Cat. # 9765

For Research Use

TaKaRa

TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0

Product Manual

v201306Da

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I. Description

TaKaRa MiniBEST Universal Genomic DNA Extraction Kit is designed to purify genomic DNA from a variety of sample sources including blood, gram-negative bacteria, mammalian cultured cells, plant tissue and animal tissue. This kit employs a proprietary lysis buffer in combination with Spin Column membrane to efficiently purify genomic DNA from the biological sample. 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 tissue cell lysed. Using the kit about 10 μg of highly purified genomic DNA can be extracted from 50 - 200 μl of mammalian whole blood (with anticoagulant), 1 - 10 μl of nucleated erythrocyte in whole blood (with anticoagulant), 1.0 - 5.0 x 10^9 gram-negative bacteria, 1.0 x 10^5 - 1.0 x 10^7 cultured cells, 2 - 30 mg of mammalian tissues and 25 - 100 mg plant tissues. 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. Kit Components (50 reactions)

The kit contains Reagent Set and Column Set.

Reagent Set	
Proteinase K (20 mg/ml)	1 ml
RNase A (10 mg/ml)	0.5 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

*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.

Column Set	
Spin Columns	50
Collection tubes	50

[Reagents not supplied in the kit]

1. 100% ethanol
2. Sterilized water
3. PBS

III. Storage and Shipping

1. The kit can be stored at room temperature (15 - 25℃).
2. Proteinase K can be stored at room temperature (15 - 25℃) for 6 months, and should be stored at -20℃ for long-term.
3. RNase A (10 mg/ml) can be stored at room temperature (15 - 25℃) for 6 months., and should be stored at -20℃ for long-term.
4. The kit is shipped at room temperature (15 - 25℃).

IV. Preparation before Experiment

1. Adjust a water bath to 56℃.
2. If precipitation occurs in Buffer GL, warm at 65℃ and use after standing at 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

● **Flow chart**

Materials

Cut into small pieces or grind in liquid nitrogen

Add Buffer GL, Proteinase K and RNase A

56℃ water bath

Lysate

Add Buffer GB to lysate

Transfer the solution to Spin Column

Discard the flow-through

Buffer WA

Buffer WB

Discard the flow-through

Place the Spin Column to 1.5 ml Tube

Add Elution Buffer

Genomic DNA solution

The procedure after cell and tissue are lysed can be accomplished in 20 minutes. The whole protocol includes homogenization, lysis, DNA binding with column, and DNA purification.

Protocol in detail is as below.

1. Homogenization and lysisEach type of sample has different requirements for the method used to achieve efficient lysis and homogenization. Protocols are provided for each sample type.

◆ For animal and plant tissue:
(1) Transfer 2 - 30 mg of animal tissue or 25 - 100 mg of plant tissue into 2 ml microtube. Cut into small pieces. Hard tissue can be grinded in liquid nitrogen.
(2) Add 180 μl of Buffer GL, 20 μl of Proteinase K and 10 μl of RNase A and incubate it in 56℃ water bath with occasionally vortexing until the tissue has been completely lysis. (2 - 3 hours. If there is a materials difficult for lysis, it may take more time.
Note : Some plant samples contain fibrous tissue which is difficult to lysis. This kit, however, is available for these samples also.
(3) Add 200 μl of Buffer GB to the lysate and mix well.

◆ For whole blood:
(1) Transfer 1 - 10 μl of nucleated erythrocytes in whole blood (with anticoagulant), 50 - 200 μl of anucleated erythrocytes in whole blood (with anticoagulant) into 2 ml microtube. Add PBS or sterilized water up to 200 μl.
(2) Add 180 μl of Buffer GB, 20 μl of Proteinase K and 10 μl of RNase A. Mix well and incubate it in 56℃ water bath for 10 minutes.

◆ For mammalian cultured cells grown in suspension
(1) Transfer 1.0 x 10^5 - 1.0 x 10^7 cell suspension in 1.5 ml microtube. Centrifuge at 5,000 rpm for 5 minutes. Discard the supernatant.
(2) Add 200 μl of PBS or sterilized water to resuspend the cells.
(3) Add 180 μl of Buffer GB, 20 μl of Proteinase K and 10 μl of RNase A. Mix well and incubate it in 56℃ water bath for 10 minutes.

