

A METHOD OF GENERATING STERILE AND MONOSEX PROGENY

STATEMENT OF GOVERNMENT RIGHTS

5 **[0001]** Aspects of the work described herein were supported by grant award # 2018-33522-28745 from the USDA-National Institute of Food and Agriculture. The United States Government may have certain rights in these inventions.

FIELD

10 **[0002]** The present disclosure relates generally to methods of sterilizing and sex-determining freshwater and seawater organisms.

BACKGROUND

15 **[0003]** The following paragraphs are not an admission that anything discussed in them is prior art or part of the knowledge of persons skilled in the art.

20 **[0004]** Fish species have been genetically engineered (GE) to produce valuable pharmaceutical proteins or to incorporate advantageous traits for aquaculture. A variety of fish with improved growth rates, food conversion ratios, resistance to disease, and enhanced nutritional benefits, have been developed to address the future demand for seafood and the need to improve sustainability in the aquaculture industry. However, worldwide adoption of these GE fish is hampered by concerns over their accidental release into natural ecosystems. Cultured fish have been shown to reproduce and survive in natural environments, resulting in feral populations. Similarly, GE fish may have native relatives, raising the possibility that the genetic modifications will spread throughout the wild population and alter the native gene pool. Commercial GE fish therefore represent a potential threat to the environment and a challenge to policy makers and regulatory agencies tasked with risk-benefit evaluations.

25 **[0005]** One approach to address one or more of the aforementioned issues is to sterilize fish. The induction of triploidy is the most used and best studied approach for producing sterile fish. Generally, triploid fish are produced by applying temperature or pressure shock to fertilized eggs, forcing the incorporation of the second polar body and producing cells with three chromosome sets (3N). Triploid fish do not develop normal gonads as the extra chromosome set disrupts meiosis. At the industrial scale, the logistics of reliably

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applying pressure or temperature shocks to batches of eggs is complicated and carries significant costs. An alternative to triploid induced by physical treatments is triploid induced by genetics, which results from crossing a tetraploid with a diploid fish. Tetraploid fish, however, are difficult to generate due to poor embryonic survival and slow growth. In some examples, triploid males produce some normal haploid sperm cells thus allowing males to fertilize eggs, though at a reduced efficiency. Also, in some species, negative performance characteristics have been associated with triploid phenotype, including reduced growth and sensitivity to disease.

[0006] Another approach for sterilizing fish is by hormone treatment extending over several weeks. However, in many cases, including these intensive long-term treatment processes do not have a desirable efficacy of sterility, and/or have been associated with decreased fish growth performance. Furthermore, treatments involving a synthetic steroid may result in higher mortality rates.

[0007] Another approach for sterilizing fish is by using transgenic-based technologies, which include a step of integrating a transgene that induce germ cell death or disrupts their migration patterns resulting in their ablation in developing embryos. However, transgenes are subject to position effect as well as silencing. Consequently, such approaches are subject to extended regulatory review processes before being considered acceptable for commercial use.

[0008] An alternative approach for sterilizing fish is by knockdown or knockout of genes governing primordial germ cell (PGC) development. Such approaches have been reported to cause PGC loss and sterility. However, the sterile trait in these fish is not heritable. Accordingly, utilizing an approach of knockdown or knockout of genes governing PGC development may be logistically challenging and costly and thus impractical to efficiently mass produce sterile fish at commercial scale.

[0009] Mechanisms governing sexual or gonadal differentiation in teleost fish are complex processes influenced by internal (genetic and endocrine factors) and external factors, including social interaction and environmental conditions (water temperature, pH and oxygen), whose relative contributions can vary significantly depending on the species.

[0010] Improvements in generating sterile, sex-determined fish, crustaceans, or mollusks is desirable.

INTRODUCTION

[0011] The following introduction is intended to introduce the reader to this specification but not to define any invention. One or more inventions may reside in a combination or sub-combination of the instrument elements or method steps described below or in other parts of this document. The inventors do not waive or disclaim their rights to any invention or inventions disclosed in this specification merely by not describing such other invention or inventions in the claims.

[0012] One or more of the previously proposed methods used for sterilizing freshwater and seawater organisms may result in: (1) an insufficient efficacy; (2) increased difficulty to propagate the sterility trait by, for example, having to perform genetic selection to identify a subpopulation of sterile individual, and/or repeating treatment at each generation; (3) an increase in operating costs by, for example, incorporating significant changes in husbandry practices, being untransferable across multiple species, increasing production times, increasing the percentage of sterile organisms with reduced growth and increased sensitivity to disease, increasing mortality rates of sterile organisms, or a combination thereof; (4) gene flow to wild populations and colonization of new habitats by cultured, non-native species; or (4) a combination thereof.

[0013] The present disclosure provides methods of producing sex-determined sterilized freshwater and seawater organisms by disrupting their sexual differentiation and gametogenesis pathways. One or more examples of the present disclosure may: (1) increase efficacy of sterilization, by for example, allowing mass production of sterile individuals and ensuring that all individuals are completely sterile; (2) decrease operating costs by, for example, decreasing the amount of costly equipment or treatments, being commercially scalable, being transferable across multiple species, decreasing feed, decreasing production times, decreasing the percentage of organisms that attain sexually maturity, increasing the physical size of sexually mature organisms, or a combination thereof; (3) decrease gene flow to wild populations and colonization of new habitats by cultured non-native species; (4) increase culture performance by decreasing loss of energy to gonad development; or (5) a combination thereof, compared to one or more previously proposed methods used for sterilizing freshwater and seawater organisms.

[0014] One or more examples of the present disclosure may yield at least a 10% improvement in food conversion rates (FCR = amount of weight gained per quantity of food fed) and about 20% faster growth rates, compared to other lines currently used in production systems (Methyltestosterone treatment). These performance benefits may only impact feed

costs (direct reduction in feed costs) and labor (reduced labor due to shortened culture times). Based on averaged itemized costs of a U.S. tilapia farming operation producing 1000 lbs of product, savings of about \$0.23 per market sized fish (1.5 pounds) using all male sterile-Tilapia may be realized, suggesting that an operation choosing to retain its savings in production costs may experience an increase in profit margin approaching about 130%.

[0015] The present disclosure also discusses methods of making broodstock freshwater and seawater organisms for use in producing sex-determined sterilized freshwater and seawater organisms, as well as the broodstock itself.

[0016] The present disclosure provides a method of generating a sterile sex-determined fish, crustacean, or mollusk, comprising the steps of: breeding (i) a fertile hemizygous mutated female fish, crustacean, or mollusk having at least a first mutation and a second mutation with (ii) a fertile hemizygous mutated male fish, crustacean, or mollusk having at least the first mutation and the second mutation; and selecting a progenitor that is homozygous by genotypic selection, the homozygous mutated progenitor being the sterile sex-determined fish, crustacean, or mollusk, wherein the first mutation disrupts one or more genes that specify sexual differentiation, and wherein the second mutation disrupts one or more genes that specify gamete function.

[0017] The present disclosure also provides a method of generating a sterile sex-determined fish, crustacean, or mollusk, comprising the step of: breeding (i) a fertile homozygous mutated female fish, crustacean, or mollusk having at least a first mutation and a second mutation with (ii) a fertile homozygous mutated male fish, crustacean, or mollusk having at least the first mutation and the second mutation to produce the sterile sex-determined fish, crustacean, or mollusk, wherein the first mutation disrupts one or more genes that specify sexual differentiation, wherein the second mutation disrupts one or more genes that specify gamete function, and wherein the fertility of the fertile homozygous female fish, crustacean, or mollusk and the fertile homozygous mutated male fish, crustacean, or mollusk has been rescued.

[0018] The fertility rescue may comprise germline stem cell transplantation. The fertility rescue may further comprise sex steroid alteration. The alteration of sex steroid may be an alteration of estrogen, or an alteration of an aromatase inhibitor.

[0019] The germline stem cell transplantation may comprise the steps of: obtaining a germline stem cell from a sterile homozygous male fish, crustacean, or mollusk having at least the first mutation and the second mutation or a germline stem cell from a sterile

homozygous female fish, crustacean, or mollusk having at least the first mutation and the second mutation; and transplanting the germline stem cell into a germ cell-less recipient male fish, crustacean, or mollusk, or into a germ cell-less recipient female fish, crustacean, or mollusk. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or mollusk may be homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using ploidy manipulation. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created by hybridization. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using exposure to high levels of sex hormones.

[0020] The germline stem cell transplantation may comprise the steps of: obtaining a spermatogonial stem cell from a sterile homozygous male fish, crustacean, or mollusk having at least the first mutation and the second mutation or a oogonial stem cell from a sterile homozygous female fish, crustacean, or mollusk having at least the first mutation and the second mutation; and transplanting the spermatogonial stem cell into a testis of a germ cell-less fertile male fish, crustacean, or mollusk or the oogonial stem cell into an ovary of a germ cell-less fertile female fish, crustacean, or mollusk. The germ cell-less fertile male fish, crustacean, or mollusk and the germ cell-less fertile female fish, crustacean, or mollusk may be homozygous for the mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using ploidy manipulation. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created by hybridization. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using exposure to high levels of sex hormones.

[0021] The sterile sex-determined sterile fish, crustacean, or mollusk may be a sterile male fish, crustacean, or mollusk. The first mutation may comprise a mutation in one or more genes that modulates the synthesis of androgen and/or estrogen. The first mutation may comprise a mutation in one or more genes that modulate the expression of aromatase Cyp19a1a, Cyp17, or a combination thereof. The one or more genes that modulate the expression of aromatase Cyp19a1a may be one or more genes selected from the group

consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof. The one or more genes that modulate the expression of Cyp17 may be *cyp17l* or an ortholog thereof. The second mutation may comprise a mutation in one or more genes that modulate spermiogenesis. The second mutation may comprise a mutation in one or more genes that cause

5 globozoospermia. The second mutation in one or more genes that cause globozoospermia may cause sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof. The second mutation may comprise a mutation in one or more genes selected from the group consisting of *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof.

10 **[0022]** The sterile sex-determined sterile fish, crustacean, or mollusk may be a sterile female fish, crustacean, or mollusk. The first mutation may comprise a mutation in one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor. The one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor may be one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof. The second mutation may comprise a mutation in one or more genes that modulate
15 oogenesis, folliculogenesis, or a combination. The one or more genes that modulate oogenesis may modulate the synthesis of estrogen. The one or more genes that modulate the synthesis of estrogen may be FSHR or an ortholog thereof. The one or more genes that modulate folliculogenesis may modulate the expression of vitellogenins. The one or more
20 genes that modulate the expression of vitellogenins may be *vtgs* or an ortholog thereof. The one or more genes that modulate the expression of vitellogenins may be a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.

25 **[0023]** The present disclosure also provides a method of generating a sterile sex-determined fish, crustacean, or mollusk, comprising the step of: breeding (i) a fertile female fish, crustacean, or mollusk having a homozygous mutation with (ii) a fertile male fish, crustacean, or mollusk having a homozygous mutation to produce the sterile sex-determined fish, crustacean, or mollusk, wherein the mutation directly or indirectly disrupts
30 spermiogenesis, and/or directly disrupts vitellogenesis, and wherein the fertility of the fertile female fish, crustacean, or mollusk and the fertile male fish, crustacean, or mollusk have been rescued.

[0024] The mutation that directly or indirectly disrupts spermiogenesis may be a mutation in *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, or an ortholog thereof. The mutation that directly disrupts vitellogenesis may be a mutation in a gene encoding or regulating:

Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof. The fertile female fish, crustacean, or mollusk and the fertile male fish, crustacean, or mollusk may have a plurality of homozygous mutations that, in combination: directly or indirectly disrupt spermiogenesis; directly disrupt vitellogenesis; or both.

[0025] The fertility rescue may comprise germline stem cell transplantation. The fertility rescue may further comprise sex steroid alteration. The alteration of sex steroid may be an alteration of estrogen, or an alteration of an aromatase inhibitor.

[0026] The germline stem cell transplantation may comprise the steps of: obtaining a germline stem cell from a sterile homozygous male fish, crustacean, or mollusk having at least the homozygous mutation or a germline stem cell from a sterile homozygous female fish, crustacean, or mollusk having at least the homozygous mutation; and transplanting the germline stem cell into a germ cell-less recipient male fish, crustacean, or mollusk, or into a germ cell-less recipient female fish, crustacean, or mollusk. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or mollusk may be homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using ploidy manipulation. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created by hybridization. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using exposure to high levels of sex hormones.

[0027] The fertile female fish, crustacean, or mollusk and the fertile male fish, crustacean, or mollusk may have an additional homozygous mutation that specifies sexual differentiation. The mutation that specifies sexual differentiation may modulate the expression of aromatase Cyp19a1a, Cyp17, an inhibitor to aromatase Cyp19a1a, or a combination thereof. The mutation that modulates the expression of Cyp17 may be a mutation in *cyp17l* or an ortholog thereof. The mutation that modulates the expression of

aromatase Cyp19a1a inhibitor may be a mutation in *Gsdf*, *dmrt1*, *Amh*, *Amhr*, or an ortholog thereof.

[0028] The breeding step of the herein disclosed methods may comprise hybridization or hormonal manipulation and breeding strategies, to specify sexual differentiation.

[0029] The fish, crustacean, or mollusk of the herein disclosed methods may be a fish.

[0030] The present disclosure also provides a fertile homozygous mutated fish, crustacean, or mollusk for producing a sterile sex-determined fish, crustacean, or mollusk, the fertile homozygous mutated fish, crustacean, or mollusk having at least a first mutation and a second mutation, wherein the first mutation disrupts one or more genes that specify sexual differentiation, wherein the second mutation disrupts one or more genes that specify gamete function, and wherein the fertility of the fertile homozygous mutated fish, crustacean, or mollusk has been rescued. The fertility rescue may comprise germline stem cell transplantation. The fertility rescue may further comprise sex steroid alteration. The alteration of sex steroid may be an alteration of estrogen, or an alteration of an aromatase inhibitor.

[0031] The germline stem cell transplantation may comprise the steps of: obtaining a germline stem cell from a sterile homozygous male fish, crustacean, or mollusk having at least the first mutation and the second mutation or a germline stem cell from a sterile homozygous female fish, crustacean, or mollusk having at least the first mutation and the second mutation; and transplanting the germline stem cell into a germ cell-less recipient male fish, crustacean, or mollusk, or into a germ cell-less recipient female fish, crustacean, or mollusk. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or mollusk may be homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using ploidy manipulation. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created by hybridization. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using exposure to high levels of sex hormones.

[0032] The germline stem cell transplantation may comprise the steps of: obtaining a spermatogonial stem cell from a sterile homozygous male fish, crustacean, or mollusk having

at least the first mutation and the second mutation or a oogonial stem cell from a sterile homozygous female fish, crustacean, or mollusk having at least the first mutation and the second mutation; and transplanting the spermatogonial stem cell into a testis of a germ cell-less fertile male fish, crustacean, or mollusk or the oogonial stem cell into an ovary of a germ cell-less fertile female fish, crustacean, or mollusk. The germ cell-less fertile male fish, crustacean, or mollusk and the germ cell-less fertile female fish, crustacean, or mollusk may be homozygous for the mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using ploidy manipulation. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created by hybridization. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using exposure to high levels of sex hormones.

[0033] The sterile sex-determined sterile fish, crustacean, or mollusk may be a sterile male fish, crustacean, or mollusk. The first mutation may comprise a mutation in one or more genes that modulates the synthesis of androgen and/or estrogen. The first mutation may comprise a mutation in one or more genes that modulate the expression of aromatase Cyp19a1a, Cyp17, or a combination thereof. The one or more genes that modulate the expression of aromatase Cyp19a1a may be one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof. The one or more genes that modulate the expression of Cyp17 may be *cyp17l* or an ortholog thereof. The second mutation may comprise a mutation in one or more genes that modulate spermiogenesis. The second mutation may comprise a mutation in one or more genes that cause globozoospermia. The second mutation in one or more genes that cause globozoospermia may cause sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof. The second mutation may comprise a mutation in one or more genes selected from the group consisting of *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof.

[0034] The sterile sex-determined sterile fish, crustacean, or mollusk may be a sterile female fish, crustacean, or mollusk. The first mutation may comprise a mutation in one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor. The one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor may be one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog

thereof. The second mutation may comprise a mutation in one or more genes that modulate oogenesis, folliculogenesis, or a combination. The one or more genes that modulate oogenesis may modulate the synthesis of estrogen. The one or more genes that modulate the synthesis of estrogen may be FSHR or an ortholog thereof. The one or more genes that modulate folliculogenesis may modulate the expression of vitellogenins. The one or more genes that modulate the expression of vitellogenins may be *vtgs* or an ortholog thereof. The one or more genes that modulate the expression of vitellogenins may be a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.

[0035] The present disclosure also provides a fertile fish, crustacean, or mollusk having a homozygous mutation for producing a sterile sex-determined fish, crustacean, or mollusk, wherein the mutation directly or indirectly disrupts spermiogenesis, and/or directly disrupts vitellogenesis, and wherein the fertility of the fertile fish, crustacean, or mollusk has been rescued.

[0036] The mutation that directly or indirectly disrupts spermiogenesis may be a mutation in *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, or an ortholog thereof. The mutation that directly disrupts vitellogenesis may be a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof. The fertile fish, crustacean, or mollusk may have a plurality of homozygous mutations that, in combination: directly or indirectly disrupt spermiogenesis; directly disrupt vitellogenesis; or both. The fertility rescue may comprise germline stem cell transplantation. The fertility rescue may further comprise sex steroid alteration. The alteration of sex steroid may be an alteration of estrogen, or an alteration of an aromatase inhibitor.

[0037] The germline stem cell transplantation may comprise the steps of: obtaining a germline stem cell from a sterile homozygous male fish, crustacean, or mollusk having at least the homozygous mutation or a germline stem cell from a sterile homozygous female fish, crustacean, or mollusk having at least the homozygous mutation; and transplanting the germline stem cell into a germ cell-less recipient male fish, crustacean, or mollusk, or into a germ cell-less recipient female fish, crustacean, or mollusk. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or

mollusk may be homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using ploidy manipulation. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created by hybridization. The germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk may be created using exposure to high levels of sex hormones.

[0038] The fertile fish, crustacean, or mollusk may have an additional homozygous mutation that specifies sexual differentiation. The mutation that specifies sexual differentiation may modulate the expression of aromatase Cyp19a1a, Cyp17, an inhibitor to aromatase Cyp19a1a, or a combination thereof. The one or more genes that modulate the expression of aromatase Cyp19a1a may be one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof. The one or more genes that modulate the expression of aromatase Cyp19a1a inhibitor may be one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.

[0039] Producing a sterile sex-determined fish, crustacean, or mollusk may comprise a breeding step comprising hybridization or hormonal manipulation and breeding strategies, to specify sexual differentiation.

[0040] The herein disclosed fertile fish, crustacean, or mollusk may be a fish.

[0041] The present disclosure also provides a method of making a fertile homozygous mutated fish, crustacean, or mollusk that generates a sterile sex-determined fish, crustacean, or mollusk, comprising the steps of: breeding (i) a fertile hemizygous mutated female fish, crustacean, or mollusk having at least a first mutation and a second mutation with (ii) a fertile hemizygous mutated male fish, crustacean, or mollusk having at least the first mutation and the second mutation; selecting a progenitor that is homozygous by genotypic selection; and rescuing the fertility of the homozygous progenitor, wherein the first mutation disrupts one or more genes that specify sexual differentiation, and wherein the second mutation disrupts one or more genes that specify gamete function.

[0042] Other aspects and features of the present disclosure will become apparent to those ordinarily skilled in the art upon review of the following description of specific examples in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] Examples of the presently disclosed methods and organisms will now be described, by way of example only, with reference to the attached Figures.

[0044] Fig. 1 is a flowchart showing an example of a method of generating a sterile sex-determined fish, crustacean, or mollusk and propagating a mutated line.

[0045] Fig. 2 is illustrations and graphs showing an example of F0 mosaic founder mutant identification and selection strategy. Mutant alleles were identified by fluorescence PCR with genes specific primers designed to amplify the regions around the targeted loci (120–300 bp). For fluorescent PCR, both combination of gene specific primers and two forward oligos with the fluorophore 6-FAM or NED attached were added to the reaction. A control reaction using wild type DNA is used to confirm the presence of single Peak amplification at each loci. The resulting amplicon were resolved via capillary electrophoresis (CE) with an added LIZ labeled size standard to determine the amplicon sizes accurate to base-pair resolution (Retrogen). The raw trace files were analyzed on Peak Scanner software (ThermoFisher). The size of the peak relative to the wild-type peak control determines the nature (insertion or deletion) and length of the mutation. The number of peaks indicate the level of mosaicism. We selected F0 mosaic founder carrying the fewest number of mutant alleles (2-4 peak preferentially).

[0046] Fig. 3 is a graph illustrating an example Melt Curve plot visualizing the genotypes of heterozygous, homozygous mutant and wild type samples. The negative change in fluorescence is plotted versus temperature ($-dF/dT$). Each trace represents a sample. The melting temperature of the wild-type allele in this example is $\sim 81^{\circ}\text{C}$ (wild type peak), the melting temperature of the homozygous mutant product (homozygous deletion peak) is $\sim 79^{\circ}\text{C}$. The remaining trace represents a heterozygote.

[0047] Fig. 4 panels A to D are photographs of different stages of growth of a Tilapia F0 generation comprising double-allelic knockout of pigmentation genes.

[0048] Fig. 5 panels A to B are photographs of Tilapia after multi-gene targeting comprising *dead end1* (*dnd*) and *tyrosinase* (*Tyr*). Fig. 5 panel A is an F0 *Tyr* deficient albino. Fig. 5 panel B shows dissected testis from control (WT) and sterile (F0 *dnd* KO) tilapia.

[0049] Fig. 6 panels A to B are photographs of germ cell depleted testis and ovary (arrowheads point toward the gonads) from *Elavl2*-Knockout tilapia (*Elavl2* ^{$\Delta 8/\Delta 8$}). Small photo inserts show the urogenital papillae. *Elavl2* mutants were produced by microinjecting

engineered nucleases targeting *Elavl2* coding sequence into one cell stage tilapia embryos. One of the resulting founder males was mated with a wild-type female and produced heterozygous mutants in the F1 generation. Mating of these F1 mutants *Elavl2*^{Δ8/+} produced an F2 generation with approximately 25% of the clutch being sterile homozygous mutant of both sexes.

[0050] Fig. 7 panels A to C are illustrations of selected mutant alleles at the tilapia *cyp17* loci. Fig. 7 panel A is a schematic of the *cyp17* gene. Exons (E1-8) are shown as shaded boxes; translational start and stop sites as ATG and TAA, respectively. Arrows point to targeted sites in the first exon. Fig. 7 panel B is the wild-type reference sequence (SEQ ID NO: 60) with the selected germ-line mutant allele (SEQ ID NO: 61) from an offspring of Cyp17 F0 mutated tilapia. This 11nt+5 nt deletion is predicted to create a truncated protein that terminates at amino acid 44 rather than position 521. Fig. 7 panel C is the predicted protein sequences of WT (SEQ ID NO: 62) and mutant *cyp17* allele (SEQ ID NO: 63) in which the first 16 amino acids are identical to those of the wild-type Cyp17 protein and the 44 amino acids are miscoded. Altered amino acids are highlighted.

