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ISOLATION OF COMMON CARP β-ACTIN PROMOTER

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ABSTRACT

Promoter in the transgene construct plays an important role on regulating of transgene expression level in transgenic fish. In fish transgenesis, researcher convinced that use all-fish promoter more safety and prospective. This study was performed to isolate β-actin promoter, - the promoter which has ubiquitous, constitutive, house keeping characteristics, from common carp. -actin promoter from common carp (ccBA) was isolated using PCR method with FBP1, RBP1 and RBP2 primers. Sequencing was performed using ABI PRISM 3100 machine and analysis of sequences was conducted using GENETYX version 7 software. The results of sequence analysis showed that the length of DNA fragment obtained approximately 1,5 kb and containing the evolutionary conserved sequences of transcription factor for β-actin promoter including CCAT, CArG and TATA box. The results demonstrated that isolated sequence was a common carp β-actin promoter.

Key words: Homologous promoter, GFP, carp transgenic

INTRODUCTION

Gene expression effectivity's in transgenesis is depend on the promoter, which ligation at upstream position is from target gene in a construction. Promoter is DNA sequent site at upper course (upstream; 5'-flanking sequences), which initiation, regulates the where, when and level gene expression (Toha, 2001; Beaumont and Hoare, 2003). First development fish transgenesis, researcher use promoter in gene construction from mammalian and virus, but for the food safety reason, so that it will developed gene construction with promoter from fish, or mention as all-fish gene construction. Better if it is use promoter from the same or a related-species with transgenic fish (Hwang et al., 2003).

Some kind promoters that have been isolated and examined for some fish species by researcher, are *cytomegalovirus* promoter (CMV) from human's virus, elongation factor-1α (EF-1α) and β-actin from medaka, myosin light chain-2 (Mylz-2) from zebra fish (Alimuddin, 2003). Based on Alimuddin's research (2003) in zebra fish, β-actin and Mylz-2 promoters indicates the strongest activities than EF-1α promoter, while CMV indicate the weakest activity because CMV promoter from human virus which possible to divide all cis-acting element are knew by zebra fish's trans acting factor, while another promoter from fish show advanced activity. Furthermore β-actin is a shape of promoter which has house-keeping characteristic; always active as long as organism live. In addition, β-actin promoter also has ubiquitous characteristic (Hackett, 1993), i.e. this promoter will active everywhere and constitutive (Volckaert et al., 1994) that promoter can active without any trigger from outside like temperature and hormone. This promoter always use in transgenic research like in common carp (Liu et al., 1990), zebra fish (Higashijima et al., 1997), medaka (Hamada et al., 1998), mud loach (Nam et al., 2001), tilapia (Hwang et al., 2003) and red sea bream (Kato et al., 2007).

Based on result of research, that heterologous promoter (the promoter from fish have difference with experimental fish) has different effectivity with homologous promoter (the promoter from the same fish with experimental fish). Like zebra fish uses β-actin promoter from red sea bream to indicate survival rate level only 63% than use this promoter in same species survival rate to achieve 95% (Kato et al., 2007). Because of that within scheme result homologous promoter, so in this result was performed to isolate β-actin promoter from common carp in order to develop a transgene construct which was 'all common carp' in origin. Common carp is one of the fresh water fish has high economy values.

