Identification of species by genes

Kobe High School Sougourigaku course

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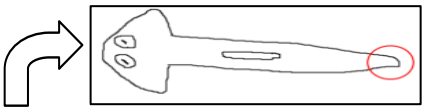
Motive/Purpose

Now, there are twenty-four native species and three introduced species of "Planaria" in Japan. However, we do not know what species live in the rivers around Rokko Mountain where our school is located.

So, our purpose is to identify the species of Planaria that live in the places near our school.

↑This is a Planaria used in our experiment

Experimental procedures



①Extract　Cut the tail of the Planaria, and extract DNA

②PCR　Reproduce and multiply COI field of DNA, using each primer

③Electrophoresis　Check whether the DNA has multiplied or not

④PCR　using primer of 18s, conduct ②&③　again

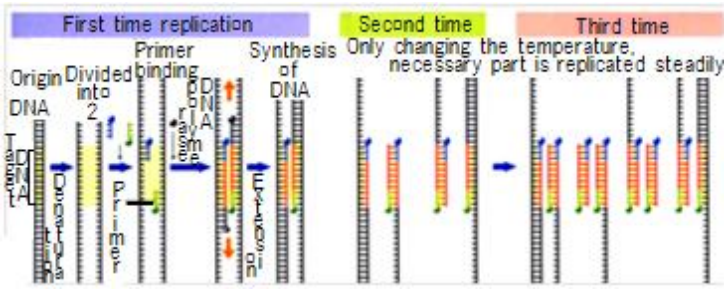
⑤P.F.　increase the purity of Mixture with finished PCR using the High Pure PCR Product Purification Kit

⑥sequence　examine the sequence of DNA（⑤）

Analyze and identify the species of Planaria with MEGA6.05

Experimental method

PCR procedure of 18s



①94℃　1min

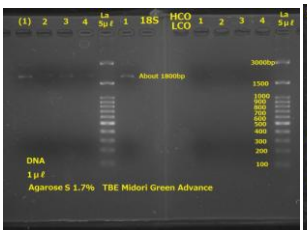
②94℃　30sec→50℃　30sec→68℃　1min　35cycles

③68℃　10min

④4℃　stay

The findings of the experiment

Illustration 1 is the effect of electrophoresis using four kinds of primers. Illustration 2 is the effect of electrophoresis of higher purity DNA .



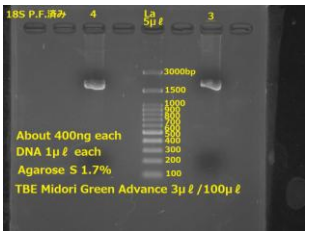


Illustration 1　Illustration 2

As a result of this gene sequencing,

We found the Planaria to be

Dugesia gonocephala(Duges,1830)

Dugesia cretica(Mexiner,1928)

Dugesia japonica(Ichikawa&Kawakatsu,1964)

(In order of the high likelihood from the top)

Discussion

According to this experiment, we can narrow down to three species of the Planaria which we caught. *Dugesia japonica* is a native species. *Dugesia gonocephala* and *Dugesia cretica* are both introduced species. They have not been officially reported to have settled in Japan now. If the Planaria we caught is *Dugesia gonocephala* or *Dugesia cretica*, it is an unusual situation. We do not know why they are in Mukogawa River. But, one reason we could think of is that Rokko Pasture is located near Mukogawa River. Rokko Mountain Pasture imports many animals from foreign countries. So, we can say that when these animals were imported , Planaria were brought by chance .

Considerations

We need to research *Dugesia gonocephala* and *Dugesia cretica* in more detail because we have not got enough information about them yet.

References

・World Register of Marine Species HP

・Illustrated book with primary color of Planarias (Kawakatsu Masaharu) HP

・Planarian class in flat waters in Japan (Kawakatsu Masaharu and others) 2008