[0051] Fig. 8 panels A to C are graphs, illustrations, and photographs showing *cyp17* loss of function produces all-male offspring with no secondary sex characteristics. Fig. 8 panel A is a graph showing Cyp17 mutant fish exhibiting complete male biased. A founder male with germline mutations at the *cyp17* loci was bred with a wild type female, and the male and female F1 progeny carrying the null $\Delta 16$ -*cyp17* allele were selected and crossed to produce F2 generation of wild type (WT) homozygous (-/-) and hemizygous mutants (+/-). The graph shows the count of males and females for a given genotype. Fig. 8 panel B shows an undetectable level of testosterone in *cyp17* loss of function mutants. Blood was collected from the caudal vein and centrifuged at 3000 rpm for 10 min. Plasma was separated and frozen at -80° C and free plasmatic testosterone level was measured by enzyme linked immunosorbent assay (ELISA) (Cayman Chemical, Michigan, USA). Plasma samples were analyzed in triplicate. Fig. 8 panel C shows photographs of two *cyp17* F0 KO (-/-) males with underdeveloped UGP compared to an age matched non-treated male (right image).

[0052] Fig. 9 panels A to E are illustrations showing Cyp17 loss of function mutants are sexually delayed with smaller testes and oligospermia. F2 progeny from hemizygous *cyp17* mutants were raised to 5 months of age, weighted (Fig. 9 panel C), and genotyped. Fig. 9 panel A shows males were sacrificed, and their testes exposed (Fig. 9 panel A) and

dissected (Fig. 9 panel B) revealing a gradient of color and size (Fig. 9 panel D) with WT being the most mature gonad and homozygous appearing as sexually delayed. Fig. 9 panel E shows volume of strippable milt from 8 homozygous and WT males and Fig. 9 panel F shows spectrophotometric comparison of sperm concentration (absorbance at 600nm).

5 **[0053]** Fig. 10 panels A to C are illustrations of selected mutant alleles at the tilapia Tight junction protein 1 (*Tjp1a*) loci. Fig. 10 panel A is a schematic of the *Tjp1a* gene. Exons (E1-32) are shown as shaded boxes; translational start and stop sites as ATG and TAA, respectively. Arrows point to targeted exons 15 and 17. Fig. 10 panel B is the wild-type reference sequence (SEQ ID NO: 71) with the selected germ-line mutant allele (SEQ ID NO: 72) from an offspring of *Tjp1a* F0 mutated tilapia. This 7 nt deletion is predicted to create a truncated protein that terminates at amino acid 439 rather than position 1652. Fig. 10 panel C is the predicted protein sequences of WT (SEQ ID NO: 73) and mutant *Tjp1a* allele (SEQ ID NO: 74) in which the first 439 amino acids are identical to those of the wild-type *Tjp1a* protein.

15 **[0054]** Fig. 11 panels A to C are illustrations of selected mutations at the tilapia Hippocampus abundant transcript 1a (*Hiat1*) loci. Fig. 11 panel A is a schematic of the tilapia *Hiat1* gene. Exons (E1-12) are shown as shaded boxes; 5' and 3' untranslated regions are shown as open boxes. Arrows point to targeted exons 4 and 6. Fig. 11 panel B is the wild-type reference sequence (SEQ ID NO: 75) with the sequence of the selected germ-line mutant allele (SEQ ID NO: 76) from an offspring of *Hiat1* F0 mutated tilapia. Location of the 17 nucleotides deletion is shown by dashes. This frameshift mutation is predicted to create a truncated protein that terminates at amino acid 234 rather than position 491. Fig. 11 panel C shows the predicted protein sequences of WT (SEQ ID NO: 77) and truncated mutant *Hiat1* protein (SEQ ID NO: 78) in which the first 218 amino acids are identical to those of the wild-type and the following 16 amino acids are miscoded.

25 **[0055]** Fig. 12 panels A to C are illustrations of selected mutations at the tilapia Small ArfGAP2 (*Smap2*) loci. Fig. 12 panel A is a schematic of the tilapia *Smap2* gene. Exons (E1-12) are shown as shaded boxes, and 3' untranslated region is shown as open box. Arrows point to targeted exons 2 and 9. Fig. 12 panel B is the wild-type reference sequence (SEQ ID NO: 79) with the sequence of the selected germ-line mutant allele (SEQ ID NO: 80) from an offspring of *Smap2* F0 mutated tilapia. Location of the 17 nucleotides deletion is shown by dashes. This frameshift mutation is predicted to create a truncated protein that terminates at amino acid 118 rather than position 429. Fig. 12 panel C shows the predicted protein

sequences of WT (SEQ ID NO: 81) and truncated mutant Smap2 protein (SEQ ID NO: 82) in which the first 53 amino acids are identical to those of the wild-type and the following 63 amino acids are miscoded.

[0056] Fig. 13 panels A to C are illustrations of selected mutant alleles at the tilapia Casein kinase 2, alpha prime polypeptide a (*Csnk2a2*) loci. Fig. 13 panel A is a schematic of the *Csnk2a2* gene. Exons (E1-11) are shown as shaded boxes; translational start and stop sites as ATG and TGA, respectively. Arrows point to targeted exons 1 and 2. Fig. 13 panel B is the wild-type reference sequence (SEQ ID NO: 83) with the selected germ-line mutant allele (SEQ ID NO: 84) from an offspring of *Csnk2a2* F0 mutated tilapia. This 22 nt deletion is predicted to create a truncated protein that terminates at amino acid 31 rather than position 350. Fig. 13 panel C is the predicted protein sequences of WT (SEQ ID NO: 85) and mutant *Csnk2a2* allele (SEQ ID NO: 86) in which the first 31 amino acids are miscoded.

[0057] Fig. 14 panels A to C are illustrations of selected mutant alleles at the tilapia Golgi-associated PDZ and coiled-coil motif (*Gopc*) loci. Fig. 14 panel A is a schematic of the *Gopc* gene. Exons (E1-9) are shown as shaded boxes; translational start and stop sites as ATG and TAA, respectively. Arrows point to targeted exons 1 and 2. Fig. 14 panel B is the wild-type reference sequence (SEQ ID NO: 87) with the selected germ-line mutant allele (SEQ ID NO: 88) from an offspring of *Gopc* F0 mutated tilapia. This 8 nt deletion is predicted to create a truncated protein that terminates at amino acid 30 rather than position 444. Fig. 14 panel C is the predicted protein sequences of WT (SEQ ID NO: 89) and mutant *Gopc* allele (SEQ ID NO: 90) in which the first 9 amino acids are identical to those of the wild-type *Gopc* protein and the following 21 amino acids are miscoded.

[0058] Fig. 15 panels A and B are photographs and graphs showing tilapia spermiogenesis specific gene knockouts phenocopy human and mice deficiencies. Fig. 15 panel A shows malformation of spermatozoa in F0 deficient tilapia for the five candidate genes. Microscopic images of spermatozoa collected from wild-type (WT) and from *Tjp1a*, *Gopc*, *Smap2*, *Hiat1* and *Csnk2a2* F0 mutant fish respectively. Black arrowheads point to WT size sperm head and yellow arrowheads indicate enlarged round spermatozoa head. Scale bars: 100µm. Fig. 15 panel B shows the fertilization success rate from hand-stripped gametes, followed by in vitro fertilization in which dry gametes (200 eggs and stripped milt) were mixed together and immediately activated with 2mL of hatching water. Data are means +/- SD, n=3 replicates.

[0059] Fig. 16 panels A to C are images and graphs showing expression levels of SMS genes in fertile and germ cell free testes. Fig. 16 panel A shows testes dissected from 4 months old *dnd1* Knockout and wild type aged match control. Fig. 16 panel B illustrates that the relative expression level of *vasa*, a germ cell specific gene is reduced to undetectable level in testis from *dnd1* KO fish but strongly expressed in wild type testis, while the Sertoli specific gene *Dmrt1* is expressed at the same level in testes from wild-type and sterile tilapia. β -actin was used as the reference gene to normalize expression level of *vasa* and *Dmrt1*. Fig. 16 panel C illustrates the relative expression level of SMS genes *Tjp1a*, *Hiat1*, *Gopc* and *Csnk2a2* in testes from wild type and sterile tilapia. *Dmrt1* was used as the reference gene to normalize expression level of SMS genes. In all cases, value represent average of 3 biological replicates, +/- SD.

[0060] Fig. 17 panels A to C are illustrations of the selected mutation at the *Cyp9a1a* loci. Fig. 17 panel A is a schematic of the tilapia *Cyp9a1a* gene. Exons (E1-9) are shown as shaded boxes. Arrows point to targeted exons 1 and 9. Fig. 17 panel B is the wild-type reference sequence (SEQ ID NO: 65) with the sequences of the selected germ-line mutant alleles from *Cyp19a1a* F0 mutated tilapia (SEQ ID NOs: 66 and 67). The 7 nt (del 8 and ins1) and 10 nt deletions are indicated by dashes. These frameshift mutations are predicted to create truncated proteins that terminate at amino acid 12 and 11 rather than position 511. Fig. 17 panel C is the predicted protein sequences of WT (SEQ ID NO: 68) and truncated mutant proteins (SEQ ID NOs: 69 and 70), in which the first 7 and 5 amino acids are identical to those of the wild-type *Cyp19a1a* protein and the following 5 and 6 amino acids are miscoded. Altered amino acids are highlighted.

[0061] Fig. 18 is an illustration and table showing an example of the breeding scheme and anticipated genotypes of mutant progeny from double heterozygote parents. m1, 2, 3 symbols indicate different mutations at the *Tjp1a* locus in F0 mosaic female. Each column in the table shows the frequency of an expected F2 progeny for each combination of *cyp17* and *Tjp1a* alleles, as well as the projected sex ratio and fertility status. The progeny anticipated to be all-male and sterile is circled.

[0062] Fig. 19 panels A to C are illustrations of the selected mutation at the *Dmrt1* loci. Fig. 19 panel A is a schematic of the tilapia *Dmrt1* gene. Exons (E1-9) are shown as shaded boxes. Arrows point to targeted exons 1 and 3. Fig. 19 panel B is the wild-type reference sequence (SEQ ID NO: 91) with the sequences of the selected germ-line mutant

alleles from Dmrt1 F0 mutated tilapia (SEQ ID NOs: 92 and 93). The 7 nt and 13 nt deletions are indicated by dashes. These frameshift mutations are predicted to create truncated proteins that terminate at amino acid 40 and 38 rather than position 293. Fig. 19 panel C is the predicted protein sequences of WT (SEQ ID NO: 94) and truncated mutant proteins (SEQ ID NOs: 95 and 96), in which the first 16 amino acids are identical to those of the wild-type Dmrt1 protein and the following 24 and 22 amino acids are miscoded. Altered amino acids are highlighted.

[0063] Fig. 20 panels A to C are illustrations of the selected mutation at the growth/differentiation factor 6-B-like loci (*Gsdf*). Fig. 20 panel A is a schematic of the tilapia *Gsdf* gene. Exons (E1-5) are shown as shaded boxes. Arrows point to targeted exons 2 and 4. Fig. 20 panel B is the wild-type reference sequence (SEQ ID NO: 97) with the sequences of the selected germ-line mutant alleles from *Gsdf* F0 mutated tilapia (SEQ ID NOs: 98 and 99). The 5 nt and 22 nt deletions are indicated by dashes. These frameshift mutations are predicted to create truncated proteins that terminate at amino acid 56 and 46 rather than position 213. Fig. 20 panel C is the predicted protein sequences of WT (SEQ ID NO: 100) and truncated mutant proteins (SEQ ID NOs: 101 and 102), in which the first 52 and 46 amino acids are identical to those of the wild-type *Gsdf* protein and the following 4 and 0 amino acids are miscoded. Altered amino acids are highlighted.

[0064] Fig. 21 panels A to C are illustrations of selected mutations at the tilapia Folliculogenesis stimulating hormone receptor (*FSHR*) loci. Fig. 21 panel A is a schematic of the tilapia *FSHR* gene. Exons (E1-15) are shown as shaded boxes; 5' and 3' untranslated regions are shown as open boxes. Arrows point to targeted exons 11 and 15. Fig. 21 panel B is the wild-type reference sequence (SEQ ID NO: 103) with the sequence of the selected germ-line mutant allele (SEQ ID NO: 104) from an offspring of *FSHR* F0 mutated tilapia. Location of the 5 nucleotides deletion is shown by dashes. This frameshift mutation is predicted to create a truncated protein that terminates at amino acid 264 rather than position 689. Fig. 21 panel C shows the predicted protein sequences of WT (SEQ ID NO: 105) and truncated mutant *FSHR* protein (SEQ ID NO: 106) in which the first 258 amino acids are identical to those of the wild-type and the following 6 amino acids are miscoded.

[0065] Fig. 22 panels A to C are illustrations of the selected mutations at the Vitellogenin Aa (*VtgAa*) loci. Fig. 22 panel A is a schematic of the tilapia *VtgAa* gene. Exons (E1-35) are shown as shaded boxes. Arrows point to targeted exons 7 and 22. Fig. 22 panel B is the wild-type reference sequence (SEQ ID NO: 107) with the sequences of the selected

germ-line mutant alleles from *VtgAa* F0 mutated tilapia (SEQ ID NOs: 108 and 109). The 5 nt and 25 nt deletions are indicated by dashes. These frameshift mutations are predicted to create truncated proteins that terminate at amino acid 279 and 301 rather than position 1657. Fig. 22 panel C is the predicted protein sequences of WT (SEQ ID NO: 110) and truncated mutant proteins (SEQ ID NOs: 111 and 112), in which the first 278 and 269 amino acids are identical to those of the wild-type *VtgAa* protein and the following 1 and 32 amino acids are miscoded. Altered amino acids are highlighted.

[0066] Fig. 23 panels A to C are illustrations of selected mutations at the tilapia Vitellogenin Ab (*VtgAb*) loci. Fig. 23 panel A is a schematic of the tilapia *VtgAb* gene. Exons (E1-35) are shown as shaded boxes; 5' untranslated region is shown as open boxes. Arrows point to targeted exons 5 and 22. Fig. 23 panel B is the wild-type reference sequence (SEQ ID NO: 113) with the sequence of the selected germ-line mutant allele (SEQ ID NO: 114) from an offspring of *VtgAb* F0 mutated tilapia. Location of the 8 nucleotides deletion is shown by dashes. This frameshift mutation is predicted to create a truncated protein that terminates at amino acid 202 rather than position 1747. Fig. 23 panel C shows the predicted protein sequences of WT (SEQ ID NO: 115) and truncated mutant *VtgAb* protein (SEQ ID NO: 116) in which the first 270 amino acids are identical to those of the wild-type *VtgAb* protein and the following 32 amino acids are miscoded. Altered amino acids are highlighted.

[0067] Fig. 24 panels A and B is a photograph and graph showing that females deficient for *VtgAa* fail to produce viable progeny. Fig. 24 panel A is a photograph of 8 hours post fertilization embryos incubation in hatching water containing methylene blue (Roth, 0.01% of stock solution in hatching water). Blue staining indicates unfertilized eggs and dead embryos. Embryos were inspected daily under a light stereomicroscope and dead embryos counted and removed. Fig. 24 panel B shows survival percentage in the progeny from F0 *VtgAa* males and females outcrossed with wild type fish. Data are means $\pm$ SD, n=2x3 replicates.

[0068] Fig. 25 is an illustration that shows breeding scheme and genotype of mutant progeny from double heterozygous parents. m1-n and m1 symbols indicate mosaic mutations in F0 and one specific mutation selected for each targeted loci. F1 genotypes shown correspond to one of the four combinations of alleles we plan to establish. Each column in the table indicates the relative frequency of expected F2 progeny for each combination of alleles, as well as the projected sex ratio and fertility status. The progeny anticipated to be all-female and sterile is circled in red.

[0069] Fig. 26 are photographs showing the impact of *FSHR* deficiency on ovarian development. Siblings 12 months old fertile control (WT body color-bottom panel) and albino F0 *FSHR* mutant female (*FSHR* ^{-/-}, *tyr* ^{-/-}; top panel) of similar body size were dissected for morphological analysis of their gonads. Left images show dissected ovaries in the peritoneal cavity of control and mutant females. The white arrows point to the gonads and the black arrows point to the urogenital papillae. Mutation of *FSHR* resulted in complete folliculogenesis arrest and atrophic string like gonad. Wild type female displays a large and prominent urogenital papilla while albino F0 *FSHR* ^{-/-} female show a significantly smaller papilla.

[0070] Fig. 27 is an illustration showing a germ cell transplantation strategy to allow mass production of donor derived gametes carrying mutations in FEM (*cyp17*, *Cyp19a1a*), SMS (*Tjp1a*, *Csnk2a2*, *Gopc*, *Smap2*, *Hiat1*), MA (*Dmrt1*, *Gsdf*) and FLS genes (*Vtgs*, *FSHR*). In the mutant donor, the defective gene causes the development of monosex male (FEM genes) or female (MA genes) populations or render spermatozoa (SMS genes) or oocytes (FLS genes) non-functional. As such, mass production of these homozygous mutant is not possible. To circumvent this limitation, we only targeted genes whose mutant phenotypes is caused by defective function in the soma and not in germ cells and produced chimeric embryos using the “germ cell transplantation” techniques. To produce chimera, ovarian or testicular cell suspension obtained from juvenile homozygous mutant fish were transplanted into the peritoneal cavity of germ cell-free recipient embryos that are wild type for the targeted gene(s). With this strategy, the wild type host chimeric embryo has normal somatic cells but a mutant germline. These chimeric recipients restore the normal sex ratio and/or sterility as they possess functional somatic gene(s). These recipient fish can be used as commercial broodstock for mass production of monosex and/or sterile fish.

[0071] Fig. 28 is an illustration showing a germ cell transplantation method to mass produce functional sperm carrying a spermiogenesis deficient gene (SMS (-)). No defects are found during the generation of primordial germ cells (PGCs) and spermatogonia in SMS-null fish progenies obtained from heterozygous SMS mutant parents. At maturity however, SMS mutant males only produce round headed, immotile sperm and are infertile. Female SMS-mutants are fertile. The SMS gene is expressed in somatic cells surrounding the germ cells (Sertoli and Leydig cells) where it exerts its activity. The lack of SMS protein causes a defective microenvironment where sperm maturation is impaired. To restore spermiogenesis, a germline stem cell can be isolated from juvenile SMS mutant and transplanted into

recipient embryos depleted of their own PGCs but carrying a functional SMS gene. Transplanted SMS *-/-* spermatogonial stem cell will colonize the recipient gonad and since SMS is dispensable for their continued development, the recipient somatic cells will nurse transplanted germ cell, restore spermiogenesis and allow production of functional

spermatozoa, all of which carrying the mutant SMS gene.

[0072] Fig. 29 is an illustration showing a germ cell transplantation method for production of functional eggs carrying a Vitellogenin deficient gene (*Vtg* *-/-*). No defects are found during the generation of primordial germ cells (PGCs) and oogonia in *Vtg*-null fish progenies obtained from heterozygous *Vtg* mutant parents. At maturity however, *Vtg* mutant female only produce oocyte lacking *Vtg* protein resulting in female sterility. *Vtg* deficient male develop normally and are fertile. The *Vtg* gene(s) are normally expressed in liver cells and *Vtg* protein(s) transported to the oocyte through the blood stream. The lack of *Vtg* protein cause the eggs to lack critical nutrient necessary to sustain early embryo or larvae development, resulting in developmental arrest. As such, *Vtg* *-/-* female are child-less. To restore vitellogenesis, a germline stem cell can be isolated from juvenile *Vtg* null-mutant and transplanted into recipient embryos depleted of their own PGCs but carrying a functional *Vtg* gene. Transplanted *Vtg* *-/-* germline stem cell will colonize the recipient gonad and the liver cells of the surrogate mother will ensure that nutrients supporting early development are properly loaded into the eggs. These recipient females crossed with *Vtg* *-/-* male will produce viable *Vtg* *-/-* offspring.

[0073] Fig. 30 is an illustration showing a germ cell transplantation method for production of viable *FSHR*-mutant eggs (*FSHR* *-/-*). No defects are found during the generation of primordial germ cells (PGCs) and oogonia in *FSHR*-null fish progenies obtained from heterozygous *FSHR* mutant parents. At maturity however, *FSHR* mutant female fail to respond to FSH-mediated signaling, resulting in folliculogenesis arrest and female. *FSHR* knock-out males develop normally and are fertile. Since *FSHR* is solely expressed in somatic follicular cells, transplantation of germline stem cells from juvenile *FSHR* null-mutant into recipient embryos depleted of their own PGCs but carrying a functional *FSHR* gene will restore normal oocyte development and allow production of viable eggs. These recipient females crossed with *FSHR* *-/-* males will only produce *FSHR* *-/-* offspring.

[0074] Fig. 31 is an illustration showing a germ cell transplantation method for production of functional FEM-mutant eggs (FEM: *Cyp19a1a*, and *cyp17*). We found no

defects during the generation of primordial germ cells (PGCs) and oogonia in FEM-null fish progenies obtained from heterozygous FEM mutant parents. At maturity however, FEM mutant female do not convert androgen into estrogen resulting in reprogramming of ovarian somatic supporting cells (Thecal and granulosa cells) into testicular somatic supporting cells (Leydig and Sertoli cells) and reversion of genetic female into phenotypic male. FEM deficient male develop normally and are fertile. The FEM gene(s) are normally expressed in ovarian somatic cells. To allow mass production of oocytes carrying FEM deficient gene, a germline stem cell can be isolated from juvenile FEM null-mutant and transplanted into recipient embryos depleted of their own PGCs but carrying a functional FEM gene.

Transplanted FEM $-/-$ germline cells will colonize the recipient gonad. The somatic cells surrounding the donor oocyte will produce normal amount of estrogen allowing progression of folliculogenesis and maintenance of female fate. These recipient females crossed with FEM $(-/-)$ males will produce only FEM $-/-$ offspring.

[0075] Fig. 32 is a schematic representation of a strategy to mass-produce all male sterile fish population. Double KO parents (e.g. SMS and *cyp17*) can be propagated by germ cell transplantation technique as described in Figs. 27-32. These broodstock parents only produce donor derived gametes carrying the mutated genes. Natural or artificial mating of this broodstock only produce an all-male sterile population.

[0076] Fig. 33 panels A and B show a germ cell transplantation experiment demonstrating successful colonization and production of donor derived tilapia gametes. Fig. 33 panel A show a graphical illustration of germ cell transplantation into newly hatched germ cell free tilapia larvae. Donor spermatogonial stem cells (SSCs) carrying mutations were transplanted into the peritoneal cavity of the hatchling depleted of endogenous germ cells. Two groups of SSCs were transplanted simultaneously, one carrying an in frame $\Delta 3nt$ deletion in the reference gene and a 6 nt insertion in the pigment gene (*tyr*^{*j6/i6*}) and the other carrying an out of frame 4 nt deletion in the reference gene and a 22 deletion in the pigment gene (*tyr* ^{$\Delta 22/\Delta 22$}). The 3 nt deletion is not expected to alter the gene function and thus, served as positive control. The transplanted cells migrate and colonize the genital ridges of the recipient. After attaining sexual maturation, the recipient fish gametes were collected, and their DNA analyzed by PCR fragment sizing assay utilizing PCR primers that flank the mutation region of donor derived gamete. The amplification products were sized and detected using capillary electrophoresis. The percentage of female and male recipients

producing functional eggs and sperm derived from donor cells after the transplantation of spermatogonial stem cells were provided. Fig. 33 panel B shows capillary fragment length analysis of sperm DNA from a wild type control and from a transplanted fertile tilapia. The bottom trace show only donor derived $\Delta 3\text{nt}$ and $\Delta 4\text{nt}$ deletion fragments from the reference gene, together with a 6nt insertion and $\Delta 22\text{nt}$ deletion fragment in the pigment gene. A negative control with wild-type sized gene specific fragments (268bp) for the test gene and 467nt for the *tyr* gene is shown for reference.

