

## Detection of PCR products from *Legionella pneumophila* using zinc finger protein and firefly luciferase fusion protein

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### INTRODUCTION

The species-specific detection of pathogenic bacteria is a pressing problem ranging from food safety to the detection of biowarfare agents. Therefore, we need simple, rapid and sensitive method to detect pathogens. In recent years, Polymerase Chain Reaction (PCR) technology has been widely used to detect pathogens. PCR amplification of pathogen-specific DNA offers a promising means of avoiding complicated procedure, time consuming and achieving the rapid and reliable identification of bacteria.

We have previously developed a novel detection system to detect pathogen specific PCR products using Zinc finger protein (ZFP), which recognizes dsDNA in sequence-specific manner [1]. We have detected specific PCR products by Enzyme-Linked ImmunoSorbent Assay (ELISA) or fluorescence depolarization measurement. ELISA is sensitive but it is time consuming, and fluorescence depolarization measurement is rapid but needs special device to detect PCR products.

Therefore, in order to construct rapid, sensitive and inexpensive system to detect PCR products, we tried to construct the fusion protein of ZFP and Luciferase. Luciferase is one of the enzyme that have high activity and emits light by catalyzing reaction with luciferine, ATP and O<sub>2</sub> [2]. This feature enables us to detect PCR products sensitive without extra source of light. Unlike ELISA, we can also detect PCR products more simply without an use of antibody. These advantages enable us to construct the rapid, sensitive and inexpensive detection system. In this work, we tried to detect *Legionella pneumophila*, which is one of the pathogen, as a model, using fusion protein of ZFP and Luciferase (Fig. 1).

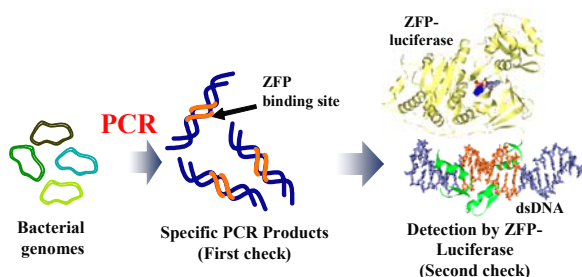


Fig. 1 Scheme of detection system of PCR products from pathogen bacteria by fusion protein of ZFP & luciferase.

### RESULTS AND DISCUSSION

The strategy to determine the target PCR region of *L. pneumophila* is described previously [1].

In order to confirm the DNA binding ability and Luciferase activity of ZFP-Luciferase, we first detected synthetic oligonucleotide, which has the sequence of target PCR region of *L. pneumophila*. For assays, three kinds of synthetic oligonucleotide, which has

ZFP-binding sequence, 1 mutation sequence, random sequence, were immobilized onto microtiter plates that were precoated with streptavidin. After the immobilization of oligonucleotide, ZFP-Luciferase was added and immobilized on each well. PicaGene® (TOYO INK) was added on each well. Luminescence was measured by fluorescence plate reader after one minute incubation (Fig. 2). The luminescence was observed in the presence of ZFP binding sequence and not in 1 mutation sequence or random sequence. This result suggests ZFP-Luciferase has both activity to bind the target DNA and emits luminescence.

We next tried to detect PCR products from *L. pneumophila* genome DNA. Using the designed primers and *L. pneumophila* genome DNA, we amplified the target sequence by PCR. We also used other bacterial genome DNAs (*Escherichia coli* DH5a genome DNA and *Proteus vulgaris* genome DNA) as controls. Amplified band of the target sequence was observed only with *L. pneumophila* PCR products and not with other genomes by gel electrophoresis. We were also able to detect the *L. pneumophila* PCR products specifically using ZFP-Luciferase by DNA binding assay.

Now we can detect specific dsDNA which contains Zinc finger binding site by Luciferase activity. When we use our system to detect bacteria specific PCR products, it is possible to detect many samples at once by luminescence using high sensitive CCD camera, like DNA microarray. Therefore our system may provide high-throughput detection system to detect PCR products from bacteria.

In conclusion, we developed a novel detection system for PCR products from *L. pneumophila* using fusion protein of Zinc finger protein and Luciferase. This system would be a rapid and sensitive method to detect the pathogens. Additionally, this system might be applicable to detect most of pathogens. We believe that our strategy will allow the rapid detection of bacteria.

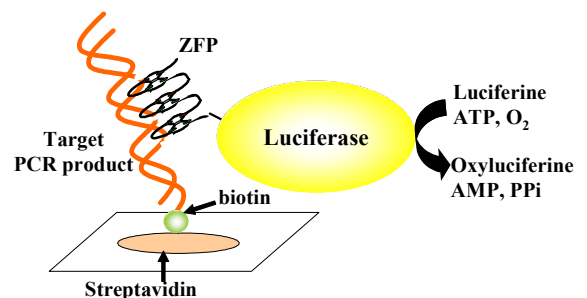


Fig. 2 DNA binding assay using ZFP-Luciferase.

### ACKNOWLEDGEMENT

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### REFERENCES

1. Yuko Osawa, Kazunori Ikebukuro, Hiroaki Motoki, Takafumi Matsuo, Michio Horiuchi and Koji Sode *Nucleic Acids Research*, *inpress*
2. Naoki Kajiyama, Tsutomu Masuda, Hiroki Tatsumi and Eiichi Nakano *Biochimica et Biophysica Acta*, 1120 (1992) 228-232