Red Fluorescent Protein Expression in Transgenic Founder of Angelfish (*Pterophyllum* sp) Driven by Zebrafish Myosin Light Chain 2 Promoter

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Abstract

Angelfish (*Pterophyllum* sp.) are attractive fish popular with aquarists. The introduction of fluorescent protein genes into angelfish has been reported, but specific techniques have not been revealed. This study aimed to develop a strategy to produce red fluorescent protein (RFP) transgenic angelfish driven by the myosin light chain 2 (mylz2) promoter from zebrafish. A 1999 bp Mylz2 promoter fragment was isolated from zebrafish muscle genomic DNA. This promoter fragment was then cloned into the plasmid pDsred2-1 open-loop at restriction enzyme *SacI* and *AgeI* sites to create the final transgene construct pMylz2-RFP. Angelfish embryos at one cell stage were microinjected with approximately 100 pg of the plasmid pMylz2-RFP. From 524 microinjected embryos, 16 successfully hatched, while 12 showed red fluorescence signals. Two larvae survived to 2 months of age. They showed significant red fluorescence expression in the muscles, suggesting that the angelfish could be used as potential transgenic founders to evaluate the next generation of stable red fluorescence expression transgenic fish.

Introduction

Zhu et al. developed the first transgenic fish in the early 1980s [1]. Since then, transgenic fish have continued to be studied in many basic studies and applications for various purposes, such as creating fish with fast growth, high feed efficiency, resistance to disease and changes in environmental conditions, and gene transfer as a model for human disease research [2–4]. Zhang and colleagues used salmon growth hormone genes to create a transgenerational carp that was 22% larger than the control [5]. Zebrafish embryos were transfected with the GFP gene under the hfe2 promoter in 2011, and researchers reported that GFP was exhibited in developing vertebrae, skeletal muscles, and liver cells [6]. Thuy et al., 2021 employed an alpha-actin promoter to control GFP gene expression in medaka fish [7]. Despite the success of some transgenic fish, these technologies are confronting significant food safety challenges and the influence of transgenic fish on ecosystems once they escape into the external environment [8].

A fundamental purpose of generating fluorescent transgenic fish is for recreational usage. Because transgenic aquarium fish are kept in aquariums, they will not pose health dangers to people or general concerns about biodiversity or hybrid species [8, 9]. Glofish is a fluorescent transgenic fish that has been available since 2004 [10]. Studies that use transgenic fish as aquarium fish to suit recreational requirements have since been conducted and have reached specific outcomes. Specifically, fluorescent protein genes transfer in zebrafish [11, 12], medaka fish [7, 13, 14], and white skirt tetra [15] has been successful. As reported in 2003, Gong and colleagues employed fluorescent protein genes of different hues (GFP, CFP, and RFP) and the promoter Mylz2 to make multicolored zebrafish visible [11]. Pan et al. (2008) generated a red fluorescent white skirt tetra transgenic fish driven by the zebrafish Mylz2 promoter. In 2014, Vu and colleagues discovered a red fluorescent protein expression in transgenic mekada (*Oryzias dancena*) regulated by its promoter, myosin light chain 2 [14]. A crucial aspect of fluorescent transgenic fish study methods is choosing a suitable promoter. Five types of promoters have been studied to these points, including alpha-actin [7, 13, 16, 17], beta-actin [18–20], myosin light chain 2 [12, 14, 15, 21, 22], myosin light chain 3 [23], and muscle-type creatine kinase b (CKMb) [24]. Angelfish

genetic information has yet to be examined, so using its promoters to conduct transgenic research will entail various complications. However, zebrafish myosin light chain 2 (mylz2) promoters have ruled other species as well as themselves [11, 12].

Angelfish is currently widely popular in aquarium hobbies around the world. The fish is native to the Amazon, Guyana, and Orinoco rivers. In natural environments, angelfish reach a maximum length of 15 cm, a maximum height of 25 cm, and lays up to 1000 eggs [25]. Although the transfer of a fluorescent red fluorescent protein gene precisely regulating CKMb muscle expression has been reported in angelfish [24], it remains unknown how gene transfer occurs in these fish. This study presents a technique for generating a transgenic angelfish displayed a red fluorescent protein expression under the control of the zebrafish mylz2 promoter. The transgenic construct pMylz2-RFP was microinjected into the one-cell stage of angelfish eggs to preferentially express red fluorescent protein in the muscular section of the embryo or larva. RFP expression was clearest when this founder's angelfish was at an adult stage.