[0077] Fig. 34 panels A to D are illustrations showing different methods for propagating monosex sterile populations. FEM-/- and MA-/- represent femaleness and maleness null genes. SMS-/- and FLS-/- represent spermiogenesis and folliculogenesis null genes. Males and females Seedstock are produced thru steroid hormone manipulation and by germ cell transplantations (Fig. 34 panels A and B) or thru germ cell transplantation only (Fig. 34 panels C and D). A limited number of seedstock can be crossed to mass-produce millions of all-male sterile embryos (Fig. 34 panels A and C) or all-female sterile embryos (Fig. 34 panels B and D) for use in aquaculture systems.

DETAILED DESCRIPTION

[0078] Generally, the present disclosure provides a method of generating a sterile sex-determined fish, crustacean, or mollusk. The method comprises the steps of: breeding (i) a fertile hemizygous mutated female fish, crustacean, or mollusk having at least a first mutation and a second mutation with (ii) a fertile hemizygous mutated male fish, crustacean, or mollusk having at least the first mutation and the second mutation; and selecting a progenitor that is homozygous by genotypic selection, the homozygous mutated progenitor being the sterile sex-determined fish, crustacean, or mollusk. The first mutation disrupts one or more genes that specify sexual differentiation. The second mutation disrupts one or more genes that specify gamete function.

[0079] The present disclosure also provides a method of generating a sterile sex-determined fish, crustacean, or mollusk. The method comprises the step of: breeding (i) a fertile homozygous mutated female fish, crustacean, or mollusk having at least a first mutation and a second mutation with (ii) a fertile homozygous mutated male fish, crustacean, or mollusk having at least the first mutation and the second mutation to produce the sterile sex-determined fish, crustacean, or mollusk. The first mutation disrupts one or more genes

that specify sexual differentiation. The second mutation disrupts one or more genes that specify gamete function. The fertility of the fertile homozygous female fish, crustacean, or mollusk and the fertile homozygous mutated male fish, crustacean, or mollusk having been rescued.

5 **[0080]** The present disclosure also provides a method of generating a sterile sex-determined fish, crustacean, or mollusk. The method comprises the step of: breeding (i) a fertile female fish, crustacean, or mollusk having a homozygous mutation with (ii) a fertile male fish, crustacean, or mollusk having a homozygous mutation to produce the sterile sex-determined fish, crustacean, or mollusk. The mutation directly or indirectly disrupts
10 spermiogenesis, and/or that directly disrupts vitellogenesis. The fertility of the fertile female fish, crustacean, or mollusk and the fertile male fish, crustacean, or mollusk have been rescued.

[0081] The present disclosure also provides method of making a fertile homozygous mutated fish, crustacean, or mollusk that generates a sterile sex-determined fish, crustacean,
15 or mollusk. The method comprises the steps of: breeding (i) a fertile hemizygous mutated female fish, crustacean, or mollusk having at least a first mutation and a second mutation with (ii) a fertile hemizygous mutated male fish, crustacean, or mollusk having at least the first mutation and the second mutation; selecting a progenitor that is homozygous by genotypic selection; and rescuing the fertility of the homozygous progenitor. The first
20 mutation disrupts one or more genes that specify sexual differentiation. The second mutation disrupts one or more genes that specify gamete function.

[0082] The present disclosure further provides a fertile homozygous mutated fish, crustacean, or mollusk for producing a sterile sex-determined fish, crustacean, or mollusk. The fertile homozygous mutated fish, crustacean, or mollusk having at least a first mutation
25 and a second mutation, where the first mutation disrupts one or more genes that specify sexual differentiation, and the second mutation disrupts one or more genes that specify gamete function. The fertility of the fertile homozygous mutated fish, crustacean, or mollusk having been rescued.

[0083] The present disclosure further provides a fertile fish, crustacean, or mollusk
30 having a homozygous mutation for producing a sterile sex-determined fish, crustacean, or mollusk, wherein the mutation directly or indirectly disrupts spermiogenesis, and/or directly disrupts vitellogenesis, and wherein the fertility of the fertile fish, crustacean, or mollusk has been rescued.

[0084] In the context of the present disclosure, a fish refers to any gill-bearing craniate animal that lacks limbs with digits. Examples of fish are carp, tilapia, salmon, trout, and catfish. In the context of the present disclosure, a crustacean refers to any arthropod taxon. Examples of crustaceans are crabs, lobsters, crayfish, and shrimp. In the context of the present disclosure, a mollusk refers to any invertebrate animal with a soft unsegmented body usually enclosed in a calcareous shell. Examples of mollusks are clams, scallops, oysters, octopus, squid and chitons.

[0085] A sterile fish, crustacean, or mollusk refers to any fish, crustacean, or mollusk with a diminished ability to generate progeny through breeding or crossing as compared to its wild-type counterpart; for example, a sterile fish, crustacean, or mollusk may have an about 50%, about 75%, about 90%, about 95%, or 100% reduced likelihood of producing viable progeny. In contrast, a fertile fish, crustacean, or mollusk refers to any fish, crustacean, or mollusk that possesses the ability to produce progeny through breeding or crossing. Breeding and crossing refer to any process in which a male species and a female species mate to produce progeny or offspring.

[0086] A sex-determined fish, crustacean, or mollusk refers to any fish, crustacean, or mollusk progenitor in which the sex of the progenitor has been pre-determined by disrupting the progenitor's sexual differentiation pathway. In some examples, sex-determined progenitor of the same generation are monosex.

[0087] Gamete function refers to the process in which a gamete fuses with another gamete during fertilization in organisms that sexually reproduce.

[0088] A mutation that disrupts one or more genes that specify sexual differentiation refers to any genetic mutation that directly or indirectly modulates gonadal function. Directly or indirectly affecting gonadal function refers to: (1) mutating the coding sequence of one or more gonadal genes; (2) mutating a non-coding sequence that has at least some control over the transcription of one or more gonadal genes; (3) mutating the coding sequence of another gene that is involved in post-transcriptional regulation of one or more gonadal genes; or (4) a combination thereof, to modulate gonadal function. Modulating gonadal function refers to specifying that the gonad produces female gametes or produces male gametes.

Examples for when masculinization is preferred include modulating one or more genes that modulate the synthesis of androgen and/or estrogen, for example, modulating the expression of aromatase Cyp19a1a, Cyp17, or a combination thereof. Genes involved in modulating the expression of aromatase Cyp19a1a include *cyp19a1a*, *FoxL2*, *sf1* (steroidogenic factor

1), and an ortholog thereof. Genes involved in modulating the expression of Cyp17 include *cyp17l* or an ortholog thereof. Examples for when feminization is preferred include modulating one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor. Genes involved in modulating the expression of an aromatase Cyp19a1a inhibitor include *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.

[0089] Alternatively, sexual differentiation may be specified without one or more genetic mutations. Examples of non-genetic mutational methods of specifying sexual differentiation include utilizing sex reversal (hormonal manipulation) and breeding, progeny testing, androgenesis, and gynogenesis, which can produce monosex male or female populations that are homozygous XX, YY or ZZ (see for example [21]; Dunham 2004, which is incorporated by reference). In some examples according to the present disclosure, the step of breeding (i) a fertile female fish, crustacean, or mollusk having a homozygous mutation with (ii) a fertile male fish, crustacean, or mollusk having a homozygous mutation to produce the sterile sex-determined fish, crustacean, or mollusk comprises a non-genetic mutational method of specifying sexual differentiation. In some examples according to the present disclosure using Atlantic salmon, creating and crossing a neomale (XX) with a female produces a monosex progeny of females. In another example according to the present disclosure, specifying sexual differentiation can be achieved by interspecific hybridization (see for example Pruginin, Rothbard et al. 1975, Wolters and DeMay 1996, which is incorporated by reference).

[0090] A mutation that disrupts one or more genes that specify gamete function refers to any genetic mutation that directly or indirectly modulates spermiogenesis, oogenesis, and/or folliculogenesis to produce a sterile fish, crustacean, or mollusk. Directly or indirectly modulating spermiogenesis, oogenesis, and/or folliculogenesis refers to: (1) mutating the coding sequence of one or more gamete genes; (2) mutating a non-coding sequence that has at least some control over the transcription of one or more gamete genes; (3) mutating the coding sequence of another gene that is involved in post-transcriptional regulation of one or more gamete genes; or (4) a combination thereof, to produce a sterile fish, crustacean, or mollusk.

[0091] A mutation that directly or indirectly disrupts spermiogenesis, and/or directly disrupts vitellogenesis refers to any genetic mutation that directly or indirectly modulates spermiogenesis, and/or directly disrupts vitellogenesis to produce a sterile fish, crustacean, or mollusk. Directly or indirectly modulating spermiogenesis refers to: (1) mutating the coding

sequence of one or more gamete genes involved in spermiogenesis ; (2) mutating a non-coding sequence that has at least some control over the transcription of one or more gamete genes involved in spermiogenesis; (3) mutating the coding sequence of another gene that is involved in post-transcriptional regulation of one or more gamete genes involved in

spermiogenesis; or (4) a combination thereof, to produce a sterile fish, crustacean, or mollusk. Directly modulating vitellogenesis refers to: (1) mutating the coding sequence of one or more gamete genes involved in vitellogenesis; (2) mutating a non-coding sequence that has at least some control over the transcription of one or more gamete genes involved in vitellogenesis; or (3) a combination thereof, to produce a sterile fish, crustacean, or mollusk.

[0092] Examples for when producing a sterile male fish, crustacean, or mollusk is preferred include modulating one or more genes that modulate spermiogenesis. Examples of one or more genes that modulate spermiogenesis may cause globozoospermia, sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof. Examples of genes that cause globozoospermia include *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof. Examples for when producing a sterile female fish, crustacean, or mollusk is preferred include modulating one or more genes that modulate oogenesis, folliculogenesis, or a combination. Examples of one or more genes that modulate oogenesis include one or more genes that modulate the synthesis of estrogen. Examples of one or more genes that modulate the synthesis of estrogen include FSHR or an ortholog thereof. Examples of one or more genes that modulate folliculogenesis include one or more genes that modulate the expression of vitellogenins. Examples of one or more genes that modulate the expression of vitellogenins include *vtgs* or an ortholog thereof. Examples of mutations that directly or indirectly disrupt spermiogenesis are mutations in *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, or an ortholog thereof. Examples of mutations that directly disrupts vitellogenesis are mutations in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; cytochrome p450, family 1, subfamily a; Zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.

[0093] A mutation may be any type of alteration of a nucleotide sequence of interest, for example, nucleotide insertions, nucleotide deletions, and nucleotide substitutions.

[0094] Rescuing sterility or fertility refers to any process in which a sterile fish, crustacean, or mollusk is converted into a fertile fish, crustacean, or mollusk. In some examples, an aromatase inhibitor is provided to the sterile fish, crustacean, or mollusk to restore fertility. In

other examples, germline stem cell transplantation of the sterile fish, crustacean, or mollusk restores fertility. Germline stem cell transplantation refers to any process in which reproductive stem cells from a sterile fish, crustacean, or mollusk is transplanted into a fertile fish, crustacean, or mollusk and restores fertility. In some examples according to the present disclosure, the germline stem cell transplantation is a process comprising: obtaining a germline stem cell from a sterile homozygous male fish, crustacean, or mollusk having at least the first mutation and the second mutation or a germline stem cell from a sterile homozygous female fish, crustacean, or mollusk having at least the first mutation and the second mutation; and transplanting the germline stem cell into a germ cell-less recipient male fish, crustacean, or mollusk, or into a germ cell-less recipient female fish, crustacean, or mollusk. A recipient male or female fish, crustacean, or mollusk is any embryo depleted of their own germ cells but carrying functional copies of genes targeted that specify sexual differentiation and gamete function. Alternatively, the germ cell depleted recipient can be a juvenile or adult fish carrying functional copies of genes targeted. Preferably, the recipient species is the same as the donor species (allogenic recipient) but other species may be used (Xenogeneic recipient). The recipient after transplantation is a chimeric fish, crustacean or mollusk with normal somatic cells but a mutant germline. These chimeric recipients restore the normal sex ratio and/or sterility as they possess functional somatic gene(s). A germ cell-less recipient may be created using ploidy manipulation, hybridization strategies, or exposure to high levels of sex hormones. Exposure of juvenile aquatic species to high levels of sex hormones may result in sterility in the exposed animals. This technique has been demonstrated (Hunter et al, 1982; Solar et al, 1984; Piferrer et al, 1994), but has not been used at a commercial scale. While the technique may be effective in creating sterile fish, it has never been demonstrated effective at inducing sterility in 100% of the treated fish. Treated fish may be suitable for research, or as recipients for germ cell transfer, but the technique may not be adequate for creating sterile fish for commercial farming (see also Hunter, G.A., E.M. Donaldson, F.W. Goetz, and P.R. Edgell. 1982. Production of all-female and sterile Coho salmon, and experimental evidence for male heterogamety. Transactions of the American Fisheries Society 111: 367-372; Piferrer, F, M Carillo, S. Zanuy, I.I. Solar, and E.M. Donaldson. 1994. Induction of sterility in Coho salmon (*Oncorhynchus kisutch*) by androgen immersion before first feeding. Aquaculture 119: 409-423; and Solar, I., E.M. Donaldson, and G.A. Hunter. 1984. Optimization of treatment regimes for controlled sex

differentiation and sterilization in wild rainbow trout (*Salmo gairdneri* Richardson) by oral administration of 17 α -methyltestosterone. Aquaculture 42: 129-139.

[0095] In some examples, the germline stem cell transplantation is a process comprising: obtaining a spermatogonial stem cell from a sterile homozygous male fish, crustacean, or mollusk or a oogonial stem cell from a sterile homozygous female fish, crustacean, or mollusk, and transplanting the spermatogonial stem cell into the peritoneal cavity of a germ cell-less embryo or into a germ cell-less differentiated testis or ovary of a fish, crustacean, or mollusk. Optionally, in addition to germline stem cell transplantation, an exogenous sex steroid is provided to the sterile fish, crustacean, or mollusk, for example, estrogen to restore fertility. In other examples, an aromatase inhibitor is provided to the sterile fish, crustacean, or mollusk to restore fertility.

[0096] Fig. 1 illustrates a flowchart according to the present disclosure of how to make a male and female broodstock, i.e. a fertile homozygous mutated male and female fish, crustacean, or mollusk for use in producing a sterile sex-determined fish, crustacean, or mollusk.

[0097] Fig. 1 illustrates genetic pathways governing sex differentiation and gametogenesis and gene KO strategies to produce monosex sterile populations.

[0098] One or more mutations in the gene *cyp19a1a*, *Foxl2*, or a combination thereof, results in low or decreased estrogen expression causing testis formation and the production of a male fish, crustacean, or mollusk. Similarly, one or more mutations in the gene *cyp17* results in low or decreased estrogen and androgen expression producing a male fish, crustacean, or mollusk. One or more additional mutations in a gene that disrupts spermiogenesis (SMS) causes the male fish, crustacean, or mollusk to be sterile. Accordingly, a sterile homozygous mutated male fish, crustacean, or mollusk is produced.

[0099] In an additional step used to propagate the line, the fertility of the sterile homozygous mutated male fish, crustacean, or mollusk may be rescued with treatment of estrogen. Following treatment, a fertile homozygous mutated female fish, crustacean, or mollusk is generated. In this sex reversal process, the phenotypic female is carrying the one or more mutations disrupting spermiogenesis and should be fertile, and oocytes carrying the one or more mutations disrupting spermiogenesis should be produced and allow for propagation of the line. Alternatively, and as described in Example 10, the fertility of the sterile homozygous mutated male fish, crustacean, or mollusk may be rescued by implanting a germ cell from the sterile homozygous mutated male fish, crustacean, or mollusk into a

fertile wild-type male testis cell to generate a fertile homozygous mutated male fish, crustacean, or mollusk, which allows for propagation of the line.

[00100] On the flip side of Fig. 1, one or more mutations in the gene *Gsdf*, *Dmrt1*, or a combination thereof, results in inactivation of Cyp19a1a inhibitors and causes high or increased estrogen expression resulting in ovarian formation and the production of a female fish, crustacean, or mollusk. One or more additional mutations in a gene that modulates oogenesis, folliculogenesis (FLS), or a combination thereof causes the female fish, crustacean, or mollusk to be sterile. Accordingly, a sterile homozygous mutated female fish, crustacean, or mollusk is produced.

[00101] In an additional step used to propagate the line, the fertility of the sterile homozygous mutated female fish, crustacean, or mollusk may be rescued with treatment of an aromatase inhibitor. Following treatment, a fertile homozygous mutated male fish, crustacean, or mollusk is generated. In this sex reversal process, the phenotypic male is carrying the one or more mutations disrupting oogenesis, folliculogenesis, or a combination and should be fertile, and sperm carrying the one more mutations disrupting oogenesis, folliculogenesis, or a combination should be produced and allow for propagation of the line. Alternatively, and as described in Example 10, the fertility of the sterile homozygous mutated female fish, crustacean, or mollusk may be rescued by implanting a germ cell from the sterile homozygous mutated female fish, crustacean, or mollusk into a fertile wild-type female ovary cell to generate a fertile homozygous mutated female fish, crustacean, or mollusk, which allows for propagation of the line.

EXAMPLES

[00102] Example 1 – Materials and Methods

[00103] Animal used and ethical statement: All experiments complied with US regulations ensuring animal welfare and animal husbandry procedures were performed according to IACUC-approved animal protocol CAT-004. Tilapia (*Oreochromis niloticus*) lines used in this study are derived from a Brazilian strain obtained from a US commercial producer.

[00104] Generation of nucleases and strategies: Generation of F0 mutants: Tilapia orthologs of the *cyp17*, *Cyp19a1a*, *Tjp1a*, *Csnk2a2*, *Hiat1*, *Smap2*, *Gopc*, *Gsdf*, *Dmrt1*,

FSHR and vitellogenin genes (*VtgAa* and *VtgAb*) were identified in silico from genomic databases.

[00105] To create DNA double strand breaks (DSBs) at specific genomic site, we used engineered nucleases. In most applications, a single DSB was produced in the absence of a repair template, leading to the activation of the non-homologous end joining (NHEJ) repair pathway. The NHEJ can be an imperfect repair process, generating insertions or deletions (indels) at the target site. Introduction of an indel can create a frameshift within the coding region of the gene resulting in abnormal protein products with an incorrect amino acid sequence. To enhance the frequency of generating null mutations in the gene of interest, we targeted 2 separate exons simultaneously apart from those targeting *cyp17*. Alongside the gene of interest, we co-targeted a pigmentation gene to serve as a mutagenesis selection marker. Typically, mutagenic frequency between the pigment gene and the gene of interest are correlated. Thus, embryos showing complete lack of pigmentation (albino phenotype) were preferentially selected compare to mosaic pigment phenotype (partial gene inactivation). To confirm functionality of the newly designed nuclease, five albino embryos from each treated batch were quantitatively assayed for genome modifications at the loci of interest by PCR fragment analysis. Treated embryos of the same batch were eliminated if all five embryos tested showed no indels at the targeted loci. Furthermore, we preferentially raised batches of embryos in which mutations are produced at the one or two cell stage, (i.e. detection of 2 or 4 mutant alleles per targeted loci by fragment analysis assay).

[00106] The template DNA coding for the engineered nuclease were linearized and purified using a DNA Clean & concentrator-5 column (Zymo Research). One microgram of linearized template was used to synthesize capped RNA using the mMESSAGE mMACHINE T3 kit (Invitrogen), purified using Qiaquick (Qiagen) columns and stored at -80° in RNase-free water at a final concentration of 800 ng/μl.

[00107] Embryo injections: Embryos were produced from *in vitro* fertilization. Approximately 10 nL total volume of solution containing the programmed nucleases were co-injected into the cytoplasm of one-cell stage embryos. Injection of 200 embryos typically produce 10-60 embryos with complete pigmentation defect (albino phenotype). Embryo/larvae survival was monitored for the first 10-12 days post injection.

[00108] Selection of founders: A minimum of 10 albino embryos were raised to 3 months of age and quantitatively assayed for genome modifications by fluorescence PCR fragment analysis (see Table 1 for gene specific genotyping primers columns 8 and 11). We

preferentially selected founders in which mutations were produced at the one or two cell stage (detection of 2 or 4 mutant alleles per target loci by fragment analysis (Fig. 2).

[00109] F1 genotyping: The selected founders were outcrossed with wild-type lines. Their F1 progeny were raised to 2 months of age, anesthetized by immersion in 200mg/L MS-222 (tricaine) and transferred onto a clean surface using a plastic spoon. Their fin was clipped with a razor blade, and placed onto a well (96 well plate with caps). Fin clipped fish were then placed in individual jars while their fin DNA was analyzed by fluorescence PCR. In brief, 60 µl of a solution containing 9.4% Chelex and 0.625mg/ml proteinase K was added to each well for overnight tissue digestion and gDNA extraction in a 55°C incubator. The plate was then vortexed and centrifuged. gDNA extraction solution was then diluted 10× with ultra-clean water to remove any PCR inhibitors in the mixture. Typically, we analyzed 80 juveniles/founder to select and raised batches of approximately 20 juveniles carrying identical size mutations.

[00110] Fluorescence PCR (see Fig. 2): PCR reactions used 3.8 µL of water, 0.2 µL of fin-DNA and 5 µL of PCR master mix (Quiagen Multiplex PCR) with 1 µl of primer mix consisting of the following three primers: the Labeled tail primer with fluorescent tag (6-FAM, NED), amplicon-specific forward primer with forward tail (SEQ ID NO: 117: 5' - TGTAACGACGGCCAGT-3' and SEQ ID NO: 118: 5' -TAGGAGTGCAGCAAGCAT-3') amplicon-specific reverse primer (Fluorescent PCR gene-specific primers are listed in Table 1). PCR conditions were as follows: denaturation at 95°C for 15 min, followed by 30 cycles of amplification (94°C for 30 sec, 57°C for 45 sec, and 72°C for 45 sec), followed by 8 cycles of amplification (94°C for 30 sec, 53°C for 45 sec, and 72°C for 45 sec) and final extension at 72°C for 10 min, and an indefinite hold at 4°C.

[00111] One-two microliters of 1:10 dilution of the resulting amplicons were resolved via capillary electrophoresis (CE) with an added LIZ labeled size standard to determine the amplicon sizes accurate to base-pair resolution (Retrogen Inc., San Diego). The raw trace files were analyzed on Peak Scanner software (ThermoFisher). The size of the peak relative to the wild-type peak control determines the nature (insertion or deletion) and length of the mutation. The number of peak(s) indicate the level of mosaicism. We selected F0 mosaic founder carrying the fewest number of mutant alleles (2-4 peak preferentially).

