Hair and Nail DNA Extraction Kit

ISOHAIR

Manual (11th edition)

Code No. 315-03403 for 10 extractions

Code No. 319-03401 for 100 extractions

NIPPON GENE CO., LTD.

Table of contents

I	Product description	1
II	Contents of kit	1
III	Storage	1
IV	Protocol	2
V	Troubleshooting	5
VI	Data collection	6
	1. Examples of experiments using human hair, nail and mucous membrane of oral cavity	6
	2. Examples of experiments using human hair	7
VII	References	12

Precautions

○ This product is a reagent for research and cannot be used for medical or other objectives. Also, 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.

I Product description

DNA extraction from human hair and nail is a very important technique in the field of forensic medicine. Allowing simple, rapid DNA extraction from such samples is highly valuable compared to the conventional test in which blood or tissue samples are utilized. DNA extraction from hair and nail is also highly useful for its unnegligible characteristics: easy sampling and tolerance to viral infection from the conventional samples in the field of molecular biology.

ISOHAIR is a kit for DNA extraction from human hair and nail.

The major component of hair and nails is keratin which is a protein difficult to decompose. In order to dissolve hair completely, it normally requires incubation in a period of long time in a buffer containing a protein degrading enzyme.

Complete degradation of hair can be achieved in about 30 min using this kit. This kit further enables simple, rapid DNA extraction from hair in about 1 hr in an efficient way especially when human genomic DNA is urgently needed in a short period of time.

ISOHAIR is also applicable to DNA extraction from mouse hair or nail.

II Contents of kit

	(For 100 extractions)	(For 10 extractions)
Extraction Buffer *1	20 ml	1 ml × 2
Enzyme Solution	1 ml	100 µl
Lysis Solution	0.8 ml	80 µl
Ethachinmate	0.2 ml	20 µl
3 M Sodium Acetate (pH 5.2)	2 ml	200 µl
TE (pH 8.0)	1 ml × 2	200 µl

*1 White crystals may appear in the Extraction Buffer but this will not affect the quality. In that case, ensure to dissolve the crystals in a water bath about at 50°C before use. The Extraction Buffer contains a protein denaturant and should be handled with care. If the product enters eyes or adheres to skin, immediately wash well with large amounts of water.

This product does not include phenol/chloroform/isoamyl alcohol, ethanol, primers and the like.

III Storage

−20℃

IV Protocol

Hair [*1)]

Wash with ethanol [*2)]
Cut hair sample into 4-5 mm pieces with scissors or the like.
← Extraction Buffer 200 µl [*3)]
← Enzyme Solution 5 µl
← Lysis Solution 8 µl
Mix [*4)]
Incubate, 55°C, 20 min [*5)]
← Enzyme Solution 5 µl
Mix [*4)]
Incubate, 55°C, 5-10 min [*6)]
← Phenol/chloroform/isoamyl alcohol (25:24:1) 200 µl
Mix by inversion [*7)], 5 min
11 K × g, room temperature, 5 min

Aqueous phase

← 3 M Sodium Acetate (pH 5.2) 20 µl
← Ethachinmate 2 µl [*8)]
Mix [*4)]
← Ethanol 400 µl [*9)]
11 K × g, room temperature, 15 min

Precipitate

← 70% ethanol 1 ml
Wash by gentle mixing by inverting several times.

Precipitate

Dry [*10)]
← TE (pH 8.0) 20 µl

DNA solution [*11)]

*1) Required volume of sample is as follows.

Human hair: 1 cm hair root sample cut at 1 cm distance from the root end OR
4 cm hair shaft sample cut at distances of 2 cm and 6 cm from the root end.
[See VI. “Amount of DNA extracted from hair” (p. 10)]

Human nail: 2 pieces of nail tip cut into 1 x 1mm squares (total of 0.5 mg)

Mouse body hair: a pinch of mouse hair (about 5 mg) cut off with scissors.