◆ For mammalian adherent cells
(1) Discard the supernatant as much as possible. Add 1 ml of PBS to each 10 cm^2 of Adherent cells. Pipette up and down several times to pull off the adherent cells, and then transfer the cell suspension to 1.5 ml microtube. Centrifuge at 5,000 rpm for 5 minutes. Discard the supernatant. Add 200 μl of PBS or sterilized water to resuspend the cells.
(2) Add 180 μl of Buffer GB, 20 μl of Proteinase K and 10 μl of RNase A. Incubate it in 56℃ water bath for 10 minutes.

◆ For gram-negative bacteria such as *E. coli*
(1) Transfer 1.0 - 5.0 x 10^9 of bacteria into 1.5 ml microtube. Centrifuge at 12,000 rpm for 2 minutes. Discard the supernatant.
(2) Add 180 μl of Buffer GL, 20 μl of Proteinase K and 10 μl of RNase A. Mix well and incubate it in 56℃ water bath for 10 minutes.
(3) Add 200 μl of Buffer GB. Mix well.

◆ For aquatic organism such as fish (rich at small size DNA molecules)

(1) Transfer 25 - 30 mg of tissue into 2 ml microtube. Cut into small pieces or grinded in liquid nitrogen.

(2) Add 180 μl of Buffer GL, 20 μl of Proteinase K and 10 μl of RNase A and incubate it in 56℃ water bath until the tissue has been completely lysis. (about 2 - 3 hours)

Note : The sample may occasionally be taken out from water bath and vortexed to accelerate tissue lysis.

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

◆ For a processed product (rich at fragmented genomic DNA)

(1) Transfer 25 - 50 mg of a processed product into 2 ml microtube. Cut into small pieces.or grinded in liquid nitrogen.

(2) Add 180 μl of Buffer GL, 20 μl of Proteinase K and 10 μl of RNase A and incubate it in 56℃ water bath until the tissue has been completely lysis. (about 2 - 3 hours)

Note : The sample may occasionally be taken out from water bath and vortexed to accelerate tissue lysis.

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

2. Transfer the solution to Spin Column. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.
3. Add 500 μl of Buffer WA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
4. Add 500 μl of Buffer WB into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.

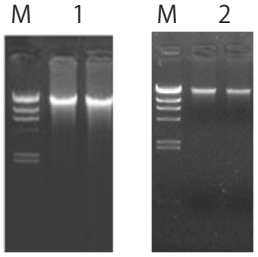
   Note : Make sure the amount of 100% ethanol specified on the bottle label has been added to Buffer WB.
   Add Buffer WB along the wall of Spin Column to wash off any residual salt.
5. Repeat Step 4.
6. Place Spin Column into Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
7. Place Spin Column into a new 1.5 ml microtube. Add 50 - 200 μl of Elution Buffer or sterile distilled water to the center of the Spin Column membrane. Let it stand for 5 minutes at room temperature.

   Note : Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve recovery efficiency.
8. Centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
   If higher yield is required, the flow-through can be re-applied into the center of the membrane and let it stand for 5 minutes at room temperature and centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
9. Analysis of the genomic DNA.

VI. Experimental Examples

Example1 : Purification of Genomic DNA from Mammalian Tissues

About 15 μg of high-purity genomic DNA was extracted from 25 mg of mouse liver tissue. 10 μg of high-purity genomic DNA has been extracted from 1.2 cm of mouse tail tip, respectively (Fig. 1).



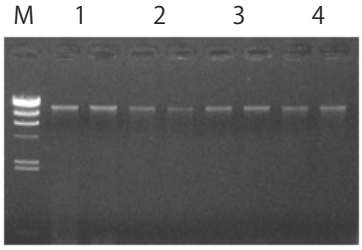
1% Agarose gel electrophoresis

M: λ-*Hin*d III digest
1: Mouse liver genomic DNA
2: Mouse tail tip genomic DNA

Fig. 1 Electrophoresis of Genomic DNA from mammalian tissues

Example 2 : Purification of Genomic DNA from Plant Tissues

About 2.5 μg, 1.5 μg, 3 μg and 2 μg of highly purified genomic DNA was extracted from 25 mg of spinach, rape, crown daisy Chrysanthemum, and Chinese white cabbage, respectively (Fig. 2).



