Soil DNA Extraction Kit

ISOIL

Manual (Second edition)

Code No. 316-06211

NIPPON GENE CO., LTD.

ISOIL Manual（ver.2）
110830KH

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I Product description

ISOIL is a kit for extracting DNA from 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.

With ISOIL, DNA is isolated with a heat extraction method in the presence of a surface-active agent. Since soil DNA is extracted without applying physical force, high molecular weight DNA can be obtained. Therefore, soil DNA extracted with ISOIL is suitable for applications involving gene resources, e.g., the construction of a metagenomic library.

However, please note that microorganisms having strong cell walls may not be destroyed.

Please use ISOIL for Beads Beating, which uses disruption by beads beating to perform group structure analysis of soil microorganisms through PCR-DGGE analysis and estimation of soil biomass by quantitative determination of soil DNA.

II Contents of kit

Lysis Solution HE	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 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 a 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 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 **>**

- 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 2 ml tube.

(2) Add 950 µl of Lysis Solution HE and 50 µl of Lysis Solution 20S, and, after mixing well by inversion, incubate at 65°C for 1 h.(Note 1)

(3) Centrifuge (12,000 x g, 1 min, room temperature).

(4) Transfer 600 µl of the supernatant to a new tube, add 400 µl of Purification Solution, and mix well.

(5) Add 600 µl of chloroform, vortex for 15 sec, and then centrifuge (12,000 x g, 15 min, room temperature).

(6) 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 2)

(7) 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 2),(Note 3)

(8) 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 2),(Note 4)

(9) Discard the supernatant, air dry the precipitates and then dissolve the precipitates in 100 µl of TE (pH 8.0).

(Note 1) The yield of soil DNA is increased by mixing by inversion in the middle of the process (see the data collection 5). If a rotator or the like is available, we recommend incubating while gently rotating.

(Note 2) 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 3) 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 (7) can be skipped.

(Note 4) Soil DNA can be recovered in a stable manner by adding Ethachinmate to 70% ethanol. However, if for some reason Ethachinmate is not added, avoid vortexing and gently wash the precipitates, mixing by inversion.

< Scale-up >

Scale-up of soil DNA extraction with ISOIL is easy. We recommend scale-up extraction when a large amount of high molecular weight soil DNA is required for cloning, construction of a library, or the like. The following points should be noted for scale-up.

1. The ratio of Lysis Solution HE and Lysis Solution 20S must be 95 : 5.
2. The ratio of the supernatant of the centrifugation at step (4) of the standard protocol and the Purification Solution must be 6 : 4.
3. The chloroform to be added must be equal to the amount to the supernatant from the centrifugation at step (4) of the standard protocol.
4. The Precipitation Solution to be added must be equal to the amount of the aqueous layer recovered after the chloroform treatment.

Example of scale-up: DNA extraction from 5 g of soil (ten-fold scale-up)

(1) Put 5 g of soil sample in a 50 ml centrifuge tube.

(2) Add 9.5 ml of Lysis Solution HE and 0.5 ml of Lysis Solution 20S, mix well by inversion and then incubate at 65°C for 1 hr while gently shaking.

(3) Centrifuge (5,000 x g, 10 min, 25°C).

(4) Transfer 6 ml of the supernatant to a new tube, add 4 ml of Purification Solution, and mix well.

(5) Add 6 ml of chloroform, mix well by inversion until homogeneous, and then centrifuge (5000 x g, 15 min, 4°C).

(6) Carefully transfer 8 ml of the aqueous layer to a new tube so that no intermediate layer contaminates the aqueous layer, mix well with 8 ml of Precipitation Solution, and centrifuge (9,000 x g, 30 min, 4°C).

(7) Discard the supernatant, add 10 ml of Wash Solution, mix by inverting a few times, and centrifuge (9,000 x g, 10 min, 4°C).

(8) Discard the supernatant, add 10 ml of 70% ethanol and 10 µl of Ethachinmate, mix by inversion, and then centrifuge (9,000 x g, 10 min, 4°C).(Note 1)

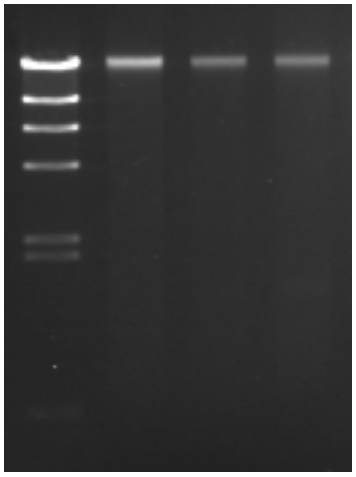
(9) Discard the supernatant, and, after air-drying, dissolve the precipitates in 500 µl of TE (pH8.0).

(Note 1) 1-2 µl of Ethachinmate is sufficient for 100 µl of the final solution. Adding too much Ethachinmate increases the viscosity and may cause problems in subsequent operations.

IV Data collection

1. DNA extraction from various soil samples

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

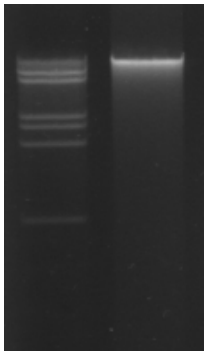


Lane 1. OneSTEP Marker 1 (λ/*Hin*d III digest)
Lane 2. Control soil from the Yayoi agricultural field, the University of Tokyo (allophone andosol soil/volcanic ash soil)
Lane 3. Saitama Agricultural Testing Station, agricultural field soil (gray lowland soil/non-volcanic ash soil)
Lane 4. Hyogo Agricultural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

1/10 amount of DNA extracted from 0.5 g of soil was electrophoresed in 1% Agarose S.

