Protocol for Advanced laboratory skills in field biology

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Abstract

In this study, our aim is to understand the procedure of analysing DNA extracted from animals and humans. We have extracted DNA from buccal swab and survey individual difference of androgen receptor gene which is reported to be related with personality evaluation by questionnaire survey. This gene encodes poly-glutamine and poly-glycine amino acid sequences in the exon region and repeat numbers are polymorphic. The papers reported the repeat length of poly-glutamine was related with aggressiveness in humans and dogs. For the procedure, we have extracted DNA we have taken from buccal swab and then measured the concentration. Then we amplified the targeted sequence by PCR. Once we finished amplifying DNA, we have measured the number it was repeated in our genes, and the result differed in participants. Comparing the survey results with the PCR amplification, we found that participants with fewer repeats exhibited lower levels of Dominance, Neuroticism, and Harm, while participants with numerous repeats exhibited higher levels of these categories respectively.

On the second day, we have used same DNA to extract genotype ALDH2 gene. This is to determine weather the participants carry genes that indicates tolerance to alcohol or not. Before we begin the PCR amplification, we have conducted a small experiment by using 70% ethanol and distilled water. Two cloths, one moistened with ethanol and the other with distilled water, were applied to the arm and left in place for several minutes. By conducting this small experiment at the beginning, we could assume our body's tolerance to ethanol. While the PCR amplification was in progress, we prepared an agarose gel for electrophoresis. After creating the gel, we loaded the PCR products into wells in the gel and ran it at 100V for 20minutes until the dye migrated to the centre of the gel. We observed that participants who exhibited ethanol tolerance in the patch test had the ALDH R gene as homozygous, while those who did not were a heterozygous combination of ALDH R and ALDH R2 or were homozygous of ALDH R2. It is intriguing we can predict alcohol tolerance only through PCR amplification but also via the patch test, and the results are remarkably consistent.

Introduction

Personality analysis and comparison of poly-Q, poly- G

In contemporary studies, there is a growing emphasis on examination of personality through DNA analysis, signifying its increasing relevance in current research discussions (Sanchez-Roige et al., 2018; Weiss et al., 2009). Personality describes behaviours, emotions and cognitive styles that are stable throughout life (McCrae & Costa, 2003). Five factors have been identified as essential in the study of personality – Neuroticism, Extraversion, Openness to Experience, Agreeableness, and conscientiousness, which are collectively referred to as Five-Factor Model (FFM) [Digman, 1990]. Later research suggests that FFM is human universal and are heritable (Bouchard & Loehlin, 2001). Early laboratory primatologists recognised the significance of personality in chimpanzees, although subsequent efforts to draw parallel between

nonhuman species and humans were criticised as anthropomorphic (Povinelli, 1997). Nonetheless, recent evidence suggests that analogues of the five human personality factors are present in various nonhuman species, exhibiting strong inter-rater reliability, construct validity and internal consistencies (Gosling, 2021; Gosling & Vazire, 2002). In 1997, King and Figueredo conducted a study on chimpanzee personality by utilizing a questionnaire derived from the FFM (Goldberg, 1990). They have identified five chimpanzee personality factors resembling the FFM and a chimpanzee specific factor called Dominance (Goldberg, 1990). To explore cross – cultural differences in the perception of chimpanzee personality, a study was conducted in Japan with the translated questionnaires used in the US. The study aimed to address questions regarding interrater reliabilities, culture- specific trait intercorrelations, and the impact of culture on the perception of sex, age, and subjective well-being in relation to chimpanzee personality (King & Figueredo, 1997). To evaluate FFM numerically, genetic regions linked to specific behaviours were extracted and the extent to which these regions were repeated was verified. This experiment centred around studying aggression and involved comparing the subject's responses to FFM questionnaire with their DNA results. Aggression was the primary focus, and it was investigated in the context of intraspecific polymorphism. This was related to the length variability on the poly- glutamine (poly- Q) and poly-glycine (poly- G) regions within the androgen receptor. The association between androgens and various traits has been documented in several animal species such as Old-world primates, as well as in various mammals, for instance dogs (Konno et al., 2011). Several studies indicate shorter polyglutamine repeats were linked to reports of increased aggression (Konno et al., 2011; Aluja et al., 2011).

