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ISOLATION OF B-ACTIN PROMOTER DERIVED FROM WALKING CATFISH (*Clarias batrachus*)

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ABSTRACT

Fish growth improvement as economic traits can be solved through fish transgenic production. Growth hormone gene is inserted into transgenic vector construction to over-express fish growth. The promoter as a part of the expression vector has an important role in its regulation. The use of promoter which is derived from mammalian or virus (such as CMV/ *cytomegalovirus*) in the expression vector, in specific goal as food material, has customer resistant rather than a promoter which is derived from in-sibling species. Beside of it, transgene expression level when using in-sibling promoter showed higher than using mammalian or viral promoter. The β-actin promoter is screened from walking catfish pituitary genome DNA using primers: pBA-cy-F (5'-GTGWGTGACGCYGGACCAAATC-3') as *forward* primer and pBA-cy-R (5'-CCATRTCRTCCCAGTTGGTSACAAT-3') as *reverse* primer, produced an amplicon of 1,7 kb in length. Sequence analysis using TF Bind™ indicated transcription factor elements: TATA box, CCAAT box, enhancer (CAAT), and CarGG (CAAATGG) motif. This result showed that promoter which is obtained from this research is useful in construction of all catfish growth hormone vector expression catfish transgenic production.

Keywords: β-actin promoter, growth hormone, expression vector, walking catfish (*Clarias batrachus*)

INTRODUCTION

Generally, the gene construct contains an promoter sequence, protein encoding gene and polyadenilation signal that is used for fish transgenic production. The expression regulator element which is inserted and can active in many types of fish tissues is β-actin promoter (Fletcher *et al*. 2011). The β-actin promoter is effective to promote fish transgenic expression . These promoter was also evidence to regulate the expression of the gene encoding 6-desaturase enzyme in zebrafish (Alimuddin *et al*. 2005) and the gene encoding growth hormone in tilapia (Kobayashi *et al*. 2007).

The use of promoters which are derived from mammalian or virus is known has lower expression level rather than promoter which is derived from in-sibling species (Alam *et al*., 1996). In zebrafish transgenic production, the viral promoter (*cytomegalovirus* / CMV) has lower expression level compared with β-actin promoter from zebrafish, because not all of *cis-acting* elements of viral promoter are recognized by *trans-acting* elements of zebrafish (Alimuddin 2003). The use of in-sibling species promoters in the construction of transgenic fish also sounds safely in customers perception rather than when using mammalian or viral promoters (Maclean & Laight 2000).

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β-actin promoter has known to be active within the same, in-sibling or different species of origin source of promoter, like within tilapia (Kobayashi *et al*. 2007), catfish (Ath-thar 2007), and zebrafish (Alimuddin *et al*. 2005). This promoter is an ubiquitous regulator in fish transgenic development (Higashijima *et al*. 1997). The β-actin is a highly conserved protein which is involved in cell motility, structure, and integrity. B-actin promoter sequence walking catfish (C. *batrachus*) were isolated using yellow catfish β-actin promoter primer (Ge *et al*. 2012), useful for the production of growth hormone expression vector "all catfish" of construction of transgenic fish.

MATERIALS AND METHODS

1. Isolation of Genomic DNA of Walking Catfish

The genomic DNA was isolated using Wizard genomic purification kit (Promega) from pituitary gland of C. *batrachus*. The gland was placed in a 1.5 mL microtube, grinded and added the chilled 300 µl nuclei lysis solution. The solution was then incubated on waterbath at 65 °C for 15 minutes, and then 1.5 µl RNase was added to the tube and be incubated on waterbath at 37 °C for 20 minutes.

