Soil DNA Extraction Kit

ISOIL for Beads Beating

Manual (Second edition)

Code No. 319-06201

NIPPON GENE CO., LTD.

ISOIL for Beads Beating Manual（ver.2）
110830KH

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I Product description

ISOIL for Beads Beating is a kit for extracting DNA from the soil samples.
By using an extracting solution with a special composition, DNA can be extracted not only from non-volcanic ash soil but also from volcanic ash soil, something which has been considered difficult to achieve up to now.
ISOIL for Beads Beating uses both chemical lysis by a surface-active agent and physical disruption of cells by beads beating as the DNA extraction method. As a result, DNA can be extracted from microorganisms having strong cell walls, and soil DNA reflecting actual soil microorganism mass structure can be obtained. Thus, the extracted soil DNA is suitable for mass structure analysis of soil microorganisms, e.g., using PCR-DGGE analysis, soil diagnosis, and estimation of soil biomass by quantitative determination of soil DNA.
However, users should be aware that DNA extracted by ISOIL for Beads Beating may have been subjected to physical shearing from beads beating.
Use ISOIL when extraction of high molecular weight soil DNA is required, e.g., when using the soil DNA as a gene resource.

II Contents of kit

Beads Tubes	50 Tubes	
Lysis Solution BB	50 ml	× 1
Lysis Solution 20S*	1.25 ml	× 2
Purification Solution*	20 ml	× 1
Precipitation Solution	40 ml	× 1
Wash Solution	50 ml	× 1
TE (pH8.0)	5 ml	× 1
Ethachinmate	100 µl	× 1
Manual		× 1

* : Crystal deposition may take place in the Lysis Solution 20S and the Purification Solution, but this will not affect quality or performance. In such cases, use after completely dissolving the crystals by incubating the whole container at about 65°C (mix occasionally).

III Storage

All the reagents included in ISOIL for Beads Beating can be stored at room temperature. However, for the Precipitation Solution, the Wash Solution and the Ethachinmate, we recommend that care be taken to prevent contamination at the time of use (contamination by fungi and bacteria), with storage at a low temperature (2-10°C) after opening.

IV Precautions

- This product is a reagent for research and cannot be used for medical or other objectives.
- This product should be handled only by persons having basic knowledge of reagents.
- Handle this product in accordance with the descriptions in the manual.
- We are not responsible for problems caused if this product is not handled in accordance with the manual.
- A patent has been filed for soil DNA extraction with ISOIL for Beads Beating by the University of Tokyo TLO. Nippon Gene has been licensed to practice the soil DNA extraction method by the University of Tokyo TLO.

V Protocol

< Reagents, instruments, etc., required in addition to this product **>**

- Beads Beating disruption apparatus (for 2 ml tube)
- 70% ethanol
- Chloroform
- Micropipette
- Pipette tip
- 2 ml microtube
- Incubator
- Microcentrifuge
- Vortex mixer

<Standard protocol>

(1) Put 0.5 g of soil sample in a Beads Tube.
(2) Add 950 µl of Lysis Solution BB and 50 µl of Lysis Solution 20S.(Note 1)
(3) Perform beads beating (4-6 m/sec or 4,200-6,800 rpm for 30-45 sec).(Note 2)
(4) Centrifuge (12,000 x g, 1 min, room temperature).
(5) Transfer 600 µl of the supernatant to a new tube, add 400 µl of Purification Solution, and mix well.
(6) Add 600 µl of chloroform, vortex for 15 sec, and then centrifuge (12,000 x g, 15 min, room temperature).
(7) Transfer 800 µl of the aqueous layer to a new tube while taking care not to transfer the intermediate layer, add 800 µl of Precipitation Solution, mix well, and then centrifuge (20,000 x g, 15 min, 4°C).(Note 3)
(8) Discard the supernatant, add 1 ml of Wash Solution, mix by inverting a few times, and then centrifuge (20,000 x g, 10 min, 4°C).(Note 4)
(9) Discard the supernatant, add 1 ml of 70% ethanol and 2 µl of Ethachinmate, vortex, and then centrifuge (20,000 x g, 5 min, 4°C).(Note 3)(Note 5)
(10) Discard the supernatant, air dry the precipitates and then dissolve the precipitates in 100 µl of TE (pH 8.0).

