

Original article

Development of the detection methods of the new fluorescent protein genes that are introduced in GloFish

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Introduction

In the United States, colorful transgenic zebrafish (*Danio rerio*), tiger barb (*Puntius tetrazona*) and black tetra (*Gymnocorymbus ternetzi*), in which various fluorescent protein genes are introduced, are commercially available as GloFish [1]. In contrast, the use and handling of such transgenic organisms are regulated under the “Cartagena Biosafety Protocol” in Japan. The Japan Fisheries Research and Education Agency is authorized to conduct on-site inspections under the Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulation on the Use of Living Modified Organisms (LMOs). Accordingly, it is mandatory to develop effective detection methods of LMOs such as GloFish, in order to monitor the introduced LMOs in Japan in case that they are illegally imported, as mentioned in [2]. Here we describe the detection methods of new GloFish fluorescent protein genes, i.e., JRed, MCitrine, Venus, Emerald, CyPet and Cerulean [3].

Materials and methods

The nucleotide sequence of each fluorescent protein gene was obtained from NCBI nucleotide database. The plasmid containing the positive control of each gene was synthesized by GenScript Japan Inc. For PCR, 0.5 pg of each plasmid was used. PCR primers were designed by the on-line software Primer3 [4].

Twenty nanogram of zebrafish genome DNA was used as the negative control (non-transgenic). For the positive control, the length of the PCR products was designed to be 517 bp for JRed, 506 bp for MCitrine, 710 bp for Venus and Emerald, 557 bp for CyPet, and 575 bp for Cerulean, respectively.

Results and discussion

The PCR product of each positive control was observed by agarose-gel electrophoresis as a single band at the expected DNA length (Figs. 1-3). On the other hand, no PCR product was observed for the lane of negative control (Figs. 1-3).

From these results, we conclude that PCR detection systems of these fluorescent protein genes, i.e., JRed, MCitrine, Emerald, Venus, CyPet, and Cerulean, were established.

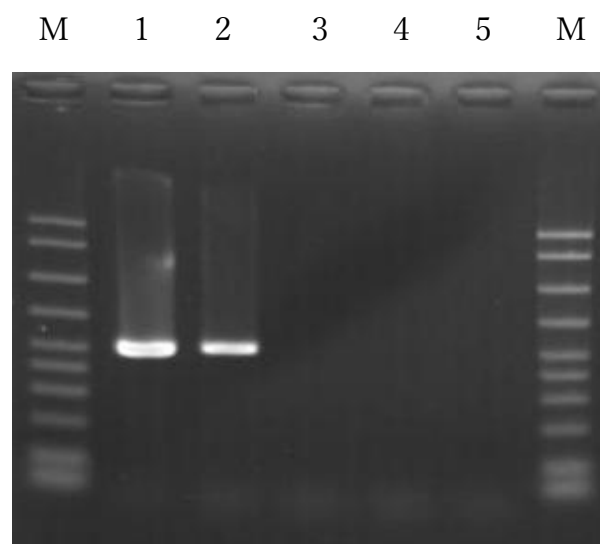


Fig. 1. PCR detection of JRed fluorescent protein gene. Lane M: molecular weight marker, from the bottom, 50, 100, 200, 300, 400, 500, 700, 1,000, 1,500, and 2,000 bp, lanes 1, 2: positive control of JRed gene (0.5 pg), lanes 3, 4: negative control of non-transgenic zebrafish genome DNA (20 ng), lane 5: negative control (D.W.). In lanes 1 and 2, positive bands with the length of 517 bp are observed.

References

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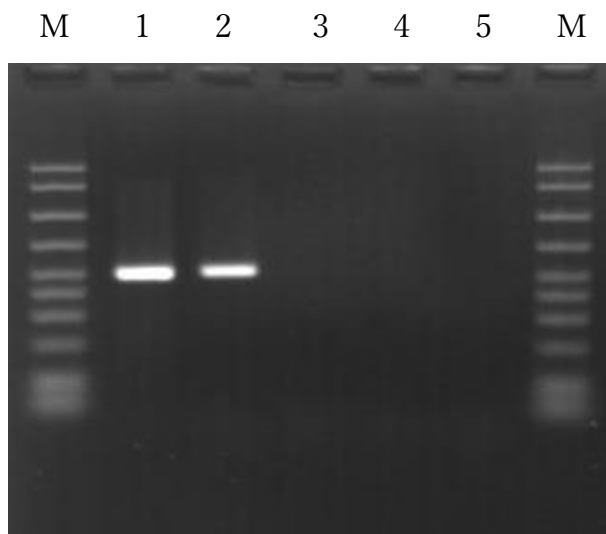


Fig. 2. PCR detection of MCitrine fluorescent protein gene. Lane M: molecular weight marker, from the bottom, 50, 100, 200, 300, 400, 500, 700, 1,000, 1,500, and 2,000 bp, lanes 1, 2: positive control of MCitrine gene (0.5 pg), lanes 3, 4: negative control of non-transgenic zebrafish genome DNA (20 ng), lane 5: negative control (D.W.). In lanes 1 and 2, positive bands with the length of 506 bp are observed.

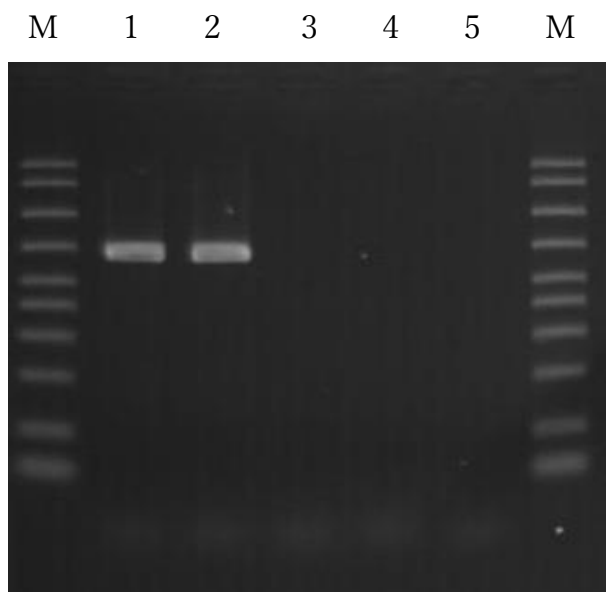


Fig. 3. PCR detection of Venus and Emerald fluorescent protein genes. Lane M: molecular weight marker, from the bottom, 50, 100, 200, 300, 400, 500, 700, 1,000, 1,500, and 2,000 bp, lane 1: positive control of Venus gene (0.5 pg), lane 2: positive control of Emerald gene (0.5 pg), lanes 3, 4: negative control of non-transgenic zebrafish genome DNA (20 ng), lane 5: negative control (D.W.). In lanes 1 and 2, positive bands with the length of 710 bp are observed.