In this study, the results obtained from the FFM were compared to the DNA analysis with shorter poly- glutamine repeats in the participants DNA would exhibit higher scores on the FFM aggression item.

Material and Methods

Materials

Tools
Micro pipettor
Heat block
Vortex mixer
Tube stand
Timer
Cooling tube stand for 1.5ml
Cooling tube stand for PCR tubes
Centrifuge for 1.5ml
Centrifuge for 8 PCR tubes
Thermal Cycler for PCR

Reagents

DNeasy Blood & Tissue Kit (QIAGEN)
0.9% NaCl
H_2O (deionised)
Ethanol 99.5%
LA Taq with GC buffer (-20 ℃)
Primers (-20 ℃)

Control DNA samples (4℃)
Size standard (4 ℃)
HDFA (-20 ℃)

Others
Cotton buds
Paper towel
Marker pen
Filter tip (1000µl, 200µl, 10µl)
Tube (1.5ml)
94 well PCR plate and cap
94 well plate for the sequencer

Methods
DNA extraction
Buccal swab
1.Add 1000µl saline (0.9% NaCl) to a new 1.5ml tube.
2.Rub buccal mucous membrane with a cotton bud.
3.Wash the cotton bud in Step 1 tube.
4.Centrifuge at 14000rpm for 1 min.
5.Discard supernatant. Around 100µl remains.

Extraction of DNA
6.Mix 100µl saline (0.9% NaCl) and 20µl protease per sample. Add 120µl of mixture to Step5 and mix thoroughly.
7.Add 200µl of Buffer AL and mix thoroughly.
8.Incubate at 56℃ for 10 min. Mix thoroughly every 3 min.
9.Add 200µl of ethanol and mix thoroughly.
10.Pipet solution in Step 9 to spin column tube and centrifuge at 14000rpm for 1 min.
11.Place spin column a new collection tube, add 500µl of Buffer AW1, and centrifuge at 8000rpm for 1 min.
12. Place spin column a new collection tube, add 500µl of Buffer AW2, and centrifuge at 14000rpm for 4 min.
13.Place spin column in a new 1.5ml tube, add 50µl of incubated H_2O, and incubate at 56℃ for 5 min.
14.Centrifuge at 8000rpm for 1 min.
15. Discard column and store DNA at 4℃.

Measuring DNA concentration
1µl of step 15 is used for Nano drop

PCR amplification
For glutamine (Q) repeat
ARhFF: 5’-TCCAGAATCTGTTCCAGAGCGTGC-3’ (FAM labelled)
ARhR: 5’-GCTGTGAGGGTTGCTGTTCCTCAT-3’ around 280bp

For glycine (G) repeat
ARGFH: 5’-CAGTGCCGGCTATGGGGACCTGGCGA-3’ (HEX labelled)
ARGR: 5’-GGACTGGGATAGGGCACTCTGCTCACC-3’ around 320bp

PCR mixture

2x GCI buffer	5.0
H2O	1.8
sNTP	1.6
Primer1(20μM)	0.25
Primer2(20μM)	0.25
LATaq	0.1
Total	9.0μl
Template DNA	1.0μl

Mix reagents (x samples +1) except DNA in 1.5ml tube, and distribute 9μl into each PCR tube, then add template DNA. PCR reaction (LA60C30):95℃ 2min (95℃ 30s, 60/65℃ 30s, 74℃ 1min)x 30/35, 74℃ 10min, 10℃∞

Genotyping
1.Diluting PCR product: 100μl water and 1μl PCR product in a new 96 well plate.
2.Mix the following reagents in a tube
Mixture of Hi-Di and size standard (for 1 run: 16 sample):
The amount should be changed for No. of samples
Hi-Di Formamide 160μl
Size Standard 1μl
3. Add 10μl of the mixture above to 96 well plate.
4.Add 1μl of Step1 into the mixture in 96 well plate.
5.Heat the sample for 5 min, at 95℃.
6.Immediately chill on ice box for 5min.
7.Set the plate in a sequencer and run.