Materials And Methods

Angelfish maintenance and egg collection

Angelfish were raised in the laboratory of the Aquaculture Biotechnology Division, Biotechnology Center of Ho Chi Minh City. The larval angelfish stage was fed brine shrimp Nauplius and NRD2/3 (INVE, Thailand), whereas the adult stage was fed beef heart flesh and worms. The fish were fed twice a day, between 8 a.m. and 4 p.m.

Before spawning, the male fish spread mucus on the substrate, and the female fish lay eggs on top of this mucus. A long plastic straw (3 mL volume) was used to aspirate the eggs on the following substrate and place them in a Petri dish with 50 mL water to collect eggs for transgenic micromanipulation.

Isolation of the mylz2 promoter

Phenol–chloroform-isoamyl alcohol techniques were used to isolate genomic DNA from zebrafish. The following procedure was used: Fixing fish muscle samples in 750 µL TNES-Urea buffer [14]. After incubating the sample for 4 hours at 55°C, 750 µL of phenol–chloroform was added and gently shaken at room temperature for 15 minutes. Next, the sample was centrifuged for 5 minutes at 13000 rpm, the supernatant was transferred to new 1.5 mL Eppendorf tubes, 700 µL of chloroform-isoamyl alcohol was added, and the mixture was gently mixed for 15 minutes at room temperature. Next, the sample was centrifuged for 5 minutes at 13000 rpm to extract 650 µL of supernatant and transferred to new 1.5 mL Eppendorf tubes. Then, 650 µL of isopropanol was added, and the sample was inverted for 5 minutes until a precipitate developed. After centrifuging the sample for 2 minutes at 13000 rpm and collecting the pellet, 500 µL of 70% ethanol was added. Centrifuge the sample for 2 minutes at 13000 rpm, then remove the supernatant and allow the pellet to dry naturally before adding 20 µL of 1X TE buffer. To obtain the solute DNA, quickly centrifuge for 5 seconds and incubate for 40 minutes at 37°C. Using 1% agarose gel

electrophoresis and OD 260/280 spectrophotometers, the quality of the genomic DNA was confirmed. Keep the samples at -20°C.

Based on sequencing data of the Mylz2 promoter from Ju et al. 2003 and the NCBI Gene Bank (with access code NC007114.6), the primers Mylz2-*SacI*-Fv and Mylz2-*AgeI*-Rv (Table 1) were designed and used for PCR to amplify the Mylz2 promoter fragment. In addition, two restriction enzymes, *SacI* and *AgeI*, were introduced (Table 1, underline). The PCR procedures were described in Thuy et al. 2021.

Table 1
Primers used for this study

Primer name	Sequencing	Purpose
Mylz2- <i>SacI</i> -Fv	5'-AGGGAGCTCAATTCGCCACAGAGGAATGAGC-3'	Isolation of Mylz2 promoter fragment
Mylz2- <i>AgeI</i> -Rv	5'-AGGACCGGTTGTCGAGACGGTATGTGTGAAGTCT-3'	
Mylz2-Fv2	5'-GCTATTTTGGTCACCACAGCTGTTC-3'	Check ligation plasmid pMylz2-RFP
DsRed-Rv	5'-TGGAAGTGGGGGGACAGG-3'	
pJET1.2-Fv	5'-CGACTCACTATAGGGAGAGCGGC-3'	Check ligation plasmid pJET1.2-Mylz2
pJET1.2-Rv	5'-AAGAACATCGATTTTCCATGGCAG-3'	

Generation of cloning vector pJET1.2-Mylz2

After being amplified by PCR, the Mylz2 promoter fragment was introduced into the cloning vector pJET1.2/blunt (Thermo Fisher Scientific, US) to generate the plasmid pJET1.2-Mylz2. The ligation reaction of the Mylz2 fragment to the pJET1.2/blunt plasmid was performed in a total reaction volume of 20 µL, containing 10 µL of T4 ligase buffer, 1 µL of pJET1.2/blunt cloning vector (50 ng/mL), 1 µL of Mylz2 fragment, 1 µL of T4 DNA ligase, and 7 µL of water. The ligation procedure was conducted at 22°C for 5 minutes.