[00112] The allele sizes were used to calculate the observed indel mutations. Mutations that are not in multiples of 3 bp and thus predicted to be frameshift mutations were selected for further confirmation by sequencing. Mutations of size greater than 8bp but

smaller than 30bp were preferentially selected to ease genotyping by QPCR melt analysis for subsequent generations. For sequence confirmation, the PCR product of the selected indel was further submitted to sequencing. Sequencing chromatography of PCR showing two simultaneous reads are indicative of the presence of indels. The start of the deletion or insertion typically begins when the sequence read become divergent. The dual sequences were carefully analyzed to detect unique nucleotide reads. The pattern of unique nucleotide read is then analyzed against series of artificial single read patterns generated from shifting the wild type sequence over itself incrementally.

[00113] QPCR genotyping of F1 and F2 generations: Real-time qPCR was performed on a ROTOR-GENE RG-3000 REAL TIME PCR SYSTEM (Corbett Research). 1- μ L genomic DNA (gDNA) template (diluted at 5-20ng/ μ l) was used in a total volume of 10 μ L containing 0.15 μ M concentrations each of the forward and reverse primers and 5 μ L of QPCR 2x Master Mix (Apex Bio-research products). qPCR primers used are presented in Table 2 (Genotyping RT-PCR primers columns 11- 14). The qPCR was performed using 40 cycles of 15 seconds at 95°C, 60 seconds at 60°C, followed by melting curve analysis to confirm the specificity of the assay (67°C to 97°C). In this approach, short PCR amplicons (approx 120–200 bp) that include the region of interest are generated from a gDNA sample, subjected to temperature-dependent dissociation (melting curve). When induced indels are present in hemizygous gDNA, heteroduplex as well as different homoduplex molecules are formed. The presence of multiple forms of duplex molecules is detected by Melt profile, showing whether duplex melting acts as a single species or more than one species. Generally, the symmetry of the melting curve and melting temperature infers on the homogeneity of the dsDNA sequence and its length. Thus, homozygous and wild type (WT) show symmetric melt curved that are distinguishable by varied melting temperature. The Melt analysis was performed by comparison with reference DNA sample (from control wild type DNA) amplified in parallel with the same master mix reaction. In short, variation in melt profile distinguishes amplicons generated from homozygous, hemizygous and WT gDNA (see Fig. 3).

[00114] Assessment of sterility in males: The volume of strippable sperm and sperm density was measured from 10 males (5 months of age) for each genotype. Sperm were counted using a Neubauer hemocytometer slide, as well as by spectrophotometry (optical density (O.D) at 600nm) of serially diluted samples. Sperm motility was measured in terms of percent motile spermatozoa in field of view [4]. Morphology of the sperm cells stained with eosin-nigrosin was analyzed under light microscopy at 400x. Fertilization capacity of sperm

was assayed by in vitro fertilization of wild type eggs from 3 different females at the optimal sperm to egg ratio (100 eggs for 5.10^6 spermatozoa). Wild type egg quality was tested in parallel using sperm from WT males. Fertilization rates was expressed as a percentage of surviving embryos to total eggs collected at 24hrs post fertilization. The mean values
5 obtained from these studies was compared across mutant genotypes using an unpaired t-test.

[00115] Assessment of sterility in females: We recorded the body weight of all fish sampled. A minimum of six females for each genotype was dissected at 4 and 6 months of age and their gonads photographed in situ before dissection. The mean total gonadosomatic
10 index was statistically compared across all genotypes (unpaired T-test). Survival of eggs, embryos and larvae produced from a minimum of six mutant females outcrossed with wild-type males were statistically analyzed (unpaired T-test) and compared to controls (wild-type females crossed with mutant males).

[00116] Donor cell isolation and germ cell transplantation: Germ cell stem cells were
15 harvested from the gonads of 3-4 months old fish (~ 50-70g) through enzymatic digestion as described by Lacerda [5]. In brief, the freshly isolated gonads were minced and incubated in 1 ml of 0.5 % trypsin (Worthington Biochemical Corp., Lakewood, NJ) in PBS (pH 8.2) containing 5 % fetal bovine serum (Gibco Invitrogen Co., Grand Island, NY) and 0.05 % DNase I (Roche Diagnostics, Mannheim, Germany) for 3-4 h at 25 °C. During incubation,
20 gentle pipetting was applied to physically disrupt any remaining intact portions of the gonads. The resulting cell suspension was filtered through a nylon screen with a pore size of 42 µm (N-No.330T; Tokyo Screen Co. Ltd., Tokyo, Japan) to remove any undissociated cell clumps and then resuspended in L-15 medium (Gibco Invitrogen Co.) before storage on ice until transplantation.

[00117] Germ cell-free recipient larvae (5-7dpf) were anesthetized with 0.0075 % ethyl
25 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich Inc.) and transferred to a Petri dish coated with 2 % agar. Cell transplantation was performed by injecting approximately 15,000 testicular cells into the peritoneal cavity of approximately 80 larvae progeny from *Elavl2* hemizygous mutant parents. Alternatively, PGC-free embryos were obtained from a cross
30 between MSC homozygous female and wild type male [6]. After transplantation, recipient larvae were transferred back to aerated embryo hatching water and raised to adulthood.

full gene name	Tilapia homolog gene (alias)	NCBI & Ensembl Accession #	Targeted exon								
				Label	Forward primer	SEQ ID NO	Forward primer	Reverse primer	SEQ ID NO	Reverse primer	Amplicon size (bp)
cytochrome P450, family 17, subfamily A, polypeptide 1	Cyp17	Acc: ZDB-GENE-040213-2	1	FAM	ttgaagtgctcactataaag	SEQ1	ttgaagtgctcactataaag	1	SEQ2	TGGTTGATGACAAATCACA	357
		ENSONG50000000000108	1	FAM	ttgaagtgctcactataaag	SEQ1	ttgaagtgctcactataaag	1	SEQ2	TGGTTGATGACAAATCACA	357
		Acc:NM_00179586	1	NED	tggtacacatcacatcccttc	SEQ3	tggtacacatcacatcccttc	1	SEQ4	AGCAGACAGACGAGCAGTATCAG	169
cytochrome P450, family 19, subfamily A, polypeptide 1a gene	Cyp19a1a	ENSONG50000000000155	9	FAM	TGATGGAGAGCTTCACTACGAA	SEQ5	TGATGGAGAGCTTCACTACGAA	9	SEQ6	GTTCCAGGTTAAATTTGATTG	186
Tight junction protein 1a	Tjp1a	Acc: ZDB-GENE-031001-2	15	NED	ggcgatttgctgacattttac	SEQ7	ggcgatttgctgacattttac	15	SEQ8	acatttacccctGAGAATCTGG	216
		ENSONG50000000000621	17	FAM	GAAAAAGGATGgtgaggaggagac	SEQ9	GAAAAAGGATGgtgaggaggagac	17	SEQ10	GAGTGTGTCTACCAACACGGA	239
Casein Kinase II subunit alpha	Csnk2a2	Acc: 100690548	1	FAM	gtaattggaaggcgtgtgaagtc	SEQ11	gtaattggaaggcgtgtgaagtc	1	SEQ12	CAGTTTGGCACATGAGCATCGTA	153
		ENSONG5000000000015898	2	NED	ATGCTCATGTGCCAAACTG	SEQ13	ATGCTCATGTGCCAAACTG	2	SEQ14	cCTTCAGGATTTTACCACCACT	222
Hippocampus abundant transcript 1a	Hlat1	Acc:100705862	4	FAM	tactgacatcacagcagcgctt	SEQ15	tactgacatcacagcagcgctt	Intron4-5	SEQ16	cagcactgagcgcgtgactttat	211
		ENSONG5000000000018605	6	NED	TGGAGCCTACCTGTCTCTGAG	SEQ17	TGGAGCCTACCTGTCTCTGAG	6	SEQ18	tactcacAGCGAAGGGGTCT	182
small ArfGAP2	Smap2	Acc: ZDB-GENE-060503-374	2	NED	gtccctcgtgtgaagctctc	SEQ19	gtccctcgtgtgaagctctc	2	SEQ20	aagactctgcagctCGGACTTGGT	211
		ENSONG500000000004622	9	FAM	AGAGAGGGCACAGTCAAGAAAC	SEQ21	AGAGAGGGCACAGTCAAGAAAC	10	SEQ22	TTGATATCCCATTTGGTTTCAT	226
Gdfr-associated PDZ and coiled-coil motif containing gene	Gopc	Acc:100692751	1	NED	tttaacgggtgtggacagagtt	SEQ23	tttaacgggtgtggacagagtt	1	SEQ24	AGATCCACATCCAGAAAGCCT	207
		ENSONG500000000001868	2	FAM	tgccctcttaaacacactta	SEQ25	tgccctcttaaacacactta	2	SEQ26	CTCAGCTTGGCCTTGCTTGACAT	207
doublesex and mab-3 related transcription factor 1	Dmrt1	Acc: ZDB-GENE-050511-1	1	NED	tgpcagagaccCATGAGCCAG	SEQ27	tgpcagagaccCATGAGCCAG	1	SEQ28	AGACACGTATCCGTGATTCTAC	135
		ENSONG5000000000014201	3	FAM	cctctacatcgttggtctac	SEQ29	cctctacatcgttggtctac	3	SEQ30	GGGTTTCCAGCAGGAGGTGAGA	140
growth differentiation factor 6-B-like	Gsdf	Acc:100710262	2	NED	ttatgttcagGTGCCAAGGTG	SEQ31	ttatgttcagGTGCCAAGGTG	2	SEQ32	TGGCTGTGTGTGAGAAACGATGCTG	156
		ENSONG500000000007633	4	FAM	agATCTGGGCTGGGACA	SEQ33	agATCTGGGCTGGGACA	4	SEQ34	tgtaactatacCTGTGTGTGG	145
Follicle stimulating hormone receptor	FSHR	Acc: ZDB-GENE-020423-5	11	NED	ttttctcgttcgtctgc	SEQ35	ttttctcgttcgtctgc	11	SEQ36	AAAGAGCTGAATAGGAGGAAGTT	137
		ENSONG5000000000015917	15	FAM	CATCTTGGCGTTCCTCTGTGT	SEQ37	CATCTTGGCGTTCCTCTGTGT	15	SEQ38	CTTGAGGGCAGCTGAGATGCG	181
Vitellogenin Aa	VtgAa	Acc: ZDB-GENE-001201-1	7	NED	GCAATCTTGATGCTCCTTGAC	SEQ39	GCAATCTTGATGCTCCTTGAC	7	SEQ40	CTGAGACTCTATGCTGTTGATA	163
		ENSONG500000000007395	22	FAM	AGAAGATCATGAAACATCATCAG	SEQ41	AGAAGATCATGAAACATCATCAG	22	SEQ42	GACTTTGTGAGCAGTTGCATCAA	227
		Acc: ZDB-GENE-001201-1	5	NED	ttttgttgatcgtTCTGGAG	SEQ43	ttttgttgatcgtTCTGGAG	5	SEQ44	gctcttacAGCTTCACAATCAT	183
Vitellogenin Ab	VtgAb	ENSONG500000000007386	22	FAM	CTTCTGAGCACTGCAITTAG	SEQ45	CTTCTGAGCACTGCAITTAG	22	SEQ46	AGACTTTGTGAGCTAGAG	227

Table 1: Primers

full gene name	Tilapia homolog gene (alias)	Genotyping RT-PCR primers						Reverse primer sequence	Amplicon size (bp)
		Forward primer exon	SEQ ID NO	Forward primer sequence	Reverse primer exon	SEQ ID NO			
cytochrome P450, family 17, subfamily A, polypeptide 1	Cyp17	1	SEQ47	GAACCAAAACCCCTCTGTCTACTG	1	SEQ48	GTAATTCACCTCCGCAGGCTCAG	184	
		1	SEQ47	GAACCAAAACCCCTCTGTCTACTG	1	SEQ48	GTAATTCACCTCCGCAGGCTCAG	184	
		1	SEQ49	ggcgATGAATCCTGTAG	1	SEQ50	ATGCATTTGAGGTACACAGA	63	
cytochrome P450, family 19, subfamily A, polypeptide 1a gene	Cyp19a1a	9	SEQ5	TGATGGAGAGCTTCATCTACGAA	9	SEQ6	GTCCAGGTTAAATTGATTG	186	
		15	SEQ51	GTTCAAGAAAGGGAGAGAGT	15	SEQ52	AAAAATTCACACATCGTT	61	
Tight junction protein 1a	Tjp1a	17	SEQ53	tgcttggtccagTGTATC	17	SEQ54	AATGCGTTTCGAATGTAGAA	71	
Casein Kinase II subunit alpha	Csnk2a2	5'UTR	SEQ11	gtaattagaaggcggtgaaggic	1	SEQ12	CAGTTTGGCACATGAGCATCGTA	153	
		1	SEQ13	ATGCTCATGTGCCAAACTG	2	SEQ14	cCTTCAGGATTTTCACCACCACCT	222	
Hippocampus abundant transcript 1a	Hiat1	Intron3-4	SEQ15	tactgacacatcagcagcgict	Intron4-5	SEQ16	cagcactgagcgcgicgatttct	211	
		6	SEQ55	CATCTGCTTCATCCTGGTGCTG	6	SEQ18	tactaacAGCCAAAGGGGTCT	110	
small ArfGAP2	Smap2	2	SEQ56	AATTTGGGCATCTTCATCTGTAT	2	SEQ57	GACAGACTTGACCTTGGAGATG	81	
		9	SEQ21	AGAGGAGGGCACAGTCAAGAAAC	10	SEQ22	TTGGATATCCCATTTTGGTTTCAT	226	
Golgi-associated PDZ and coiled-coil motif containing gene	Gopc	1	SEQ58	ATGTCCTGCTTCGACTGGATGC	1	SEQ59	GCCATCGAAACATGSGACATACTG	76	
		Intron1-2	SEQ25	tgccctcttaaaaccactta	2	SEQ26	CTCAGCTTGGCCTTGCTTGACAT	207	
doublesex and mab-3 related transcription factor 1	Dmrt1	5'UTR-1	SEQ27	ttgccagagaccATGAGCCAG	1	SEQ28	AGACACGTATCCGTGATTTTCTAC	135	
		Intron2-3	SEQ29	ctcttcactctgtgtgtctac	3	SEQ30	GGGTTTCCAGCAGGAGGT CAGA	140	
growth/differentiation factor 6-B-like	Gsdf	2	SEQ31	ttaigttagGTGCCAAGGTG	2	SEQ32	TGGCTGTGTGAGAAACGATGCTG	156	
		4	SEQ33	agATCTGGGCTGGGACA	4	SEQ34	tgttaactatacCTGTGTGTTGG	145	
Follicle stimulating hormone receptor	FSHR	Intron10-11	SEQ35	ttttctcgcgtctgtctgtgc	11	SEQ36	AAAGAGCTGAATAGGAGGAAAGTT	137	
		15	SEQ37	CATCTTGGCGTTTCTTCTGTGT	15	SEQ38	CTTGAGGGCAGCTGAGATGGC	181	
Vitellonin Aa	Vrg4a	7	SEQ39	GCAATCCTTGATGCTCCTTGAC	7	SEQ40	CTGAGACTCTATGTGTTGATA	163	
		22	SEQ41	AGAAGATCATCAAAACACATCACG	22	SEQ42	GACTTGTGAGCAGTTGCATCAA	227	
Vitellonin Ab	Vrg4b	5	SEQ43	tttttgatctagTCTGGAG	5	SEQ44	gdtctaacGCTTCAACAATCAT	183	
		22	SEQ45	CTTCTGGACCAAGTCATTGAG	22	SEQ46	AGACTTGTGGAGCTAGAG	227	

Table 2: Primers

[00118] Example 2 - Use of a gene editing tool to induce double-allelic knockout in Tilapia F0 generation

[00119] We have independently targeted two genes involved in pigmentation, namely the genes encoding tyrosinase (*tyr*) [2] and the mitochondrial inner membrane protein MpV17 (*mpv17*) (Krauss, Astrinides et al. 2013) [8]. We found that 50% and 46% of all injected embryos showed a high degree of mutation at the *tyr* and *mpv17* loci respectively (Fig. 4). Loss-of-function alleles cell-autonomously lead to unpigmented melanophores in the embryo body (Fig. 4 panel B) and in the retinal pigment epithelium (Fig. 4 panel C), producing embryonic phenotypes ranging from complete to partial loss of melanine and iridophore pigmentation that are readily identifiable against wild type phenotype (Fig. 4 panels A and C). Embryos showing a complete lack of pigmentation (10-30% of treated fish) were raised to 3 months of age and all lacked wild type *tyr* and *mpv17* sequences. These fish display transparent and albino phenotypes (Fig. 4 panel D), indicating that functional studies can be performed in F0 tilapia.

[00120] Example 3 – Multi-gene targeting in Tilapia

[00121] We tested whether multiple genomic loci can be targeted simultaneously and whether mutagenic efficiency measured at one loci is predictable of mutation at other loci in the tilapia genome. To test our hypothesis, we co-targeted *tyr* and *Dead-end1 (dnd)*. Dnd is a PGC-specific RNA binding protein (RBP) that maintains germ cell fate and migration ability [3]. Following injection of programmed nucleases, we found that mutations in both gene targets *tyr* and *dnd* were highly correlated. Approximately 95% of abino (*tyr*) mutants also carried mutations at the *dnd* loci, demonstrating the suitability of the pigmentation defect as a selection marker (Fig. 5 panel A). Upon further analysis of the gonads from 10 albino fish, 6 were translucent germ cell-free testes (Fig. 5 panel B). Expression of *vasa*, a germ cell specific marker strongly expressed in wild type testes, was strikingly not detected in *dnd* mutant testes. This result indicates that zygotic *dnd* expression is necessary for the maintenance of germ cells and that maternally contributed *dnd* mRNA and/or protein cannot rescue the zygotic loss of this gene.

[00122] Example 4 – Producing germ cell free gonads

[00123] We produced sterile tilapia by implementing transient silencing of the *dnd* gene in embryos via microinjection of antisense modified oligonucleotides (dnd-Morpholino as well as dnd-AUM oligos). We produced sterile tilapia following bath-immersion of embryos exposed to a small molecule initially discovered in a screen to ablate PGCs in zebrafish [10].

5 We further generated sterile tilapia using gene knockout strategies as describe for *dnd* in the section above (Example 3). We also found that breeding *Elavl2* heterozygous mutant lines and selecting the homozygous-mutant progeny allow production of germ cell-free adult of both sexes (Fig. 6). These gene KO approaches, along with others mentioned above, produce infertile tilapia, displaying either female urogenital papillae (UGP) and a string-like
10 gonad or male UGP and a translucent tube-like gonad (Fig. 6). These methodologies, however, are not viable solutions for commercial production of sterile fish because only a 25% of progeny from heterozygous mutant parent are sterile and other knock down approaches are insufficiently robust and reliable to ensure complete sterilization of each fish in every batch treatment. In the present invention mass-production of sterile fish rely on
15 broodstock surrogate parents that start as germ cell free fish, then receive germline stem cell transplant and ultimately produce donor derived sperm or eggs. Sterilization of these recipient broodstock in our approach preferentially use knockout strategies (e.g. *elavl2*-null progeny from heterozygous parents; see Example 11). Knockout strategies other than *Elavl2* may be used to produce sterile recipient, including a null mutant for *dead-end1*, *vasa*,
20 *nanos3* or *piwi*-like genes. Such a knockout recipient ensures that only donor derived gametes are produced after transplantation. Depending on the species of fish, crustacean or mollusk, alternative strategies to produce sterile recipient can be used, including hybridization and triploidization (Benfey et al.,1984; Felip et al., 2001).

25 **[00124] Example 5 – Cyp17I is necessary for female development in Nile tilapia**

[00125] The balance of steroidogenic hormones may govern sex differentiation and maturation of the gonads in teleost fish, with estrogen playing an essential role for female differentiation. However, gonadal differentiation and gametogenesis in the absence of both androgen and estrogen has not been investigated. To this end, we produced an *in vivo* tilapia
30 model lacking the *cyp17I* gene (hereafter referred to as *cyp17*).

[00126] In Nile tilapia, this enzyme is exclusively expressed in Theca cells and produces androgens in response to luteinizing hormone (LH) [13]. Androgens are then converted into estrogen by follicle stimulating hormone (FSH)-induced aromatase (*cyp19a1a*)

in the neighboring granulosa cells of growing follicles. Accordingly, *cyp17* loss of function (via gene editing knockout) should simultaneously block androgen and estrogen synthesis.

Consistent with this model, we found that 20 of the 22 selected F0 albino/*cyp17* mutants developed as phenotypic males, which all displayed minuscule UGP (Fig. 8 panel C). The atrophy of the genitalia is not unexpected given the relationship between androgens and genital papilla [14]. These F0 males remained fertile however, possibly due to a partial loss of function phenotype in the mosaic F0 context. For a complete phenotypic analysis, we generated individuals carrying the same null $\Delta 16$ -*cyp17* mutation in all cells of their body by selective breeding of F1 progeny (Fig. 7). Intercrossing between F1 heterozygotes (*cyp17*+/-) produced ~360 F2 progeny and a typical Mendelian segregation of wildtype (n = 110; *cyp17*+/+), hemizygous (n = 159; *cyp17*+/-) and homozygous animals (n = 91; *cyp17*-/-). A total of 155 F2 progeny were sexed at 6 months of age, based on the morphological characteristics of their urogenital papillae (UGP). We found that all 33 homozygotes fish developed as phenotypic males, with atrophic UGP (Fig. 8 panel A). Our results indicate that Cyp17 is indispensable for female development.

[00127] We then quantified the amount of free plasma testosterone by ELISA in wild-type and *cyp17*-mutant tilapia. A mean of 86pg/mL of testosterone was measured in wild-type (*cyp17*+/+) and heterozygous mutant tilapia (*cyp17* +/-) whereas no detectable level of testosterone was found in homozygous mutant (*cyp17* -/-) (Fig. 8 panel B). This confirm the essential role of this enzyme in androgens production.

[00128] We further examined the morphology and functionality of the gonads in Cyp17 deficient fish. Sibling 5-month-old males *cyp17*+/+ , *cyp17*+/- and *cyp17*-/-, of identical size were dissected and all organs except the gonads were removed from their body cavity (Fig. 9 panel A). WT and hemizygous mutants showed pink colored testes typically found in sexually mature fish, while homozygous mutants exhibited translucent testes (Fig. 9 panels A and B). Furthermore, mutant testes were 50% smaller than controls (Fig. 9 panel D) and strippable milt volume was less than 20% of WT (Fig. 9 panel E). In addition, sperm concentration in homozygous *cyp17* mutants was reduced 20 and 6-fold at 5 and 6 months of age respectively (Fig. 9 panel F). We found no defect in sperm morphology, motility or functionality, as evidenced by the successful fertilization of WT eggs with milt collected from 10 null mutants.

[00129] The fact that *cyp17* null mutants can undergo spermatogenesis suggests that androgens are not strictly necessary for this process in Nile tilapia. Thus, a loss of function

mutation in this gene may not be sufficient to produce all-sterile male populations. To identify the regulatory mechanism responsible for the formation of functional spermatozoa, we investigated additional genes associated with male infertility in mammals.