Genotype analyses
Check and select one or two peaks for each sample, create and export table.
Change the value to the integer.

Results
Personality analysis

The self-assessment results exhibited significant variations among the participants (Table 1). To measure these variations, specific behavioural traits, such as aggression, were quantified. next, the lengths of poly-Q and poly- G regions were determined from the extracted DNA. Since androgen receptors are encoded on the X chromosome, the results exhibited gender-based differences. In males, a single peak was observed in the results, whereas females displayed two distinct peaks (Figure 1 and 2).

Subsequently, the frequency of repeats for both poly-Q and poly-Q regions was determined, resulting in two parameters: ARQ and ARG. ARQ values ranged from 24 to 26, and ARG values ranged from 21.5 to 23. For female participants, ARQ and ARG values were

calculated as averages, allowing the of the length of the glutamine and glycine regions within the androgen receptor gene for each individual (Table 2 and Table 3). Participants were categorised into two groups based on the length of their ARQ repeat regions, specifically those with short (24.00) and long (25.00 or more) repeats. A comparison was conducted by assessing the mean scores on the FMM items within these groups. Notably, individuals with shorter repeat regions in Dominance exhibited lower scores compared to those with linger repeat regions. Additionally, participants with shorter genes scored slightly higher on the Neuroticism item (Table 2).

Discussion

The results of the study yielded diverse outcomes, and no clear patterns or tendency could be discovered. Even when the number of repetitions of the ARQ domains was the same, there were significant individual differences reflected in the values of the FFM items. These differences did not align with the expected outcomes based on the prior research mentioned in the introduction. One possible reason for this discrepancy could be the limited sample size of the participants in the current experiment.

The primary aim of this study was to explore the degree to which an individual's DNA influences their personality by combining personality assessments with DNA diagnostics. It is worth nothing that as the accuracy of DNA diagnostics advances in the future, it may become feasible to quantitatively express aspects of one's personality and unnoticed habits that cannot be effectively assessed through traditional personality tests. However, the enhanced precision in personality diagnosis through DNA analysis also raises certain ethical concerns. There is a risk that using another person's DNA could inadvertently reveal their personality traits without their consent, potentially resulting in the inadvertent disclosure of personal information. Therefore, it is imperative to handle such information, along with other biometric data like fingerprints and voiceprints, with great care and consideration for privacy and consent.

Tables and figures

Table 1. The quantified results of FMM questionnaire survey

	Dominance	Neuroticism	Harm
S 2,3,6	1.84	-0.11	39.20
L 1,4,5	2.22	-0.22	60.00

S, L represents participants with Short and Long ARG repeat, respectively.