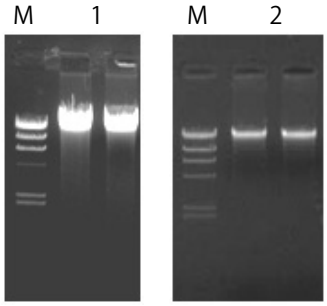
1% Agarose gel electrophoresis

M: λ-*Hin*d III digest
1: Spinach genomic DNA
2: Crown daisy genomic DNA
3: Rape genomic DNA
4: Chinese white cabbage genomic DNA

Fig. 2 Electrophoresis of Genomic DNA from plant tissues

Example 3 : Purification of Genomic DNA from Whole Blood

About 12 μg and 2 μg of high-purity genomic DNA was extracted from 5 μl of fish whole blood (containing EDTA anticoagulant) and 200 μl of horse whole blood (containing EDTA anticoagulant), respectively (Fig. 3).



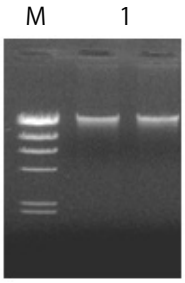
1% Agarose gel electrophoresis

M: λ-*Hin*d III digest
1: Fish whole blood genomic DNA
2: Horse whole blood genomic DNA

Fig. 3 Electrophoresis of Genomic DNA from whole blood

Example 4 : Purification of Genomic DNA from gram-negative bacteria *E. coli*

About 10 μg of high-purity genomic DNA was extracted from 2.0 x 10^9 of *E. coli* JM109 (Fig. 4).



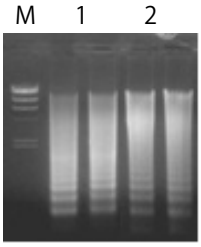
1% Agarose gel electrophoresis

M: λ-*Hin*d III digest
1: Genomic DNA purified from JM109

Fig. 4 Electrophoresis of Genomic DNA from JM109

Example 5 : Purification of Genomic DNA from carp fin and gills

About 10 μg of high-purity genomic DNA was extracted from 25 mg of carp fin and gills (Fig. 5).

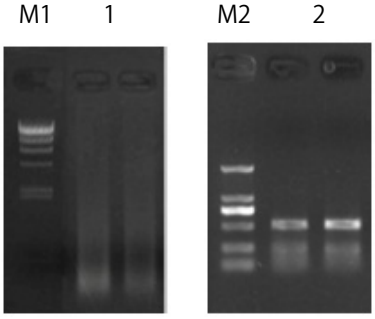


1% Agarose gel electrophoresis
M: λ-*Hin*d III digest
1: carp gills genomic DNA
2: carp fin genomic DNA

Fig. 5 Electrophoresis of Genomic DNA from carp fin and gills

Example 6 : Purification of Genomic DNA from processed products, beef jerky.

About 5 μg of high-purity genomic DNA was extracted from 50 mg of beef jerky. A region (528 bp) at cattle mitochondrial genome (GenBank; V00654.1) was amplified by PCR using the obtained genomic DNA as a template (Fig. 6).

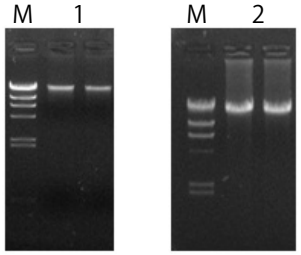


1% Agarose gel electrophoresis
M1 : λ-*Hin*d III digest
1: Genomic DNA purified from beef jerky
M2 : DL2,000 DNA Marker
2 : 528 bp PCR product

Fig. 6 Electrophoresis of Genomic DNA from beef jerky and PCR fragment.

Example 7 : Purification of Genomic DNA from HL60 cells.

About 10 μg of high-purity genomic DNA was extracted from 2.0 x 10^6 of cultured cells HL60. Using the genomic DNA as template, 17.5 kb of β-globin gene fragment was amplified by PCR (Fig. 7).