Mouse nail: 1-2 mm mouse nail tip

Using of fresh samples is recommended to obtain high DNA yields. [See VI. “Elapsed time after hair removal and DNA yield” (p. 11)]

*2) Hair cosmetics may inhibit PCR reaction. Wash hair samples thoroughly with ethanol as follows before proceeding to the next step when they are adhered with hair cosmetics, dirt, or dust.

(1) Add about 1 ml of ethanol to a 1.5 ml plastic tube.
(2) Put uncut hair sample into (1) using forceps.
(3) Invert (2) several times.
(4) Take out the sample with forceps, place on filter paper or the like and remove as much ethanol as possible.

If a hair sample is clean and if it is not adhered with foreign subjects, there is no need to wash with ethanol. It is also recommend that mouse body hair is washed with ethanol before starting the procedure. When using a manicured nail sample, remove manicure paint first.

*3) White crystals may appear in the Extraction Buffer but this will not affect the quality. In that case, ensure to dissolve the crystals in a water bath at about 50°C before use and to homogenize by stirring.

The Extraction Buffer contains a protein denaturant and should be handled with care. If the product enters eyes or adheres to skin, immediately wash well with large amounts of water.

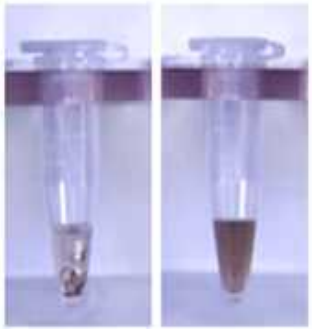
Perform all of the following steps before ethanol precipitation at room temperature unless otherwise noted.

*4) Mix by tapping tube several times with a finger. Avoid vigorous mixing such as vortexing.

*5) Hair starts degrading during this incubation

Reaction temperature can be 37°C or room temperature. The reaction time at 37°C is 2-3 times longer than at 55°C, and 3-5 times longer at room temperature.

*6) Small pieces of digested hair sample deposit at the bottom of tube during dissolution step.



After *5) After *6) (Complete dissolution)

Mix by tapping the bottom of the tube gently with a finger every 2-3 min during the reaction. Dissolution time varies depending on the type and volume of the hair.
If undigested pieces of hair remain after incubation (after *6)), add 5 µl of the Enzyme Solution and incubate at 55 °C for 5-10 min again. If the hair still cannot be dissolved completely even after second incubation with additional Enzyme Solution, continue the procedure with the obtained solution as it should contain eluted DNA.
NOTE: mouse hair normally cannot be dissolved completely.
In the case of using human or mouse nail sample, there is only a little visible change in appearance (or seemingly no change) but continue the procedure with the obtained solution as it should contain eluted DNA.

*7) Mix gently by inversion. Avoid vigorous mixing such as vortexing.

*8) Addition of Ethachinmate during ethanol precipitation enhances efficiency in DNA extraction. For the feature of Ethachinmate, it can accelerate the DNA extraction process by omitting incubation at -20°C in the conventional ethanol precipitation.

*9) When the final DNA yield is low, it may be increased by incubation at -20°C for 30 min after the step 9).

*10) Air dry or briefly vacuum dry pellet for 5-10 min. It is important not to over-dry the pellet as it may become harder to resuspend.

*11) When DNA is extracted from a hair root sample, there is a risk of RNA contamination. Treat the solution with RNase to eliminate RNA as necessary.

V Troubleshooting

● Hair sample does not dissolve.

- After the step *6) , add another 5 µl of the Enzyme Solution and incubate at 55°C.
- Even if hair cannot be dissolved completely, continue the procedure with the obtained solution as it should contain eluted DNA.

● No band or faint band in gel electrophoresis.

- Amount of DNA extracted from hair varies between individuals as well as between individual hair strands from the same person. Even in an identical hair sample, it also varies in sample types of hair: hair root and shaft. Since only a small amount of DNA can be extracted from a hair sample (especially from hair shaft), a band may not be observed on a gel after electrophoresis. In that case, it is recommended to proceed to the PCR step. → See VI. “Amount of DNA extracted from hair” (p. 10).