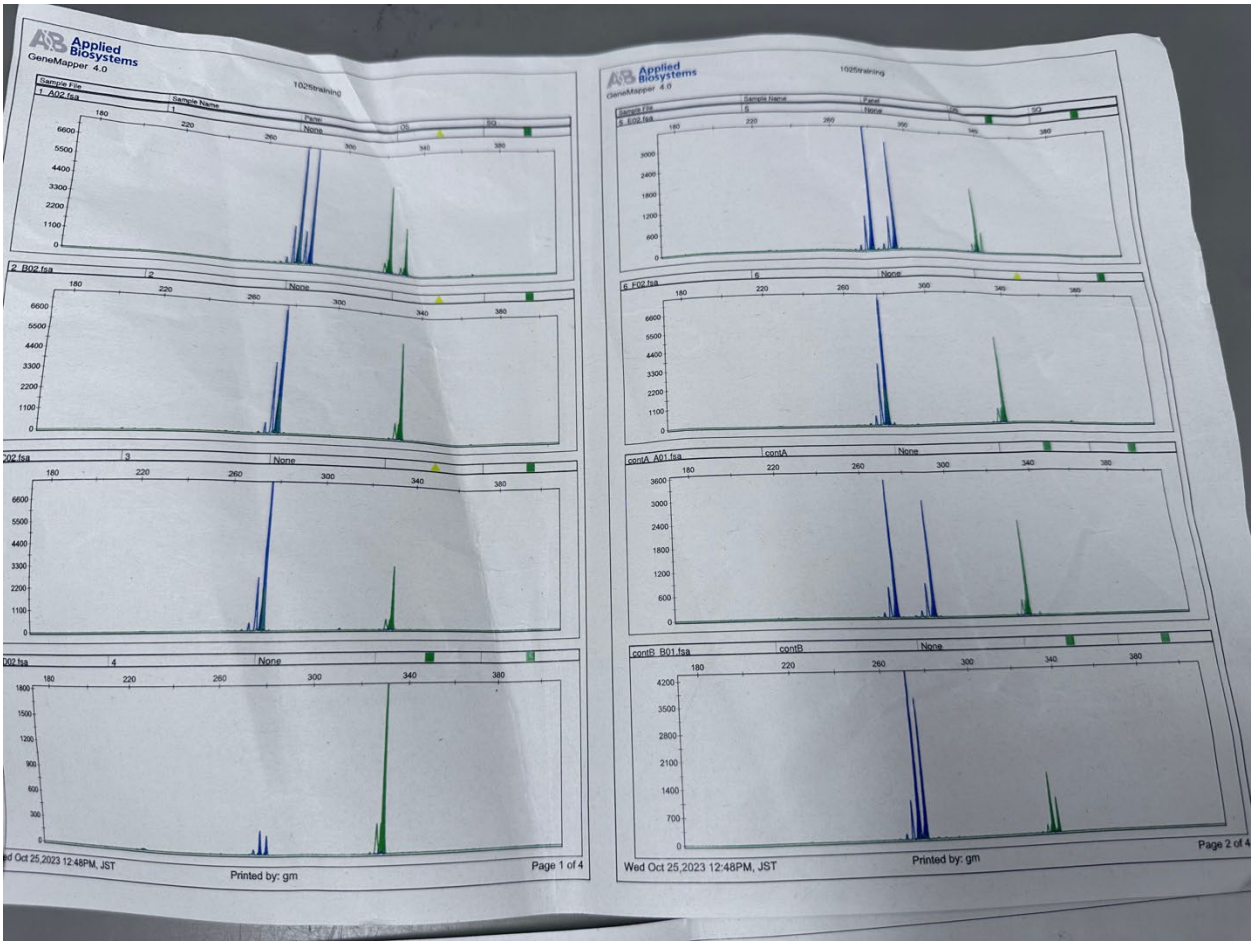
Table 2. The result of DNA amplification of participants

ID	Sex	ARQ_1	ARQ_2	ARG_1	ARG_2	ARQ_1_Repeat_Number	ARQ_2_Repeat_Number	ARG_1_Repeat Number	ARG_2_Repeat Number	ARQ average	ARG average	Dominance	Neuroticism	Harm avoidence
1	F	279.00	285.00	323.00	332.00	25.00	27.00	20.00	23.00	26.00	21.50	2.00	-0.33	80.00
2	M	276.00	—	332.00	—	24.00	—	23.00	—	24.00	23.00	2.18	-0.33	
3	F	276.00	276.00	332.00	332.00	24.00	24.00	23.00	23.00	24.00	23.00	1.35	-0.44	49.00

4	F	279.00	282.00	332.00	332.00	25.00	26.00	23.00	23.00	25.50	23.00	2.53	-0.11	40.00
5	F	276.00	288.00	332.00	332.00	24.00	28.00	23.00	23.00	26.00	23.00	2.12	-0.22	40.00
6	M	276.00	—	332.00	—	24.00	—	23.00	—	24.00	23.00	2.00	-0.44	68.60

Table 3. Repeat number of ARQ/ARG relative to the number of codons in the gene.