The ligation products were transformed into *E. coli* DH5α competent cells. Colonies were screened on LB plate media with ampicillin (HiMedia, India; 50 µg/mL). The pJET1.2-Fv and pJET1.2-Rv primers (Table 1) were used for PCR of colonies to test the success of ligation of pJET1.2-Mylz2. The bacterial cells successfully transformed with the plasmid pJET1.2-Mylz2 were maintained at -80°C in LB medium with 50% glycerol (Bio Basic, Canada). An ISOLATE II Plasmid Mini Kit (Bioline, England) was used to isolate the plasmid pJET1.2-Mylz2. After isolation, the plasmid quality was validated by employing electrophoresis on a 1% agarose gel and OD 260/280 ratio.

Generating the pMylz2-RFP expression vector

The plasmids pJET1.2-Mylz2 and pDsRed-1 (Clontech Laboratories, US) were digested with 2 restriction enzymes, *SacI*-HF (NEB, US) and *AgeI*-HF (NEB, US), to acquire the Mylz2 gene fragment and open-loop the plasmid pDsRed-1. The digestion process contained 10 µL of 10X CutSmart Buffer, 1 µg vector, 10-unit restriction enzymes, and water in a total reaction volume of 50 µL. The digestive response lasted 1 hour at 37°C. The digestion products were purified using the MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON, Korea). Finally, the Mylz2 fragment was introduced into pDsred2-1 to create the expression vector plasmid pMylz2-RFP.

The plasmid pMylz2-RFP was then transformed into *E. coli* DH5 competent cells. Colonies were screened on LB plate media with 50 g/mL kanamycin (HiMedia, India). PCR was performed on plasmid-transformed pMylz2-RFP colonies using the primers Mylz2-Fv2 and DsRed-Rv (Table 1). Purification of the plasmid pMylz2-RFP was performed in the same manner as described in the creation of pJET1.2-Mylz2.

Microinjection and expression analysis of founder transgenic embryos

The plasmid pMylz2-RFP was diluted with 1X TE buffer to produce a final 100 µg/mL concentration for injection into angelfish embryos. The plasmid was kept at -20°C until microinjection.

The procedure of microinjection into one-cell stage fish embryos was mentioned previously by Thuy et al. 2021. In brief, approximately 100 pg of the pMylz2-RFP plasmid was directly injected into angelfish embryos at the one-cell stage using a Carl Zeiss microinjection micromanipulator microinjection system (Gottinge, Germany). The eggs were placed in a separate Petri dish containing 50 mL of system water after microinjection. The eggs will be incubated at 28°C after being microinjected with the pMylz2-RFP plasmid.

To confirm the effective delivery of plasmid pMylz2-RFP into embryos, embryos at 1 day postfertilization (dpf) will be evaluated for red fluorescence protein expression using a Nikon ECLIPSE TS100 fluorescence microscope (Nikon, Japan) with a G-2A filter (EX 510–560 nm, DM 575 nm, BA 590 nm).

Result

Mylz2 promoter isolation and generation of the pJET1.2-Mylz2 cloning vector

To isolate the Mylz2 promoter from zebrafish, we first extracted genomic DNA from the muscular tissue of mature zebrafish. The overall optical density (OD_{260/280} ratio) of all 4 samples was between 1.7 and 1.8 (Fig. 1A), and the DNA concentration was more prominent than 150 µg/mL after extraction. Therefore, sample 3 delivered a clean result, and the DNA quality was better than that of the other samples (Fig. 1A, 1B). For this reason, we selected sample 3 for PCR amplification of the 1999 bp Mylz2 promoter fragment with primer pairs Mylz2-*SacI*-Fv and Mylz2-*AgeI*-Rv (Table 1).

These fusion products were transformed into DH5a-competence cells and disseminated onto LB agar plates to generate colonies containing the plasmid pJET1.2-Mylz2 (Fig. 2A, 2B). No colonies grew on the negative control plate (Fig. 2A). Fig. 2B shows multiple single colonies forming on a plate with the selective antibiotic ampicillin.

To identify colonies containing the plasmid pJET1.2-Mylz2, we performed PCR on 10 colonies (Fig. 2B) using the primer pairs pJET1.2-Fv and pJET1.2-Rv (Table 1). The electrophoresis results (Fig. 2C) show that a 2 kb in the band of well number 8 corresponds to the expected size of the Mylz2 promoter fragment. This result suggested that Mylz2 promoter fragment success inserted into the pJET1.2/blund vector.

