Development of a simple method for investigating genetic diversity using PCR and electrophoresis

PCR と電気泳動法を用いた遺伝的多様性の簡易調査法の開発

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概要

生物多様性の保全は持続可能な社会の構築に重要な対策の一つであるため、その根本的なメカニズムである遺伝的多様性に関する理解を大学教育で促すことが本研究の目標である。具体的に、遺伝子情報解析の最も基本的な技術である PCR と電気泳動法を用いて、ヒト集団内の遺伝情報が多様であることを実証する。本研究の内容は、武蔵大学における自然科学教育の一環として、全学対象総合科目「サイエンスラボ講座（生物学）」（2025 年度）において実践された。実験操作の簡易性と実験結果の可読性・可視性は教育効果の向上につながる重要な要素であると認識しているため、本稿では、アルコール代謝関連遺伝子 *Aldh2* および運動能力に関与する遺伝子 *ACTN3* の一塩基多型（SNP）を調べるための簡素化プロトコルを記述する。

1. Introduction

Genetic diversity is a universal phenomenon in all the organisms that results from genetic mutations randomly occurring in the process of DNA replication or caused by environmental factors such as radiation and chemicals. This molecular mechanism is the most fundamental driving force for biological evolution and generates millions of species,

contributing to the biodiversity that sustains the whole ecosystem. In this study, an experimental method for easy understanding of genetic diversity was established by using PCR and electrophoresis, which allows the students to visualize the results immediately and at the meanwhile analyzes their own genotypes.

The investigated genes were *Aldh2* and *ACTN3*, which are well-known examples of single nucleotide polymorphism (SNP). *Aldh2* encodes aldehyde dehydrogenase 2 (ALDH2), which is required for detoxification of alcohol in the liver. The point mutation of guanine (G) to adenine (A) causes lysine-for-glutamate substitution at position 487 (E487K), leading to the formation of the inactive ALDH2*2 variant, which is thought to be the genetic reason for "Asian flush" after alcohol drinking and may be associated with the increasing risk of chemical-induced liver tumor development[1]. Interestingly, this SNP is found only in East Asia, with approximately 40% of the population inheriting this mutation, indicating a diverse adaptive evolution of *Homo sapiens* across continents.

Alpha-actinin 3 (ACTN3) is an actin-binding protein, which helps the antiparallel orientation of actin filaments to form contractile bundles. However, the point mutation of cytosine (C) to uracil (U) leads to the substitution of arginine (R) to termination codon (X) at position 577 (R577X). This X allele in X/X genotype results in ACTN3 deficiency and alters muscle function from fast- to slow-twitch muscle fibers, which apparently reduces the performance in power and sprint activities[2,3], but may meanwhile contributes to the adaptive response in endurance training by increasing oxidative enzyme activity and altering fiber type adaptation[4]. It was reported that the frequency of the X/X genotype in both Black and White elite-level bodybuilders and strength athletes is under-represented in comparison to the general population[5]. While on the other hand, in Japanese elite-level middle- and long-distance runners, the X/X genotype showed a lower proportion than the general population[6], suggesting that ACTN3 might also be crucial for endurance performance, and the compensation mechanisms for ACTN3 loss may vary among different biological populations.

Polymerase Chain Reaction (PCR) is a widely applied molecular biology technique that

can amplify a specific DNA segment in millions to billions of times within hours. This easy-to-operate method has contributed to the evolutional development in numerous fields including medicine, agriculture, and archaeology. A well-known example is the PCR test for viral infections during the SARS-CoV-2 pandemic, which provides reliable and definitive data not only for diagnostic purposes but also as a vital clue in epidemiological studies. Therefore, learning about its principle is also an important educational goal to equip students with the latest understanding of modern science and technology.

Electrophoresis is applied to detect the DNA fragments amplified by PCR, which is a fundamental biotechnique usually introduced in the college science education. It utilizes the porous agarose gel to separate negatively charged DNA molecules by size in an electric filed. In traditional laboratory teaching, agarose gel preparation is one of the training themes, which is time-consuming and requires extreme caution when handling hazardous DNA dyes. In this study, precast agarose gel incorporated with DNA dyes (i.e., E-Gel™ EX Agarose Gels) was used to omit this step. The EX Agarose gel is designed for a compact electrophoresis device that includes an integrated blue-light transilluminator and camera for real-time visualization and gel imaging. Therefore, by employing this versatile system (i.e., E-Gel™ Power Snap Plus Electrophoresis System), the DNA electrophoresis procedures can be largely simplified and completed by liberal arts students within one class period (i.e., 105 min) to meet the educational needs.