As the next step, 100 µl of protein precipitation solution was added into the tube, and placed it on-ice for 5 minutes. The tube was then centrifuged at 12000 rpm for 4 minutes. The supernatant was transferred into a new sterile 1.5 mL microtube, which has contained 300 µl of isopropanol, and mixed it with gently inverting. The tube was then centrifuged at 12000 rpm for 1 minute. The supernatant removed and 300 µl of chilled etanol 70 % added into the tube and it be centrifuged 12000 rpm for 1 minute. The ethanol is removed and lets the tube dry. The DNA then be rehydrated by putting 50 µl of rehydration solution into the tube and incubate it on waterbath at 65 °C for 1 hour.

2. PCR Amplification

The β-actin promoter sequence was amplified by PCR method using primers: pBA-cy-F (5'-GTGWGTGACGCYGGACCAAATC-3') as *forward* primer and pBA-cy-R (5'-CCATRTCRTCCCAGTTGGTSACAAT-3') as *reverse* primer (Ge *et al*. 2012). The PCR cocktails contained: 12.5 µl of fast start pcr master mix (Roche); 1.5 µl of pBAYc-F (10 µM); 1.5 µl of pBAYc-R (10 µM); 2.5 µl of genomic DNA and 7 µl of nuclease free water.

The PCR program used was: 95 °C for 5 minutes (pre-denaturation), 94 °C for 1 minute (denaturation); 62 °C for 45 seconds (annealing); 72 °C for 2 minutes (extension) in 30 cycles and 72 °C for 10 minutes (final extension).

3. Sequence Analysis

Analysis of β-actin promoter sequence was performed using TF Bind™ to verify the presence of transcription factor elements which are contained within the sequence (Alimuddin *et al*. 2008; Kato *et al*. 2007). DNA sequencing was done at 1st Base (Singapore). Verification of sequence was conducted by aligning the sequence with Genbank databases of Indian catfish (*Heteropneustes fossilis*) and yellow catfish (*Pelteobagrus fulvidrac*o) using GENETYX version 7.

RESULTS AND DISCUSSION

1. Genomic DNA Isolation

The isolation was successfully to obtain the pituitary genomic DNA of C. *batrachus* on 1 % agarose gel electrophoresis (Figure 1). Based on the size of the marker fragments 1 kb DNA ladder, DNA containing exons and introns, so the size of the fragments is relatively long. Thus the DNA fragments of the local catfish detected by 1% agarose gel electrophoresis.

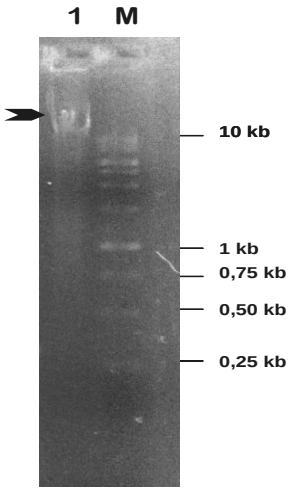

Figure 1. Electroferogram of pituitary genomic DNA of walking catfish, *Clarias batrachus* (▶); 1 = Pituitary genomic DNA of C. *batrachus*. M= 1 kb DNA Marker

2. PCR Amplification of β-actin Promoter

Amplification of β-actin promoter from pituitary genomic DNA of walking catfish showed an amplicon of 1,7 kb in length (Figure 2). Ge *et al*. (2012) also successfully obtained a 1,65 kb of β-actin promoter isolated from yellow catfish.

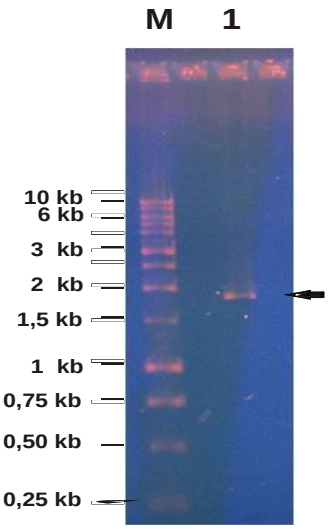

Figure 2. PCR amplification product of β-actin promoter from pituitary genomic DNA of walking catfish, C. *bratachus* ( ) = β-actin promoter (1,7 kb). M = 1 kb DNA Marker