5 **[00130] Example 6 – Gene candidates for targeting spermiogenesis**

10 **[00131]** There are significant differences in the morphology and function of mammalian and fish sperm. In particular, fish sperm lack an acrosome and are immotile in seminal fluid, while mammalian spermatozoa possess an acrosome (a key organelle necessary to penetrate the egg chorion) and is mobile in seminal fluid. Globozoospermia is a rare and severe form of human infertility characterized by sperm defective in both morphology and function. Fish models of this disease, however, have not been developed, likely because fish sperm lack an acrosome. Using genomic databases, we identified in silico the tilapia orthologs of the following mammalian genes: *Csnk2a2* [15] *Gopc* [16, 17], *Hiat1* [18], *Tjp1a*, *Smcp2* [21]. To explore their function in tilapia, we targeted 2 separate exons for each gene (see Figs. 10 to 14). A pigmentation gene (tyrosinase) was co-targeted and used as a mutagenesis selection marker.

15 **[00132]** In conjunction with non-treated controls, approximately 20 embryos per candidate gene displaying pigmentation defects were raised to adulthood. At 5 months of age, milt from F0 males and WT controls were stripped to assay sperm density, motility and morphology. Compared to controls, all F0 mutant males produced diluted sperm. Under microscopy, mutant spermatozoa largely produced only a trembling movement and we found wide-ranging frequencies (25% - 95%) of abnormally shaped sperm heads, characteristic of the defects seen in human and mice with globozoospermia (Fig. 15 panel A). These mutations caused significant decreases in fertilization rates (Fig. 15 panel B). Furthermore, we found a positive correlation between the severity of the sterility phenotype and the observed frequency of the sperm deformities, with the lowest fertilization rate found in *Tjp1a* mutants where 95% of sperm were deformed (Fig. 15 panels A and B). We found that all females in these F0 mutant lines are fertile.

20 **[00133]** Our results point to the existence of an evolutionarily conserved pathway controlling spermiogenesis in fish and mammals. These results support the idea that the targeted disruption of these corresponding genes will cause a sterility phenotype in many other teleost species, and possibly more broadly in other taxa as well.

[00134] Example 7 - Sterile all-male fish in *cyp17* KO background.

[00135] To engineer male sterility, we first evaluated the effect of null mutations in the *cyp17* gene, which controls an important branch point in steroid hormone synthesis,

regulating both androgen and estrogen production. We found that all *cyp17*^{-/-} fish develop as

5 male. Surprisingly, milt produced by *cyp17*^{-/-} contained a small number of mature spermatozoa that could fertilize oocytes by *in vitro* fertilization. We then investigated the possibility of blocking spermiogenesis. Our preliminary screens focused on five genes associated with globozoospermia (collectively termed spermiogenesis specific genes or SMS-genes: *Smad2*, *Cnsk2a2*, *Gopc*, *Hiat1* and *Tjp1a*), whose mutations caused subfertility
10 in F0 males with severe oligo-astheno-teratozoospermia, while F0 mutant females were fully fertile. Previous genetic characterizations of F0 KO fish indicate that they typically carry mosaic mutations at the corresponding targeted loci, some of which are often in-frame causing partial rescue of the phenotype. Thus, to measure the full loss-of function phenotype, we performed additional phenotypic characterization on homozygous SMS-null-
15 mutants. We further established lines of tilapia carrying double homozygous mutations to interrogate the effect of simultaneously impairing spermiogenesis and steroid hormone synthesis.

[00136] Experiment: To assess *in vivo* function of double gene knockouts in *cyp17* and one of the 5 SMS gene, we outcrossed F0 SMS mutant females with *cyp17*^{Δ16/+} males.

20 Offspring (120 to 180 fish) were genotyped at each target locus by PCR fragment analysis (as described in Fig. 2) [22]. We only raised individuals carrying an identical mutant allele, hereafter referred to as m1 (Fig. 18), at the selected SMS locus (typically 12-50% of the F1 progeny population share the same genotype). A minimum of 10 double heterozygotes (e.g. *cyp17*^{Δ16/+}; *SMS*^{m1/+}) were raised to adulthood. These double heterozygotes were inter-
25 crossed, and their progeny genotyped at 1 month of age by QPCR melt analysis. For each of the 9 ensuing possible F2 genotypes (see Fig. 9), a minimum of 30 fish are currently being raised to adulthood and will be assayed for fertility. Females *cyp17*^{+/+}; *SMS*^{+/m1} (e.g. *cyp17*^{+/+}; *Tjp1a*^{+/m1}) were set aside for further studies described in section 2 below. Fig. 9 summarizes this experimental scheme, using *Tjp1a* as an example of an SMS gene target.

30 **[00137]** Without being bound by theory, we believe that in finfish, as in mammals, null mutations in all 5 conserved spermiogenesis specific genes will result in oligo-astheno-teratozoospermia and cause infertility. We expect that all double homozygous mutants

(*cyp17*^{-/-}; *SMS*^{-/-}) will develop as sterile males with even lower sperm counts than any single KO male defective in spermiogenesis (*SMS*^{-/-}). Indeed, *cyp17*^{-/-} fish should be deficient in 11-ketotestosterone, a positive regulator of spermatogenesis. Consistent with the idea that androgen plays an intra-testicular paracrine role in spermatogenesis, *cyp17*^{-/-} tilapia have previously been shown to display low sperm counts. Fig. 9 shows the nine genotypes along with four different corresponding phenotypes with the expected percentages: 1) ~56% fertile for both sexes, 2) ~19% fertile female and sterile male, 3) ~19% all fertile male; and 4) ~ 6% all-sterile male. Looking at each trait individually, we expect a progeny population of 62% male with 25% of these males being sterile.

[00138] Example 8 – Sterile all-male fish in *cyp19a1a* KO background

[00139] An alternative strategy to generate all-male population is to inactivate the *Cyp19a1a* aromatase (hereafter referred to as *Cyp19*). We created out of frame mutations in the coding sequence of the tilapia *cyp19* gene (Fig. 17). This enzyme is produced by the somatic gonad and convert testosterone into estrogen. Consistent with this model, we found a strong male bias amongst the 25 F0 *Cyp19* mutants selected, with 20 mutants developing as phenotypic males (Table 3). Notably these mutant males displayed normally appearing male urogenital papillae, indicating that androgen production is not impaired and secondary male sexual characteristics develop normally. This stand in contrast to *cyp17* KO males, which lack androgen and accordingly develop atrophic urogenital papillae. The generation of all-male sterile tilapia populations, which either express or do not express androgens (as in *cyp19* KO and *cyp17* KO backgrounds respectively), will allow us to interrogate the influence of male sex steroid hormone on tilapia growth performance. The stimulatory action of testosterone on GH secretion and responsiveness is well documented in mammals. For a complete phenotypic analysis, we generated individuals carrying the same null mutations in all cells of their body. Heterozygous *cyp19* F1 offspring with a $\Delta 10$ -*cyp19* deletions in the first exon were selected to breed the F2 generation. This frame-shift mutation is expected to create a truncated protein lacking >98% of its wild type amino acid sequence (Fig. 17). This F2 generation was genotyped and sexed. As expected, we found that homozygous $\Delta 10$ -*cyp19* tilapia all develop as males (n=38) while hemizygous (n=97) and wild-type (n=40) had a normal sex ratio. We further established lines of tilapia carrying double homozygous mutations to interrogate the effect of simultaneously impairing spermiogenesis and steroid hormone synthesis.

[00140] Experiment: We first outcrossed heterozygous F1 males $\Delta 10$ -cyp19a1a with the heterozygous mutant females from Example 7 (*Gopc* ^{$\Delta 8/+$} ; *Smap2* ^{$\Delta 17/+$} ; *Tjp1a* ^{$\Delta 7/+$} ; *Csnk2a2* ^{$\Delta 22/+$} ; *Hiat1* ^{$\Delta 17/+$}). Only SMS genes that cause male sterility when disrupted in a

Cyp17 null background (results from Example 7) will be selected. The progeny will be genotyped and at least 10 double heterozygous will be raised to adulthood, sexed, and inter-crossed. The resulting progeny will be assayed for fertility as described in Example 7. A maximum of 5 different double KO males will be generated. Without being bound by theory, we expect double KO *cyp19*^{-/-}; *SMS*^{-/-} fish to develop as sterile males and anticipate a progeny population of 62% male, with 25% of them being sterile.

10

Gene categories	Gene names	phenotype in F0 albino	Selected F1 genotypes (hemizygous)	Selected double hemizygous mutant	F2 homozygous
FEM	<i>Cyp17</i> <i>Cyp19a1a</i>	80% male (n=20) 75% male (n=23)	<i>Cyp17</i> ^{$\Delta 16/+$} <i>Cyp19</i> ^{$\Delta 7/+$} , <i>Cyp19</i> ^{$\Delta 10/+$}		<i>Cyp17</i> ^{$\Delta 16/\Delta 16$} <i>Cyp19</i> ^{$\Delta 10/\Delta 10$}
SMS	<i>Tjp1a</i> <i>Csnk2a2</i> <i>Hiat1</i> <i>Smap2</i> <i>Gopc</i>	Reduced fertility in males (70-90%)	<i>Tjp1a</i> ^{$\Delta 7/+$} <i>Csnk2a2</i> ^{$\Delta 22/+$} <i>Hiat1</i> ^{$\Delta 17/+$} <i>Smap2</i> ^{$\Delta 17/+$} <i>Gopc</i> ^{$\Delta 8/+$}	<i>Tjp1a</i> ^{$\Delta 7/+$} and <i>Cyp19</i> ^{$\Delta 10/+$} <i>Csnk2a2</i> ^{$\Delta 22/+$} and <i>Cyp17</i> ^{$\Delta 16/+$} <i>Hiat1</i> ^{$\Delta 17/+$} and <i>Cyp17</i> ^{$\Delta 16/+$} <i>Smap2</i> ^{$\Delta 17/+$} and <i>Cyp19</i> ^{$\Delta 10/+$}	<i>Tjp1a</i> ^{$\Delta 7/\Delta 7$} <i>Csnk2a2</i> ^{$\Delta 22/\Delta 22$} <i>Hiat1</i> ^{$\Delta 17/\Delta 17$} <i>Smap2</i> ^{$\Delta 17/\Delta 17$} <i>Gopc</i> ^{$\Delta 8/\Delta 8$}
MA	<i>Dmrt1</i> <i>Gsdf</i>	50% female (n=24) 95% female (n=20)	<i>Dmrt1</i> ^{$\Delta 7/+$} , <i>Dmrt1</i> ^{$\Delta 13/+$} <i>Gsdf</i> ^{$\Delta 5/+$} , <i>Gsdf</i> ^{$\Delta 22/+$}		
FLS	<i>FSHR</i> <i>VtgAa</i> <i>VtgAb</i>	Sterile females (2 in 6 tested) Sterile females (3 in 5 tested) Reduced fertility in females (70-90%)	<i>FSHR</i> ^{$\Delta 5/+$} <i>VtgAa</i> ^{$\Delta 5/+$} , <i>VtgAa</i> ^{$\Delta 25/+$} <i>VtgAb</i> ^{$\Delta 8/+$}	<i>FSHR</i> ^{$\Delta 5/+$} and <i>Dmrt1</i> ^{$\Delta 7/+$} <i>VtgAa</i> ^{$\Delta 25/+$} and <i>Gsdf</i> ^{$\Delta 22/+$} <i>VtgAb</i> ^{$\Delta 8/+$} and <i>VtgAa</i> ^{$\Delta 25/+$}	<i>VtgAa</i> ^{$\Delta 25/\Delta 25$} <i>VtgAb</i> ^{$\Delta 8/\Delta 8$}

Table 3: Description of single gene mutant alleles, double hemizygous mutant alleles and homozygous mutant alleles generated in this study. Genes names are listed based on their specific role in feminization (FEM), spermiogenesis (SMS), masculinization (MA) and folliculogenesis (FLS). Phenotypes observed in selected F0 mutant are described.

15

[00141] **Example 9 - Evaluate two genes targeting male differentiation in conjunction with two other genes controlling oogenesis to produce a sterile all-female population.**

[00142] The transcriptional inhibitor Gonadal soma-derived factor (*Gsdf*) is a TGF- β superfamily member expressed only in the gonads of fish, predominantly in the Sertoli cells. Similarly, the transcription factor *Dmrt1* is preferentially expressed in pre Sertoli and Sertoli cells as well as in epithelial cells of the testis. Both genes are necessary for normal testis development ([23, 24]).

[00143] To produce all-female tilapia populations, we generated null mutations in either *Dmrt1* or *Gsdf* genes (maleness genes or MA) (Fig. 19 and Fig. 20). We found that 19 out of 20 *Gsdf* mutated albino tilapia developed as females (Table 3). In contrast, F0 mutant showing mosaic pigment defect had normal sex ratio. Postulating a positive correlation of mutagenic frequency between co-targeted *tyrosinase* and *Gsdf* genes, our result suggests that high-mutation-rate in *Gsdf* cause XY male to sex reverse into female. Surprisingly we did not observe a female sex bias amongst selected F0 *Dmrt1* mutant (Table 3).

[00144] To engineer sterility in females, we targeted genes involved in the maturation of ovarian follicle. We have identified two genes in the molecular pathway controlling folliculogenesis: 1) *FSHR* which acts upstream of ovarian estrogen synthesis and. 2) vitellogenins (*Vtgs*) which act downstream of ovarian estrogen synthesis. Vitellogenins are preferentially produced by the liver while *FSHR*, the follicle-stimulating hormone (FSH) receptor is expressed in Theca cells surrounding the developing oocytes. To test the necessity of *FSHR* and *Vtgs* in normal ovarian development (folliculogenesis specific genes or FLS) we produced loss-of-function mutations in those genes in independent F0 lines (Figs. 22-24).

[00145] We found that *FSHR* is indispensable to folliculogenesis and the disruption of the *FSHR* gene resulted in a complete failure of follicle activation and female sterility (Fig. 26 and Table 3). In tilapia, *FSHR* mutation was not followed by masculinization of genetic females into males, as previously described in zebrafish [29]. However, we found that F0 *FSHR* mutant females had significantly smaller urogenital papillae when compared to control female. This observation likely reflects a reduced level of estrogen in *FSHR* mutant, consistent with a role of *FSHR* in locally up-regulating aromatase expression and estrogen production. We found no significant reproductive phenotype in F0 *FSHR* mutant male.

[00146] Nile tilapia only possess 3 *Vtg* genes [25], two forms of complete *Vtgs* (*VtgAa* and *VtgAb*) and one form of incomplete C-type teleost vitellogenin, lacking three protein domains (*VtgC*). Since *VtgAa* and *VtgAb* are expressed at higher level than *VtgC* and assumed to be critical to early embryo development, we targeted those two genes

individually as well as jointly (Figs. 22, 23, and Table 3). Consistent with functions in oocyte maturation and nutritional support for embryogenesis, we found that 3 F0 females mutated in *VtgAa* out of 4 tested failed repeatedly to produce viable progeny (Fig. 24). We also found that one F0 female carrying mutations in *VtgAb* out of 5 produced embryos progeny that died before hatch (data not shown).

[00147] For a complete phenotypic characterization, it is essential to generate identical mutations in every cell of the animal. Thus, we will establish and characterize 4 lines of tilapia deficient in both masculinization and vitellogenesis.

[00148] At 6 months of age, mosaic F0 XX MA m_{1-n} female (e.g. *Dmrt1* m_{1-n} or *Gsdf* m_{1-n}) were outcrossed to mosaic F0 *FLS* m_{1-n} males (*FSHR* m_{1-n} or *Vtgs* m_{1-n}) and their F1 progeny genotyped to identify double heterozygous mutants (e.g. *Dmrt1*^{Δ7/+}-*FSHR*^{Δ5/+}) carrying the same gene specific indel at each locus (Table 3).

[00149] Experiment: A minimum of 10 double heterozygotes (for each of the four gene combinations) are currently being raised to adulthood. The WT alleles should ensure that these F1 fish develop as both fertile males and females. These double heterozygous mutants will then be incrossed, and their progeny genotyped at 1 month of age by QPCR melt analysis. For each of the 9 ensuing possible genotypes (see Fig.25), a minimum of 30 fish will be raised to adulthood, then sexed, and assayed for fertility.

[00150] Fig. 25 shows nine genotypes and the corresponding four different phenotypes we expect with the following fractional ratios: 1) ~56% fertile for both sexes, 2) ~19% fertile female and sterile male, 3) ~19% all fertile male; and 4) ~ 6% all-sterile female. Looking at each trait individually, we expect a progeny population of 62% female with 25% of these females being sterile.

[00151] Our phenotypic investigations in F0 mutant lines (Table 3) mostly agree with our initial hypothesis and we fully expect corroborating genotype-phenotype relationships in subsequent generations. We found that *Gsdf* deficiency caused feminization while *FSHR* and *Vtgs* inactivation resulted in female sterility. These results strongly suggest that double *FSHR-Gsdf* KOs will develop into monosex sterile female populations characterized by atrophic ovaries containing follicles arrested at the previtellogenic stage. The lack of a sex differentiation phenotype in F0 *Dmrt1* mutant likely reflects incomplete editing, regional mosaicism and compensation by non-mutated cells. Without being bound by theory however, we believe that double *FSHR-Dmrt1* KOs in which the mutations have been inherited through

the germline, will develop into all female sterile populations. In our F0 mutagenesis screen we observed that blocking the precursor of major yolk proteins (as in Vtg KOs), compromises egg quality and impairs the development and survival of embryos. As such, we expect that double KOs *Gsdf-Vtgs* and *Dmrt1-Vtgs* will develop into monosex sterile female populations.

5

[00152] Example 10 - Propagation of all-male and all-female sterile lines by germline transplantation into sterile surrogate adults

10 **[00153]** Examples 8 and 9 above illustrate how to generate monosex sterile fish by breeding double hemizygous mutant and by individually selecting the subpopulation of double KO progeny. This approach however may not be sufficiently efficient and may be too expensive to be used in industrial settings. Intracytoplasmic sperm injection in assisted reproduction offers a solution to propagate male broodstock that are defective in spermiogenesis. However, this approach is also not scalable for mass production of commercial stocks (as it requires conducting methods on 'one fish at a time'). The key to
15 larger scale production is to generate male and female broodstock that only produce mutant gametes so that no selection is needed to identify the double KO progeny. Importantly, those mutant gametes should also be functional so that natural mating of these broodstock can be used to produce a viable population of monosex sterile progeny. This is only possible if sex ratio and gamete functionality are rescued in the broodstock. We speculated that this can be
20 achieved by germline stem cell transplantation from a double KO mutant fish to a germ cell free recipient not mutated for the same genes. Such transplanted broodstock have normal somatic cells but a mutant germline (see Figs. 27-32). These chimeric recipients possess functional MA or FEM somatic gene(s) that ensure normal sex ratio (Fig. 34 panels C and D) and functional SMS or FLS somatic genes to rescue spermiogenesis (Fig. 28) or oogenesis
25 (Figs. 29 and 30) assuming the mutated genes do not function in germ cells.

[00154] Since spermatogenic failure can result from defects in germ cells or in their somatic environment we analyzed the SMS genes expressions to identify those preferentially not expressed in germ cells (Fig. 16). Our SMS gene expression study in sterile testes point to a role of gonad somatic cells in supporting germ cell development. For example, we found
30 that *Tjp1a* is a highly expressed in sterile testes at level above wild type testes, while *Hiat1* and *Gopc* expression levels are only slightly reduced compare to fertile testes (Fig. 16).

[00155] These results suggest that mutant of those genes develop a testicular microenvironment, where spermiogenesis is impaired due to Sertoli and/or Leydig-specific

defects (Fig. 28). Consequently, we expect that transplantation of spermatogonial stem cells from the male knockout infertile donors to a permissive wild type testicular environment will restore sperm functionality and fertility (Fig. 28).

[00156] Likewise, *FSHR* and *Vtgs*, are strictly expressed in somatic cells (Theca and liver cells respectively). Thus, oocytes carrying null alleles of these genes should retain their intrinsic capacity to proliferate and differentiate, ensuring that oogonial stem cells from a sterile female mutant donor can re-populate the ovaries and differentiate into functional eggs upon transplantation into a WT/permissive recipient (Figs. 29 and 30). Thus, we believe that recipient males or females can produce gametes that carry the donor genotype.

10 [00157] **Example 11 – *Elavl2* KO recipients can produce functional gametes**

[00158] To confirm that sterile *Elavl2* KO recipients can produce functional gametes from donor-derived germ cells, we harvested spermatogonial stem from the testes of albino (*tyr*^{-/-}) male tilapia carrying mutations (in-frame and out-of-frame) in a reference gene (Fig. 33 panel A). We transplanted the testicular cell suspension from both mutant lines, into germ cell depleted recipient embryos progeny from *Elavl2*^{-/+}, *tyr*^{+/+} parents. We genotyped transplanted fish to select homozygous *Elavl2*^{-/-} mutant and raised them to adulthood. At 5 months of age, between 31-50% of transplanted *Elavl2*^{-/-} male and 40% of six months old transplanted *Elavl2*^{-/-} female produced exclusively albino progeny when outcrossed with albino male and female. Non-transplanted *Elavl2*^{-/-} controls were sterile. Thus, *Elavl2*^{-/-} recipients can produce donor-derived gametes after germline stem cell transplantation illustrating the feasibility to create a tilapia that produced only donor derived gametes. Using albinism to assay for gametes carrying *tyr* alleles provided an easy quantifiable high-throughput assay for germline transmission efficacy of mutant alleles, but these experiments do not demonstrate that the null mutations was successfully propagated. To this end, we extracted and analyzed the sperm DNA from one fertile recipient by PCR fragment sizing assay. The amplification products were sized using capillary electrophoresis (Fig. 33 panel B). Results reveal that the recipient fish only produces sperm containing donor derived in-frame and out-of-frame (3 nt and 4 nt) deletions fragments suggesting that the null allele (4 nt deletion) can colonize the gonad and proliferate as efficiently as the positive control mutation (3 nt deletion) (Fig. 33 panel B).

[00159] Experiment: Spermatogonial and oogonial stem cells (SSCs, OSCs) will be isolated from all-male and all-female juvenile tilapia lines (developed as per Examples 7, 8,

and 9). After harvest, these germline stem cells will be transplanted into *Elavl2* KO recipient hatchlings as described above. Without being bound by theory, we expect production of functional spermatozoa and oocytes carrying the donor genotypes. To evaluate the functionality of donor-derived gametes produced after transplantation, in vitro fertilization assays will be performed. Moreover, we expect only albino progeny to arise from a cross between the naturally pigmented recipient carrying albino donor gametes and albino lines. We will genotype 10 progenies for mutations in donor-derived spermatogenesis and vitellogenesis specific genes.

[00160] As illustrated in Fig. 34 panel B, crossing surrogate mothers with double KO sex reversed males, obtained from treatment with aromatase inhibitors, will produce all-female sterile progeny. Alternatively, crossing surrogate fathers with double KO sex reversed female mutants rescued after estrogen treatment, will produce all-male sterile populations (Fig. 34 panel A). Sex reversal of double KO with estrogen (as in Fig. 34 panel A) or androgen inhibitor (as in Fig. 34 panel B) can otherwise be substituted by germ line transplantation method to produce the female broodstock (Fig. 34 panel C) or male broodstock (Fig. 34 panel D).

