Soil DNA Extraction Kit

ISOIL Large for Beads ver.2

Manual (First edition)

Code No. 312-06791

NIPPON GENE CO., LTD.

ISOIL Large for Beads ver.2 Manual（ver.1）
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I Product description

ISOIL Large for Beads ver.2 is a kit for extracting DNA from soil sample of 5 g.
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, which has been considered difficult to achieve up to now.
In the protocol of ISOIL Large for Beads ver.2, DNA is extracted by thermal extraction method under the presence of surface-active agent and physical disruption of cells by beads beating. Also, by precipitating DNA under special conditions, highly purified soil DNA can be extracted at a higher concentration compared to the conventional method.

II Contents of kit

(For 8 reactions)

Beads Tubes	8 tubes	
Lysis Solution BB	80 ml	× 1
Lysis Solution 20S*	4 ml	× 1
Purification Solution*	45 ml	× 1
Precipitation Solution	90 ml	× 1
Wash Solution	45 ml	× 1
TE (pH 8.0)	10 ml	× 1
Ethachinmate	100 µl	× 1
Manual		× 1

* : Crystal may form in the Lysis Solution 20S and in 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 37-65°C (mix occasionally).

III Storage

All the reagents included in ISOIL Large for Beads ver.2 can be stored at room temperature. Care must be taken to protect the Precipitation Solution, the Wash Solution, and the Ethachinmate from contamination at time of use, and it is recommended to store at 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 Large for Beads ver.2 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
- Messpipette
- Micropipette
- Pipette tip
- Centrifuge tube
- Vortex mixer
- Incubator
- Centrifuge

<Standard protocol>

(1) Put 5 g of soil sample in a Beads Tube.

(2) Add 9.5 ml of Lysis Solution BB and 0.5 ml of Lysis Solution 20S.

(3) After vortexing at the highest speed for 10 min, incubate at 65°C for 60 min. (Note 1) (Note 2) (Note 3)

(4) Centrifuge (4,000 x g, 5 min, room temperature).

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

(6) Add 6 ml of chloroform, vortex for 1 min, and then centrifuge (4,000 x g, 15 min, room temperature). (Note 1)

(7) Transfer the aqueous layer (upper layer) to a new centrifuge tube while taking care not to transfer the intermediate layer, add an equal volume of the Precipitation Solution, mix well and centrifuge (8,000 x g, 60 min, 4°C). (Note 5) (Note 6)

(8) Discard the supernatant, rinse the precipitates and the wall of the centrifuge tube by adding 5 ml of the Wash Solution, and then centrifuge (8,000 x g, 10 min, 4°C). (Note 6) (Note 7) (Note 8)

(9) Discard the supernatant, add 5 ml of 70% ethanol and 10 µl of Ethachinmate, vortex, and then centrifuge (8,000 x g, 10 min, 4°C). (Note 6) (Note 8) (Note 9)

(10) Discard the supernatant, air dry the precipitates and then dissolve the precipitates in an appropriate amount of TE(pH 8.0) (about 0.3-1 ml). (Note 10)

(Note 1) Make sure that the lid of the centrifuge tube is tightly closed so as not to cause leak.

(Note 2) The yield of soil DNA is increased by mixing by inversion during the incubation.

(Note 3) When the beads disruption apparatus for 50 ml centrifuge tubes is used instead of the vortex, the beating time may be shortened. Optimize conditions (speed, time).

(Note 4) When the water content of the soil sample is low (when the sample is dry), the volume of the supernatant after the centrifugation (4) may be less than 6 ml. In such cases, continue the experiment by adjusting the ratio among the volume of the supernatant after the centrifugation, the volume of the Purification Solution and the volume of chloroform to 3 : 2 : 3. This volume ratio is very important.

(Note 5) When the supernatant after the centrifugation of (4) is 6 ml, the Purification Solution is 4 ml and chloroform is 6 ml, the aqueous phase (upper layer) after the centrifugation (6) will be a little more than 8 ml. Carefully collect 8 ml

so that the intermediate layer is not mixed, and proceed to the next step.

(Note 6) At Nippon Gene, centrifugation is performed at 8,000 x g for 60 min using conical disposable centrifuge tubes and a corresponding centrifuge rotor. Make sure to set the centrifugal force to be not more than the allowable maximum centrifugal force of the centrifuge tubes to be used. Also, adjust the centrifugal force and time in accordance with the centrifuge tubes to be used.

(Note 7) Normally DNA is not visible at this step. Take care not to touch the wall of the tube where DNA may be precipitated with a pipette or the like. The precipitates become visible at (9) after addition of Ethachinmate and vortexing.