● Colored DNA solution

- The dye melanin contained in hair may have eluted out. Melanin is known to inhibit PCR reaction but addition of T4 gene 32 protein may moderate the inhibition. →See VI 2. “Moderation of PCR inhibition using T4 gene 32 protein” (p. 8, 9).

● No amplification in PCR.

- Use a hair root or a part that is as close to the hair root as possible. → See VI. “Position in hair and amount of DNA” (p. 10).
- Use a fresh sample. → See VI. “Elapsed time after hair removal and DNA yield” (p. 11)
- If the obtained DNA solution is colored, it may contain the PCR inhibitor melanin. Add T4 gene 32 protein to moderate the inhibition. → See VI. “Moderation of PCR inhibition using T4 gene 32 protein” (p. 8, 9).
- Optimize PCR conditions.
  Change the primer design, increase the amount of *Taq* DNA polymerase, change the denaturing temperature, etc.

● Smeared PCR amplified product.

- Reduce the amount of *Taq* DNA polymerase.
- Increase the amount of the template.
- Set the denaturing temperature higher.
- Make the primers longer. → See VI. “PCR condition” (p. 11)

● Nonspecific amplification.

- Change the primer design.
- Extract after washing hair with ethanol thoroughly.

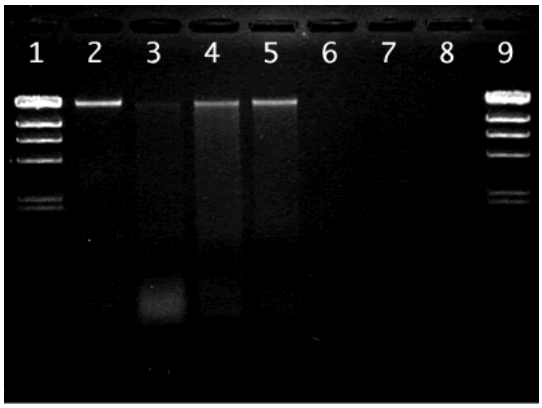
VI Data collection

1. Examples of experiments using human hair, nail and mucous membrane of oral cavity

DNA was extracted using the ISOHAIR from hair root, nail, and mucous membrane of the oral cavity.

Oral mucous membrane sample was collected with a cotton-tipped swab and DNA sample was collected in different methods: (1) collecting pellet after the sample was suspended in 1 x PBS and centrifuged; (2) forming suspension by directly inserting the cotton-tipped swab into the Extraction Buffer. (See the Nippon Gene website for more detailed experimental procedures).

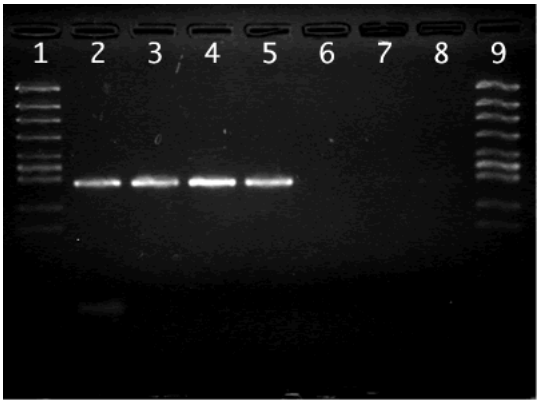
Electrophoresis was performed using 1/4 of amount of DNA obtained from each sample.



Marker 1 (λ/*Hin*d III digest)
0.8% Agarose S

Lane 1: Marker
Lane 2: Hair root, 1 cm ×3
Lane 3: Nail, 1 mm cube ×2
Lane 4: Oral mucous membrane (1)
Lane 5: Oral mucous membrane (2)
Lane 6: Control: cotton swab (1)
Lane 7: Control: cotton swab (2)
Lane 8: Negative control
Lane 9: Marker

Human *p53* gene (Exon 10; 279 bp) was amplified by PCR using 1/10 of amount of DNA solution obtained from each sample and electrophoresed.