[00161] Example 12 – Tank grow-out trials

[00162] There is a direct trade-off between growth and reproduction, as energy channeled into the gonads detracts from somatic growth. Nile tilapia mature precociously and can reproduce throughout the year, with short vitellogenic periods [26], and a physiological process that demands a high metabolic rate. Furthermore, Tilapia species can suppress growth to maintain their reproductive capacity [27], and in other fish species the onset of puberty can have a major impact on important production parameters in fish farming such as appetite, growth rate, feed conversion efficiency, flesh quality traits, external appearance, health, welfare and survival rates. Thus, delaying or blocking sexual maturation is likely to confer significant benefits to commercial aquaculture producers. In our efforts to develop sterile monosex populations, we have targeted genes whose mutations block or delay the onset of puberty. However, genes targeted for these effects might also have pleiotropic effects, detrimental to the line, acting via unknown hormonal, physiological or behavioral changes.

[00163] Experiment: To generate groups used for growth performance trials, embryos from single paired crossings (at least three separate crosses) will be produced for each line

of interest. Treatment and control embryos will be reared separately using established hatchery procedures. At the feeding stage, half of the control animals will be sex reversed using appropriate exogenous hormone treatment protocols (i.e. feeding methyl testosterone or DES). When fish within a group (treatment and control) reach a mean weight of 60g, they will be PIT tagged and divided into six 1000L tanks (3 control and 3 treatment tanks, with 50 fish/tank). All fish will be fed three times daily, to satiation.

[00164] Each fish will be individually weighed, and the length of each fish measured at 4-week intervals over a period necessary to reach market size (680g Sdv: 77g, 8 months). At the end of the experiment, fish will be sacrificed and sexed based on the structure of the urogenital orifice. We will record the individual weights of dissected gonads and carcass for calculation of gonadosomatic index (GSI) and carcass index (n=60 per group). Specific growth rate (G) will be calculated according to the formula of Houde & Sheckter [28]

[00165] Without being bound by theory, we believe that most, if not all, double KO fish created in Examples 7, 8, and 9 will develop as monosex and be sterile with no other biological processes impaired. Thus, selected mutations should not negatively impact the overall fish performance. On the contrary, we expect to find an improved growth rate and feed conversion ratios inversely correlated to gonad weight. Mutant lines should be sexually delayed (male sterile) or immature (female arrested at the previtellogenic stage). In the unlikely event that we achieve only partial sterilization of monosex populations, we expect improvement in productivity in tilapia to be proportional to the fraction of sterile fish in the population, as a result of reduced energy expenditure. In all cases, we anticipate sterile fish and fish with atrophic gonads to out-perform their fully fertile counterparts (e.g. monosex populations derived from exogenous hormone treatments) in regard to growth characteristics.

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SEQUENCE LISTING

10

SEQ ID NO 1

LENGTH: 38

TYPE: DNA

ORGANISM: Artificial Sequence

15 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 1

TGTAAAACGACGGCCAGTttgaagttgctacataaaaag

SEQ ID NO 2

20 LENGTH: 22

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 2

25 TGGTTGATGACAATCACACTGT

SEQ ID NO 3

LENGTH: 41

TYPE: DNA

30 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 3

TAGGAGTGCAGCAAGCATtgttctacatcatcacccttctc

35 **SEQ ID NO 4**

LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 4

5 AGCAGACAGACGAGCAGTATCAG

SEQ ID NO 5

LENGTH: 41

TYPE: DNA

10 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 5

TGTAAAACGACGGCCAGTTGATGGAGAGCTTCATCTACGAA

15 **SEQ ID NO 6**

LENGTH: 20

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

20 SEQUENCE: 6

GTTCCAGGTTAAATTGATTG

SEQ ID NO 7

LENGTH: 41

25 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 7

TAGGAGTGCAGCAAGCATgcgtgatttgctgaccttttac

30

SEQ ID NO 8

LENGTH: 22

TYPE: DNA

ORGANISM: Artificial Sequence

35 OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 8

acacttacCCTGAGAATCTGG

SEQ ID NO 9

LENGTH: 41

TYPE: DNA

ORGANISM: Artificial Sequence

5 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 9

TGTAACACGACGGCCAGTGAAAAAGGATGgtgagggatgac

SEQ ID NO 10

10 LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 10

15 GAGTGTGTCTACCACACGGAAAA

SEQ ID NO 11

LENGTH: 41

TYPE: DNA

20 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 11

TGTAACACGACGGCCAGTgtatttagaaggcgggtaagggtc

25 **SEQ ID NO 12**

LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

30 SEQUENCE: 12

CAGTTTGGCACATGAGCATCGTA

SEQ ID NO 13

LENGTH: 37

35 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 13

TAGGAGTGCAGCAAGCATATGCTCATGTGCCAAACTG

SEQ ID NO 14

5 LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 14

10 cCTTCAGGATTTTCACCACCACT

SEQ ID NO 15

LENGTH: 41

TYPE: DNA

15 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 15

TGTAAAACGACGGCCAGTtactgacacatccagcagcgtct

20 **SEQ ID NO 16**

LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

25 SEQUENCE: 16

cagcactgagccgtcagtattct

SEQ ID NO 17

LENGTH: 37

30 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 17

TAGGAGTGCAGCAAGCATTGGAGCCTACCTGTCTGAG

35

SEQ ID NO 18

LENGTH: 20

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 18

tactcacAGCGAAGGGGTCT

5

SEQ ID NO 19

LENGTH: 38

TYPE: DNA

ORGANISM: Artificial Sequence

10 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 19

TAGGAGTGCAGCAAGCATgctcctctgcgaagactctc

SEQ ID NO 20

15 LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 20

20 aagacctccgacCTGGACTTGCT

SEQ ID NO 21

LENGTH: 41

TYPE: DNA

25 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 21

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30 **SEQ ID NO 22**

LENGTH: 22

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

35 SEQUENCE: 22

TTGGATATCCCATTGTTTCAT

SEQ ID NO 23

LENGTH: 40

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

5 SEQUENCE: 23

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SEQ ID NO 24

LENGTH: 22

10 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 24

AGATCCACATCCACGAAAGCCT

15

SEQ ID NO 25

LENGTH: 37

TYPE: DNA

ORGANISM: Artificial Sequence

20 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 25

TGTAAAACGACGGCCAGTtgtcccctttaaccaccta

SEQ ID NO 26

25 LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 26

30 CTCAGCTTGGCCTTGCTTGACAT

SEQ ID NO 27

LENGTH: 39

TYPE: DNA

35 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 27

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SEQ ID NO 28

LENGTH: 23

5 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 28

10 AGACACGTATCCGTGATTTCTAC

SEQ ID NO 29

LENGTH: 41

TYPE: DNA

ORGANISM: Artificial Sequence

15 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 29

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SEQ ID NO 30

20 LENGTH: 22

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 30

25 GGGTTTCCAGCAGGAGGTCAGA

SEQ ID NO 31

LENGTH: 39

TYPE: DNA

30 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 31

TAGGAGTGCAGCAAGCATttatgttcagTGCCAAGGTG

35 **SEQ ID NO 32**

LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 32

TGGCTGTGTGAGAAACGATGCTG

5 **SEQ ID NO 33**

LENGTH: 35

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

10 SEQUENCE: 33

TGTAAAACGACGGCCAGTagATCTGGGCTGGGACA

SEQ ID NO 34

LENGTH: 23

15 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 34

tgtaactatacCTGTGTGTTGG

20

SEQ ID NO 35

LENGTH: 38

TYPE: DNA

ORGANISM: Artificial Sequence

25 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

SEQUENCE: 35

TAGGAGTGCAGCAAGCATttttctccgcttgcttctgc

SEQ ID NO 36

30 LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 36

35 AAAGAGCTGAATAGGAGGAAGTT

SEQ ID NO 37

LENGTH: 39

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 37

5 TGTAACGACGGCCAGTCATCTTGGCGTTCTTCTGTGT

SEQ ID NO 38

LENGTH: 21

TYPE: DNA

10 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 38

CTTGAGGGCAGCTGAGATGGC

15 **SEQ ID NO 39**

LENGTH: 40

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

20 SEQUENCE: 39

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SEQ ID NO 40

LENGTH: 22

25 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 40

CTGAGACTCTATGTCGTTGATA

30

SEQ ID NO 41

LENGTH: 41

TYPE: DNA

ORGANISM: Artificial Sequence

35 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 41

TGTAACGACGGCCAGTAGAAGATCATCAAACACATCACG

SEQ ID NO 42

LENGTH: 23

TYPE: DNA

5 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 42

GACTTGTTGAGCAGTTGCATCAA

10 **SEQ ID NO 43**

LENGTH: 38

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (NED)

15 SEQUENCE: 43

TAGGAGTGCAGCAAGCATttttgatctagTCTGGAG

SEQ ID NO 44

LENGTH: 22

20 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 44

gctcttacAGCTTCACAATCAT

25

SEQ ID NO 45

LENGTH: 41

TYPE: DNA

ORGANISM: Artificial Sequence

30 OTHER INFORMATION: Description of Artificial Sequence: Forward tailed Primer (FAM)

SEQUENCE: 45

TGTAAAACGACGGCCAGTAGAAGATCATCAAACACATCACG

SEQ ID NO 46

35 LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 46

GACTTGTTGAGCAGTTGCATCAA

5 **SEQ ID NO 47**

LENGTH: 22

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

10 SEQUENCE: 47

GAACCAAACCCCTCTGTCACTG

SEQ ID NO 48

LENGTH: 22

15 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 48

GTAATTCCTCCGAGGCTCAG

20

SEQ ID NO 49

LENGTH: 17

TYPE: DNA

ORGANISM: Artificial Sequence

25 OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 49

ggcgATGAATCCTGTAG

SEQ ID NO 50

30 LENGTH: 22

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 50

35 ATGGCATTGAGGTCACAGAGA

SEQ ID NO 51

LENGTH: 19

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 51

GTTCAAGAAGGGAGAGAGT

5

SEQ ID NO 52

LENGTH: 18

TYPE: DNA

ORGANISM: Artificial Sequence

10 OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 52

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SEQ ID NO 53

15 LENGTH: 20

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 53

20 tgcttggtcagTGTATC

SEQ ID NO 54

LENGTH: 19

TYPE: DNA

25 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 54

AATGCGTTCGAATGTAGAA

30 **SEQ ID NO 55**

LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

35 SEQUENCE: 55

CATCTGCTTCATCCTGGTGGCTG

SEQ ID NO 56

LENGTH: 23

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 56

5 AATTTGGGCATCTTCATCTGTAT

SEQ ID NO 57

LENGTH: 22

TYPE: DNA

10 ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 57

GACAGACTTGACCTTGGAGATG

15 **SEQ ID NO 58**

LENGTH: 21

TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

20 SEQUENCE: 58

ATGTCTGCTTCGACTGGATGC

SEQ ID NO 59

LENGTH: 23

25 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 59

GCCATCGAAACATGGACATACTG

30

SEQ ID NOs 60 and 62 (wild-type Cyp17a1)

LENGTH: 1563bp and 521aa

TYPE: cDNA (SEQ ID NO: 60) and Protein (SEQ ID NO: 62)

ORGANISM: Nile tilapia

35

1	GAACCAAACCCCTCTGTCACTGATATGGCTTGGTTTTTGTGTCTGTGCGTGTTCATGGCG	60
1	-E--P--N--P--S--V--T--D--M--A--W--F--L--C--L--C--V--F--M--A--	20

40

61	GTGGGCCTCACTTTGTTAGCACTGCAGTTCAAGTTCAGGATGTCTGCACATGGTTCTGGG	120
21	-V--G--L--T--L--L--A--L--Q--F--K--F--R--M--S--A--H--G--S--G--	40

	121	GAGCCGCCACACCTCCCTGCACTACCACTGATTGGCAGCCTGCTGAGCCTGCGGAGTGAA	180
	41	-E--P--P--H--L--P--A--L--P--L--I--G--S--L--L--S--L--R--S--E-	60
5	181	TTACCACCGCATGTGCTTTTCAAAGAACTGCAGGTAAAATACGGACATACATACTCGCTG	240
	61	-L--P--P--H--V--L--F--K--E--L--Q--V--K--Y--G--H--T--Y--S--L-	80
	241	ATGATGGGCTCCACAGTGTGATTGTCATCAACCAGCATGTGCACGCCAAAGAAGTCTTG	300
10	81	-M--M--G--S--H--S--V--I--V--I--N--Q--H--V--H--A--K--E--V--L-	100
	301	CTCAAGAAGGAAAGACGTTTGCAGGAAGACCTAGAAGTGTAAACCACAGATATTCTGACT	360
	101	-L--K--K--G--K--T--F--A--G--R--P--R--T--V--T--T--D--I--L--T-	120
	361	AGAGATGGGAAGGACATTGCATTTGGAGACTACAGTGCTACGTGGAAGTTCCACAGGAAG	420
15	121	-R--D--G--K--D--I--A--F--G--D--Y--S--A--T--W--K--F--H--R--K-	140
	421	ATAGTCCATGGAGCCCTGTGCATGTTTGGAGAAGTTCTGCCTCTATTGAGAAGACCATT	480
	141	-I--V--H--G--A--L--C--M--F--G--E--G--S--A--S--I--E--K--T--I-	160
20	481	TGTGCAGAGGCCAGTCTCTGTGCTCCGTGCTGTCTGAGGCAGCAGATGTGGGACTGGCC	540
	161	-C--A--E--A--Q--S--L--C--S--V--L--S--E--A--A--D--V--G--L--A-	180
	541	CTGGATCTTGCTCCTGAGCTGACTCGCGTGTCACCAACGTTATCTGTTCTCTCTGCTTC	600
25	181	-L--D--L--A--P--E--L--T--R--A--V--T--N--V--I--C--S--L--C--F-	200
	601	AACTCGTCTACTGCCGAGGCGACTCAGAGTTTGAGACAATGCTGCAGTACAGCCAGGGC	660
	201	-N--S--S--Y--C--R--G--D--S--E--F--E--T--M--L--Q--Y--S--Q--G-	220
	661	ATTGTGGACACTGTGGCTAAAGACAGCCTGGTAGACATTTTCCCCTGGCTTCAGATCTTT	720
30	221	-I--V--D--T--V--A--K--D--S--L--V--D--I--F--P--W--L--Q--I--F-	240
	721	CCTAATGCGGACCTACGTCTCCTAAAACATTGTGTTTCCATCAGAGACAACTTCTACAG	780
	241	-P--N--A--D--L--R--L--L--K--H--C--V--S--I--R--D--K--L--L--Q-	260
35	781	AGGAAATTTGATGAACACAAGGTGAATTACAATGATCACGTGCAGAGAGACTTGATAGAC	840
	261	-R--K--F--D--E--H--K--V--N--Y--N--D--H--V--Q--R--D--L--I--D-	280
	841	GCTCTGCTAAGAGCCAAGCGCAGTGCAGGAGAACAACAACACATCAGAGATAAGTGCAGAG	900
40	281	-A--L--L--R--A--K--R--S--A--E--N--N--N--T--S--E--I--S--A--E-	300
	901	TCTGTGGGCTGAGTGATGACCACATTCTCATGACAGTGGGAGACATCTTTGGCGCTGGC	960
	301	-S--V--G--L--S--D--D--H--I--L--M--T--V--G--D--I--F--G--A--G-	320
	961	GTGGAAACCACTACCACTGTGCTCAAATGGGCCATAACGTACCTCATTCATCACCCAGAG	1020
45	321	-V--E--T--T--T--T--V--L--K--W--A--I--T--Y--L--I--H--H--P--E-	340
	1021	GTGCAAAGACGTATCCAGGATGAGCTGGACAGGACGGTGGGTGACAGCCGCTCTCCTAAA	1080
	341	-V--Q--R--R--I--Q--D--E--L--D--R--T--V--G--D--S--R--S--P--K-	360
50	1081	CTCACCAGACAGAGGCAGTCTGCCTTATCTGGAGGCCACCATTAGGGAAGTATTGCGGATT	1140
	361	-L--T--D--R--G--S--L--P--Y--L--E--A--T--I--R--E--V--L--R--I-	380
	1141	CGCCCCGTGGCACCCTACTCATCCCCATGTGGCTCTCTGTGACACCAGCATTTGGAGAT	1200
55	381	-R--P--V--A--P--L--L--I--P--H--V--A--L--C--D--T--S--I--G--D-	400
	1201	TTCACAGTGAGAAAAGGAACTCGAGTCATTATCAACCTGTGGGCTCTGCACCATGATGAG	1260
	401	-F--T--V--R--K--G--T--R--V--I--I--N--L--W--A--L--H--H--D--E-	420
	1261	AAGGAGTGAAGAACCAGAGCGGTTTGACCCTGGCCGGTTCTTGAAAAGTGAAGGCACA	1320
60	421	-K--E--W--K--N--P--E--R--F--D--P--G--R--F--L--K--S--E--G--T-	440

	1321	GGACTGACAATCCCATCACCCAGCTACCTGCCCTTTGGTGTCTGGGCTGAGAGTATGTTTA	1380
	441	-G--L--T--I--P--S--P--S--Y--L--P--F--G--A--G--L--R--V--C--L-	460
5	1381	GGTGAGGCCTTGCCCAAGATGGAGCTCTTTCTCTTCCTGTCTGGATCCTGCAGCGCTTC	1440
	461	-G--E--A--L--A--K--M--E--L--F--L--F--L--S--W--I--L--Q--R--F-	480
	1441	ACTCTGTCTGTCCCACCAGGCCACAGTCTGCCAGTCTGGAGGGAAAGTTGGAGTGGTC	1500
10	481	-T--L--S--V--P--P--G--H--S--L--P--S--L--E--G--K--F--G--V--V-	500
	1501	CTGCAGACAGCCAAGTACAAGGTGAATGCCACAATCAGACCAGACTGGGCAAGACATAAG	1560
	501	-L--Q--T--A--K--Y--K--V--N--A--T--I--R--P--D--W--A--R--H--K-	520
15	1561	TGC	1563
	521	-C-	521

SEQ ID NOs 61 and 63 (Cyp17a1 mutant allele- 16nt deletion)

LENGTH: 1563bp and 44aa

TYPE: cDNA (SEQ ID NO: 61) and Protein (SEQ ID NO: 63)

20 ORGANISM: Nile tilapia

	1	GAACCAAACCCCTCTGTCACTGATATGGCTTGGTTTTTGTGTCTGTGCGGTGGGCCTCAC	60
	1	-E--P--N--P--S--V--T--D--M--A--W--F--L--C--L--C--G--G--P--H-	20
25	61	TTTGTTAGCACTGCAGTTCAAGTTCAGGATGTCTGCACATGGTTCTGGGGAGCCACCTCC	120
	21	-F--V--S--T--A--V--Q--V--Q--D--V--C--T--W--F--W--G--A--T--S-	40
	121	CTGCACTACCACTGATTGGCAGCCTGCTGAGCCTGCGGAGTGAATTACCACCGCATGTGC	180
30	41	-L--H--Y--H--*-	44

SEQ ID NOs 65 and 68 (wild-type Cyp19a1a)

LENGTH: 1707bp and 511aa

TYPE: cDNA (SEQ ID NO: 65) and Protein (SEQ ID NO: 68)

35 ORGANISM: Nile tilapia

	1	GCGATGAATCCTGTAGGCTTAGACGCCGTGGTGGCAGATCTCTCTGTGACCTCAAATGCC	60
		...M--N--P--V--G--L--D--A--V--V--A--D--L--S--V--T--S--N--A-	19
40	61	ATCCAATCGCATGGGATATCAATGGCAACCAGAACGCTGATACTGCTCGTCTGTCTGCTG	120
	20	-I--Q--S--H--G--I--S--M--A--T--R--T--L--I--L--L--V--C--L--L-	39
	121	TTGGTTGCCTGGAGTCACACGGACAAGAAAATTGTGCCAGGTCCTTCTTTCTGTTTGGGT	180
45	40	-L--V--A--W--S--H--T--D--K--K--I--V--P--G--P--S--F--C--L--G-	59
	181	TTGGGCCCCACTTCTGTCTATCTGAGATTTATCTGGACTGGCATAGGCACAGCCAGCAAC	240
	60	-L--G--P--L--L--S--Y--L--R--F--I--W--T--G--I--G--T--A--S--N-	79
50	241	TACTACAATAACAAGTATGGAGACATTGTTAGAGTCTGGATCAACGGAGAAGAGACGCTC	300
	80	-Y--Y--N--N--K--Y--G--D--I--V--R--V--W--I--N--G--E--E--T--L-	99
	301	ATACTAAGCAGATCTTCAGCAGTGCACCATGTGCTGAAGAACGGAAACTATACTTCACGT	360
	100	-I--L--S--R--S--S--A--V--H--H--V--L--K--N--G--N--Y--T--S--R-	119

	361	TTTGGGAGCATCCAGGGACTCAGCTGCCTCGGCATGAACGAGAGAGGCATCATATTCAAC	420
	120	-F--G--S--I--Q--G--L--S--C--L--G--M--N--E--R--G--I--I--F--N-	139
5	421	AACAACGTAACCTCTGTGGAAAAAGATACGCACCTATTTTGCTAAAGCTCTGACAGGCCCA	480
	140	-N--N--V--T--L--W--K--K--I--R--T--Y--F--A--K--A--L--T--G--P-	159
	481	AATTTGCAGCAGACGGCGGATGTTTGCCTCTCCTCCATACAGGCTCACCTGGACCACCTG	540
10	160	-N--L--Q--Q--T--A--D--V--C--V--S--S--I--Q--A--H--L--D--H--L-	179
	541	GACAGCCTGGGACACGTTGATGTCTCAATTTGCTGCGTGCACCGTGCTGGACATCTCT	600
	180	-D--S--L--G--H--V--D--V--L--N--L--L--R--C--T--V--L--D--I--S-	199
15	601	AACCGACTCTCCTGGACGTACCTCTCAATGAGAAAGAGCTGATGCTGAAGATTCAAAAG	660
	200	-N--R--L--F--L--D--V--P--L--N--E--K--E--L--M--L--K--I--Q--K-	219
	661	TATTTTCACACATGGCAGGATGTGCTTATCAAACCTGACATCTACTTCAAGTTCGGCTGG	720
	220	-Y--F--H--T--W--Q--D--V--L--I--K--P--D--I--Y--F--K--F--G--W-	239
20	721	ATTCACCACAGGCACAAGACAGCAACCCAGGAGTTACAAGATGCCATTAAACGTCTTGTA	780
	240	-I--H--H--R--H--K--T--A--T--Q--E--L--Q--D--A--I--K--R--L--V-	259
	781	GATCAAAAGAGGAAAAATATGGAGCAGGCAGATACGCTGGACAACATCAACTTCACGGCA	840
25	260	-D--Q--K--R--K--N--M--E--Q--A--D--T--L--D--N--I--N--F--T--A-	279
	841	GAGCTCATATTTGCACAAAACCCACGGTGAGCTGTCTGCTGAGAATGTGACGCAGTGCCTG	900
	280	-E--L--I--F--A--Q--N--H--G--E--L--S--A--E--N--V--T--Q--C--V-	299
30	901	CTGGAGATGGTGATTGCAGCTCCGGACACTCTGTCCCTCAGTCTCTTCTTCATGCTTCTG	960
	300	-L--E--M--V--I--A--A--P--D--T--L--S--L--S--L--F--F--M--L--L-	319
	961	CTCCTCAAACAAAACCCGCACGTGGAGCCGCAGCTGCTACAGGAGATAGACGCTGTTGTG	1020
	320	-L--L--K--Q--N--P--H--V--E--P--Q--L--L--Q--E--I--D--A--V--V-	339
35	1021	GGTGAGAGACAGCTTCAGAACCAGGATCTTCACAAGCTGCAGGTGATGGAGAGCTTCATC	1080
	340	-G--E--R--Q--L--Q--N--Q--D--L--H--K--L--Q--V--M--E--S--F--I-	359
	1081	TACGAATGCTTGCGCTTCCACCCAGTGGTGGACTTCACCATGCGTCGAGCCCTGTCTGAT	1140
40	360	-Y--E--C--L--R--F--H--P--V--V--D--F--T--M--R--R--A--L--S--D-	379
	1141	GACATCATAGAAGGCTACAGGATCTCGAAGGGCACAAACATCATTTCTGAACACAGGCCGA	1200
	380	-D--I--I--E--G--Y--R--I--S--K--G--T--N--I--I--L--N--T--G--R-	399
45	1201	ATGCACCGCACCGAGTTTTTCCTCAAAGCCAATCAATTTAACCTGGAACACTTTGAAAAC	1260
	400	-M--H--R--T--E--F--F--L--K--A--N--Q--F--N--L--E--H--F--E--N-	419
	1261	AATGTTCTCGGCGCTACTTTTCAGCCGTTCCGGTTCAGGCCCTCGCGCATGCATTGGCAAG	1320
	420	-N--V--P--R--R--Y--F--Q--P--F--G--S--G--P--R--A--C--I--G--K-	439
50	1321	CACATCGCCATGGTGATGATGAAATCCATTTTGCTGACACTGCTGTCTCAGTACTCTGTT	1380
	440	-H--I--A--M--V--M--M--K--S--I--L--V--T--L--L--S--Q--Y--S--V-	459
	1381	TGTACTCACGAGGGCCCGATCCTGGACTGCCTCCACAAACCAACAACCTTTCCAGCAG	1440
55	460	-C--T--H--E--G--P--I--L--D--C--L--P--Q--T--N--N--L--S--Q--Q-	479
	1441	CCTGTAGAGCACCAGCAGGCGGAGACTGAACATCTCCACATGAGGTTCTTACCCAGGCAG	1500
	480	-P--V--E--H--Q--Q--A--E--T--E--H--L--H--M--R--F--L--P--R--Q-	499
60	1501	AGAAGCAGCTGTCAAACCTCCGAGACCCGAACCTTTAGCTGTACCTGCACTTTTGTATA	1560
	500	-R--S--S--C--Q--T--L--R--D--P--N--L--*--.....	511

5
1561 CTTAATTTGTATAATCTTATAACGACACACAGCTAGCCTTTATATTTTGATAGACGCAA 1620
.....
1621 GATTGTATTTGTACTCAAACCTGTATGCATGATGTGAAATGTACATTTAAACCTGCTAACA 1680
.....
1681 CTGAAATAAATGTAAGTTATTGTGTCA 1707
.....