(Note 1) When DNA is extracted from soil containing a large quantity of allophones, perform extraction using Lysis Solution BB SP1 (sold separately: Code No. 313-06221) in place of Lysis Solution BB. Lysis Solution BB SP1 is an optional solution exclusively for ISOIL for Beads Beating for extracting DNA from the soil containing a large quantity of allophone.

(Note 2) Make sure that the lid of the Beads Tube is tightly sealed. A loose lid may cause liquid to leak during beads beating.

(Note 3) If the maximum centrifugal force of the available centrifuge is not more than 20,000 x g, then spin at the maximum centrifugal force (but not less than 12,000 x g).

(Note 4) Remove as much of the supernatant as possible. The colored substance (humic substance) in the supernatant is known to inhibit PCR. Also, if contamination by humic substance is minimal (less coloring of the aqueous layer after the chloroform treatment), step (8) can be skipped.

(Note 5) Soil DNA can be recovered in a stable manner by adding Ethachinmate to 70% ethanol. However, if Ethachinmate is not added, avoid vortexing and gently wash the precipitates, mixing by inversion.

<Protocol to obtain maximum yield>

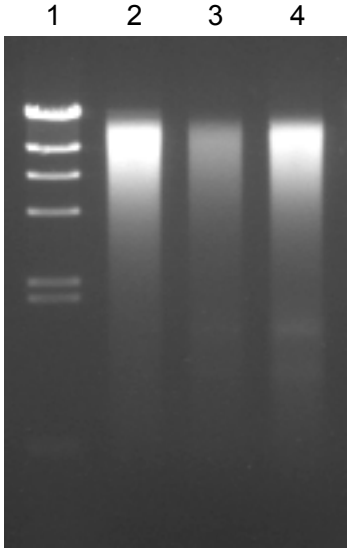
This is a protocol for obtaining the maximum yield with ISOIL for Beads Beating. Use this method when the extraction yield of soil DNA is important, e.g., when estimating biomass through quantitative measurement of soil DNA.

(1) Put 0.5 g of soil sample in a Beads Tube.
(2) Add 950 µl of Lysis Solution BB and 50 µl of Lysis Solution 20S prewarmed at 65°C.
(3) After performing beads beating (4-6 m/sec or 4,200-6,800 rpm for 30-45 sec), incubate at 65°C for 30 min to 1 hr.
(4) Centrifuge (12,000 x g. 1 min, room temperature).
(5) The remaining steps are the same as steps (5)-(10) of the standard protocol (5)-(10).

VI Data collection

1. DNA extraction from various soil samples

DNA was extracted with this kit from three kinds of soils, and the results indicated that DNA could be extracted from any of these soils.

