NIPPON Genetics EUROPE

Application

SNP analysis of aldehyde dehydrogenase by PCR

Product: KAPATaq EXtra PCR Kit (Cat No. KK3009)
Manufacturer: KAPA BIOSYSTEMS

Product: Midroi Green Advance (Cat No. MG04)
Manufacturer: NIPPON Genetics EUROPE

Product: FAS-Nano (Cat No. GP-06LED)
Manufacturer: NIPPON Genetics EUROPE

The following data was posted due to the courtesy of Mr Naoharu Takano and Shota Moriya, Biochemistry Dept., Tokyo Medical University, Japan

Introduction

Here, we analyze the SNP (*1) of aldehyde dehydrogenase (ALDH2) gene which determines “strong” or “weak alcohol tolerance.

*1: What is SNP?
99.9% of the human DNA sequences are the same and it is considered that the difference of genetic information of 0.1% is creating human diversity, such as difference in face, body shape, constitution and character among individuals.
Single nucleotide polymorphism (SNP) refers to a state in which the nucleotide sequence of a gene differs by one site and it is known that the function of in vivo proteins such as enzymes changes slightly depending on the type of SNP.
It is known that SNP is susceptible to diseases and the reaction to medicine is different. In the medical field a development of “tailor-made medical” , which offers the optimal treatment for individual patients based on SNP, is expected.

Experiment

In this experiment, genomic DNA is extracted from buccal mucosa cells and the ALDH2 gene which is involved in alcohol metabolism is amplified by PCR. After that, amplified fragments are treated with restriction enzymes and the genotype of ALDH2 is determined by agarose electrophoresis, thereby confirming the tollerance of alcohol.

References: Helminen A, Väkeväinen S, Salaspuro M.
ALDH2 genotype has no effect on salivary acetaldehyde without the presence of ethanol in the systemic circulation.
PLoS One. 2013;8:e74418.
(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3772811/)

Sample collection
↓
DNA extraction
↓
PCR
↓
Restriction enzyme treatment
↓
Electrophoresis
↓
Gel photography

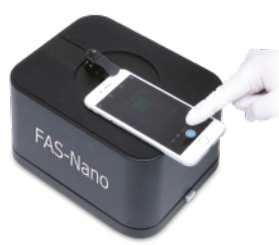
KAPATaq EXtra PCR Kit (Cat No. KK3009)

- A new enzyme that combines “amplification” , “accuracy” and elongation.
- Excellent in versatility, it can be used in all rounds from standard PCR to amplification of long sizes.
- Using TAQ for standard PCR, it can be used without optimization.
- The accuracy is 4 times higher than of Taq polymerase.

Midori Green Advance (Cat No. MG04)

- Safer DNA fluorescent stain, replacing ethidium bromide (EtBr)
- Overwhelmingly lower mutagenic and non-carcinogenic substances than EtBr
- Excelent compatibility with LED light, but can be used with sufficient sensitivity even with UV

Gel Documentation System Fas-Nano (Cat No. GP-06LED)



- Gel shooting device that can be easily taken with your smartphone or tablet
- Advanced Blue/Green LED (500 nm) that demonstrates outstanding compatibility with a wide range of nucleic acid staining reagents.
- Since UV illuminator is not used, gel shooting can be performed safely together with Midori Green.

Note: Because it becomes a simple photographing device, the photographed image will depend on the performance of the smartphone.

Experimental method

● Sample

Oral mucosa was collected with physiological saline from human who obtained informed consent and genomic DNA was extracted with InstaGene (BIO-RAD)

PCR reaction

● PCR reaction composition

KAPATaq EXtra DNA Polymerase (5 U/μL)	0.2	μL
5×KAPATaq EXtra buffer（Mg^{2+} free）	8	μL
25mM $MgCl_2$	2.8	μL
dNTP Mix（10 mM each）	1	μL
PrimerF（10 μM）	2	μL
PrimerR（10 μM）	2	μL
Template DNA	20	μL
PCR grade Water	4	μL
Total	40	μL

● Thermal cycler conditions

Taq activation, denaturing	94℃	5min	
↓			
Denaturing	94℃	30sec	40 cycles
↓			
Annealing	60℃	30sec	
↓			
Extension	72℃	60sec	
↓			
Final extension	72℃	2min	

Thermal cycler: BIO-RAD MyCycler

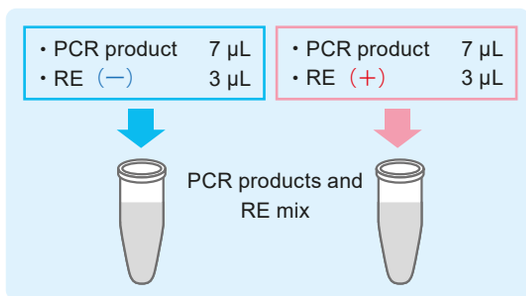
● Primer sequence:

Forward Primer: TCAAATTACAGGGTCAACTGCT
Reverse Primer: GGCTGGGTCTTTACCCTCTC

References: Helminen A, Väkeväinen S, Salaspuro M. ALDH2 genotype has no effect on salivary acetaldehyde without the presence of ethanol in the systemic circulation. PLoS One. 2013;8:e74418.