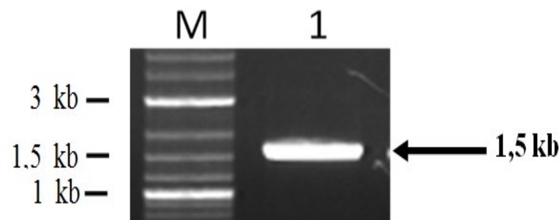
MATERIALS AND METHODS

β-actin common carp promoter was isolated use PCR methods with printing like genom DNA result of extraction from common carp's liver tissue. DNA extraction was done with use DNA isolation kit (Gentra). The primer sequence are forward "F-BP1" (5'-GTGWGTGA-CGCY GGACCAATC-3') and reverse "R-BP1" (5'-TAGAAGGTGTGRTG CCAGATCTTC-3') also nested primer "R-BP2" (5'-TTGCACATRCCRGAKCCGTTGTC-3'). PCR amplification result was separated with electroforesis use agarosa gel 0,7%. Target DNA fragment with length approximately 1500 bp was purified from agarosa gel uses gel/PCR DNA Gene Mate Purification kit (ISC BioExpress) and then was ligated to pGEM-T Easy vector. Plasmid of ligation result was named as pT-ccBA. pT-ccBA Plasmid was transformed to competent cell *Escherichia coli* DH5α. Bacteria clone which carry pT-ccBA was known by see it's colony color; white blue colony bacteria usually carry insertion plasmid, while blue colony color not carry insertion plasmid. White colony bacteria clone was supposed carry pT-ccBA was reconfirmed using cracking method. Cracking was explained by bacteria which carry pT-ccBA was cultured in liquid media 2xYT for plasmid duplicated. pT-ccBA plasmid was isolated from bacteria using Flexiprep (Amersham Bioscience) kit. ccBA sequent in pT-ccBA plasmid was analyzed use ABI PRISM

3100 machine. Characteristic and homology of ccBA sequen with β-aktin promoter sequen there in Gen Bank was analyzed use GENETYX versi 7 software.

RESULTS AND DISCUSSION

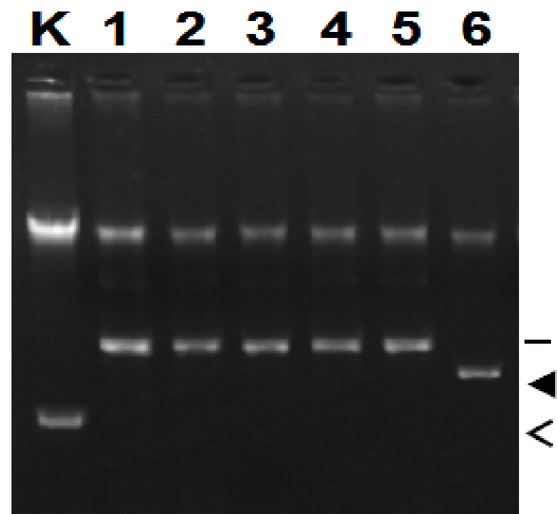
Amplification product of PCR has the DNA fragment length range to 1,5 kb (picture 1). The sequent result showed us that the DNA length is 1528 bp (picture 2). Sequent analysis showed that there is a transcription factor CCAAT, CArG ($CC(A/T)_6GG$) and TATA box which has vital element for the β-actin promoter effectiveness. The function of CArG sequent was as a responsive element to the serum (Liu *et al*., 1990), the position is between the CCAAT and TATA boxes. TATA boxes is an element that is common seen to the promoter sequent, as a place for polymerase RNA to stick when the transcription process of RNA is still going on (Glick and Pasternak, 2003). Cooperation between the three element will caused the active of promoter and controlled the transgene expression in the right time and place.



Picture 1. Electroforesis of PCR amplification result (1) and (M) marker measurement DNA fragment 2-log ladder (Biolabs, New England). Target DNA fragment was shown with bow sign.

Alignment analysis of common carp β-actin gene sequencing result with common carp β-actin data from gene bank (No. Gene Bank Accession: M24113) can be found of high identical that's 97,52% (Picture 2). From analysis result of sequence and alignment were balanced that isolation result sequent is common carp β-actin promoter. Alignment of common carp β-actin promoter sequencing result with grass carp (No. Gene Bank Accession: M2503), medaka fish (No. Gene Bank Accession: 574868) and megalobrama fish (No. Gene Bank Accession: AY170122) were showed in picture 3. Based on alignment result was known that sequent position of CCAAT, CArG motif and TATA box common carp β-actin promoter equal relative with grass carp, medaka fish and megalobrama fish have been reported before. So, conclusion of this result that cloning result is common carp β-actin promoter sequence.