(Note 8) 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 9) Soil DNA can be recovered in a stable manner by adding Ethachinmate to 70% ethanol.

(Note 10) When the obtained soil DNA is colored or PCR inhibition is observed, perform the repurification of the soil DNA by the optional protocol① described below.

<Optional protocol① Repurification of soil DNA>

When the obtained soil DNA is colored or PCR inhibition is observed, perform the repurification of the soil DNA by the following protocol.

(1) Transfer 300 µl of the soil DNA obtained by the standard protocol and optional protocol② to a new 1.5 ml tube, add 200 µl of Purification Solution, and mix well.
(2) Add 300 µl of chloroform, vortex for 15 sec, and then centrifuge (12,000 x g, 15 min, room temperature).
(3) Transfer 400 µl of the aqueous layer (upper layer) to a new 1.5 ml tube while taking care not to transfer the intermediate layer, add 400 µl of Precipitation Solution, mix well, and then centrifuge (20,000 x g, 10 min, 4°C).
(4) Discard the supernatant, rinse the precipitates and the wall of the tube by adding 500 µl of the Wash Solution, and then centrifuge (20,000 x g, 10 min, 4°C). (Note 1) (Note 2)
(5) Discard the supernatant, add 500 µl of 70% ethanol and 1 µl of Ethachinmate, vortex, and then centrifuge (20,000 x g, 5 min, 4°C). (Note 2) (Note 3)
(6) Discard the supernatant, air dry the precipitates and then dissolve the precipitates in an appropriate amount of TE(pH 8.0) (about 100-300 µl).

(Note 1) Normally DNA is not visible at this step. Take care not to touch the wall of the tube where DNA may be precipitated with a pipette or the like. The precipitates become visible at (5) after addition of Ethachinmate and vortexing.

(Note 2) 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 (4) can be skipped.

(Note 3) Soil DNA can be recovered in a stable manner by adding Ethachinmate to 70% ethanol.

<Optional protocol② Scaling up to recover a minute amount of DNA>

When the amount of microorganisms included in a soil sample is small and a sufficient amount of DNA for analysis is thought to be difficult to recover from 5 g of the soil sample, extract DNA from 20 g of the sample by the following protocol.

(1) Put 5 g each of the soil samples in 4 Beads Tubes (20 g total).
(2) Add 9.5 ml of Lysis Solution BB and 0.5 ml of Lysis Solution 20S to each tube.
(3) After vortexing at the highest speed for 10 min, incubate at 65°C for 60 min. (Note 1) (Note 2) (Note 3)
(4) Centrifuge (4,000 x g, 5 min, room temperature).
(5) Transfer 6 ml each of the supernatant to 4 new 50 ml tubes so that no precipitates are mixed, and add 4 ml of the Purification Solution to each tube and mix well. (Note 4)
(6) Add 6 ml of chloroform to each tube, vortex for 1 min, and then centrifuge (4,000 x g, 15 min, room temperature). (Note 1)
(7) Transfer the aqueous layer (upper layer) from 2 tubes to a new 50 ml tube while taking care not to transfer the intermediate layer, add an equal volume of the Precipitation Solution to each tube, mix well and centrifuge (8,000 x g, 60 min, 4°C). (Note 5) (Note 6) (Combine the sample from 4 tubes into 2 tubes).
(8) Discard the supernatant, rinse the precipitates and the wall of the centrifuge tube by adding 5 ml of the Wash Solution to each tube, and then centrifuge (8,000 x g, 10 min, 4°C). (Note 6) (Note 7) (Note 8)
(9) Discard the supernatant, add 5 ml of 70% ethanol and 10 µl of Ethachinmate to each tube, vortex, and then centrifuge (8,000 x g, 10 min, 4°C). (Note 6) (Note 8) (Note 9)
(10) Discard the supernatant, air dry the precipitates and then dissolve the precipitates in an appropriate amount of TE(pH 8.0) (about 0.3-1 ml). (Note 10)

(Note 1) Make sure that the lid of the centrifuge tube is tightly closed so as not to cause leak.
(Note 2) The yield of soil DNA is increased by mixing by inverting during the incubation.
(Note 3) When the beads disruption apparatus for 50 ml centrifuge tubes is used instead of the vortex, the beating time may be shortened. Optimize conditions (speed, time).
(Note 4) When the water content of the soil sample is low (when the sample is dry), the volume of the supernatant after the centrifugation (4) may be less than 6 ml. In such cases, continue the experiment by adjusting the ratio among the volume of the supernatant after the centrifugation, the volume of the

Purification Solution and the volume of chloroform to 3 : 2 : 3. This volume ratio is very important. If the supernatant of (4) after the centrifugation is much greater, about 7.5 ml may be recovered.