Marker 5 (φX174/*Hin*c II digest)
3% Agarose 21

＜PCR mixture＞

Template DNA	2 µl
10×Gene *Taq* Universal Buffer	5 µl
dNTP mixture (2.5 mM each)	4 µl
primer-forward (20 pmol/µl)	1 µl
primer-reverse (20 pmol /µl)	1 µl
Gene *Taq* NT (5 units/µl)	0.5 µl
H_2O	36.5 µl
Total	50 µl

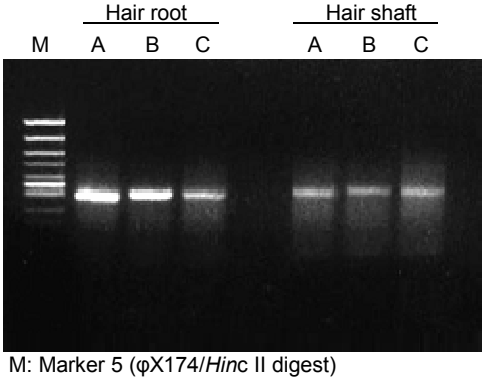
＜PCR condition＞

94°C 1 min.
94°C 30 sec.
55°C 30 sec. } 35 cycles
72°C 1 min.
72°C 5 min.

2. Examples of experiments using human hair

Detection of mitochondrial DNA

Using the ISOHAIR, DNA was extracted from 1 cm hair root and 6 cm hair shaft from three different human subjects (A, B, C). Using 1/4 amount of the obtained DNA, human mitochondrial DNA (D loop region; 280 bp) was amplified by PCR and electrophoresed.



M: Marker 5 (φX174/*Hinc* II digest)
3% Agarose 21

＜PCR mixture＞

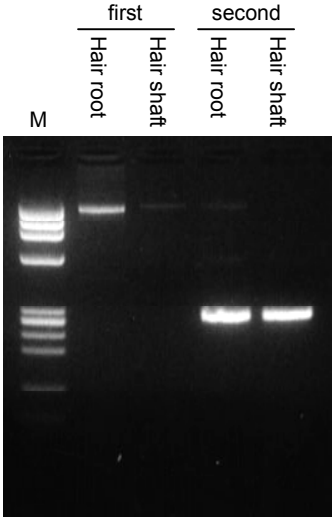
Template DNA	5 µl
10×Gene *Taq* Universal Buffer	5 µl
dNTP mixture (2.5 mM each)	4 µl
primer (20 pmol each/µl)	1 µl
Gene *Taq* NT (5 units/µl)	0.5 µl
H_2O	34.5 µl
Total	50 µl

＜PCR condition＞

94°C 1 min.
98°C 15 sec. ⎫
55°C 15 sec. ⎬ 40 cycles
72°C 30 sec. ⎭
72°C 5 min.

Detection of *p53* gene

DNA was extracted from 1 cm hair root and 6 cm hair shaft using the ISOHAIR. Using 1/4 amount of the obtained DNA, the *p53* gene (exon 11) was amplified by semi-nested PCR (first PCR product 1,296 bp, second PCR product 265 bp)[1)] and electrophoresed.



M: Marker 4 (φX174/*Hae* III digest)
3% Agarose 21

Using the first PCR product amplified from the human *p53* gene, cycle sequencing was performed, and sequencing for both hair root and hair shaft was observed successfully.

＜PCR Mixture＞

Template DNA	
10×Gene *Taq* Universal Buffer	5 µl
dNTP mixture (2.5 mM each)	4 µl
primer-forward (20 pmol/µl)	1 µl
primer-reverse (20 pmol/µl)	1 µl
Gene *Taq* NT (5 units/µl)	0.25 µl
H_2O	
Total	50 µl

＜first PCR condition＞ * 5 µl was used as a template.