To confirm the successful isolation of the Mylz2 promoter, the plasmid pJET1.2-Mylz2 was sequenced with primer pairs pJET1.2-Fv and pJET1.2-Rv (Table 1). According to the sequencing results, the Mylz2 promoter from the isolated zebrafish was 1999 bp in size (Fig. 2D, 2E). Furthermore, the TATA box, E-box, and MEF2 box sequence regions were identified (Fig. 2D). To summarize, we successfully created the plasmid pJET1.2-Mylz2, which contains the Mylz2 promoter segment from zebrafish and can be used as a template to clone the following transgenic structure.

Generating the expression plasmid pMylz2-RFP

The Mylz2 promoter fragment from plasmid pJET1.2-Mylz2 was inserted into the open-loop pDsRed2-1 containing the RFP red fluorescent gene to create the pMylz2-RFP transgene. Both plasmids were digested simultaneously with 2 restriction enzymes, *SacI* and *AgeI*. These digestion products were tested on a 1% agarose gel. Two restriction enzymes, *SacI* and *AgeI*, cleaved 4974 bp of plasmid pJET1.2-Mylz2 at the first fragment was a 2988 bp pJET1.2/blunt vector band, and the second was a 1986 bp Mylz2 promoter fragment band (Fig. 4A). Because the product is completely separated, it is ideal for gel purification with the Mylz2 gene fragment. Similarly, in well 4 (Fig. 3A), a pDsRed2-1 plasmid with a size of 4107 bp was also successfully digested with 2 restriction enzymes, *SacI* and *AgeI*, at the precise cutting point on the plasmid to create a small fragment with a size of 196 bp and a significant fragment with a size of 3911 bp (Fig. 3A). Wells 1 and 3 (Fig. 3A) are 2 control wells without restriction enzyme digestion of 2 plasmids, pJET1.2-Mylz2 and pDsRed2-1, respectively. The bands in well 1 (Fig. 3B, with band 1986 bp) and well 2 (Fig. 3B, with band 3911 bp) are the electrophoresis results of the Mylz2 promoter fragment and the pDsRed2-1 open-loop plasmid, respectively, post purification (Fig. 3B). The ligation pMylz2-RFP plasmid products were transformed into competent *E. coli* DH5a cells and disseminated for culture on LB agar treated with the antibiotic kanamycin (50 µg/ml) (Fig. 3C; D).

Colonies (Fig. 3D) will be confirmed by PCR using primer pairs Mylz2-Fv2 and DsRed-Rv (Table 1) to assess whether the Mylz2 promoter segment is efficiently concatenated into the pDsRed2-1 plasmid. The results of PCR product electrophoresis showed that colonies from 11 to 24 (excluding colony number 18) had the correct bands with the predicted size of 1200 bp (Fig. 3E). Colonies of the band 20 were then randomly selected and isolated. This refined plasmid product was subsequently cleaved by 2 restriction enzymes, *SacI* and *AgeI*. The electrophoresis results in Fig. 4F (well 1) reveal 2 bands with a size of 1999

bp: the promoter Mylz2 and a band of 3911 bp, plasmid pDsRed2-1 open-loop. These results revealed that the Mylz2 promoter fragment was effectively integrated into the pDsRed2-1 plasmid and formed the pMylz2-RFP transgene (Fig. 3G).

Characterization of Mylz2-RFP transgenic founder angelfish

Of 524 single-cell embryos microinjected with the pMylz2-RFP plasmid, 16 developed normally (Table 2). Of those 16 developing embryos, 12 showed red fluorescence (Fig. 4). Compared to eggs that were not microinjected (56 percent), fish following microinjection had a poor hatch rate (an average of 2.3 percent). After 2 dpf, embryos microinjected with the pMylz2-RFP plasmid exhibited red fluorescence in the trunk muscle region with variable phenotypes and RFP expression levels (Fig. 4A1; B1; C1; and D1). After 5 dpf, angelfish egg embryos emerged into larvae and showed apparent red fluorescence in the trunk muscle region (Fig. 5A1; B1; C1) under fluorescence. Intriguingly, at 1 month postfertilization, distinct expression of RFP was discernible in the fish body muscle region to the naked eye. The expression features will be changed in diverse individuals (Fig. 6A, B, and C). By the time the fish reached the age of 2 months, the expression of RFP was widespread throughout the body and was apparent under blue LED light (Fig. 6D). The results demonstrated that the transgenic pMylz2-RFP construct was effectively transplanted into angelfish eggs and strongly expressed red fluorescent protein in the fish muscle up to 2 months of age. These founder strains could have the ability to establish stable RFP-expressing transgenic fish lines in succeeding generations.

