Indonesia's Stage on Gene Editing Research (Livestock and Fish)

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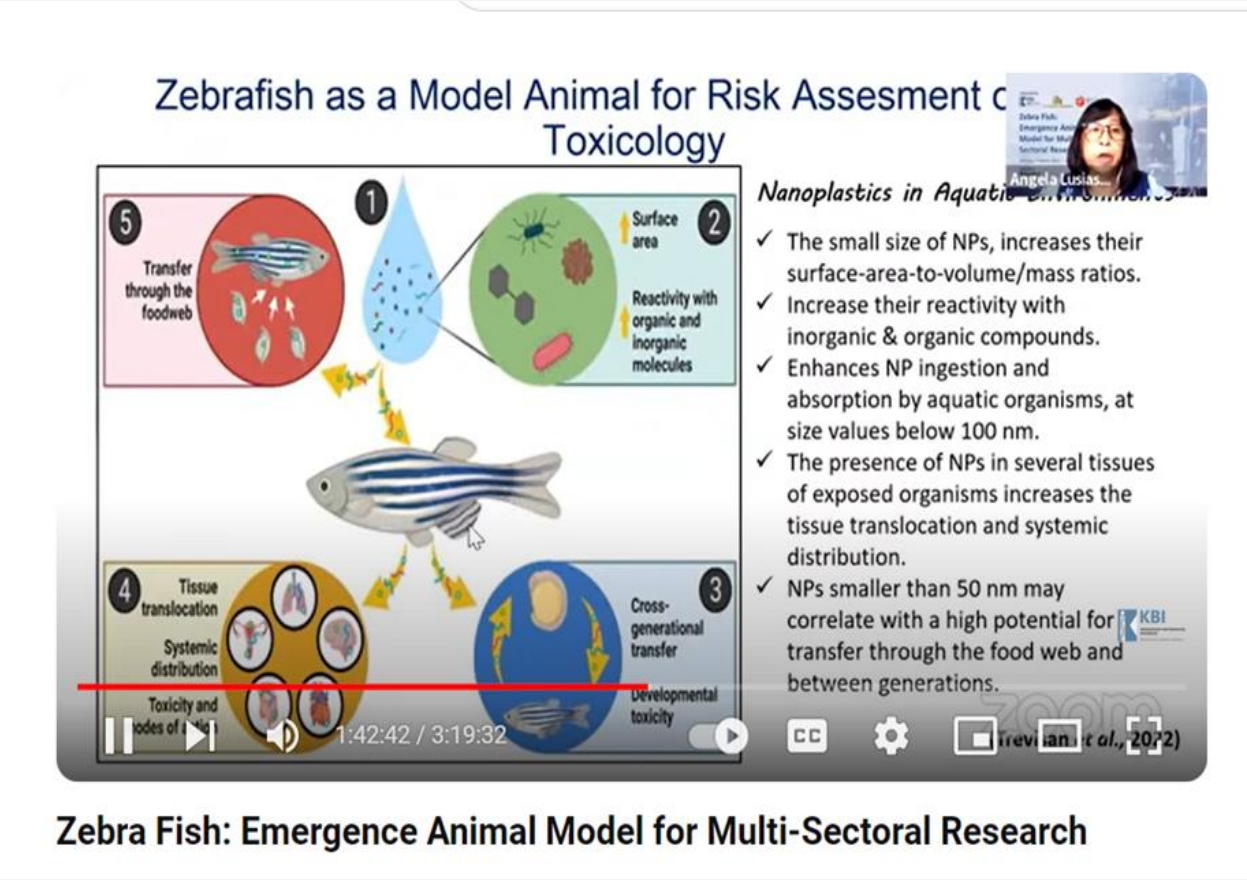
1. GMO in Animal Research

1. Research on zebra fish



https://www.youtube.com/live/vQnr5Rs34Rs?feature=shared

2. GH Gene insertion in catfish

KONSTRUKSI VEKTOR EKSPRESI GEN HORMON PERTUMBUHAN LELE DUMBO (*Clarias* sp.) UNTUK PRODUKSI IKAN LELE LOKAL (*Clarias batrachus*) TRANSGENIK