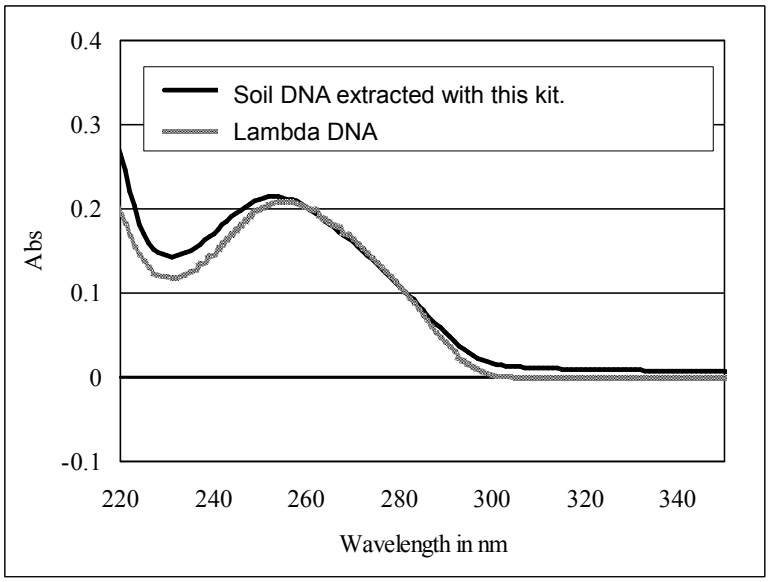


Lane 1. OneSTEP Marker 1 (λ/*Hin*d III digest)
Lane 2. Control soil from the Yayoi agricultural field, the University of Tokyo (allophane andosol soil/volcanic ash soil)
Lane 3. Saitama Agricultural Testing Station, agricultural field soil (gray lowland soil/non-volcanic ash soil)
Lane 4. Hyogo Agricultural Testing Station, forest soil (brown forest soil/non-volcanic ash soil)

One-twentieth of the amount of DNA extracted from 0.5 g of soil was electrophoresed in 1% Agarose S.

2. Absorption spectra of soil DNA

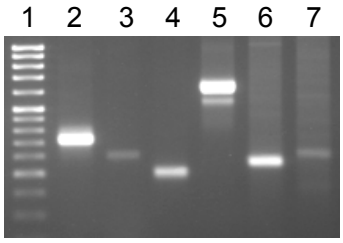
Absorption spectra of DNA extracted from the control soil from the Yayoi agricultural field, the University of Tokyo, using this kit was compared with that of highly purified Lambda DNA (Code No.318-00414).



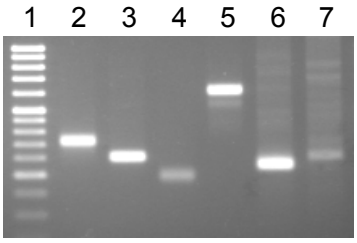
The soil DNA extracted with this kit was determined to be high purity.

3. Detection of phyletic group by PCR

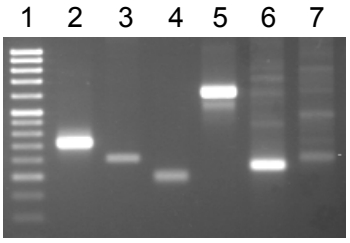
Soil DNA extracted with this kit was analyzed by PCR using primers for phyletic group detection, and the results indicated that DNA fragments derived from various phyletic groups were detected.



Control soil from the Yayoi agricultural field, the University of Tokyo (allophone andosol soil)



Saitama Agricultural Testing Station, agricultural field soil (gray lowland soil)



Hyogo Agricultural Testing Station, forest soil (brown forest soil)

Lane 1. OneSTEP Ladder 100
Lane 2. Bacteria（723 bp）
Lane 3. *Bacillus* species and relatives（600 bp）
Lane 4. High-G+C gram-positive bacteria（542 bp）
Lane 5. *Streptomyces* species and related taxa（1,243 bp）
Lane 6. Fungi, protists, and green algae（555 bp）
Lane 7. Plants（597 bp）

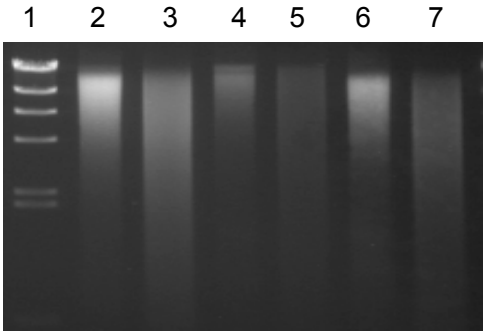
A portion of the PCR product was electrophoresed in 2% Agarose S.

Reference for primers:

Small-Scale DNA Sample Preparation Method for Field PCR Detection of Microbial Cells and Spores in Soil.
Kuske CR, Banton KL, Adorada DL, Stark PC, Hill KK, Jackson PJ.
Appl Environ Microbiol. 1998 Jul 1; 64(7): 2463-72.

4. Restriction enzyme digestion of soil DNA

Soil DNA extracted with this kit was digested with restriction enzyme *Eco*R I.