94°C 1 min.
98°C 15 sec. ⎫
55°C 15 sec. ⎬ 40 cycles
72°C 30 sec. ⎭
72°C 5 min.

＜second PCR condition＞ * 1 µl of the first PCR product was used as a template.

94°C 1 min.
98°C 15 sec. ⎫
60°C 15 sec. ⎬ 30 cycles
72°C 30 sec. ⎭
72°C 5 min.

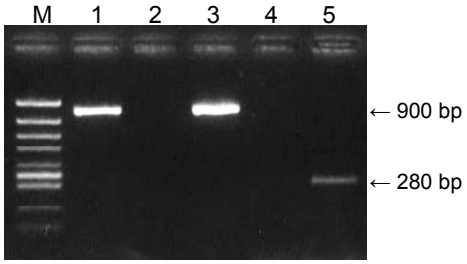
Relief of PCR inhibition with T4 gene 32 protein

DNA solution extracted from hair may have a dark brown or black coloring. This is especially conspicuous when DNA is extracted from the tip end of hair that is 15 cm or longer, or bleached hair. The causative substance is considered to be the dye melanin contained in hair which is reported to inhibit PCR[3)].

Using the ISOHAIR, DNA was extracted from hair with high melanin content.

To the reaction mixture, for which PCR amplification had been already confirmed (Lane 1), 1/20 amount of the obtained DNA was added, and no amplification was observed (Lane 2). T4 gene 32 protein was then added to the mixture, and amplification was successfully observed (Lane 3).

Also, no amplification of human mitochondria DNA (280 bp) was observed (Lane 4) in PCR using the obtained DNA as a template, but amplification was observed when T4 gene 32 protein was added (Lane 5).



M: Marker 5 (φX174/*Hinc* II digest)
3% Agarose 21

Lane 1: Amplification of 900 bp using ColE1 as a template
Lane 2: Lane 1 + 1/20 of the amount of 6 cm hair shaft DNA containing a significant amount of melanin
Lane 3: Lane 2 + T4 gene 32 protein (2 μg)
Lane 4: Amplification of human mitochondrial DNA (280 bp) using as a template 1/20 of the amount of DNA containing a significant amount of melanin extracted from 6 cm hair shaft.
Lane 5: Lane 4 + T4 gene 32 protein (2 μg)

＜ColE1 PCR mixture＞

Template DNA (ColE1/*Sau*96 I digest)	0.1 ng
10×Gene *Taq* Universal Buffer	5 μl
dNTP mixture (2.5 mM each)	4 μl
primer-forward (20 pmol/μl)	1 μl
primer-reverse (20 pmol/μl)	1 μl
Gene *Taq* NT (5 units/μl)	1 μl
Total (H_2O to volume)	40 μl

＜Human mitochondria DNA PCR mixture＞

10×Gene *Taq* Universal Buffer	5 μl
dNTP mixture (2.5 mM each)	4 μl
primer-forward (20 pmol/μl)	1 μl
primer-reverse (20 pmol/μl)	1 μl
Gene *Taq* NT (5 units/μl)	1 μl
H_2O	28 μl
Total	40 μl

	Lane 1	Lane 2	Lane 3
ColE1 PCR mixture	40 μl	40 μl	40 μl
DNA solution with much melanin	0 μl	1 μl	1 μl
T4 gene 32 protein (0.5 μg/μl)	0 μl	0 μl	4 μl
H_2O	10 μl	9 μl	5 μl
Total	50 μl	50 μl	50 μl

	Lane4	Lane5
Human mitochondria DNA PCR mixture	40 μl	40 μl
DNA solution (template) with much melanin	1 μl	1 μl
T4 gene 32 protein (0.5 μg/μl)	0 μl	4 μl
H_2O	9 μl	5 μl
Total	50 μl	50 μl

＜PCR condition＞

94°C 1 min.
94°C 20 sec.
55°C 20 sec.
72°C 20 sec.
) 20 cycles
72°C 5 min.