Table 2
The frequency of microinjection of pMylz2-RFP into founder angelfish eggs.

Microinjection assays	No. of microinjected eggs	The hatching rate of eggs	Profiles of RFP expression in muscle embryos and larvae
1	167	3.04% (5/164)	1.82% (3/164)
2	185	3.78% (7/185)	2.7% (5/185)
3	172	2.33% (4/172)	2.33% (4/172)
Control	200	56% (112/200)	0% (0/200)

Discussion

This paper describes the effective transfer of the pMylz2-RFP plasmid into angelfish embryos. The low survival rate of angelfish eggs following micromanipulation is a concern that needs to be addressed. After microinjection, the survival rate of angelfish eggs was only 2.3 percent, which is 60-80 percent lower than that of zebrafish eggs [26]. Angelfish are species that lay eggs on substrates and are particularly sensitive to changes in environmental circumstances [25, 27]. As a result, embryo survival is poor because of egg harvest and the influence of the micromanipulator on embryos during gene transfer.

Usually, microinjections of genes are performed at the one-cell stage of embryos to achieve the most significant effect of gene transfer. In addition, since the micromanipulation process requires fast, precise manipulation techniques, the transgenic plasmid must be microinjected into the embryos at the correct stage of one cell. Angelfish embryos develop at a single-cell stage for approximately 20 minutes [25], which is enough time to transfer a large amount of plasmid to be made at one time into multiple egg embryos. In light of this, even though embryo survival after microinjection was poor in this study, the percentage of embryos expressing red fluorescence protein expression was high (Table 2).

The characteristics of red fluorescence protein expression patterns in angelfish muscle under the control of the Mylz2 promoter in this report (Fig. 5-7) were similar to previous studies similar to zebrafish (Gong et al. 2003), white skirt tetra (Pan et al. 2008), and medaka (Vu et al. 2014; Thuy et al. 2021). This finding suggests that the Mylz2 promoter produced in this study is effective for transgenic red fluorescence-expressing angelfish.

The GFP gene is the most investigated transgenic gene in plants and animals, followed by the RFP gene (Stewart, 2006). The DsRed gene, originating from the sponge species *Discosoma sp.*, is widely employed in animal, plant, and bacterial genetic engineering as a high potential marker gene [28]. Furthermore, when the DsRed gene is overexpressed, plant or animal tissues seem pinkish-red in natural light and are clearly recognizable by the naked eye (Fig. 7).

Microinjection remains the most successful and extensively used approach for transferring DNA into fish embryos [7, 11, 14, 17, 29]. Because DNA microinjection is transferred directly into the genome of an animal cell at an early embryonic stage, it is easier to integrate into the cell's genome and pass it on to the next generation, which can also manage the amount of microinjected DNA, resulting in a higher success rate than other methods [30, 31]. A previous gene transfer study in angelfish (Chen et al. 2015), however, used the electroporation technique and gave similar results to our work. The efficacy of gene transfers and the survival rate of angelfish embryos following gene transfer remained low. This study reveals that angelfish is a demanding topic for transgenic efficiency when compared to previously examined transgenic fish.

Conclusion

Transgenic angelfish muscle tissues expressed red fluorescent protein under the control of the zebrafish myosin light chain 2 (Mylz2) promoter at embryonic and larval stages. The founder generation of red fluorescent angelfish at 2 months of age is being developed and tested for fluorescence transmission to succeeding generations.

Declarations

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Authors' contributions

V.T.N. and B.H.L. carried out the studies, while V.T.N. and V.N.H.T. created the transgenic constructs for microinjection. D.T.T. and V.N.H.T. did data analysis, and V.T.N. conceived of the study. The initial draught of the work was written by V.T.N. and D.T.T. All writers read and approved the manuscript.

Compliance with Ethical Standards:

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All of the fish experiments were done in accordance with the "Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms" and the "Cartagena Protocol on Biosafety." All experiments were also conducted in accordance with the "Guide for the Care and Use of Laboratory Animals at the Biotechnology Center of Ho Chi Minh city."

Informed consent

The manuscript was published with the consent of all authors.