3. Sequence Analysis

The sequencing result was showed in Figure 3. The Analysis of transcription factor elements of β-actin promoter was done using TF Bind™ (Hoffman *et al*., 1997; Alimuddin *et al*., 2008). The transcription factor elements of β-actin promoter are represented by TATA box, CAAT box, and CarG motif (Hew & Fletcher, 2001). TATA box has important role on transcription regulation in proximal region of promoter, CAAT box is an enhancer which is needed to increase transcription level, and CarG motif is involved in transcription regulation in basal region of promoter (Aranburu *et al*., 2006; Santoro & Walsh, 1991). These elements (TATA box, CAAT box, and CarG motif) are found within the sequence of C. *bratachus* β-actin promoter. The CAAT box was located in nucleotide (nt) of 22-29; TATA box in nucleotide of 49-54 and 296-302; and CarG motif in nucleotide of 615–623.

Other important elements are GC box (GGGCGGG) and TGACG motif, which are involved as intermediate factor of pituitary growth hormone (Argenton *et al*., 1996). The result of alignment of transcription factor elements of β-actin promoter of C. *bratachus* and *Indian catfish* (*H. fossilis*) using GENETYX version 7.0 was showed on Figure 3. TGACG motif of β-actin promoter of C. *bratachus* was found at 814 – 818 nt.

C. batrachus.txt 0:----------
---------- 0
H. fossilis.txt
1:CAAACATATCTTGCATTTCCAGACTGAAATCTTGAGAGACAGGTTTTAAGAGGAAAAGTA
60

C. batrachus.txt 0:----------
---------- 0
H. fossilis.txt
61:TTCACAGGGAGGAGCAGCCTGTGCTCTGCTGTGTTTGAATGATCAGATAAGCAGGGGG
AC 120

C. batrachus.txt 0:----------
---------- 0
H. fossilis.txt
121:TTGCTCGACAGCTTTATACAGGTTCATATTTTATATTTATTTATATTTGAACTTTGTAAA
180

C. batrachus.txt 0:----------
---------- 0
H. fossilis.txt
181:CCTCTATATGTATTAATATTTGTTCAAGAACATATATCTTAGATTAGGCCTCACATCAAT
240

C. batrachus.txt 0:----------
---------- 0
H. fossilis.txt
241:CCCTTTTCCCCTAGCCCGTTACTGTTATCGCCTCCCTCTTTCTACGCTACGCTCAGTGCA
300

C. batrachus.txt 1:----------TANGNNNNTCTTC
13

H. fossilis.txt
301:CCACAGCGTGCACCCGACGTGCCCCAGTGAGTGACGCTGGACCAATCACAGCCGCG
ATTC 360

C. batrachus.txt 14:CGAAAAGGGTCCATTT---TAGACGGCCATGT---GGAGCG--TATAAA-
ACCAGGCGCC 64
H. fossilis.txt
361:CGAAAGTTTACCTTTTATGGAAAGGGCCGGGCAACGGACGGACTATAAATACCACGC
CCA 420

C. batrachus.txt 65:CAGACTCCCACTTCACTT-
TGAGCTCCTCCACACGCAGCTAGTGCGGAATATCATCTGCC 123
H. fossilis.txt 421:CGGCTAGCAAATTCACTACTGAGCGCCGTCACAC-
CAGCTTGTGCGGA-TATCATTCGCC 478

C. batrachus.txt 124:C--
AACCAAATTTATTTTTTCTTAAGCCGACAACCCCCAAATCTTAAGGTAAGTTTTTTT 181
H. fossilis.txt 479:TGAAACCGATTCCCTTGAAACTCATGCTT----------
---------- 507