＜PCR condition＞

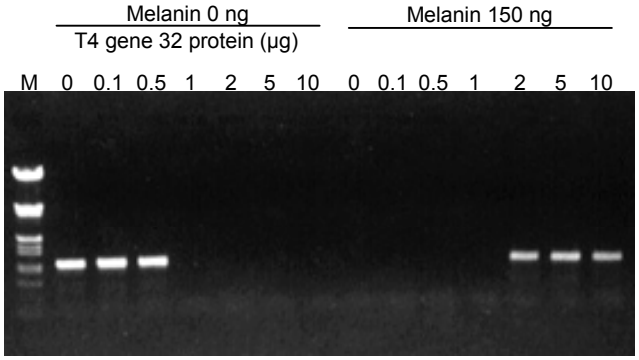
98°C 1 min.
98°C 15 sec.
60°C 15 sec.
72°C 30 sec.
) 35 cycles
72°C 5 min.

<Reference data>

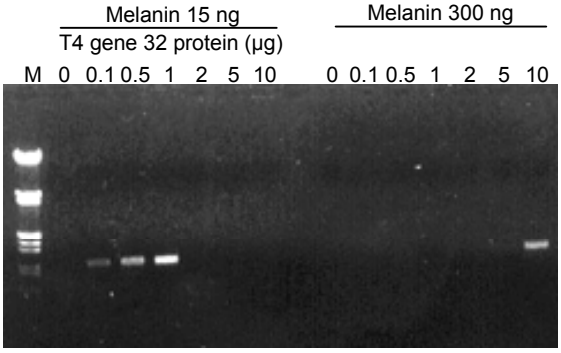
Examples are shown to indicate the relationship between the application volumes of melanin and of T4 gene 32 protein.

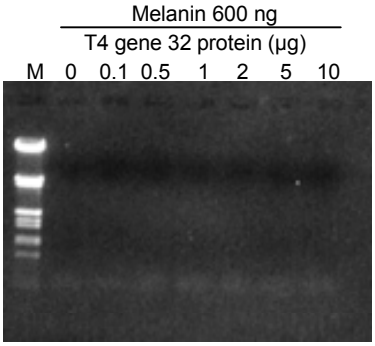
Relief of PCR inhibition by melanin with T4 gene 32 protein is described in the previous page, but T4 gene 32 protein itself can inhibit PCR reaction if an excessive amount is added.

Also, in the case of very high melanin content, it is expected that the addition of T4 gene 32 protein cannot relief the inhibition.



M: Marker 2
(λ/*Hin*d III・*Eco*R I double digest)
0.8% Agarose S





＜ColE1 PCR mixture＞

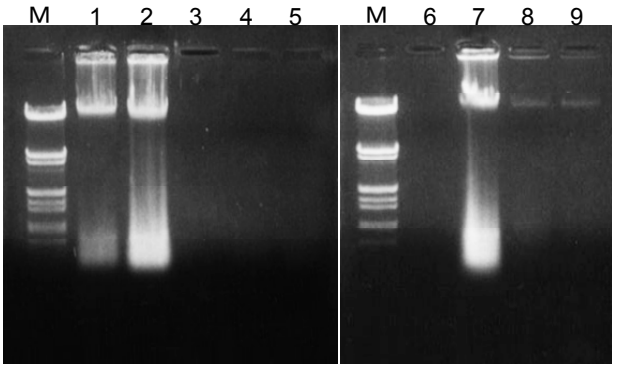
Template DNA (ColE1/*Sau* 96 I digest)	0.1 ng
10×Gene *Taq* Universal Buffer	5 μl
dNTP mixture (2.5 mM each)	4 μl
primer-forward (20 pmol/μl)	1 μl
primer-reverse (20 pmol/μl)	1 μl
Gene *Taq* NT (5 units/μl)	1 μl
Melanin	0, 15, 150, 300, 600 ng
T4 gene 32 protein	0, 0.1, 0.5, 1, 2, 5, 10 μg
H_2O	
Total (H_2O to volume)	50 μl

＜PCR condition＞

94°C 1 min.
94°C 20 sec.
55°C 20 sec. 20 cycles
72°C 1 min.