Gene Bank Accession: AY170122). CCAAT sequent position, CArG motif and TATA box were showed above sequent. CCAAT sequent position, CArG motif and TATA box in sequent primer F for common carp identical with another fish were seen from S terminal end (were seen from on the left).



Picture 4. Electrophoresis result of cracking blue colony bacteria (K) and white (no. 1-6). No. 1-5: bacteria which carry pCCBA-EGFP gene construction, no. 6: bacteria which carry pEGFP-N1 plasmid. (–): DNA plasmid measurement to contain insertion DNA or pccBA-EGFP, (◄): DNA pEGFP-N1 plasmid, while (<): plasmid from blue colony bacteria.

To know β-actin promoter was isolated from common carp can active of not, so that's promoter was ligated with target gene EGFP. Checking of transformation bacteria cracking result was showed in picture 4. DNA measurement for bacteria of transformation result with pCCBA-EGFP plasmid most target than pEGFP-N1 plasmid (Picture 4, coulomb 6) and control plasmid that's blue bacteria (Picture 4, K). This case show that pCCBA-EGFP gene construction to succeed make and bacteria which carry plasmid succeed was identified (no. 1-6 in Picture 4).

Promoter activity assay was conducted to prove in vivo that the isolation of DNA sequences able to regulate the expression of foreign genes. In this study used EGFP marker gene, a gene encoding green fluorescent protein when given to ultraviolet light, to facilitate testing. As shown in Picture 5, the gene expression seen in the head, yolk and muscle tissue of common carp larvae.

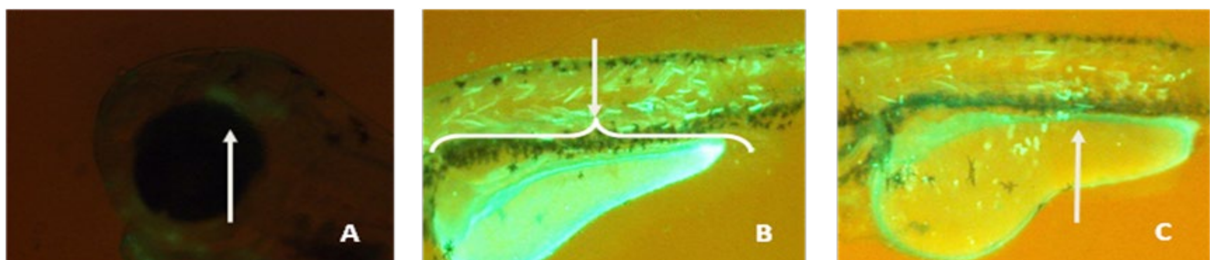


Figure 5. GFP gene expression using ccBA-GFP in carp embryos in the head (A), yolk (B), and muscle (C).