C. batrachus.txt
182:CCCCCTTTCCTCCTGGTATTGTTATTACTGTTAGCAATAGTAATTGCAGTAACAATAGTA
241
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
242:ACATTGCTATTTATGTATGCAAGGGTTTTAATTGTAAAAACTATATATATATTTTTATAA
301
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
302:ATTAATGAATGACTGCAAAAAGAACAATACGTTTTCCCTTTATCATGCAGCGATATTAAA
361
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
362:CTAGACAGGAATTATTTTTGTAAATTTTTACCTCAGGTTTTTTTTTTTTTTTCCCTGGGG
421
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
422:GCTAAAAGCCCCGTAAAACGGCGGGGAGGGGGGGGTTTTTTTTTATTTCTATAAATGAA
A 481
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
482:AAACCGTTTTTTGCCCTGATTATTAAAAAGCGGAGGAAGACCCGTATTTGTCGACGATAA
541
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
542:TCACAAAAAAAGCGG**GGGCGGGGG**GCCTCCCCCGTTTTTTCCACCTCCCCCCCGGAGA
GG 601
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
602:GTAGGATTTCAC**CCAAATTGG**GACAACGAGACCCCTGGGGTAATTCGCTGCCTACCCC
TG 661
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
662:GGCACTTGAAAAGGGGGGCCCAGTCGTGCTTAAGGCTGTCAAGCCAAGAAGGCTCC
CCCA 721
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
722:TTTTTTTCTCGCTAGGGGGGGGGGCCAGAAAAAATAATATATTTTTTTTATTTTATACCT
781
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
782:ACTTCCTCACAACTGTACAAAAAAAAGCCAC**TGACG**AGCTCTTGTTATTACAACACCCAG
841
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
842:AGATATTGTCCGTCTTATAATTTTTTTTTCTAAAGGCGCCACAAAGCGGACCACACACTT
901
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
902:AAATCATAGTTAAGTAAGGCCGCCTTGTTGGTGAGCAAGTATACAGGACCGGGAAGTA
GA 961
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt
962:GATAGAATGAGTTGATAAAATTAAAATATAAATTTGTTCAGNNTGAACGGAGGCCTGCC
C 1021
H. fossilis.txt 507:----------
---------- 507

C. batrachus.txt 1022:GGGCTCTGCAGAT 1034
H. fossilis.txt 507:---------- 507

Figure 3. Alignment result of of β-actin promoter of C. *bratachus* and *Indian catfish* (*H. fossilis*, genbank : AY 531754.1) (Meenakumari *et al*. 2004)

CAAT box = GGCCAATCT
CarG = CC(A/T)$_6$GG
TATA box = TATAAA
GC box = GGGCGGG

In the alignment result of β-actin promoter of C. *bratachus* and *yellow catfish* (*Pelteobagrus fulvidraco*) also indicate these transcription factor elements (TATA box, CAAT box, and CarG motif) (Figure 4).

C. batrachus.txt 0:----------
---------- 0
yellow catfish.txt
1:GTGAGTGCGCCGGAC**CAAT**CAGACGAAGCGATGCCGAAAGTTTACCTTATATGGAAGTT
G 60

C. batrachus.txt 0:----------
---------- 0
yellow catfish.txt
61:CCGGCCACAGTGCCGGG**TATAAA**TACAGCGTCGCCCCGGTTAAGCTGCCACTCTGAGT
TT 120

C. batrachus.txt 0:----------
---------- 0
yellow catfish.txt
121:GCCTGTGCACGAGTCTAGAAGGACATTAATTCCAGCTATATTCCTGTTGAACCACTGATT
180

C. batrachus.txt 0:----------
---------- 0
yellow catfish.txt
181:CTTTGGTAAGATCAGCTTTATCTTTGTCTACATACGATTGTGATTATAACTACATCCTCT
240

C. batrachus.txt 1:----------
TANGNNNNTCTTCCGAAAAGGGTCCATTTTAGAC**GGCCATGT** 42
yellow catfish.txt
241:TTCTACCTTAAATCTATTTTTATTTGTTTTATTAACGATGTACCTGATTTATATCGAGTT
300