(Note 5) When the supernatant after the centrifugation of (4) is 6 ml, the Purification Solution is 4 ml and chloroform is 6 ml, the aqueous phase (upper layer) after the centrifugation (6) will be a little more than 8 ml. Carefully transfer 16 ml of the aqueous phase (collected from 2 tubes) to a new 50 ml tube so that the intermediate layer is not mixed.

(Note 6) At Nippon Gene, centrifugation is performed at 8,000 x g for 60 min using conical disposable centrifuge tubes and a corresponding centrifuge rotor. Make sure to set the centrifugal force to be not more than the allowable maximum centrifugal force of the centrifuge tubes to be used. Also, adjust the centrifugal force and time in accordance with the centrifuge tubes to be used.

(Note 7) Normally DNA is not visible at this step. Take care not to touch the wall of the tube where DNA may be precipitated with a pipette or the like. The precipitates become visible at (9) after addition of Ethachinmate and vortexing.

(Note 8) 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.

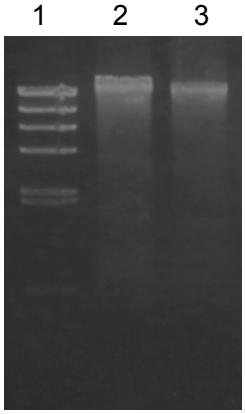
(Note 9) Soil DNA can be recovered in a stable manner by adding Ethachinmate to 70% ethanol.

(Note 10) When the obtained soil DNA is colored or PCR inhibition is observed, perform the repurification of the soil DNA by the optional protocol① described above.

VI Data collection

1. DNA extraction from various soil samples

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

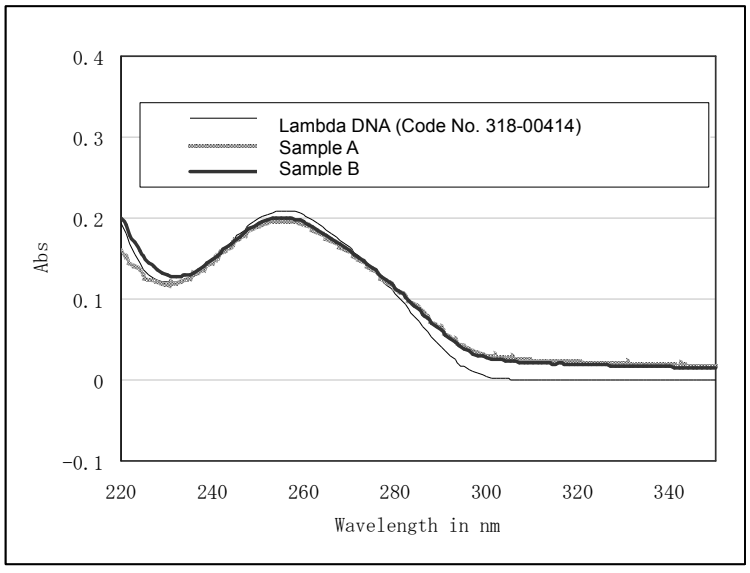


Lane 1. OneSTEP Marker 1 (λ/*Hin*d III digest)
Lane 2. Control soil from the Yayoi agricultural field, the University of Tokyo (allophone andosol soil/volcanic ash soil)
Lane 3. Experimental field, rice paddy soil, the Akita Prefectural University (gray lowland soil/non-volcanic ash soil)

1/200 of the amount of DNA extracted from 5 g of soil was electrophoresed in 1% Agarose S.

2. Absorption spectra of soil DNA

Absorption spectra of DNA extracted from the soil using this kit was compared with highly purified Lambda DNA (Code No.318-00414).

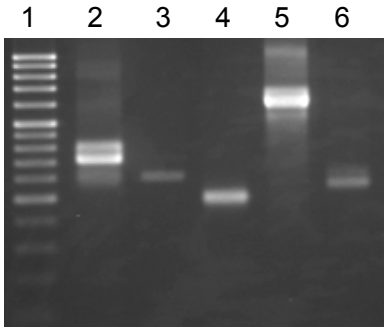


Sample A:
Control soil from the Yayoi agricultural field, the University of Tokyo (allophone andosol soil/volcanic ash soil)

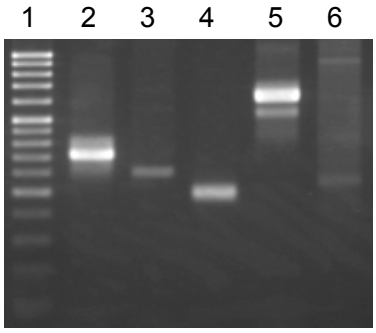
Sample B:
Experimental field, rice paddy soil, the Akita Prefectural University (gray lowland soil/non-volcanic ash soil)