SEQ ID NOs 66 and 69 (Cyp19a1a mutant allele- 7nt deletion)

10 LENGTH: 1707bp and 12aa
TYPE: cDNA (SEQ ID NO: 66) and Protein (SEQ ID NO: 69)
ORGANISM: Nile tilapia

15
1 GCGATGAATCCTGTAGGCTTAGACTGGCAGATCTCTCTGTGACCTCAAATGCCATCCAAT 60
...-M--N--P--V--G--L--D--W--Q--I--S--L--*- 12

SEQ ID NOs 67 and 70 (Cyp19a1a mutant allele- 10nt deletion)

20 LENGTH: 1707bp and 11aa
TYPE: cDNA (SEQ ID NO: 67) and Protein (SEQ ID NO: 70)
ORGANISM: Nile tilapia

25
1 GCGATGAATCCTGTAGGCTGGTGGCAGATCTCTCTGTGACCTCAAATGCCATCCAATCGC 60
...-M--N--P--V--G--W--W--Q--I--S--L--*- 11

SEQ ID NOs 71 and 73 (wild-type Tip1a)

LENGTH: 6674bp and 1652aa

TYPE: cDNA (SEQ ID NO: 71) and Protein (SEQ ID NO: 73)

5 ORGANISM: Nile tilapia

	1	AAAGAGGAAAACAATGCATCATATAACTTTATAAGTAAGAGTGC GGCGATGGAGGAAACC	60
	1	-K--E--E--N--N--A--S--Y--N--F--I--S--K--S--A--A--M--E--E--T-	20
10	61	GTCATATGGGAACAGCACACAGTTACCCCTTCACAGGGCCCCAGGATTTGGGTTTGGCATT	120
	21	-V--I--W--E--Q--H--T--V--T--L--H--R--A--P--G--F--G--F--G--I-	40
	121	GCCATCTCGGGTGGGCGAGACAACCCTCATTTCAGAGTGGTGAAACATCTATTGTAATA	180
15	41	-A--I--S--G--G--R--D--N--P--H--F--Q--S--G--E--T--S--I--V--I-	60
	181	TCGGATGTGCTGAAAGGAGGTCTGCAGAGGGTCTGCTACAAGAAAATGATCGAGTAGTA	240
	61	-S--D--V--L--K--G--G--P--A--E--G--L--L--Q--E--N--D--R--V--V-	80
	241	ATGGTCAATGCAGTCTCTATGGACAATGTAGAGCATGCCTATGCTGTTCAACAACCTCGA	300
20	81	-M--V--N--A--V--S--M--D--N--V--E--H--A--Y--A--V--Q--Q--L--R-	100
	301	AAGAGTGGCAAAAATGCAAAGATAACTATTTCGAGAAAAAGGAAAGTACAAATCCCAGCG	360
	101	-K--S--G--K--N--A--K--I--T--I--R--R--K--R--K--V--Q--I--P--A-	120
25	361	TCACGGCACGGGGACAGGGAGACGATGTCTGAGCACGAGGAGGAGACAGCGATGAGGCT	420
	121	-S--R--H--G--D--R--E--T--M--S--E--H--E--E--E--D--S--D--E--A-	140
	421	GATGCTTACGATCACCGCAGTGGACGTGGTGGACAAAACGATCGGGAGCGTAGCAGCAGT	480
30	141	-D--A--Y--D--H--R--S--G--R--G--G--Q--N--D--R--E--R--S--S--S-	160
	481	GGGAGGCGGGATCACAGTGCCTCACAGGAGAGGAGCATCTCACCACGCTCCGATCGCCGA	540
	161	-G--R--R--D--H--S--A--S--Q--E--R--S--I--S--P--R--S--D--R--R-	180
	541	TCACAAGCCTCTTCTGCTCCACCCAGGCCCTCCAAGGTCACCTCTTGTCAAGTCCCGCAA	600
35	181	-S--Q--A--S--S--A--P--P--R--P--S--K--V--T--L--V--K--S--R--K-	200
	601	AACGAAGAATATGGACTGCGGCTGGCCAGCCATATCTTTGTGAAGGACATCTCTCCAGAG	660
	201	-N--E--E--Y--G--L--R--L--A--S--H--I--F--V--K--D--I--S--P--E-	220
40	661	AGCCTTGCAGCCAGAGATGGAAACATTCAGGAGGGAGATGTTGTACTTAAGATTAACGGC	720
	221	-S--L--A--A--R--D--G--N--I--Q--E--G--D--V--V--L--K--I--N--G-	240
	721	ACAGTTACAGAGAACCTATCACTGACAGATGCCAAGAAGCTGATTGAGAGGTCAAAGGGC	780
45	241	-T--V--T--E--N--L--S--L--T--D--A--K--K--L--I--E--R--S--K--G-	260
	781	AAGCTGAAGATGGTAGTGCAGAGAGACGAGCGGGCCACGCTGCTCAATATTCCTGACCTT	840
	261	-K--L--K--M--V--V--Q--R--D--E--R--A--T--L--L--N--I--P--D--L-	280
	841	GACGACAGCATCCCATCAGCCAATAACTCAGACAGAGATGACATTTTCAGAGATACATTCA	900
50	281	-D--D--S--I--P--S--A--N--N--S--D--R--D--D--I--S--E--I--H--S-	300
	901	CTGACATCCGATCATTCCAATCGATCCCATGGGAGAGGAAGTCAATCCCATTTCGCCTGAC	960
	301	-L--T--S--D--H--S--N--R--S--H--G--R--G--S--Q--S--H--S--P--D-	320
55	961	AGGGTTGAAACATCCGAGCATCTCCGCCACTCACCGCGCAGATCAGCAATGGCAGTAAT	1020
	321	-R--V--E--T--S--E--H--L--R--H--S--P--R--Q--I--S--N--G--S--N-	340

	1021	GGATTTCTCTGGGAAAGAGCTGAGGAATTAATCAAACAGGAATGGGTGGTGAACAGGAA	1080
	341	-G--F--L--W--E--R--A--E--E--L--I--K--Q--E--W--V--V--K--Q--E-	360
5	1081	TGTTATTTTGCCTGTGCCCATACTATAAAATGTGTAATAACCGTGACAGTTTGGGCAAAA	1140
	361	-C--Y--F--A--C--A--H--T--I--K--C--V--I--T--V--T--V--W--A--K-	380
	1141	AAACCCCAAAACAGTAACATGCCAGAACCAAAGCCAGTTTATGCACAGCCTGGTCAGCCT	1200
	381	-K--P--Q--N--S--N--M--P--E--P--K--P--V--Y--A--Q--P--G--Q--P-	400
10	1201	GACGTGGACCTGCCTGTCAGCCCATCTGATGCCCTGTACCCAGTGCTGCACATGATGAC	1260
	401	-D--V--D--L--P--V--S--P--S--D--A--P--V--P--S--A--A--H--D--D-	420
	1261	AGCATTCTCAGACCAAGTATGAAGCTGGTCAAGTTCAAGAAGGGAGAGAGTGTCCGTCTG	1320
15	421	-S--I--L--R--P--S--M--K--L--V--K--F--K--K--G--E--S--V--G--L-	440
	1321	AGGTTAGCAGGCGGAAACGATGTGGGAATTTTGTGGCAGGAGTTTGGAAAGACAGCCCC	1380
	441	-R--L--A--G--G--N--D--V--G--I--F--V--A--G--V--L--E--D--S--P-	460
20	1381	GCAGCCAAGGAGGGGCTGGAAGAGGGAGACCAGATTCTCAGGGTGAACAACGTGGACTTT	1440
	461	-A--A--K--E--G--L--E--E--G--D--Q--I--L--R--V--N--N--V--D--F-	480
	1441	GCTAACATCATCCGGAAGAGGCTGTGCTTTTCTGCTCGATCTTCCAAAAGGAGATGAC	1500
	481	-A--N--I--I--R--E--E--A--V--L--F--L--L--D--L--P--K--G--D--D-	500
25	1501	GTTACTATTCTGGCTCAGAAGAAAAAGGATGTGTATCGAAGGATAGTGAATCAGACGTG	1560
	501	-V--T--I--L--A--Q--K--K--K--D--V--Y--R--R--I--V--E--S--D--V-	520
	1561	GGTGACTCCTTCTACATTGGAACGCATTTTGAATATGAAAAAGAGTCACCGTATGGGCTG	1620
30	521	-G--D--S--F--Y--I--R--T--H--F--E--Y--E--K--E--S--P--Y--G--L-	540
	1621	AGCTTTAACAAGGGAGAGGTTTCCGTGTGGTAGACACACTCTATAATGGCAAATTAGGC	1680
	541	-S--F--N--K--G--E--V--F--R--V--V--D--T--L--Y--N--G--K--L--G-	560
35	1681	TCCTGGCTCGCTATCCGTATCGGCAAGAACCACCAGGAAGTGGAAGAGGCATAATCCCC	1740
	561	-S--W--L--A--I--R--I--G--K--N--H--Q--E--V--E--R--G--I--I--P-	580
	1741	AACAAGAATAGAGCCGAGCAGCTATCCAGTGTGCAGTACACCTTCCTAAAACGCCTGGG	1800
	581	-N--K--N--R--A--E--Q--L--S--S--V--Q--Y--T--L--P--K--T--P--G-	600
40	1801	GGCGACAGAGCTGACTTCTGGAGGTTTCAGAGGGCTGCGGAGTTCCAAGAGGAATTTGCGG	1860
	601	-G--D--R--A--D--F--W--R--F--R--G--L--R--S--S--K--R--N--L--R-	620
	1861	AAAAGCAGGGAGGACCTGTGCGCCAGCCTGTTTCAGACCAAGTTCCCTGCCTATGAGAGG	1920
45	621	-K--S--R--E--D--L--S--A--Q--P--V--Q--T--K--F--P--A--Y--E--R-	640
	1921	GTGGTGTCTGAGGGAAGCTGGGTTCCTGAGGCCTGTGGTTATCTTTGGGCCGATTGCAGAC	1980
	641	-V--V--L--R--E--A--G--F--L--R--P--V--V--I--F--G--P--I--A--D-	660
50	1981	GTGGCCCCGAGAGAACTGGCCAGGGAGGTGCCCGAAGTGTGAGCTAGCCAAGAGTGAA	2040
	661	-V--A--R--E--K--L--A--R--E--V--P--E--V--F--E--L--A--K--S--E-	680
	2041	CCCAGGGATGCAGGAACAGACCAGAAGAGCTCTGGCATCATCCGCCTGCACACCATTAAG	2100
	681	-P--R--D--A--G--T--D--Q--K--S--S--G--I--I--R--L--H--T--I--K-	700
55	2101	CAGATCATTGATCGAGACAAGCATGCAGTGTGGATATAACCCCGAATGCAGTGGACCGA	2160
	701	-Q--I--I--D--R--D--K--H--A--V--L--D--I--T--P--N--A--V--D--R-	720
	2161	CTGAACTACGCTCAGTGGTATCCAATTGTGGTGTCTTCTCAACCCGGACACCAAGCAGGGC	2220
60	721	-L--N--Y--A--Q--W--Y--P--I--V--V--F--L--N--P--D--T--K--Q--G-	740

	2221	ATCAAGAACATGAGGACACGGCTCTGCCCCGAGTCTAGGAAGAGCGCGAGAAAGCTTTAT	2280
	741	-I--K--N--M--R--T--R--L--C--P--E--S--R--K--S--A--R--K--L--Y-	760
5	2281	GATCGAGCCCTCAAGTTAAGAAAGAACAACCACCACCTCTTCACCACAACCATTAACCTTG	2340
	761	-D--R--A--L--K--L--R--K--N--N--H--H--L--F--T--T--T--I--N--L-	780
	2341	AACAACATGAACGATGGTTGGTTTGGAGCACTGAAAGAAATCATCCATCAGCAGCAGAAC	2400
	781	-N--N--M--N--D--G--W--F--G--A--L--K--E--I--I--H--Q--Q--Q--N-	800
10	2401	CAGCTGGTGTGGGTTTTCAGAGGGCAAGGCTGATGGAGTTGGCGACGATGACCTGGACATC	2460
	801	-Q--L--V--W--V--S--E--G--K--A--D--G--V--G--D--D--D--L--D--I-	820
	2461	CACGACGACCGCCTTTCTACCTGTCTGGCGCCAGGCAGTGAGTATTCCATGTACAGCACC	2520
	821	-H--D--D--R--L--S--Y--L--S--A--P--G--S--E--Y--S--M--Y--S--T-	840
15	2521	GACAGCCGCCACACCTCCGATTACGAGGACACGGACACAGAGGGAGGAGCCTACACCGAC	2580
	841	-D--S--R--H--T--S--D--Y--E--D--T--D--T--E--G--G--A--Y--T--D-	860
	2581	CAGGAGCTGGATGAAACGCTGAACGATGACGTGGGTCCACCCACGGAGCCTGCCATCACG	2640
	861	-Q--E--L--D--E--T--L--N--D--D--V--G--P--P--T--E--P--A--I--T-	880
20	2641	CGGTCCTCTGAGCCTGTCCGTGAGGACCCGCTGTTCATCCAAGAGCCCCCTGGCTATGTC	2700
	881	-R--S--S--E--P--V--R--E--D--P--P--V--I--Q--E--P--P--G--Y--V-	900
	2701	AGCTACCCGCACACAGTGCAGCCGGACCCCTGAACCGCATCGACCCGGCTGGTTTCAAG	2760
	901	-S--Y--P--H--T--V--Q--P--D--P--L--N--R--I--D--P--A--G--F--K-	920
	2761	GCACCAGCGCCGCAGCAGATGTTTCAGAAGGATCCGTACAGCACAGACAACATAGGCAGA	2820
	921	-A--P--A--P--Q--Q--M--F--Q--K--D--P--Y--S--T--D--N--I--G--R-	940
30	2821	GGTGGTCACGGCATGAAGCCTGTGACGTACAACCCTCAGCAGGGGTATCACCCCGACGAG	2880
	941	-G--G--H--G--M--K--P--V--T--Y--N--P--Q--Q--G--Y--H--P--D--E-	960
	2881	CAGCCATACAGAGATTACGATCACCCACCCAGCCGGTATGACATCAGCAGCAGTGGTGTG	2940
	961	-Q--P--Y--R--D--Y--D--H--P--P--S--R--Y--D--I--S--S--S--G--V-	980
	2941	GGCGGTGGCTACCAGGAGCCAAAGTACCGTAACCTATGAGAGCTATGAGAACAGCGTGCCT	3000
	981	-G--G--G--Y--Q--E--P--K--Y--R--N--Y--E--S--Y--E--N--S--V--P-	1000
40	3001	CACTACGACCAGCAACCGTGAACCCCTACAACCAGCCGTTCTCCACTGCCAACACCCAG	3060
	1001	-H--Y--D--Q--Q--P--W--N--P--Y--N--Q--P--F--S--T--A--N--T--Q-	1020
	3061	GCCTACGATCCCCGTCCTCCTTACGGTGAGGGCCCCGACTCTCATTACACCCCTCCCCTG	3120
	1021	-A--Y--D--P--R--P--P--Y--G--E--G--P--D--S--H--Y--T--P--P--L-	1040
45	3121	CGCTACGACGAGCCGCCACCTCAGCAGGGATTTGACGGACGGCCTCGCTACGGCAAACCG	3180
	1041	-R--Y--D--E--P--P--P--Q--Q--G--F--D--G--R--P--R--Y--G--K--P-	1060
	3181	ACAGTTTTCAGCACCTGTCCGTTACGATGATCTTCCGCCTCCCCCTCAGCCGTCTGAATTG	3240
	1061	-T--V--S--A--P--V--R--Y--D--D--L--P--P--P--P--Q--P--S--E--L-	1080
50	3241	CACTATGACCCAAATTCTCACCTGAGCACATACCCCTCAGCTGCCCCGCTCACCAGAACCC	3300
	1081	-H--Y--D--P--N--S--H--L--S--T--Y--P--S--A--A--R--S--P--E--P-	1100
	3301	GCTGCCCAGCGACCCGCCTATAACCAGGGACCAGCATCGCAGCAGAAAGGTTACAAACCT	3360
	1101	-A--A--Q--R--P--A--Y--N--Q--G--P--A--S--Q--Q--K--G--Y--K--P-	1120
	3361	CAGCAGTACGATCCTGCTCCTGTGAACCTCTGAATCCAGCCCCAGCCTTCATAAAGTCGAG	3420
	1121	-Q--Q--Y--D--P--A--P--V--N--S--E--S--S--P--S--L--H--K--V--E-	1140
60			

	3421	ACGCCCTCACCTTCACCTGCTGATGTTCCAAAAGCTGCACCTGCAAGAGATGAGCAGCAG	3480
	1141	-T--P--S--P--S--P--A--D--V--P--K--A--A--P--A--R--D--E--Q--Q-	1160
5	3481	GAGGAGGATCCAGCCATGCGGCCCTCAGTCAGTACTGACGAGGGTCAAAATGTTTGAGAAC	3540
	1161	-E--E--D--P--A--M--R--P--Q--S--V--L--T--R--V--K--M--F--E--N-	1180
	3541	AAACGCTCTGTGTCCATGGACCGAGCCAGAGATGCCGGGGATTTCATTTGGGAATAAGGCA	3600
	1181	-K--R--S--V--S--M--D--R--A--R--D--A--G--D--S--F--G--N--K--A-	1200
10	3601	GCCGATTTGCCCTTGAAAGCTGGTGGAGTAATCCCTAAAGCAAATTCTCTGAGCAACCTG	3660
	1201	-A--D--L--P--L--K--A--G--G--V--I--P--K--A--N--S--L--S--N--L-	1220
	3661	GATCAAGAGAAGACCTTTAGCAGAGGGCCAGAGCCTCAGAAGCCTCAGTCCAAGGGATCC	3720
15	1221	-D--Q--E--K--T--F--S--R--G--P--E--P--Q--K--P--Q--S--K--G--S-	1240
	3721	GATGACATCGTGCCTCCAACCATTTATGACCTGATGAGGATGAGGACTACTACAGGAAA	3780
	1241	-D--D--I--V--R--S--N--H--Y--D--P--D--E--D--E--D--Y--Y--R--K-	1260
20	3781	CAGTTGTCTTACTTTGACAGACTGCAGACTGGCTCCAATAAACCCCAACCACAAGCACAG	3840
	1261	-Q--L--S--Y--F--D--R--L--Q--T--G--S--N--K--P--Q--P--Q--A--Q-	1280
	3841	TCCAGCCACAGCTTCCCCAGCCATTATACACATTTTGGATATTCAAGTGTCTTTCTTTTC	3900
	1281	-S--S--H--S--F--P--S--H--Y--T--H--F--G--Y--S--S--V--F--L--F-	1300
25	3901	TTTTCTTAATGATGGACTCTGTGGAGAAACCAAGCCCACTGGAGAAAAATATGAACCA	3960
	1301	-F--S--L--M--M--D--S--V--E--K--P--S--P--L--E--K--K--Y--E--P-	1320
	3961	GTTCCCAAGTGACACCAGCTGTGCCACCGGCCACGCTGCCCAAGCCCTCACCTGATGGT	4020
30	1321	-V--P--Q--V--T--P--A--V--P--P--A--T--L--P--K--P--S--P--D--G-	1340
	4021	AAAATTGACTGTAGTCAGGATTTTTATCTCATCTCTTTGACTGATGTGCGTTGCTCTTCC	4080
	1341	-K--I--D--C--S--Q--D--F--Y--L--I--S--L--T--D--V--R--C--S--S-	1360
35	4081	ACAGCCAAACCTCCTGCTCGAGAGGACACGGTCCAGACCAACTTTCTTCCTCACAAGAGC	4140
	1361	-T--A--K--P--P--A--R--E--D--T--V--Q--T--N--F--L--P--H--K--S-	1380
	4141	TTCCCTGAGAAGTCTCCAGTCAATGGCACCAGTGAACAGCCTCCAAAGACGGTCACTAGC	4200
	1381	-F--P--E--K--S--P--V--N--G--T--S--E--Q--P--P--K--T--V--T--S-	1400
40	4201	ACCGGGGGTTTGCCACATCCACCTACAACCGCTTTGCGCCCAAGCCCTACACCTCCTCT	4260
	1401	-T--G--G--L--P--T--S--T--Y--N--R--F--A--P--K--P--Y--T--S--S-	1420
	4261	GCCAAGCCTTTTTGCGCAAGTTCGACAGTCCATAATTCAACCACAACCTCCTGTCCAAT	4320
45	1421	-A--K--P--F--S--R--K--F--D--S--P--K--F--N--H--N--L--L--S--N-	1440
	4321	GACAAGCCTGAGAGTGCTCCCAAGGGACGGAGCTCGAGTCCGGTAAAGCCTCAGGTACCC	4380
	1441	-D--K--P--E--S--A--P--K--G--R--S--S--S--P--V--K--P--Q--V--P-	1460
50	4381	CCACAGCCCCAGAACGCAGACCAAGACAGTGGCCTGGACACTTTCACACGCACAACGGAC	4440
	1461	-P--Q--P--Q--N--A--D--Q--D--S--G--L--D--T--F--T--R--T--T--D-	1480
	4441	CCCCGATCCAAATACCAGCAGAACAACGTAAACGCCGTGCCCAAGGCCATCCCTGTGAGC	4500
	1481	-P--R--S--K--Y--Q--Q--N--N--V--N--A--V--P--K--A--I--P--V--S-	1500
55	4501	CCCAGTGCCTAGAGGATGATGAAGATGAAGACGAAGGTCACACTGTGGTGGCAACAGCT	4560
	1501	-P--S--A--L--E--D--D--E--D--E--D--E--G--H--T--V--V--A--T--A-	1520
	4561	CGTGGCATCTTCAACTCTAACGGTGGCGTTCTGAGCTCCATCGAGACAGGTGTGAGCATC	4620
60	1521	-R--G--I--F--N--S--N--G--G--V--L--S--S--I--E--T--G--V--S--I-	1540