ARQ	ARQ Repeat Number	
273	23.00	
ARG	ARG Repeat Number	
314	17.00	



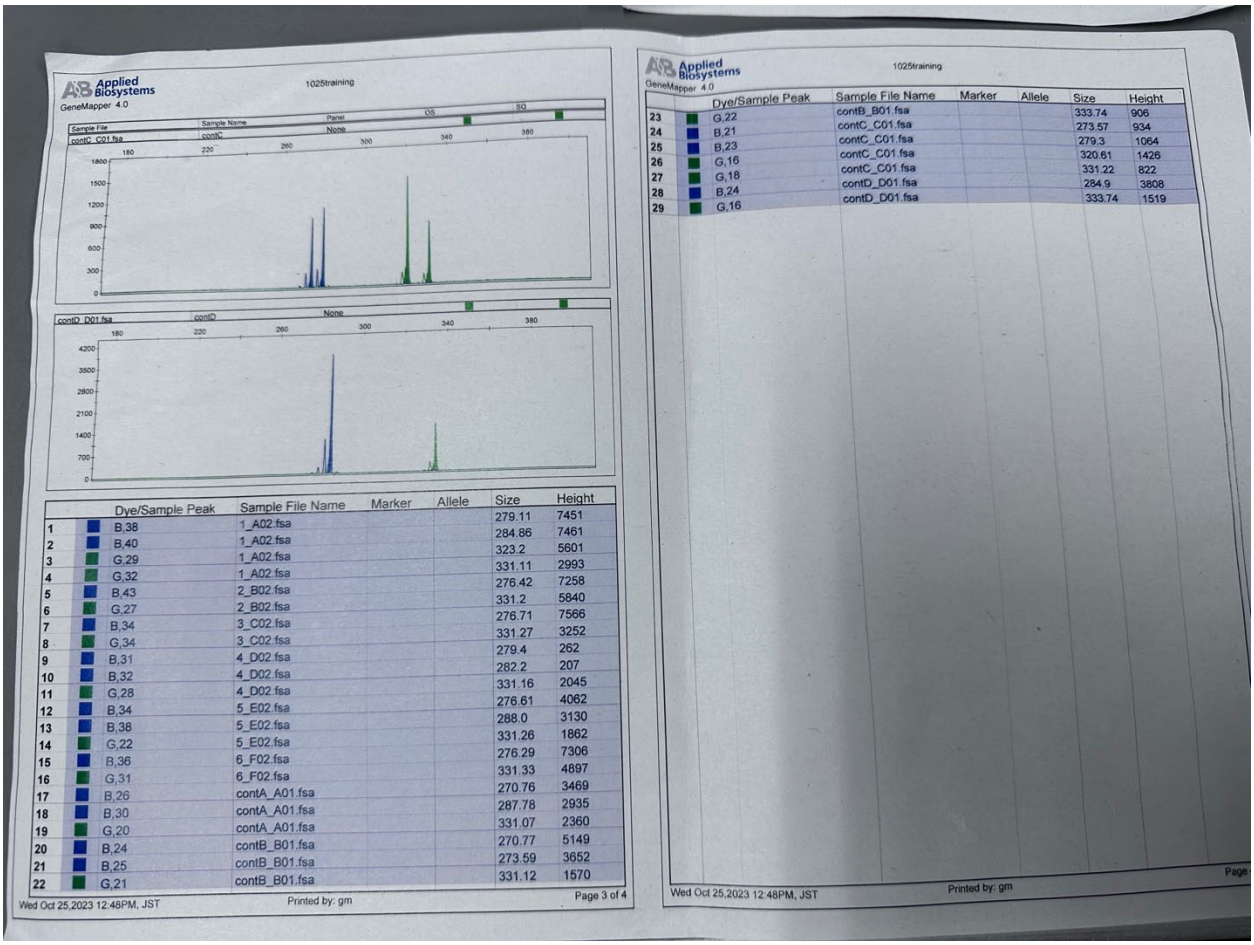


Figure 1 and 2. The result of the PCR amplification.Wave coloured in Blue and Green represents the amount of Glutamine and Glycine in the DNA fragment, respectively.