Amount of DNA extracted from hair

Using the ISOHAIR, DNA was extracted from hair roots or hair shafts of three human subjects (A, B, C), and electrophoresis was performed.
The results indicate that DNA yield varies between individuals and even between hair strands from the same individual. Based on the result of gel electrophoresis, the amount of extracted DNA is estimated to be about 0.5 µg per hair root and less than 10 ng per hair shaft. [4].



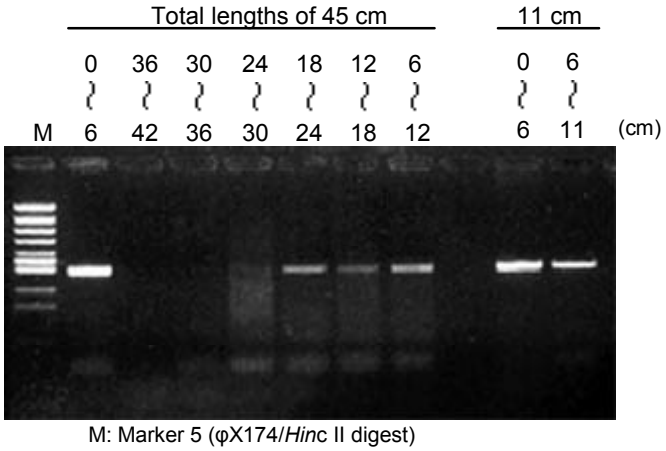
* The lower bands of Lane 1, 2, 7 (indicated by arrow) are RNA.

Lane 1: Hair root A 1 cm
Lane 2: Hair root A 1 cm×5
Lane 3: Hair shaft A 6 cm
Lane 4: Hair shaft A 18 cm
Lane 5: Hair shaft A 36 cm
Lane 6: Hair root B 1 cm
Lane 7: Hair root B 1 cm×5
Lane 8: Hair root C 1 cm
Lane 9: Hair root C 1 cm×5

M: Marker 2
(λ/*Hin*d III・*Eco*R I double digest)
0.8% Agarose S

Regional difference of hair in DNA yield

DNA yield varies depending on a region of hair sampled even for the same length of hair. Using the ISOHAIR, DNA was extracted from each region of 0-6 cm, 6-12 cm, 12-18 cm, 18-24 cm, 24-30 cm, 30-36 cm and 36-42 cm from the root end of hair with total lengths of 45 cm and 11 cm. Using 1/4 amount of the obtained DNA, human mitochondrial DNA (D loop region; 280 bp) was amplified by PCR, and electrophoresed.
Increasing trend in amount of amplified DNA was seen in the sites closer to the root end, suggesting that more DNA is contained in samples closer to the hair root.



M: Marker 5 (φX174/*Hin*c II digest)
3% Agarose 21

＜PCR mixture＞

Template DNA	5 µl
10×Gene *Taq* Universal Buffer	5 µl
dNTP mixture (2.5 mM each)	4 µl
primer (20 pmol each/µl)	1 µl
Gene *Taq* NT (5 units/µl)	0.5 µl
H_2O	34.5 µl
Total	50 µl

＜PCR condition＞

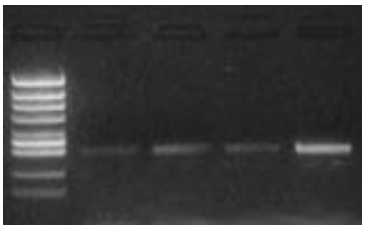
94°C 1 min.
98°C 15 sec.
55°C 15 sec. } 40 cycles
72°C 30 sec.
72°C 5 min.