C. batrachus.txt
43:GGAGCG**TATAAAA**CCAGGCGCCCAGACTCCCACTTCACTTTGAGCTCCTCCACACGCA
GC 102
yellow catfish.txt 301:GG--CGTTTATTTCAGTGCTTCC---
CTGTACATGAGCTCTGGGGTTATGA**TGACG**TTAT 355

C. batrachus.txt 103:TAGTGCGGAATATCATC-
TGCCCAACCAAATTTATTTTTTCTTAAGCCGACAACCCCCAA 161
yellow catfish.txt
356:TAATGTGTTCTATAATGATTTATAGAGAGATTCGTTACTCAGTGTAAGGAGAAACTG—A
413

C. batrachus.txt
162:ATCTTAAGGTAAGTTTTTTTCCCCCTTTCCTCCTGGTATTGTTATTACTGTTAGCAATAG
221
yellow catfish.txt
414:GACAATACAAAGATTTATTAATACAGTGAGTGTGTGTGTGTGTGTGTGTGTGTGTGTG
473

C. batrachus.txt
222:TAATTGCAGTAACAATAGTAACATTGCTATTTATGTATGCAAGGGTTTTAATTGTAAAAA
281
yellow catfish.txt 474:TGTGTGT-GTGAGAGATG---
CTGTGGTGTTTAAGTTAGATGTGGGTGTGGCTGGTGTTG 529

C. batrachus.txt
282:CTATATATATATTTT**TATAAA**TTAATGAATGACTGCAAAAAGAACAATACGTTTTCCCTT
341
yellow catfish.txt 530:ATGCAGCT-
TAGCTTCAGTTTGTCTTCAGAAACATTATAATGAAGTTTGCATGATTAATT 588

C. batrachus.txt
342:TATCATGCAGCGATATTAAACTAGACAGGAATTATTTTTGTAAATTTTTACCTCAGGTTT
401
yellow catfish.txt 589:TACC--TCAGTGAAATTAATTAATTTCTGGATTAAAAAAA-
AACCTGTTAAGTTATACAG 645

C. batrachus.txt
402:TTTTTTTTTTTTCCCTGGGGGCTAAAAGCCCCGTAAAACGGCGGGGAGG-----
GGGGGG 456
yellow catfish.txt 646:TAAAGCTACAGTTGCTGCAG—
TAAATTAAACATGATTTGGTGAGCAGGTTTTTGGAGAG 703

C. batrachus.txt
457:TTTTTTTTTATTTC**TATAAA**TGAAAAAACCGTTTTTTGCCCTGATTATTAAAAAGCGGAG
516
yellow catfish.txt 704:CAGGTTTTTTTGTGTACAA-TGTGGGACTCGATCATGGACCC---
TTCAGCAGGGCGCAT 759

C. batrachus.txt 517:GAAGACCC-GTATTTGTCGACGA-
TAATCACAAAAAAAGCGG**GGGCGGG**GGGCCTCCCCC 574
yellow catfish.txt
760:AATGAGGCTGTAAAAAAAGGGGAGTGGTCGCGTCTCACGAGCCGGTCGAACTTATTA
CCA 819

C. batrachus.txt
575:GTTTTTTCCACCTCCCCCCCGGAGAGGGTAGGATTTCAC**CCAAATTGGG**-
ACAACGAGAC 633
yellow catfish.txt
820:TATAAGGCAATAGCGAGTCTGTTGGCCACTTCCTTTGTCTCAAACCGCCCAGTTTTTGGG
879

C. batrachus.txt 634:CCCTGGGGTAATTCGCTGCCTACCCCTGGGC---------ACTTGAA--
AAGGGGG-GCC 681
yellow catfish.txt
880:CTTTGCGCATGTGCGCCACCTGCTGGTGATATATATATTTTTTTAAATAAAGGGTGTGTG
939