1% Agarose gel electrophoresis
M： λ-*Hin*d III digest
1： Genomic DNA purified from HL60 cultured cells
2： β- globin (about 17.5 kb)

Fig. 7 Electrophoresis of Genomic DNA from HL60 and PCR fragment.

Example 8. Effect of elution volume on DNA extraction

Extract genomic DNA from 2.0 x 10^6 HL60 cells. Elute DNA two times using 10 μl, 50 μl, 100 μl, and 200 μl of Elution Buffer, respectively (Fig. 8). With the elution volume increasing, the DNA yield increases but the concentration decreases.

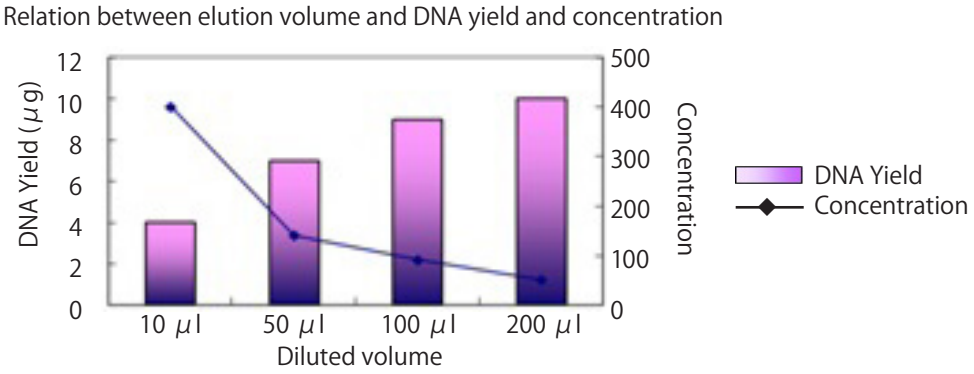


Fig. 8 Effect of elution volume on DNA extraction

Example 9. Effect of elution times on DNA extraction

Extract genomic DNA from 15 mg and 30 mg of mouse liver. Elute DNA three times using 100 μl and 200 μl of Elution Buffer, respectively (Fig. 9).

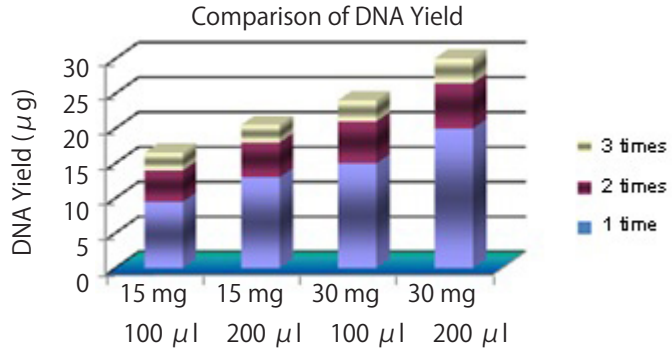


Fig. 9 Effect of elution frequency on DNA extraction

VII. Cautions

1. Use fresh material to avoid degradation of genomic DNA.
2. When the tissue is grinded by liquid nitrogen, the liquid nitrogen should be added as required.
3. Some reagents contain chemical irritants. When working with these reagents, always wear suitable protection such as safety glasses, laboratory coat and gloves and operate in fume hood. Be careful to avoid contacting with eyes and skin. In the case of such contact, wash immediately with plenty water. If necessary, seek medical advice.
4. If the genomic DNA is stored for a long time, it is recommended to store in the Elution Buffer.

VIII. DNA Extraction from Various Materials

The yields of DNA extracted are shown as follows.

Material	Tissue amount	DNA yield
Mouse Liver	25 mg	15 - 20 μg
Mouse Spleen	5 - 10 mg	20 - 40 μg
Mouse Tail	1.2 cm	10 - 15 μg
Mouse Brain	25 mg	10 - 20 μg
Mouse Kidney	25 mg	15 - 30 μg
Mouse Lung	25 mg	15 - 30 μg
Mouse Intestine	25 mg	15 - 30 μg
Mouse Ear	25 mg	10 - 20 μg
Carp Fin	25 mg	10 - 20 μg
Short Necked Clam Meat	25 mg	3 - 10 μg
HL60 Cultured Cells	2.0×10^6	10 - 15 μg
E. coli JM109	2.0×10^9	5 - 10 μg
Horse Blood	200 μl	2 - 5 μg
Fish Blood	5 μl	10 μg
Celery	25 mg	4 - 6 μg
Rape	25 mg	1 - 2 μg
Spinach	25 mg	2 - 3 μg