In summary, a simple experiment protocol to investigate *Aldh2* and *ACTN3* polymorphism was designed using PCR and electrophoresis by optimizing the PCR reagent, reducing the number of operation steps, and utilizing a multi-functional compact electrophoresis device to enable rapid detection and acquire visible results. The students were able to complete all the experimental procedures within two class periods and have successfully obtained the PCR results to analyze their own genotypes.

2. Methods

2.1. Materials and experimental devices

Phosphate-buffered saline (PBS) solution (FujiFilm), UniversAll™ Extraction Buffer II (Yeastern Biotech), TE buffer (ThermoFisher Scientific), E-Gel™ 50 bp DNA Ladder (Invitrogen), and GoTaq G2 Hot Start Green Master Mix (Promega) were purchased and preserved under the condition indicated by the manufacturers. The DNA extraction and PCR procedures for *Aldh2* genotype investigation were modified from the protocol kindly provided by Kazusa DNA Research Institute in Chiba Prefecture, Japan. The DNA primer sequences for *ACTN3* polymorphism investigation were referenced from the report of Ulucan K. *et al* (2016)[7]. The customized DNA oligomers were purchased from ThermoFisher Scientific Inc., and the DNA primer sequences used for PCR were listed in Table 1.

Table 1. The primer sets used in the PCR experiments.

Wild-type *Aldh2*1* (amplicon length 524 bp)	
Forward primer	5'-CCAGGCATAGTGGCACATACT-3'
Reverse primer	5'-CCACACTCACAGTTTTCACTCC-3'
Mutant-type *Aldh2*2* (amplicon length 209 bp)	
Forward primer	5'-AGACTTTGGGGCAATACAGG-3'
Reverse primer	5'-CCACACTCACAGTTTTCACTCT-3'
ACTN3 R allele (amplicon length 413 bp)	
Forward primer	5'-CGCCCTTCAACAACTGGCTGGA-3'
Reverse primer	5'-CATGATGGCACCTCGCTCTCGG-3'
ACTN3 X allele (amplicon length 318 bp)	
Forward primer	5'-CAACACTGCCCGAGGCTGACTG-3'
Reverse primer	5'-GATGAGCCCGAGACAGGCAAGG-3'

For rapid and easy visualization of PCR products, E-Gel™ Power Snap Plus Electrophoresis System (Invitrogen) together with precast gel E-Gel™ EX 2% agarose incorporated with DNA dye SYBR Gold II were used according to the manufacturer's instruction.

2.2. DNA extraction

For easy operation and acquisition of human DNA, oral mucosal epithelial cells were collected by gently rubbing the inside of the cheek with a cotton swab. The cells were transferred into a 200 μL PBS buffer and centrifuged using a bench-top centrifuge for 1 min. 10 μL of the cell pellet was then transferred into a 50 μL Extraction Buffer and after thorough mixing, it was heated at 95 ℃ for 5 mins to allow denaturation of cellular proteins. After centrifugation for 5 s, 10 μL of the supernatant was added to a 150 μL TE buffer to stabilize DNA.

The DNA samples were collected by students themselves and used individually. The students have understood and agreed on the usage of their own DNA for educational purpose, and the PCR results were kept by students themselves and are not allowed to be disclosed to others or the public. No personal information was recorded for any purpose. The results showed in this report were from representative samples without identifiable personal information.

2.3. PCR

PCR reagents were prepared in advance by adding 10 μL Master Mix, 2.6 μL deionized water, 0.2 μL forward primer, and 0.2 μL reverse primer into a PCR tube. There are four types of primer sets used (Table 1), which detect the wild-type *Aldh2*1*, the mutant-type *Aldh2*2*, the R allele of *ACTN3* and the X allele of *ACTN3*, respectively. 7 μL of the obtained DNA-containing TE buffer was added to each PCR tube and placed into a thermocycler. The PCR condition was set at 95℃ for 2 mins, followed by 35 cycles of DNA denaturation at 95℃ for 10 s, primer annealing at 60℃ for 10 s, and DNA extension at 72 ℃ for 10 s. At last, PCR tubes were cooled down to 4 ℃ and preserved in a freezer for one week before electrophoresis.

2.4. Electrophoresis

To reduce the effect of salts during electrophoresis, 20 μL of deionized water was added

to PCR tubes after thawing. Then, 20 μL of the diluted PCR products as well as DNA maker were loaded in a precast 2% agarose gel incorporated with DNA dye, and electrophoresis was performed for 11 mins under the preset conditions programmed by the manufacturer. Gel images were acquired using the build-in transilluminator and camera.