Elapsed time after hair removal and DNA yield

Amount of DNA that can be extracted from hair gradually decreases over time after the removal.

Using ISOHAIR, DNA was extracted from 4 cm hair shaft samples that were left standing for 76 days, 57 days, 31 days and 0 days. Using 1/4 amount of the obtained DNA, human mitochondrial DNA (D loop region; 280 bp) was amplified by PCR and electrophoresed.

Larger amount of amplified DNA was extracted from a hair sample with shorter time after removal, suggesting either a higher yield or less elution of the PCR inhibitor.



M: Marker 5 (φX174/*Hinc* II digest)
3% Agarose 21

＜PCR mixture＞

Template DNA	5 µl
10×Gene *Taq* Universal Buffer	5 µl
dNTP mixture (2.5 mM each)	4 µl
primer-forward (20 pmol/µl)	1 µl
primer-reverse (20 pmol/µl)	1 µl
Gene *Taq* NT (5 units/µl)	0.5 µl
H_2O	33.5 µl
Total	50 µl

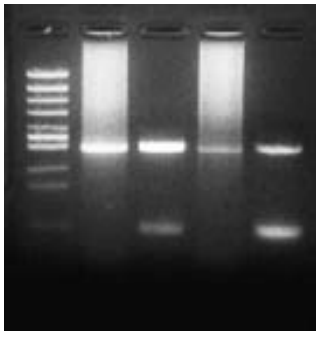
＜PCR condition＞

98°C	1 min.	
98°C	15 sec.	35 cycles
60°C	15 sec.	
72°C	30 sec.	
72°C	5 min.	

PCR condition

Since only a small amount of DNA can be extracted from a hair sample, it is significant to obtain optimal PCR conditions in order to improve reproducibility.

Using the ISOHAIR, DNA was extracted from 1 cm of hair root and 6 cm of hair shaft. Human mitochondrial DNA (D loop region; 280 bp) was amplified by PCR using 1/4 amount of the obtained DNA as a template and 20 mer or 30 mer primer and electrophoresed. The result indicates that nonspecific amplification was inhibited with the longer primer.



M: Marker 5 (φX174/*Hinc* II digest)
3% Agarose 21

<PCR mixture>

Template DNA	5 µl
10×Gene *Taq* Universal Buffer	5 µl
dNTP mixture (2.5 mM each)	4 µl
20 mer primer-forward (20 pmol/µl)	1 µl
20 mer primer-reverse (20 pmol/µl)	1 µl
Gene *Taq* NT (5 units/µl)	0.25 µl
H_2O	33.75 µl
Total	50 µl

＜PCR condition＞

94°C	1 min.	
98°C	15 sec.	40 cycles
55°C	15 sec.	
72°C	30 sec.	
72°C	5 min.	

Template DNA	5 µl
10×Gene *Taq* Universal Buffer	5 µl
dNTP mixture (2.5 mM each)	4 µl
30 mer primer-forward (20 pmol/µl)	1 µl
30 mer primer-reverse (20 pmol/µl)	1 µl
Gene *Taq* NT (5 units/µl)	0.25 µl
H_2O	33.75 µl
Total	50 µl

Other experimental examples are also shown under “ISOHAIR” on the product page of the Nippon Gene website.

List of experimental examples:

[Experimental example using human hair]
- MCT118 type test

[Experimental example using human nail]
- Detection of mitochondrial DNA
- Detection of *p53* gene
- MCT118 type test

[Experimental example using mouse body hair]
- Detection of mitochondrial DNA
- Detection of *p53* gene

[Experimental example using mouse nail]
- Detection of mitochondrial DNA and *p53* gene

etc.

Nippon Gene Co., Ltd. Website http://www.nippongene.com

VII References

1）Wilson, M. R., Polanskey, D., Butler, J., Dizinno, J. A., Replogle, J. and Budowle, B., *BioTechniques*, 18 (4), 662-669 (1995)

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