Digestion

Restriction enzyme (RE)（—）		Restriction enzyme (RE)（+）	
Ultra pure water	2.8 μL	Ultra pure water	2 μL
10xCutSmart Buffer	1.6 μL	10xCutSmart Buffer	1.6 μL
1.6mM S-adenosyl-methionine※	0.4 μL	1.6mM S-adenosyl-methionine※	0.4 μL
		Acu I	0.8 μL
total	4.8 μL	total	4.8 μL



* final concentration 40 μM

Acu I：NEB
10xCutSmart Buffer and S-adenosyl-methionine are attached to Acu I
S-adenosyl-methionine used 1.6 mM as a stock solution

Electrophoresis

Agarose: Agarose（NIPPON Genetics EUROPE: Cat No. AG01）

Nucleic acid stain reagent: Midroi Green Advance
(NIPPON Genetics EUROPE Cat No. MG04)

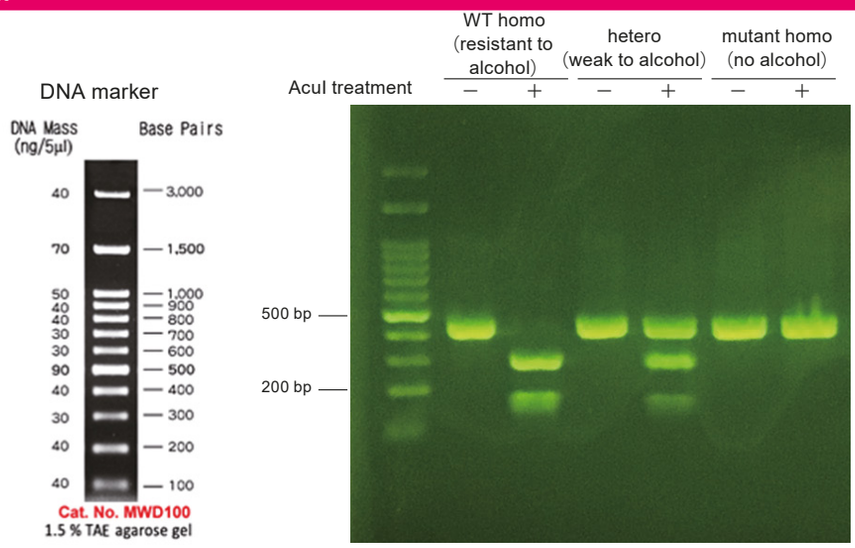
Electrophoresis conditions: 100V
Stop electrophoresis when 1/2 of the gel is proceeded

Buffer: TBE

Electrophoresis device: Mupid-2plus

Loading dye has better results with NIPPON Gene: 313-90111 (SDS containing, BPB, XC) SDS containing cleaner results.
In particular, in this experiment many impurities such as Taq and restriction enzyme are contained in the electrophoresis sample, so there is a possibily that the band pattern is disturbed unless SDS entry is used.

Experimental result



2% Gel Midori Green Advance Dyed 1:20,000
Shot with Fas-Nano

The pattern of electrophoresis results of three genotypes are listed.

Before digestion with AcuI, a PCR product of 430 bp was confirmed.
By treatment with AcuI, fragments of 296 bp and 134 bp were generated in the wild type homozygote.
On the other hand, mutant homozygote (G at the 114th base of ALDH2-1 replaced by A) was not cleaved, because restriction site was mutated.
Both of 430 bp, 296 bp and 134 bp bands were confirmed in hetero.

* The resulting electrophoresis photograph is electrophoresed with a positive control cloned to confirm all band patterns.

customer's comment

Although it was used for student´s practice, the sytem using FAS-Nano seemed to be slightly beneficial as the student took pictures with their own smartphone, brought back the data and can see the pictures at any time. However, as the quality of the photograph depends on the performance of the smartphone, it seemed there were times when it was not possible to take beautiful pictures (Mr. Takano).

For student training (120 students), I was looking for a PCR kit with excellent cost performance from the viewpoint of cost reduction.
Therefore, when I tried the KAPATaq EXtra PCR kit, good experimental results were obtained (In recent years, dNTPs, buffers, etc. have already been mixed in the market, but since this product is separate, I have chosed it from the viewpoint of education to teach the meaning of each reagent in student training).
I think the KAPATaq EXtra PCR kit is very cost effective. By using FAS-Nano, it was nice to be able to conduct ethical free and safe practice (Mr. Moriya).

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