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Introduction

In the human body, there are 19 aldehyde dehydrogenases (ALDHs) that serve various roles in preserving biological functions (Jackson et al., 2011; Marchitti et al., 2008). One of these ALDH2, plays a critical role in metabolizing aldehydes, which are by-product of alcohol (Marchitti et al., 2008). Various ALDH enzymes, apart from ALDH2, are known to be associated with impaired biological function when they are deficient (Marchitti et al., 2008). Interestingly, it is not able to distinguish from a person's phenotype whether they possess ALDH2 defects, as these defects lack distinctive outward manifestations and are generally considered to have minimal impact on the preservation of biological functions (Matsumoto et al., 2014).

While there are multiple genetic polymorphisms of ALDH2, only rs671 is notably distinct (Matsumoto et al., 2014). The wild type allele is termed to as ALDH2*1, and the mutant type allele is known as ALDH2*2. This results in three genotypes: homozygous wild type, heterozygous, and homozygous mutant type. ALDH2 mutations are primarily observed in Mongoloid populations, and in Japan, they are relatively less common in regions such as Okinawa, Kyushu, Hokkaido, and Tohoku (Goedde et al., 1992). In this experiment, we have used the DNA samples collected from participants in the previous study.

Material and Methods

Materials

Patch test
1.Prepare two 1.5cm square cut paper towel
2.Dip one towel in 70% ethanol, another one to H_2O
3.Put them on inside of arm for 5 min
4.After reaction, store at 10℃

Glu (GAA) strong
↓
Lys (AAA) weak
Detection of SNP (single nucleotide polymorphism)

F primer…….GAA……
CTT remirp R
F primer…….AAA……
TTT remirp 2R

F primer (ALDH F): CAAATTACAGGGTCAACTGCT
R primer (ALDH R): CCACACTCACAGTTTTCACTTC
R2 primer (ALDH R2): CCACACTCACAGTTTTCACTTT
Two reactions (F+R, F+R2) per sample

PCR Mixture

AmpliTaq Gold 360 Master Mix	5.0
H_2O	2.5
Primer aet A or B	
F primer	0.25
R/R2 primer	0.25
Total	8.0µl per person
Template DNA	2.0µl per person

PCR reaction

Agarose gel electrophoresis

Preparation of 1.5% agarose gel

1. Measure 60ml of 1 x TBE and 0.9g agarose
2. Melt in a microwave, add H2O to restore to original volume, cool down to 60°C
3. Wait more than 30 min until the gel hardens, add a small volume of 1 x TBE and remove the comb

Electrophoresis and taking a picture

1. Fill a tank with 400ml of 1 x TBE
2. Set the gel
3. Add 1µl of dye to parafilm, add 3µl of size marker, mix by pipetting and load all to the well within the gel
4. Add 1 µl of dye to a parafilm, add 3µl of size marker, mix by pipetting and load all to the well in the gel
5. Run at 100V for 20 min until the dye moves to the middle of the gel
6. Put the gel on the UV Trans illuminator and take a photo

Results

Patch test

Among the five participants in the patch test, one displayed a reddish discoloration in the patched area as a reaction to alcohol (Table 4). This reaction indicated that the participant likely to carry a genotype of heterozygous of ALDH2*1 and ALDH2*2 or homozygous of ALDH2*2. The other four participants, excluding the one who displayed discoloration in the patch test, did not show any such redness and were predicted to carry a genotype of homozygous of ALDH2*1.

Electrophoresis

Electrophoresis results indicated that all six participants carried the gene of ALDH2*1, while among the participants, only one participant carried the gene of ALDH2*2 (Figure 3). This

implies that five of the participants were homozygous for ALDH2*1, and one participant was heterozygous for ALDH2*1 and ALDH2*2.