C. batrachus.txt 682:CAGTCGTGCTTAAG—
GCTGTCAAGCCAAGAAGGCTCCCCCATTTTTTTCTCGCTAGGGG 739
yellow catfish.txt
940:CATTCCTACAGCAGATGCTGAATTACCCCTTGTTCTAAATGACCCTGGTCACTTAACTCC
999

C. batrachus.txt
740:GGGGGGCCAGAAAAAATAATATATTTTTTTTATTTTATACCTACTTCCTCACAACTGTAC
799
yellow catfish.txt 1000:TTTGTGTTCTCCACAGCCATGGATGATGAA-
ATTGCCGCACTGGTTGTTGACAACGGATC 1058

C. batrachus.txt 800:AAAAAAAAGCCAC**TGACG**AG-
CTCTTGTTATTACAACACCCAGAGATATTGTCCGTCTTA 858
yellow catfish.txt 1059:CGGTATGTGCAAGGCTGGATTCGCTGGAGATGATG-
CTCCCCGTGCTGTCTTCC--CATC 1115

C. batrachus.txt
859:TAATTTTTTTTTCTAAAGGCGCCACAAAGCGGACCACACACTTAAATCATAGTTAAGTAA
918
yellow catfish.txt 1116:CATTGTTGGTCGCCCAAGACACCAGGTA-CGAACCACACAATC---
TGCCTCTTATGGAA 1171

C. batrachus.txt
919:GGCCGCCTTGTTGGTGAGCAAGTATACAGGACCGGGAAGTAGAGATAGAATGAGTT
GATA 978
yellow catfish.txt 1172:G-CTTAATTGTT--TTAACTATTTTAAATATTATTTAA—
AAACCAAGGGTGCTCAAACT 1226

C. batrachus.txt
979:AAATTAAAATATAAATTTGTTCAGNNTGAACGGAGGCCTGCCCGGGCTCTGCAGAT----
1034
yellow catfish.txt
1227:ATTTTTAAGGATAA**CCTTGTTAGG**TTTGAACAATGGCACAACCTTCATCATAGGAGCTTG
1286

Figure 4. Alignment result of of β-actin promoter of C. *bratachus* and yellow catfish (*Pelteobagrus fulvidraco*, genbank : EU 161065.2) (Song *et al*., 2012)

CAAT box = GGCCAATCT
CarG = CC(A/T)$_6$GG
TATA box = TATAAA
GC box = GGGCGGG

In this alignment, CAAT boxes are found at nucleotide 35-45 (C.*batrachus*) and at nucleotide 16-19 (*P. fulvidraco*); CarG motifs are found at nucleotide 615-623 (C.*batrachus*) and at nucleotide 1232-1241 (*P. fulvidraco*); and then, TATA boxes are found at nucleotide 49-54; 296-302; 472-477 (C.*batrachus*) and at nucleotide 78-83 (*P. fulvidraco*). As additional, TGACG motifs are found at nucleotide 814-818 (C.*batrachus*) and at nucleotide 348-352 (*P. fulvidraco*). While, GC box is found only in C.*batrachus* at nucleotide 558-564. The presence of these elements (TATA box, CAAT box, and CarG motif) suggested that most likely the isolated sequence is C.*batrachus* β-actin promoter. Further more β-actin promoter of

walking catfish can be used as a regulator of the expression of protein encoding genes in the production of transgenic fish (Fletcher & Hew, 2011).

CONCLUSION

Based on the results of the research, the conclusions are: The β-actin promoter of C.*batrachus* is successfully obtained from pituitary genomic DNA by in-vitro amplification (PCR) using pBAYc-F dan pBAYc-R primers and the transcription factor elements of β-actin promoter of C.*batrachus* are represented by the presence of TATA box, CAAT box, and CarG, and also TGACG and GC box.

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