3. Results and Discussion

3.1. *Aldh2* polymorphism

As shown in Fig. 1, three genotypes of *Aldh2* polymorphism were detected from the collected samples (n=15), including wild-type homozygotes (i.e., 524 bp fragments detected only), mutant-type homozygotes (i.e., 209 bp fragments detected only), and heterozygotes (i.e., both 524 bp and 209 bp fragments detected). The wild-type homozygotes are thought to have an active alcohol metabolism, and in the alcohol patch test, these individuals showed a high tolerance to alcohol. In comparison, the heterozygotes showed an intermediate tolerance, while the mutant-type homozygotes exhibited an extremely low tolerance in the alcohol patch test. The PCR results were consistent with those of the alcohol patch test, confirming the accuracy and reliability of this method.

In this experiment, all non-science major undergraduate students have successfully obtained their own results, indicating the practicality of this simplified protocol for educational purposes.

The storage stability of DNA markers in the freezer can be affected by repeated freeze-thaw cycles. As shown in Fig. 1, a DNA ladder stored for 2 years and used for more than five times migrated faster than expected in electrophoresis.

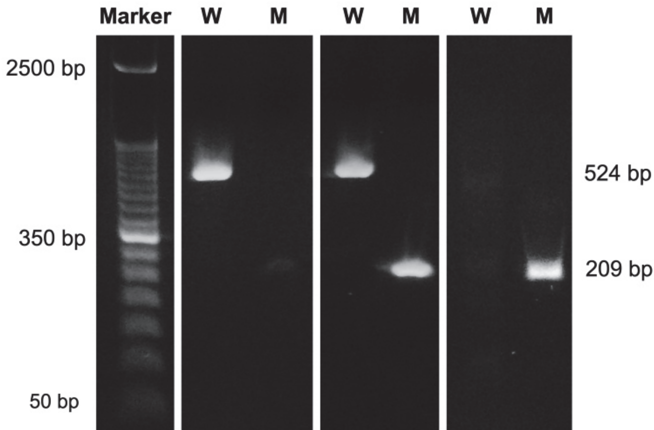

Fig. 1 Representative electrophoresis results of *Aldh2* allele investigation.
W: wild-type gene; M: mutant-type gene; bp: base pair

3.2. *ACTN3* polymorphism

Two genotypes of *ACTN3* polymorphism were detected from the collected samples (n=5), including R/R homozygotes and R/X heterozygotes (Fig. 2). Although R allele (413 bp fragments) and X allele (318 bp fragments) were successfully visualized, X/X genotype was not detected probably due to the low sample number. The experiments with all the five samples were successfully accomplished within the same class period conducted in parallel with *Aldh2* investigation, verifying the applicability of this protocol in *ACTN3* gene detection. However, the accuracy of this method needs to be further verified, for example, by increasing the sample number or changing PCR primer designs.

The concentration of Master Mix was crucial for visualization of the PCR products when using this compact electrophoresis system. If the concentration was reduced to half under the same experimental condition, no PCR products were detected (data not shown). In addition, the dilution rate of PCR products with deionized water was another important factor to ensure that the DNA bands appeared straight and not bent. However, excessive dilution can reduce the resolution of DNA bands, potentially resulting in suboptimal

results. Therefore, different dilution rates (e.g., 2x, 5x, and 10x) were tested, and it was found that 2x dilution was the most suitable condition for a clear view of PCR products.

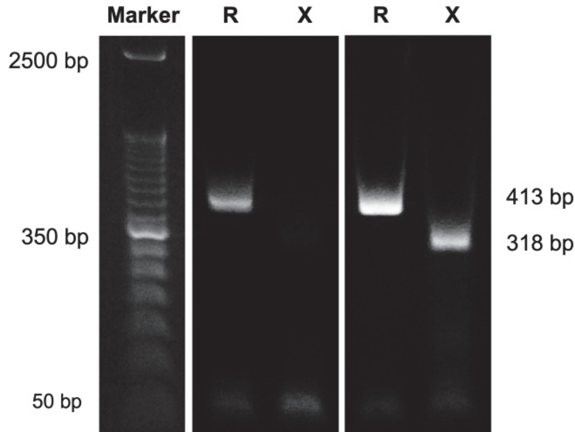

Fig. 2 Representative electrophoresis results of *ACTN3* allele investigation.
R: R allele; X: nonsense variant; bp: base pair

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