Comparing the results of the two experiments, we can predict that participants who exhibited reddish discoloration in the patch test carries the genotype of heterozygous of ALDH2*1 and ALDH2*2 genotype or the genotype of homozygous of ALDH2*2. In contrast, those who did not show reddish coloration in the patch test were found to have the genotype of homozygous of ALDH2*1 at the patch test stage.

Discussion

As mentioned in the introduction, it is believed the ALDH2 variant is predominantly found in Mongoloids. The proportion of individuals possessing this variant varies from country to country and region to region, but it is generally estimated that over 40% of the population in Japan possesses the variant. In Japan, the prevalence of the wild type of ALDH2 is reported to be higher in the Tohoku, Okinawa, and Kyushu regions in order. In the current experiment, five out of the six participants have Asian, Extreme Orient roots, which, in terms of expected values, suggests two of them carry the mutation. However, only one of these five participants possessed the heterozygous genotype. Interestingly, the participant with the heterozygous genotype was from the Tohoku region.

This variant of ALDH2 is believed to have originated in neo-Mongoloids and is widespread in specific areas of the Extreme Orient, likely spreading through invasions and ethnic migrations. Even within the Extreme Orient, this mutation is almost absent in Siberia, Oceania, North and South America, suggesting its expression may have originated in southern to central China. How this mutation entered the Japanese archipelago remains uncertain and may be a factor to consider when examining rhe migration of ancient Japanese to the archipelago.

However, if this gene was introduced, there should be much more variants in the Kyushu, and Okinawa islands, which re geographically close to the continent. Nevertheless, the variant is still abundant from Honshu to the south part of Tohoku region. This persistence might be due to the fact that alcohol-containing food/drink was not essential for survival, and individuals with the variant gene were not subject to the strong natural selection. Consequently, it accumulated in the population as a neutral gene, a phenomenon known as genetic drift.

Furthermore, the removal of restrictions on freedom of migration within Japan, starting from the Meiji era with the abolition of feudal domains, contributed to the disappearance of regional differences. These differences are believed to have persisted only in areas with limited human interaction and migration, such as remote islands.

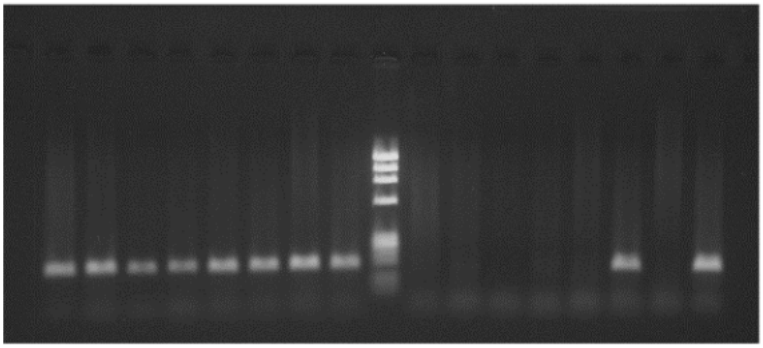
Tables and figures

Table 4. The result of the patch test and PCR amplification of all participants (1-6). Black cell represents person who did not take the patch test (or control).

participant	patch test	genotype A	genotype B	result
1	−	+	−	strong
2	−	+	−	strong
3	−	+	−	strong

4	[redacted]	+	−	strong
5	−	+	−	storong
6	+	+	+	medium
C1	[redacted]	+	−	strong
C2	[redacted]	+	+	medium

Figure 3. The result of Electrophoresis.



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Acknowledgement

This experiment was carried out smoothly under the guidance of Professor Murayama and Assistant Professor Sato. As I mainly do fieldwork, it has been a long time since I have done experiments in the lab. I also felt that it takes a lot of efforts to do both fieldwork and laboratory work. If there are any opportunity, I would be grateful to receive advice on analysing the samples gained in the fieldwork. I would like to appreciate Professor Murayama and Assistant Professor Sato again.