Lane 1. OneSTEP Marker 1 (λ/*Hin*d III digest)

Lane 2. Intact DNA of the control soil from the Yayoi agricultural field, the University of Tokyo

Lane 3. DNA/*Eco*R I of the control soil from the Yayoi agricultural field, the University of Tokyo

Lane 4. Intact DNA of agricultural field soil from Saitama Agricultural Testing Station

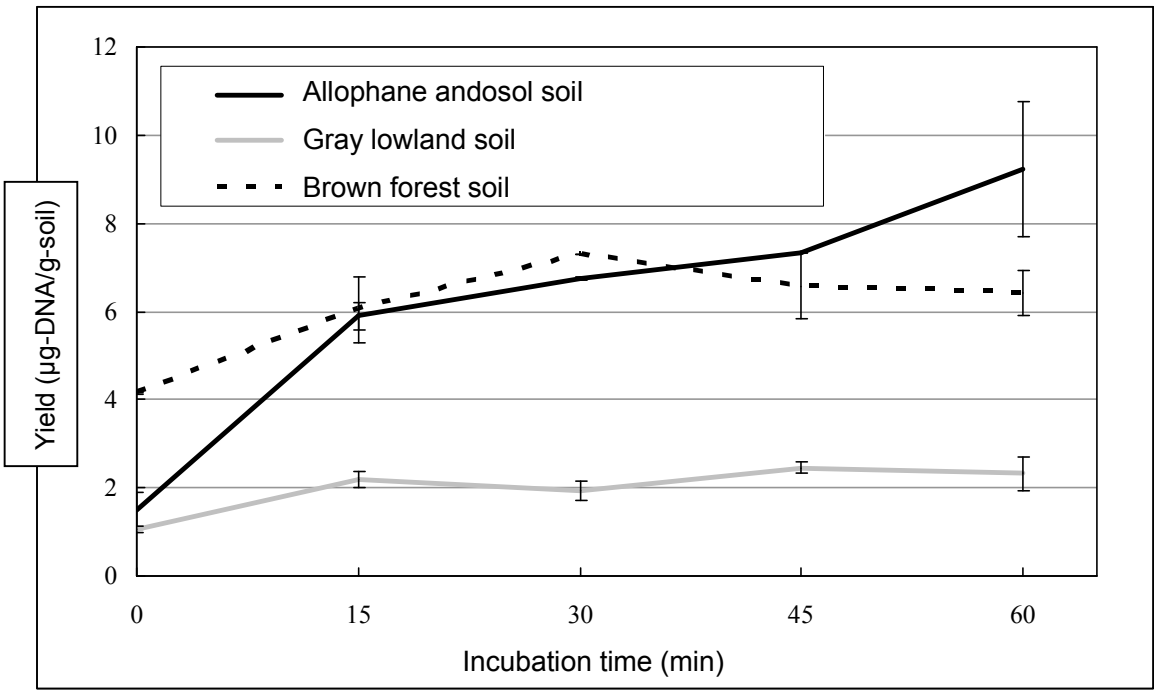
Lane 5. DNA/*Eco*R I of agricultural field soil from Saitama Agricultural Testing Station

Lane 6. Intact DNA of forest soil from Hyogo Agricultural Testing Station

Lane 7. DNA/*Eco*R I of forest soil from Hyogo Agricultural Testing Station

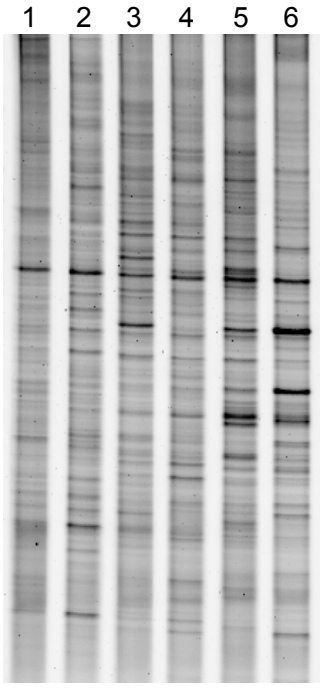
5. Incubation time at 65°C after Beads Beating and yield of soil DNA

The relationship between the incubation time at 65°C after Beads Beating and the yield of soil DNA was investigated, and the results indicated that in some cases the DNA yield was increased by incubating at 65°C.