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ABSTRAK

Penelitian ini bertujuan untuk memperoleh konstruksi vektor ekspresi (pTarget) rekombinan yang mengandung sisipan gen hormon pertumbuhan ikan lele dumbo (*Clarias* sp.) dan promoter β-aktin ikan lele lokal (*C. batrachus*) dalam upaya pembuatan ikan lele lokal transgenik. Promoter β-aktin lele lokal (p*Cb*BA) telah berhasil diisolasi dari hipofisa ikan tersebut dengan ukuran sekitar 1,7 kbp; dan memiliki elemen transkripsi *CAAT box*, *TATA box*, *GC box*, motif CArG, dan TGACG berdasar analisis program *TF Bind*. Penggantian promoter CMV (*cytomegalovirus*) yang terkandung dalam vektor ekspresi pTarget menggunakan dua enzim restriksi *Sgf*-I dan *I-Ppo* I, menghasilkan fragmen DNA berukuran 6.083 bp (pTarget-GH lele dumbo) sebagai produk *digesti*. Fragmen pTarget-GH lele dumbo yang diligasi dengan promoter β-aktin lele lokal membentuk konstruksi pTarget-p*Cb*BA-GH lele dumbo (7.783 bp) sebagai vektor ekspresi ikan lele lokal transgenik.

KATA KUNCI: **vektor ekspresi, hormon pertumbuhan, lele dumbo, lele lokal transgenik**

ABSTRACT: ***Construct of growth hormone gene expression vector of African catfish** (Clarias **sp.) to produce transgenic walking catfish** (Clarias batrachus). **By: Ibnu Dwi Buwono, Nono Carsono, Yuniar Mulyani, and M. Untung Kurnia***

*This study aims to obtain an expression vector construct (pTarget) containing recombinant growth hormone gene insertion of African catfish (**Clarias** sp.) and β-actin promoter derived from walking catfish (**Clarias batrachus**) in order to produce transgenic local catfish. β-actin promoter of walking catfish (pCbBA) have been isolated from the pituitary of the fish with a size of about 1.7 kbp, and has a transcription element: CAAT box, TATA box, GC box, CArG, and TGACG motif based on analysis result of TF Bind program. Replacement of CMV (cytomegalovirus) promoter contained in the expression vector pTarget using restriction enzymes Sgf-I and I-Ppo I, obtained a product of digestion with the fragment size of 6,083 bp (pTarget-GH African catfish). pTarget-GH fragments were ligated with the African catfish β-actin promoter to arrange a construct of pTarget-pCbBA-African catfish GH (7,783 bp) as transgenic walking catfish expression vector.*

KEYWORDS: ***expression vectors, growth hormone, African catfish, transgenic walking catfish***

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VERIFIKASI GEN HORMON PERTUMBUHAN LELE DUMBO PADA CALON INDUK HIBRID KETURUNAN PERTAMA LELE MUTIARA TRANSGENIK (*Clarias* sp.)