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Figures

Figure 1

Electrophoresis results of the isolation of the myosin light chain 2 (Mylz2) promoter from zebrafish. A: The optical density (OD 260/280 ratio) values and DNA concentration of genomic DNA. B: The genomic DNA qualities were checked under 1% agarose. The bands from wells 1, 2, 3, and 4 contained DNA sample numbers 1, 2, 3, and 4, respectively. C: An approximately 2 kb PCR product of the Mylz2 promoter fragment was amplified from sample No. 3. M: GeneRuler standard scale 1 kb DNA ladder.

Figure 2

The generation of the pJET1.2-Mylz2 cloning vector. **A** and **B**: Transformation ligation result from the PCR product of the Mylz2 promoter fragment with pJET1.2/blunt. **C**: Verification of the successful generation of the pJET1.2-Mylz2 cloning vector by colony PCR conducted using primers pJET1.2-Fv and pJET1.2-Rv (Table 1). (-): negative control; 1-11: single colony PCR from Fig. 2B colonies. **D**: The pJET1.2-Mylz2 plasmid representation map was created using Snapgene 5.3.2 software. **E**: Sequence analysis of the

1999 bp myosin light chain 2 promoter fragment. The TATA box, E-box (CATTG sequence), and MEF2 (binding site box with consensus $[(C/T)TA(T/T)_4TA(A/G)]$ are highlighted in green, gray, and red, respectively. The forward and reverse primer pair sequences are indicated in yellow. Underlined nucleotides reflect the sequences of the restriction enzymes *SacI* and *AgeI*. M: GeneRuler standard scale 1 kb DNA ladder.

Figure 3

Generation of pMylz2-RFP transgene structure. **A** and **B**: Digest products of pDsRed2-1 and pJET1.2-Mylz2 with restriction enzymes *SacI* and *AgeI*. **A**: The bands in wells 1 and 2: uncut and cut plasmid pJET1.2-Mylz2 products with the restriction enzymes *SacI* and *AgeI*. The bands in wells 3 and 4 are cut and uncut plasmid pDsRed2-1 products with the restriction enzymes *SacI* and *AgeI*. **B**: The gel-purified Mylz2 promoter fragment and plasmid pDsRed2-1 open-loop. The band in well 1 is the Mylz2 gene fragment, while that in well 2 is the open-loop pDsRed2-1 plasmid. **C** and **D** represent recombinant pMylz2-RFP plasmid transformations into competent *E. coli* DH5 cells on LB agar medium with kanamycin antibiotic (50 g.mL^{-1}). **C**: Nontransformed pMylz2-RFP negative control plate. **D**: Transform the ligation product containing the plasmid pMylz2-RFP. **E**: PCR products from Fig. 4D colonies. The band from wells 1 to 24 is the single colony PCR result from Fig. 3D. **F**: Verification of successful ligation of the Mylz2 promoter with the open-loop pDsRed2-1 plasmid to form the transgenic plasmid pMylz2-RFP by digestion of the presumptively purified plasmid pMylz2-RFP with restriction enzymes *SacI* and *AgeI*. The bands in wells 1 and 2 from the undigested and digested plasmid pMylz2-RFP with restriction enzymes *SacI* and *AgeI*. Snapgene software (version 5.3.2) was used to generate the pMylz2-RFP plasmid representation map. M: GeneRuler™ standard scale 1 kb DNA ladder.

Figure 4

Red fluorescent protein expression in angelfish embryos 4 days after fertilization. **A**, **B**, **C**, and **D**: Fluorescence microscopy images of fish embryos that were not filtered using an RFP filter. **A1**, **B1**, **C1**, and **D1**: Fluorescence microscopy images of fish embryos with an RFP filter (G-2A with EX parameters: 510–560 nm; DM: 575 nm; BA: 590 nm).

Figure 5

Red fluorescent protein expression in angelfish larvae 5–6 days postfertilization. A, B, and C: The larvae were examined using fluorescence microscopy without an RFP filter. A1, B1, and C1: Larvae were observed by fluorescence microscopy using an RFP filter (G-2A with EX parameters: 510–560 nm; DM: 575 nm; BA: 590 nm).

Figure 6

Ornamental expression of red fluorescent protein at 1 and 2 months after fertilization of founder transgenic angelfish. A, B, and C: RFP expression of 1-month postfertilization founder transgenic angelfish under normal lighting. D: RFP expression at 2 months postfertilization of founder transgene angelfish under LED blue light (wavelength 400-500 nm).

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Figure 7

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