Production of Transgenic Fish for Improvement of Culture Stock*

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Introduction

The techniques of fish culture have increased with an expanding human population. However, the productivity of many culture fish systems is restricted by natural physiochemical performances of the species. In order to improve qualities of important ornamental fish species, it is necessary to develop new techniques, other than those, which rely solely on classical genetic selection and cross hybridization methods. The anticipate growth of aquaculture will certainly require genetic manipulation improvement progresses. Among different genetic manipulation techniques are that can have tremendous impact on aquaculture is development of transgenic fish. Following the development of techniques for gene transfer in mammals (Palmiter *et. al.* 1987), Zhang *et. al.* (1990) carried out the first gene transfer experiment, in which human growth hormone, fused to mouse metallothionein gene promoter, and was microinjected into goldfish eggs, producing fish twice the size of their normal siblings. Since then, a number of laboratories around the world have become interested in application of the methodology, the various species of fish seeking to develop phenotypic characteristics such as faster growth, resistance to infection and freezing temperatures (Fletcher *et. al.1988*). Gene transfer technique will be able to produce better quality fish and prawns, which expected to grow bigger in the same period than that present-day fish takes to mature. The technique use in the study would also be applied to improving quality of other Malaysian plants and animals. This study is to identify and isolate the growth promoter gene, which can be used to produce transgenic fish.

Materials and Method

cDNA synthesis of fish pituitary gland was performed. Firstly RNA was isolated using Trizol reagent follows by selection of ploy $(A)^+$ RNA using messenger maker. The first and second strand syntheses were done using *Not* 1 and *Sal* 1 adapters followed by column chromatography. 1µl of the ligated cDNA was added to 25µl of electrotransformable cells (ELECTROMAX DH10B cells) and electroporate using 0.1 cm gap chamber at settings of 100ohms and 25µF in the Gene Pulser Electroporation at 2.5 KV. The transformed cells were plated on LB plates containing 100µg/ml amphicillin and the remaining cells were stored at -70 C in 60% LB medium and 40%v/v glycerol.

Results and Discussion

Total RNA isolated was free of protein and DNA contamination with a concentration of 117.4 ug/ml in 100 mg of fish pituitary glands. A double oligo (dT) selection yielded mRNA, which was suitable for cDNA synthesis with a concentration of 2.0772 ug/ml of mRNA. The superscript plasmid system integrated cDNA synthesis with simplified downstream technology to produce cDNA ligated to a versatile plasmid vector, ready to introduce into *E.coli*. Superscript RT shows to have yield and size of first strand cDNA transcribed from HeLa mRNA is greater than those using Avian Myeloblastosis Viral (AMV) Reverse Transcriptase (RT). This suggests that the absence of RNase H activity would be an advantage in the first strand of cDNA synthesis. Superscript RT has been further engineered to produce an improved version of the enzyme (Superscript II RT) thus it was used in synthesising cDNA from mRNA. E.coli DNA polymerase 1 in combination with E.coli RNase H and E.coli DNA ligase catalysed the second strand synthesis. Although RNase H was not essential if the first strand synthesis was catalysed by AMV or M-MLV RT, *E.coli* RNase H must be included in the second strand reaction when Superscript had been used for first strand cDNA synthesis. The first and second strand reactions produced a blunt-ended cDNA thus it was a poor substrate for T4 DNA ligase. To maximise ligation efficiency into the vector, Sal *I* and *Not* 1 adapter were added to the blunt end of the cDNA. The ligated cDNA was successfully electroporated into *E.coli* using Bio-Rad electroporator. The cells were plated in the forms of 1.0. 0.1 and 0.01 dilution onto LB plates overnight and results showed that colonies were formed on the plates. Transformation efficiency was calculated as a quantitative number that represents the total number of bacterial cells that express the protein divided by the amount of DNA used in the experiment. It gave an indication of the effectiveness of getting DNA molecules into a colony of bacterial cells. The transformation efficiency was found to be 1.4 x 104 transformant/µg. Further to this either DNA hybridisation or immunological assays will screen the cDNA library. This will identify clones that carry a specific plasmid-cDNA construct. The positive clones that will be isolated by either method must be examined further to determine which one(s) carries the complete coding sequence for the target protein. The gene product will be used in the production of transgenic fish.

Conclusions

The trangene technology will have a deeper insight into genetic regulation of the developmental process using fish as a model. This prompts the researcher to suggest that there is an urgent need

for constructing transgenes of fish origin.

Benefits from the study

The gene isolated and transgenic fish will be very useful in the study of gene expression *in vivo* and *in vitro*.

Literature cited in the text

Fletcher, G.L., Shears, M.A., King, M.J., Davies, P.L and Hew, C.L. 1988. Evidence for antifreeze protein gene transfer in fish: The first step toward a more freeze-resistant salmon. *Can J.Fish. Aquat. Sci.* 45: 352-357.

Palmiter, R.D., Behringer, R.R., Quaife, C.J., Maxwell, F., Maxwell, I.H. and Brinster, R.L. 1987. Cell lineage ablation in transgenic mice by cell-specific expression of a toxin gene. *Cell.* 50: 435-443.

Zhang,P., Hayat, M., Joyce, C., Gonzalez-Villasenor, L.I., Lin, C.M., Dunham, R.A., Chen, T. and Powers, D.A. 1990. Gene transfer, expression and inheritance of pRSV-rainbow trout-GH cDNA in the common carp, caprinus carpio (Linnaeus). *Mol. Reprod. Dev.* 25: 3-13.

Project Publications in Refereed Journals

None.

Project Publications in Conference Proceedings

None.

Graduate Research

None.

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