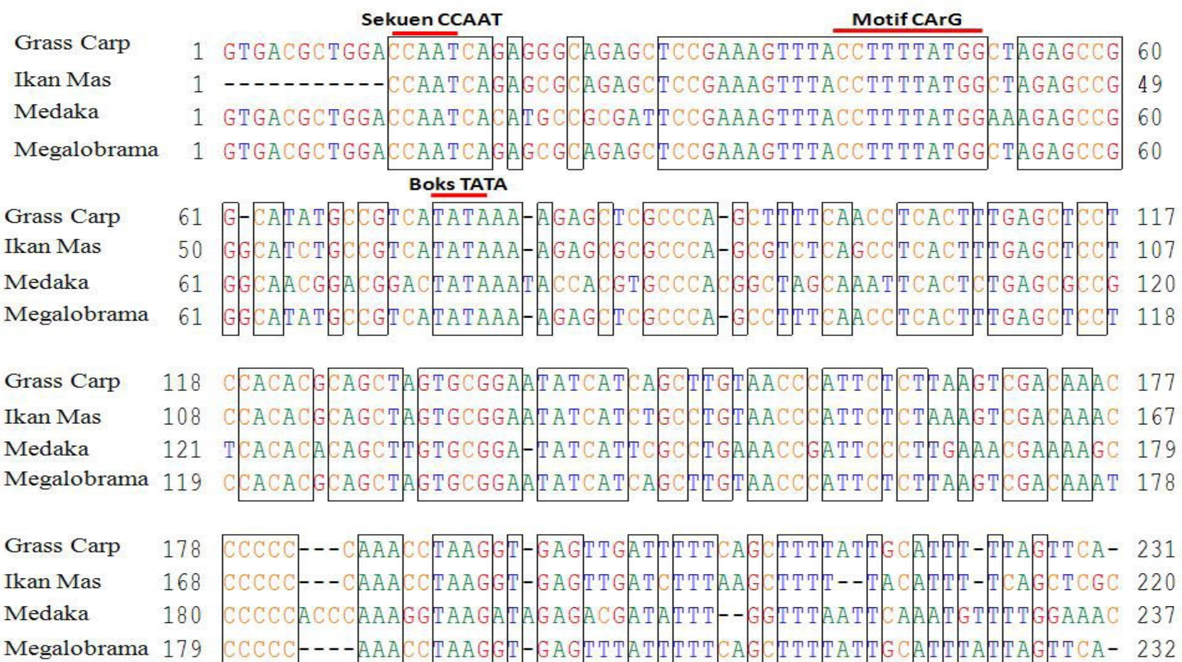
CCAAT

Motif CArG

Boks TATA

Primer Reverse 3'

Picture 2. Alignment of sequencing result common carp β-actin promoter with common carp β-actin gene (No. Gene Bank Accession: M24113). Important element there are CCAAT lying in nt. 12-16, CArG (CC(A/T)$_6$GG) lying in nt. 42-51, and TATA box in nt. 75-80.



Picture 3. Alignment of sequencing result common carp β-actin promoter, grass carp (No. Gene Bank Accession : M25013), medaka fish (No. Gene Bank Accession : S74868) and megalobrama fish (No.

GFP gene expressions seen in the carp are not specific to an organ. This is regarding the nature of the ubiquitous (found everywhere) of β-actin promoter, which means promoters can be active on all the muscle tissue. According to Iyengar et al., (1996), unequal distribution of transgene copies in poliploid tissues such as muscle cells or cells in the syncytial egg layer (YSL) may explain the high variability of transgene expression in tissues. Further explained that the difference in transgene expression may be related to the position where the transgene is integrated in the chromosome that activates the gene in muscle. GFP gene expression in larvae quite strong. This occurs presumably because of expression can appear and strengthened back to a time when growth is rapid, so many cells that divide and re-occurring increase in replication or it can also occur at the start of the formation of muscles due to β-actin promoter was isolated from muscle and can be active in all muscle tissue (Winkler et al., 1991). These events may occur given the β-actin promoter has a housekeeping nature (can be active at any time) and constitutive (active without triggering factors). However, these expressions seem no longer visible at any given time because it has been covered by skin color, flesh and so depend on the ability of the promoter in expressing the GFP gene.

Gene expression levels in each there is a strong promoter and weak there. According to Chou et al., (2001) if the DNA fragment consisting of a target gene or marker gene homologous / heterologous transferred it will be very common to find the incidence of mosaic (Mosaic), while expression (transient expresion), and the diverse expression of genes transferred in transgenic fish. This is due to unequal distribution of the transgene in embryos injected fish. Expression in each embryo partially or not spread where there are several networks that are expressed strongly while others do not exist. Most of the suspected expression levels are strongly associated with transgene copy number in each cell (Hwang et al., 2003). Alimuddin et al., (2007) explains that gene expression is weak or even nonexistent when integrated at the centromere or telomere DNA where transcription is not active experience and is positioned in heterochromatin. In addition, the possibility of gene integration occurs randomly in the chromosome, resulting in differences in gene expression levels in different tissues.

CONCLUSION

β -actin promoter has been isolated common carp with a length of 1.5 kbp and are used in the manufacture of construction is 1.3 kbp. In the isolated sequences contained a transcription factor that is common on β-actin promoter, the TATA box, CCAAT motif and CArG motif (CC (A / T) 6GG).

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