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)



Experimental field, rice paddy soil,
the Akita Prefectural University
(gray lowland soil)

Lane 1. OneSTEP Ladder 100 (0.1-2 kb)
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）

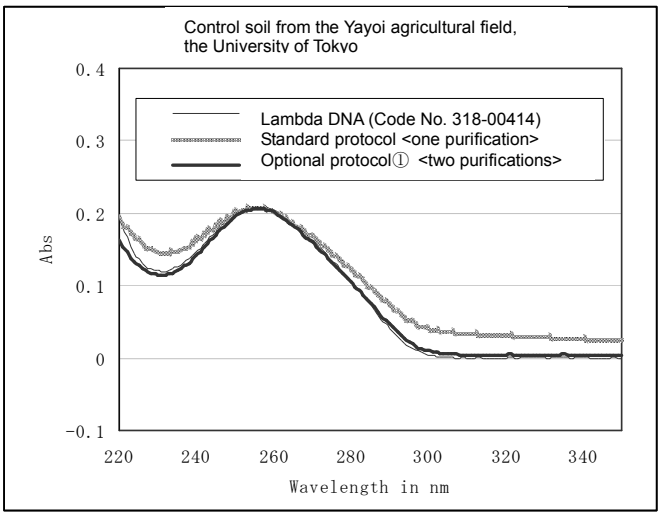
A portion of the PCR product was electrophoresed in 2% Agarose S.

Reference for primers:

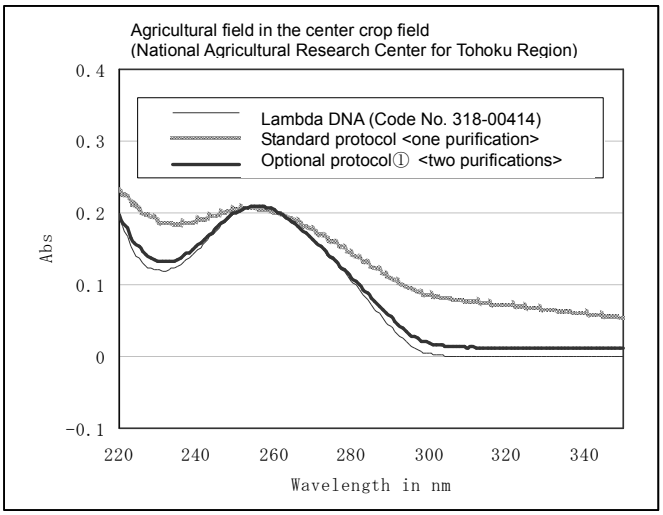
Small-Scale DNA Sample Preparation Method for Field PCR Detection of Microbial Cells and Spores in Soil.
Kuske CR, Banton KL, Adorada DL, Stark PC, Hill KK, Jackson PJ.
Appl Environ Microbiol. 1998 Jul 1; 64(7): 2463-72.

4. Repurification of soil DNA

Soil DNA purified from andosol soil with high humic substance content by the “standard protocol” is repurified by the “Optional protocol① Repurification of soil DNA” and the absorption spectra were compared.



The soil DNA purified by the Standard protocol was found to have slight coloration, but colorless clear solution was obtained by repurifying by the Optional protocol ①.



The soil DNA purified by the Standard protocol was found to have pale brown coloring, but colorless clear solution was obtained by repurifying by the Optional protocol①.

By performing repurification, the DNA was purified to a higher degree than any other soil DNA. Also, the yield in this repurification was around 70%.

VII Troubleshooting

Problem	Possible cause	Possible countermeasure
Low yield of soil DNA	Few soil microorganisms	Use fresh soil whenever possible.
	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.
	The concentration of Lysis Solution BB is not optimal.	For some soil, which does not adsorb DNA such as gray lowland soil and brown forest soil, it is known that DNA yield can be increased by using the Lysis Solution BB diluted two fold with distilled water in place of the Lysis Solution BB.
Molecular weight of soil DNA is low.	DNA is sheared by physical shock by beads crush.	When high molecular weight soil DNA is desired to be extracted, use ISOIL, which employs the thermal extraction method in the presence of a surface active agent.
White crystals deposit appear in the Lysis Solution BB, Lysis Solution 20S and 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 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 low temperature (2-10°C) after opening the package.
Floating objects in Ethachinmate	Contamination by fungi and the like	Purchase a 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 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 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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