2. Size of soil DNA

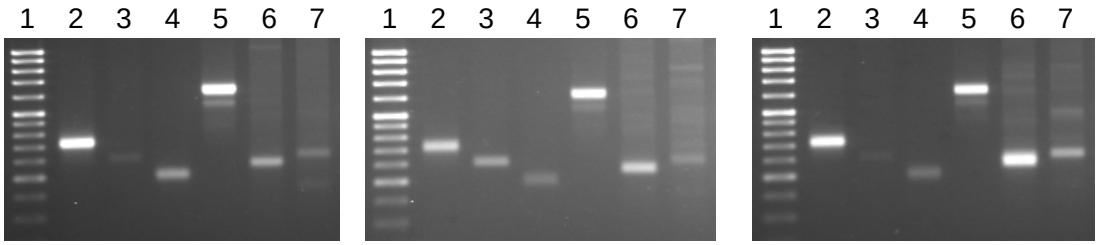
Soil DNA extracted using this kit was electrophoresed in 0.5% Agarose H. The results indicated that the extracted soil DNA was not less than 50 kb.



Lane 1. Marker 7GT (166, 50, 42, 23, 21, 18, 10, 1.3 kb)
Lane 2. DNA extracted from the control soil from the Yayoi agricultural field, the University of Tokyo

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.



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)

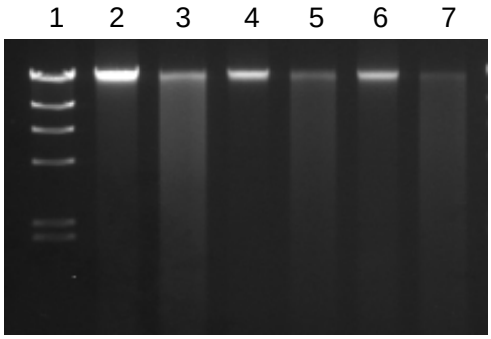
A portion of the PCR product was electrophoresed in 2% Agarose S.

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

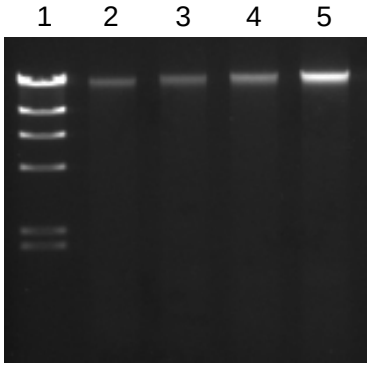
Lane 5. DNA/*Eco*R I of agricultural field soil from Saitama Agricultural Testing Station

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. Stirring during 65°C incubation and yield of soil DNA

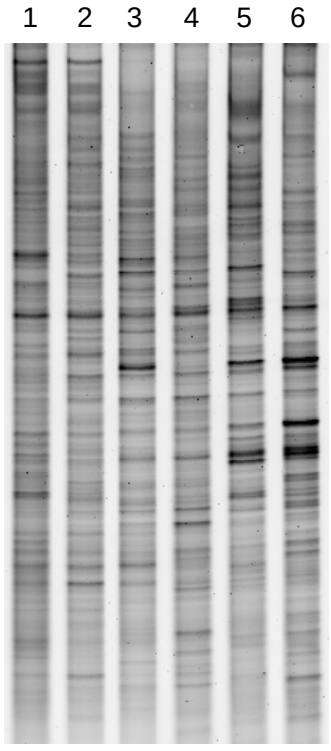
The relationship between stirring during incubation at 65°C and the yield of soil DNA was investigated, and the results indicated that the DNA yield was increased by performing mixing by inversion during incubation.



Lane 1. OneSTEP Marker 1 (λ/*Hin*d III digest)
Lane 2. Letting stand for 60 min
Lane 3. Mixing by inversion at 30 min intervals
Lane 4. Mixing by inversion at 20 min intervals
Lane 5. Mixing by inversion at 10 min intervals

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, DNA yield may increase by mixing the solution by inversion at 10 min intervals while incubating at 65°C (see data collection 5).
	DNA precipitates may be washed away.	In the step (8) of the protocol, the DNA precipitates are prone to be peeled off. Use 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.
	Microorganisms are not well disrupted.	Use ISOIL for Beads Beating which employs physical disruption of microorganisms body by beads beating. However, since the physical shearing force is applied, the extracted soil DNA is about 23 kb.
Shearing of DNA	Physical shearing occurs during pipetting and stirring.	Cut off the ends of pipette tips. Also, when stirring the solution, avoid using a vortex and mix well by inversion.
White crystal deposits appear in the Lysis Solution 20S.	Reagents are precipitated due to a 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 a low temperature.	Completely dissolve crystals by incubating at 37-65°C and then use. This will not affect quality or performance.

Problem	Possible cause	Possible countermeasure
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.
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. Also, when using, take thorough precautions against contamination. We recommend storage at a low temperature (2-10°C) after opening the package.

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 with this kit DNA can be extracted from dozens of various Japanese soil types. However, since soil can vary greatly, the yield may not be high 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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