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Abstrak

Aplikasi teknologi transgenesis untuk mempercepat pertumbuhan telah berhasil diterapkan pada ikan lele dengan dihasilkannya lele mutiara transgenik yaitu lele yang disisipi gen eksogen berupa gen hormon pertumbuhan (GH) lele dumbo yang saat ini sudah mencapai keturunan pertama. Penelitian ini bertujuan untuk memverifikasi gen GH lele dumbo pada lele mutiara transgenik keturunan pertama. Penelitian dilaksanakan di Laboratorium Basah Hatchery, Laboratorium Bioteknologi Perikanan, Laboratorium Mikrobiologi dan Bioteknologi Molekuler FPIK UNPAD. Metode penelitian menggunakan metode eksperimen eksploratif dan dianalisis secara deskriptif. Kegiatan dimulai dari isolasi DNA, amplifikasi, dan elektroforesis untuk mendeteksi GH lele dumbo. Primer Cg-F (5'ATGGCTCGAGTTTTGGTGCTGCT-3') dan Cg-R (5'-CTACAGAGTGCAGTTGGAATCCA GGG-3') digunakan untuk mengkopi sekuen gen GH lele dumbo. Ikan uji yang digunakan sebanyak 10 ekor dari 20 ekor F1 MTMNT (hasil persilangan F0 jantan mutiara transgenik dan betina lele mutiara non transgenik), 10 ekor dari 20 ekor F1 MTS (hasil persilangan F0 jantan mutiara transgenik dan betina sangkuriang), dan ikan F1 MNTS (hasil persilangan jantan mutiara non transgenik dan betina sangkuriang) sebagai kontrol. Hasil verifikasi menunjukkan munculnya pita di ikan F1 MTMNT sebanyak 2 pita pada ukuran fragmen 750bp dan 1000bp (ikan 1, 3, 5, 8, dan 10), sebanyak 3 pita pada ukuran fragmen 750bp, 1000bp, dan 1250bp (ikan 2, 4, 6, 7, dan 9). Pada ikan F1 MTS sebanyak 2 pita pada ukuran fragmen 750bp dan 1000bp (ikan 1-5), sebanyak 1 pita pada ukuran fragmen 1000bp (ikan 6-10) sementara pada kontrol tidak terdeteksi pita DNA. Analisis sekuens dengan *software BioEdit* versi 7.1.8 menunjukan bahwa sekuen GH lele dumbo (600bp) terkandung di dalam sekuen fragmen 750bp, 1000bp, dan 1250bp yang menyatakan bahwa 50% ikan uji F1 MTMNT (10 dari 20 sampel) dan 50% ikan uji F1 MTS (10 dari 20 sampel) teridentifikasi sebagai ikan lele mutiara transgenik.

Kata Kunci : Biometrik , fragmen , lele mutiara, primer, sekuensing , sisipan,.

Abstract

Application of transgenesis technology to accelerate the growth has been successfully applied to the catfish with the production of transgenic mutiara catfish is a catfish inserted exogenous gene in the form of growth hormone gene (GH) dumbo catfish which is now reached the first generation. This study aims to verify the growth hormone dumbo catfish in first generation (F1) transgenic mutiara catfish. The research was conducted at Basah Hatchery Laboratory, Fisheries Biotechnology Laboratory, Microbiology and Molecular Biotechnology Laboratory of FPIK UNPAD. The research method used explorative experimental method and analyzed descriptively. Activity starts from DNA isolation, amplification, and electrophoresis to detect growth hormone dumbo catfish. Primers Cg-F (5'ATGGCTCGAGTTTTGGT GCTGCT-3 ') and Cg-R (5'-CTACAGAGTGCAGTTGGAATC CAGGG-3') were used to copy the growth hormone dumbo catfish sequences. The test fish used were 10 of 20 F1 MTMNT fish (result of crosses between F0 male transgenic mutiara and female non transgenic mutiara), 10 of 20 F1 MTS fish (result of crosses between F0 male transgenic mutiara and female sangkuriang), and F1 MNTS fish (result of crosses between male non transgenic mutiara and female sangkuriang) as control. The results of the verification showed the presence of ribbon at fish F1 MTMNT much as 2 ribbon on fragment size 750bp and 1000bp (fish 1, 3, 5, 8, and 10), as many as 3 ribbon on fragment size 750bp, 1000bp and 1250bp (fish 2, 4, 6, 7, and 9). At F1 MTS fish as much as 2 band at 750bp and 1000bp fragment size (1-5 fish), as many as one band at 1000bp fragment size (6-10 fish) while on the control is not detected DNA tape. Sequence analysis with software BioEdit version 7.1.8 shows that sequences of growth hormone dumbo catfish (600bp) contained in sequence fragments 750bp, 1000bp and 1250bp which states that 50% F1 MTMNT fish (10 of 20 sample) and 50% F1 MTS fish (10 of 20 sample) identified as transgenic mutiara catfish.

Keywords : *Biometrics, fragment, insertion, mutiara catfish, primers, sequencing.*

2. State of the art on GE Research in animals

1. Research on GE animals is on preparation (to start) → fish, other species of livestock
2. Regulation on GE, is under development → Ministry of Environment and Forestry
3. Countries need:
   - capacity building for GE technical aspect.
   - involvement of overseas researchers in our research activity

3. Way Forward

1. Identify colleagues willing to collaborate
2.