IX. Maximum Amount of Starting Materials

Material	maximum amount	recommended amount
Cultured Cells	1.0×10^7	2.0×10^6
Nucleated erythrocytes in whole blood	10 μl	1 ～ 5 μl
Anucleated erythrocytes in whole blood	200 μl	50 ～ 200 μl
E. coli and other gram-negative bacteria	5.0×10^9	2.0×10^9
Common animal tissue	30 mg	25 mg
Tissue of high DNA content (e.g. spleen)	10 mg	5 mg
Tissue of higher DNA content (e.g. calf thymus)	2 mg	1 mg
Plant material	100 mg	25 ～ 50 mg

X. Q & A

Q1. What is the extraction yield of genomic DNA?

A1. This kit is suitable for purification of genomic DNA from plant, whole blood, cultured cells and gram-negative bacteria. The yield of genomic DNA differs by starting material. Generally, 15 μg of genomic DNA can be extracted from 25 mg of liver tissue; 10 μg of genomic DNA can be extracted from 100 mg of spinach; 0.5 μg of genomic DNA can be extracted from 100 μl of human whole blood; 10 μg of genomic DNA can be extracted from 2.0×10^6 of cultured cells; 10 μg of genomic DNA can be extracted from 2.0×10^9 of *E. coli* cells.

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 material is not enough, for instance, genomic DNA from 2×10^3 cultured cells can't be detected by electrophoresis.

(2) Incomplete grinding animal tissue or plant tissue causes incomplete release of DNA. It's recommended to extending the lysis time (up to overnight) or increasing the amount of lysis buffer.

(3) The content of genomic DNA in material is low. Increase the amount of starting material.

(4) The amount of starting material is too much so it's hard for lysis. Increase the amount of lysis buffer appropriately or divide the material into more than one.

(5) Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.

(6) Strictly follow the protocol.

Q3. Why is the extracted genomic DNA degraded?

A3. (1) The experimental materials are not fresh enough or the tissue materials are not processed in time or not stored at low temperature after collecting. It is recommended that the materials should be stored at -80℃ and shipped using dry ice.

(2) There is residue DNase on experimental materials. Wash with Buffer WA once more.

Q4. Why is there contamination with RNA in extracted genomic DNA?

A4. (1) RNase A has not been used in operation. Strictly follow the protocol to use RNase A.

(2) RNase A may be inactivated. RNase A should be stored at -20℃. RNase A is stable and not easily inactivated.

Q5. Why does the extracted genomic DNA have low biological activity?

A5. (1) The salt concentration in extracted genomic DNA is too high. When washing the Spin Column 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 lysate. Let the column stand for 2 minutes at room temperature before adding lysis solution to the column and it will improve the effect of washing.
(3) The elution buffer must be added in the center of Spin Column membrane at DNA elution and not residue on the tube wall of Spin Column.

Q6. How to extract more amount of materials is more than the amount in the protocol?

A6. This kit is designed to purify a small amount of genomic DNA. When the amount of materials is more than the amount described in the protocol, increase the Buffer GL or Buffer GB and divide the obtained homogenate into two tubes. It is important to adjust the amount of starting material in the specified range. The incomplete lysis will block the Spin Column and cause the failure of DNA purification.

Q7. How to extract genomic DNA from aquatic such as fish?

A7. The standard protocol of this kit is adapted to extract large size of genomic DNA. Most genomic DNA of fish and other aquatic animals is, however, smaller size genomic DNA than that of mammals. Then you should follow the protocol of "For lysis of aquatic organism such as fish" for homogenization and lysis.

Q8. How to extract genomic DNA from a processed product?

A8.
- The standard protocol of this kit is adapted to extract large size genomic DNA. Most genomic DNA of a processed product is degraded into small size DNA in the process. Then you should follow the protocol of "For a processed product" for homogenization and lysis.
- A processed product is low DNA content and its DNA is degraded during processed. The DNA from a processed product cannot be detected as bands by gel electrophoresis.