6. PCR-DGGE analysis of soil DNA

PCR-DGGE analyses were performed using the soil DNAs extracted with this kit.



Lane 1. Control soil from the Yayoi agricultural field, the University of Tokyo

Lane 2. Pasture land soil from Tanashi farm, the university of Tokyo

Lane 3. Forest soil from Chiba Agricultural Testing Station

Lane 4. Forest meadow soil from the Grass Field Testing Station

Lane 5. Agricultural field soil from Saitama Agricultural Testing Station

Lane 6. Forest soil from Hyogo Agricultural Testing Station

Data provided by: Hiroki Rai, Ph. D. Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo

VII Troubleshooting

Problem	Possible cause	Possible countermeasure
Low yield of soil DNA	Few soil microorganisms	Use fresh soil whenever possible. Also, in some cases, DNA yield may increase by incubating at 65°C for 30 min to 1 hr after beads beating (see Data collection 5)
	DNA precipitates may be washed away	In step (9) of the protocol, the DNA precipitates are prone to be peeled off. Use attached Ethachinmate together with 70% ethanol. Also, since the precipitates become visible by Ethachinmate, carefully remove the supernatant while visually making sure the precipitates are not washed away.
	Too much allophane in the soil	Extract using Lysis Solution BB SP1 (sold separately: Code No. 313-06221) in place of Lysis Solution BB. Lysis Solution BB SP1 is an optional solution exclusive for ISOIL for Beads Beating for extracting DNA from soil containing a large quantity of allophane.
Molecular weight of soil DNA is low.	DNA is sheared by physical impact of beads beating.	When high molecular weight soil DNA is desired to be extracted, use ISOIL, which employs the heat extraction method in the presence of a surface active agent.
White crystal deposits appear in the Lysis Solution 20S.	Reagents are precipitated due to low temperature.	Completely dissolve crystals by incubating at 37-65°C and then use. This will not affect quality or performance.
White crystal deposits appear in the Purification Solution.	Reagents are precipitated due to low temperature.	Completely dissolve crystals by incubating at 37-65°C and then use. This will not affect quality or performance.
Floating objects in the Precipitation Solution	Contamination by fungi and the like	Purchase a new kit. Since the composition of this solution allows the growth of fungi and the like, take thorough precautions against contamination. We recommend storage at a low temperature (2-10°C) after opening the package.

Problem	Possible cause	Possible countermeasure
Floating objects in Wash Solution	Contamination by fungi and the like	Purchase a new kit. However, if the contamination of humic substance is minor, step (8) of the protocol can be skipped. In such cases, the Wash Solution is not required. Since the composition of this solution allows the growth of fungi and the like, take thorough precautions against contamination. We recommend storage at a low temperature (2-10°C) after opening the package.
Floating objects in Ethachinmate	Contamination by fungi and the like	Purchase new Ethachinmate. However, when DNA yield is not important, Ethachinmate is not needed. Also, when using, take thorough precautions against contamination. We recommend storage at a low temperature (2-10°C) after opening the package.
Lysis Solution spilling out of Beads Tube	The void volume of the soil is large.	Reduce the amount of soil sample to less than 0.5 g.

Soil is composed of inorganic substances such as various minerals and clay, soil organic substances including various plants, dead plants and humic substance, and various microorganisms living therein. Therefore, it can be said that every soil is different.
Our test results until now have confirmed that DNA can be extracted with this kit from dozens of various Japanese soil types. However, since soil can vary greatly, the yield may not be high in some samples depending on the type and condition of the soil.
If the yield of soil DNA extracted with this kit is low, the problem may be that the particular soil sample has unique characteristics which other samples do not have rather than a problem with the reagents and procedures.

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