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Characterization beta actin promoter cloned from Japanese flounder and its driving growth hormone expression in Zebrafish

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Introduction

Nowadays, growth enhancement or disease resistance transgenic fish were successfully generated and did considerable contribution to meet the increasing demand of seafood in the whole world. However, it also caused many food safety concerns about transgenic fish. Therefore, in the procedure of transfer gene construction, we have to consider more about its food safety and the feeling of consumer. The transfer of gene constructs derived from the same, or closely related species, so called "all fish" transgenesis, definitely is easier to be accepted and becomes more promising, since it does not add any new gene sequences to the genome but multiply the number of copies of already existing genes.
Further more, previous researches showed us that homologous promoters performed in higher efficiency than those from the other organism (Hong et al., 1993). Hence, there should be an increasing demand to of piscine regulatory sequences to efficiently and safely drive our favorite gene expression in commercial aquaculture species. In this paper we cloned beta actin promoter from Japanese flounder (*Paralichthys olivaceus*) and did GFP transient expression. In order to confirm its promoter activity, we linked Japanese flounder growth hormone coding sequence with it and tested its expression in zebrafish.

Materials and method

After Gnomic DNA extraction from Japanese flounder fish muscle, we digest all extracted DNA by restriction enzyme *Hind* III. Then we performed nested PCR by Takara LA PCR in vitro cloning Kit. Finally, a 2.4 kb beta actin genomic fragment was amplified and sequencing. PCR products was inserted into pEGFP vector to construct the GFP reporter vector. Based on this, we ligated Japanese flounder

growth hormone coding sequence after GFP gene removed stop codon, thereby constructed the growth hormone transfer gene. Both adult fish (*Danio rerio*) and embryos were maintained at 28.5℃ on a 14-hour light and 10-hour dark cycle. Every morning we select appropriate mounts fish for mating to ensure minimum 50 embryos for injection. Purified plasmid DNA was adjusted to 100 ng/μl in distilled water and 0.1% phenol red and microinjected into one-cell stage zebrafish embryos. We recorded the microinjection GFP expression by digital camera under fluorescence microscope 12 ~ 72 hours (28.5℃) after DNA microinjection.

Result and Summary

Sequence analysis results showed us that it contained many cis-regulatory motif. The zebrafish embryos injected GFP reporter gene expressed very strong Green fluorescence under 540 nm UV light. GFP expression can be detectedin the fish whole body including head, eyes, skin, muscles, and blood cell. Flounder Growth hormone transfer zebrafish showed fast growth speed and grew up to about 1.5 time large as the control group three months after fertilization.

Our beta actin promoter drove strong expression of both the GFP transient expression vector and flounder growth hormone fusion gene in zebrafish embryos that is consistence with its sequence character. Many function motif can be predicted in its sequence and make its high activity reasonable. The growth hormone transfer accelerates zebrafish growth speed that definitely brings us a promising prospect of GH transgenic fish; at least provide one more promoter choice for fish transgenesis.

Reference

1) Hong Y, C. Winkler, G. Brem and M. Schartl, 1993. Development of a heavy metal-inducible fish-specific expression vector for gene transfer in vitro and in vivo. *Aquaculture,* 111:215-216