recommended to save in the Elution Buffer.

Q4. There is contamination with RNA in genomic DNA extracted.
A4. (1) RNase A has not been used in the procedure. Strictly follow the protocol to use RNase A.
(2) RNase A may be inactivated. RNase A should be stored at -20℃. Activity of RNase A is stable and not easily inactivated.

Q5. Why does genomic DNA extracted have low biological activity?
A5. (1) The salt concentration in extracted genomic DNA is too high. When washing the DNA preparation membrane using Buffer WA and Buffer WB, add them along the tube wall of Spin Column and let it stand for 5 minutes at room temperature to wash off any residual salt and improve the washing effect.
(2) There is residual ethanol in Genomic DNA solution. Let the column stand for 2 minutes at room temperature before adding Elution Buffer to the column and it will improve the effect of elution.
(3) The Elution Buffer must be added in the center of column membrane during DNA elution and not residue on the tube wall of Spin Column.

Q6. When the amount of experimental materials is more than the amount in the protocol?
A6. This kit is designed to purify a small amount of genomic DNA. If the amount of experimental materials is more than the amount described in protocol, increase the Lysozyme, Buffer GL and Buffer GB and divide the homogenate into more Spin Column. It's better to control the amount of starting material in the specified range and divide into more than one. The incomplete lysis will block the column and cause the failure of DNA purification.

Q7. How much is appropriate starting sample?
A7. The 0.5 ml of *Escherichia coli* JM109 cultured overnight in LB medium (OD_{600} = 3 - 4) can be used for DNA purification.

Q8. Can the kit extract gDNA from all bacteria?
A8. The kit can extract genomic DNA from most gram-negative and gram-positive bacteria, but there are some gram-positive bacteria (e.g.: *Bacillus thuringiensis*) which cell wall structure is rather specific and digested more difficult, so DNA extraction is markedly less. If some bacteria are difficult for lysis, they can be grinded in liquid nitrogen, and then follow the method for gram-negative bacterial genomic DNA extraction.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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