	4621	ATTATCCCACAGGGTGCCATCCCCGACGGCGTGGAGCAAGAGATTTACTTCAAGGTCTGT	4680
	1541	-I--I--P--Q--G--A--I--P--D--G--V--E--Q--E--I--Y--F--K--V--C-	1560
5	4681	CGAGACAACAGCATCCTGCCGCCACTCGACAAGGAGAAAGGAGAGACTCTGCTCAGCCCT	4740
	1561	-R--D--N--S--I--L--P--P--L--D--K--E--K--G--E--T--L--L--S--P-	1580
	4741	CTGGTGATGTGTGGACCTCACGGCCTAAAGTTCTGAAGCCTGTGGAGCTACGCTTACCT	4800
	1581	-L--V--M--C--G--P--H--G--L--K--F--L--K--P--V--E--L--R--L--P-	1600
10	4801	CACTGTGCGTCAATGACCCCTGATGGTTGGTCTTTTGCTCTAAAATCCTCCGACTCCTCG	4860
	1601	-H--C--A--S--M--T--P--D--G--W--S--F--A--L--K--S--S--D--S--S-	1620
	4861	TCGGGTGATCCAAAAGCTGGCAGAACAAGTCTCTACCGGAGACCCCAACTACCTGGTG	4920
15	1621	-S--G--D--P--K--S--W--Q--N--K--S--L--T--G--D--P--N--Y--L--V-	1640
	4921	GGAGCCAACCTGTGTCTCTGTGCTCATTGACCACTTTTAAAGAAGAAGCAGCAGGTGTGAT	4980
	1641	-G--A--N--C--V--S--V--L--I--D--H--F--*--.....	1652
20	4981	GTTACTGAATGTGGAAGAATGGCGGATGAAATGAAGACGATGGAAACGCACGCACGCAAA	5040
			
	5041	CACACACATATACCACTACACACACACACACTGACAGACGCACTCCAAGCAAACCAAC	5100
			
	5101	ACACAGCATAGAGTATGAAGAAGACCCAGACAGTGCTGGACGAAGGAGAGACACCAATGA	5160
25			
	5161	TCGTTACGAGCTGTTCTTTAAACTCAATTTCAAAGTTTTGATGTAAAATGATGCATGCCC	5220
			
	5221	AACGTCACCTGACGATTGACACTTATATATAAAGCAATGTTTAATGTAATTTTCTTTTTT	5280
			
30	5281	CTTTTTTTTACAAAAGTATAGATGGATGTATGGCTTTTGAGGCAGCATACATGCTTGAAAA	5340
			
	5341	ATCTGTGTCAATGTATTTATGCTATATATAGCTACAGTATATAGAGAAGATAGAGAAGA	5400
			
	5401	AATTGGACTCGAATTCGATCGCCAGTCAACATCTTGTGTGTTTTTCAGTTCAGGGGACTG	5460
			
35	5461	GATTTTTTGTGTTGTTGTTGTTGTTTTTCCCTTCCACATTGAAGGAATCTTACTGA	5520
			
	5521	AGGTTTGATACAGTTGGTTTAAGGAGGTGGCAAGACATGAGCTGGACATGAACCCAAGCA	5580
			
40	5581	GCAGCAACAGCACACTTTTAGAGACGTTCTTCCTACACTTCTCACTTTGTCTTCTCTTTT	5640
			
	5641	CTTACCTTTTGTAGCTTCCTCTCTTACTGAGCACCACCTCTCTCTTCCAGCCTGAGGGA	5700
			
	5701	GATCTATGCATGTTCTTTACTCAGGTCCAGTAGCCTCCTCGGTTCTTCTCACATCTAC	5760
			
45	5761	TTAATATCTTTTCTTTCTGTGCACTCTTGCACCTCACACAAATAAGCAGTGATGCCTT	5820
			
	5821	ATCTGCAGATTATTCACCTTTTCATTAAGAAAAAAGTAAGTTATGATAAATTATGGTAT	5880
			
50	5881	AATGTCATTTGTTTTGCCATTTTTTTGTGAACCTCTGTATAAATAAACTTGGGTTTAGC	5940
			
	5941	ACACGCAGAAACAGTCGATAAAAGATAACAAAGGTATGCTCTTCTTTTATCTGCTATGCA	6000
			
	6001	TTGCTTAAAAACAAAAACCATCAGAGAAGAAGTGGCTGTAAATAAAGCTAGCATATTGC	6060
			
55	6061	CTTGTTTCTTTTTTGTGTAATGAACCTTTTGTATGTCTTTCTTTTTTGTATAAACTT	6120
			
	6121	AGAGAAAACATGTTTTAAAAAAGCAGAAGGAAGTGAAGTGGTTATCTTTGTATTATGGG	6180
			
60	6181	CATACCTTCAAGCCTTTGAATTGTAACCTAACAATAATACATCACACCTTTTCTACCGAT	6240
			

	6241	ATGTTGCCGCCGCTATTTTACCGTCTCAAAAAGGTCGTCTTTTTTATTTTATTTCTAT	6300
	6301	TTTTATTACTTTGTCCACGTAGGGTTAAGGAAAGTATTTGCGGCTCAGATGCATTTAAAA	6360
5	6361	CATCTTCATTGGAAGGGTGTGCTCTCAAAGTGTCCCTCTCACTGATTTCTGATACTGGA	6420
	6421	TGCTATATTGTGTGCCCTTAAATGTATTTTGTACTAATAGACAATATATTACGTATGGC	6480
10	6481	ACACCAGTACTGTTTTACTTTTAGATATAACACGGCTTTATTGGATATTAGCTCTTCACT	6540
	6541	TGTGGCTGACTTTTTTTTTTTTCCCCTCTGCAACACAATTTTAAGTATACCACTGTATTA	6600
	6601	ATAAATAAAATCATTTTTTAAATTAAAGAGTGTGTAAGGATTTTTTATTTTTTTTACCCT	6660
15	6661	ACAGGGTTTTGTAT	6674

SEQ ID NOs 72 and 74 (Tip1a mutant allele- 7nt deletion)

LENGTH: 6674bp and 439aa

20 TYPE: cDNA (SEQ ID NO: 72) and Protein (SEQ ID NO: 74)

ORGANISM: Nile tilapia

	1	AAAGAGGAAAACAATGCATCATATAACTTTATAAGTAAGAGTGCGGCGATGGAGGAAACC	60
25	1	-K--E--E--N--N--A--S--Y--N--F--I--S--K--S--A--A--M--E--E--T-	20
	61	GTCATATGGGAACAGCACACAGTTACCCTTCACAGGGCCCCAGGATTTGGGTTTGGCATT	120
	21	-V--I--W--E--Q--H--T--V--T--L--H--R--A--P--G--F--G--F--G--I-	40
30	121	GCCATCTCGGGTGGGCGAGACAACCCTCATTTCCAGAGTGGTGAAACATCTATTGTAATA	180
	41	-A--I--S--G--G--R--D--N--P--H--F--Q--S--G--E--T--S--I--V--I-	60
	181	TCGGATGTGCTGAAAGGAGGTCCTGCAGAGGGTCTGCTACAAGAAAATGATCGAGTAGTA	240
	61	-S--D--V--L--K--G--G--P--A--E--G--L--L--Q--E--N--D--R--V--V-	80
35	241	ATGGTCAATGCAGTCTCTATGGACAATGTAGAGCATGCCTATGCTGTTCAACAACCTTCGA	300
	81	-M--V--N--A--V--S--M--D--N--V--E--H--A--Y--A--V--Q--Q--L--R-	100
	301	AAGAGTGGCAAAAATGCAAAGATAACTATTCGCAGAAAAAGGAAAGTACAAATCCCAGCG	360
40	101	-K--S--G--K--N--A--K--I--T--I--R--R--K--R--K--V--Q--I--P--A-	120
	361	TCACGGCACGGGACAGGGAGACGATGTCTGAGCACGAGGAGGACAGCGATGAGGCT	420
	121	-S--R--H--G--D--R--E--T--M--S--E--H--E--E--E--D--S--D--E--A-	140
45	421	GATGCTTACGATCACCGCAGTGGACGTGGTGGACAAAACGATCGGGAGCGTAGCAGCAGT	480
	141	-D--A--Y--D--H--R--S--G--R--G--G--Q--N--D--R--E--R--S--S--S-	160
	481	GGGAGGCGGGATCACAGTGCCTCACAGGAGAGGAGCATCTCACCACGCTCCGATCGCCGA	540
	161	-G--R--R--D--H--S--A--S--Q--E--R--S--I--S--P--R--S--D--R--R-	180
50	541	TCACAAGCCTCTTCTGCTCCACCCAGGCCCTCCAAGTCACTCTTGTCAAGTCCCGCAA	600
	181	-S--Q--A--S--S--A--P--P--R--P--S--K--V--T--L--V--K--S--R--K-	200
	601	AACGAAGAATATGGACTGCGGCTGGCCAGCCATATCTTTGTGAAGGACATCTCTCCAGAG	660
55	201	-N--E--E--Y--G--L--R--L--A--S--H--I--F--V--K--D--I--S--P--E-	220
	661	AGCCTTGACGCCAGAGATGGAACATTGAGAGGGAGATGTTGTACTTAAGATTAACGGC	720

	221	-S--L--A--A--R--D--G--N--I--Q--E--G--D--V--V--L--K--I--N--G-	240
	721	ACAGTTACAGAGAACCTATCACTGACAGATGCCAAGAAGCTGATTGAGAGGTCAAAGGGC	780
5	241	-T--V--T--E--N--L--S--L--T--D--A--K--K--L--I--E--R--S--K--G-	260
	781	AAGCTGAAGATGGTAGTGCAGAGAGACGAGCGGGCCACGCTGCTCAATATTCCTGACCTT	840
	261	-K--L--K--M--V--V--Q--R--D--E--R--A--T--L--L--N--I--P--D--L-	280
10	841	GACGACAGCATCCCATCAGCCAATAACTCAGACAGAGATGACATTTTCAGAGATACATTCA	900
	281	-D--D--S--I--P--S--A--N--N--S--D--R--D--D--I--S--E--I--H--S-	300
	901	CTGACATCCGATCATTCCAATCGATCCCATGGGAGAGGAAGTCAATCCCATTCGCCTGAC	960
	301	-L--T--S--D--H--S--N--R--S--H--G--R--G--S--Q--S--H--S--P--D-	320
15	961	AGGGTTGAAACATCCGAGCATCTCCGCCACTCACCGCGGCAGATCAGCAATGGCAGTAAT	1020
	321	-R--V--E--T--S--E--H--L--R--H--S--P--R--Q--I--S--N--G--S--N-	340
	1021	GGATTTCTCTGGGAAAGAGCTGAGGAATTAATCAAACAGGAATGGGTGGTGAAACAGGAA	1080
20	341	-G--F--L--W--E--R--A--E--E--L--I--K--Q--E--W--V--V--K--Q--E-	360
	1081	TGTTATTTTGCCTGTGCCATACTATAAAATGTGTAATAACCGTGACAGTTTGGGCAAAA	1140
	361	-C--Y--F--A--C--A--H--T--I--K--C--V--I--T--V--T--V--W--A--K-	380
25	1141	AAACCCCAAAACAGTAACATGCCAGAACCAGCCAGTTTATGCACAGCCTGGTCAGCCT	1200
	381	-K--P--Q--N--S--N--M--P--E--P--K--P--V--Y--A--Q--P--G--Q--P-	400
	1201	GACGTGGACCTGCCTGTGAGCCCATCTGATGCCCCTGTACCCAGTGCTGCACATGATGAC	1260
	401	-D--V--D--L--P--V--S--P--S--D--A--P--V--P--S--A--A--H--D--D-	420
30	1261	AGCATTCTCAGACCAAGTATGAAGCTGGTCAAGTTCAAGAAGGGAGAGAGTGTGCGGT	1320
	421	-S--I--L--R--P--S--M--K--L--V--K--F--K--K--G--E--S--V--G--*-	439

SEQ ID NOs 75 and 77 (wild-type Hiat1a)

35 LENGTH: 5281bp and 491aa

TYPE: cDNA (SEQ ID NO: 75) and Protein (SEQ ID NO: 77)

ORGANISM: Nile tilapia

40	1	TTCTGCTTCGCCCTTGATTAGACAGCCAATCGCTGGACGTCACCTCCGCCAGAAGGGGTG	60
	61	GGTTGACGTAGTACAGGAAGCCAGGCGAGGTGAGGTGGGGAGGAGAGATCACAAAATTGT	120
	121	TAGCTCGCTGCTAGCTGCCTCCTCCGATTTGCCCGAAGTGCGATGAGCCCAGGAGGCGAA	180
45	181	ATTTGTGGGGTTTTTTGGTTTTGATTGGCGGACGATGACCCCTCTGACCCTAAGAATGGA	240
	241	CATAAGTTAATGATGACGGGGGAGAAGAAGAAGAAGCGGCTGAACCGCAGCATTCTT	300
		-M--M--T--G--E--K--K--K--K--K--R--L--N--R--S--I--L-	17
50	301	CTTGCAAAGAAAATTATAATAAAAGATGGAGGAAGTCCTCAGGGAATCGGGGAGCCCAGT	360
	18	-L--A--K--K--I--I--I--K--D--G--G--S--P--Q--G--I--G--E--P--S-	37
	361	GTTTACCATGCTGTGGTGGTCATCTTCCTGGAGTTTTTTGCATGGGGTCTGCTCACTACC	420
55	38	-V--Y--H--A--V--V--V--I--F--L--E--F--F--A--W--G--L--L--T--T-	57
	421	CCGATGCTCACGGTATTACACCAGACATTCCCCCAACACACATTCTGATGAATGGGCTC	480

	58	-P--M--L--T--V--L--H--Q--T--F--P--Q--H--T--F--L--M--N--G--L-	77
5	481	ATTCATGGTGTGAAGGGCCTGTTATCATTTCTCAGTGCTCCGCTAATTGGAGCGTTGTCA	540
	78	-I--H--G--V--K--G--L--L--S--F--L--S--A--P--L--I--G--A--L--S-	97
	541	GACGTATGGGGACGCAAGTCCTTCCTGCTGCTAACGGTCTTCTTCACTTGCGCGCCCAT	600
	98	-D--V--W--G--R--K--S--F--L--L--L--T--V--F--F--T--C--A--P--I-	117
10	601	CCGCTGATGAAGATCAGTCCATGGTGGTACTTTGCAGTCATCTCGATGTCCGGTGTTTTT	660
	118	-P--L--M--K--I--S--P--W--W--Y--F--A--V--I--S--M--S--G--V--F-	137
	661	GCCGTACACCTTCTCTGTGATCTTTGCCATGTGGCAGACATCACACAAGAGCATGAGAGG	720
	138	-A--V--T--F--S--V--I--F--A--Y--V--A--D--I--T--Q--E--H--E--R-	157
15	721	AGCACAGCTTATGGTTTGGTATCAGCTACCTTTGCAGCAAGCCTGGTTACCAGCCCAGCC	780
	158	-S--T--A--Y--G--L--V--S--A--T--F--A--A--S--L--V--T--S--P--A-	177
	781	ATTGGAGCCTACCTGTCTGAGGCTTACAGTGACACCTTGGTTGTGATCCTGGCCACAGCC	840
20	178	-I--G--A--Y--L--S--E--A--Y--S--D--T--L--V--V--I--L--A--T--A-	197
	841	ATCGCACTGCTCGACATCTGCTTCATCCTGGTGGCTGTACCAGAGTCGCTGCCGGAGAAG	900
	198	-I--A--L--L--D--I--C--F--I--L--V--A--V--P--E--S--L--P--E--K-	217
25	901	ATGAGGCCAGCGTCATGGGGAGCGCCATCTCCTGGGAACAGGCAGACCCCTTCGCTTCT	960
	218	-M--R--P--A--S--W--G--A--P--I--S--W--E--Q--A--D--P--F--A--S-	237
	961	CTGCGTAAAGTGGGCCAGGACTCTACGGTGCTCCTCATCTGTATCACAGTGTTCCTCTCC	1020
	238	-L--R--K--V--G--Q--D--S--T--V--L--L--I--C--I--T--V--F--L--S-	257
30	1021	TACCTCCCTGAGGCCGGCCAGTACTCCAGCTTCTTCCTCTATCTCAGACAGGTCATAGGC	1080
	258	-Y--L--P--E--A--G--Q--Y--S--S--F--F--L--Y--L--R--Q--V--I--G-	277
	1081	TTCTCCTCAGAGACAGTGGCTGCCTTCATCGCTGTTGTGGGAATCCTCTCAATATTAGCT	1140
35	278	-F--S--S--E--T--V--A--A--F--I--A--V--V--G--I--L--S--I--L--A-	297
	1141	CAGACGGTCGTGTTGGGGATCCTGATGCGCTCTATAGGAAATAAGAACACAATCCTGCTC	1200
	298	-Q--T--V--V--L--G--I--L--M--R--S--I--G--N--K--N--T--I--L--L-	317
40	1201	GGCCTCGGCTTTCAGATCCTCCAGCTGGCCTGGTATGGCTTTGGATCTCAGCCCTGGATG	1260
	318	-G--L--G--F--Q--I--L--Q--L--A--W--Y--G--F--G--S--Q--P--W--M-	337
	1261	ATGTGGGCAGCTGGAGCCGTTGCTGCCATGTCAGCATCACGTTCCCCGCCATCAGCGCC	1320
	338	-M--W--A--A--G--A--V--A--A--M--S--S--I--T--F--P--A--I--S--A-	357
45	1321	ATTGTGTCCCGTAATGCGGATCCTGACCAGCAAGGTGTTGTTTCAGGGCATGATCACTGGA	1380
	358	-I--V--S--R--N--A--D--P--D--Q--Q--G--V--V--Q--G--M--I--T--G-	377
	1381	ATTCGAGGCCTCTGTAACGGTTTGGGTCCTGCTCTTTACGGCTTCGTCTTCTACTTATTC	1440
50	378	-I--R--G--L--C--N--G--L--G--P--A--L--Y--G--F--V--F--Y--L--F-	397
	1441	CACGTGGAGCTGACCGACACGGACGGCTCTGAGAAAGGTGCCAAAGGGAACATGGCCAAC	1500
	398	-H--V--E--L--T--D--T--D--G--S--E--K--G--A--K--G--N--M--A--N-	417
55	1501	CCCACTGACGAGAGTGCCATCATCCCAGGTCCTCCCTTCCTCTTTGGTGCATGCTCAGTG	1560
	418	-P--T--D--E--S--A--I--I--P--G--P--P--F--L--F--G--A--C--S--V-	437
	1561	CTGCTGTCTCTGCTGGTGGCGCTGTTTCATCCCGAGCACACTGGGCCCCGGTATGAGGCC	1620
	438	-L--L--S--L--L--V--A--L--F--I--P--E--H--T--G--P--G--M--R--P-	457
60	1621	GGCTCCTACAAGAAGCACAGCAACGGGGCACAGAGTCACTCCCACAGCCCGCAAGGCAGC	1680

	458	-G--S--Y--K--K--H--S--N--G--A--Q--S--H--S--H--S--P--Q--G--S-	477
	1681	GGGGCAGAGGGCAAGGAGCCGCTGCTGGAGGACAGCAGCGTATAACCTCAGCTCAGGGGG	1740
5	478	-G--A--E--G--K--E--P--L--L--E--D--S--S--V--*-.	491
	1741	GGCAGACTCCCTCGCTCCACCTCAAAATGCCCTGCACACATGGACAGATACACATAATTT	1800
	1801	ATCACAAGGACACACACGCACCTCAGGCACACGTCACACTCGAGTGCCGCAAAGAGATGT	1860
10	1861	TTGTCTGTTTTGCTGTCCACAGCACAAAGTTGGGCGCTCCTTCCTTAGCAACCCTTTTCT	1920
	1921	TTATAATAGCTGGGTTATTGTGAGGACTTTCTAAAGACCCTGTGTGAAGAAAGTGTGTCTG	1980
15	1981	AGCATCATCAGGGCTGCAGTGAAGACCGTGTATGTGTGTGTGTGTGTGTGTGTGTGTGT	2040
	2041	GTGTGTGTGGCTGAGCTGAGCTGAGCTGGACTCCAATCTTTGGTTTGTCTGAAGTTGTAA	2100
	2101	CAGTGGAGCACACAACAGCTGTCCCCCTCCTGGCGCGAAACAGGACTGAAGTGAATTTG	2160
20	2161	GTTTAATGTGCGAGTGGGGATATATCTCTGATACGTTACTAAATACCTGTGTGACTCTTG	2220
	2221	ATTATTCTCTTTAGTTAGCCAAGTGGCACCTTCGTTTGTGAGAGAGAGCGTGACGAAC	2280
25	2281	GCCCTCTCACATGCTAATACTTCTGTTCTGATGCTTGTCTTTATGACTACAGCTCTGTTT	2340
	2341	AGGCGTCCAAGAAGGAAACATAGTTCTTCTCTGTGTGGACAACAGGGGAGCGCAGCAGC	2400
	2401	TGTTAAACCTGTGAAAGGAGCCTGCAAACCAGTATTGGAGAGGCGCTGCCTAATTGCAGT	2460
30	2461	CAGGGTTGGCAACCAGTTCAGATACAAAAAGCTTTGTTAGGACCAGGTTTGTTCAAATA	2520
	2521	TCAAACCTTCTTACAGAGAGATGACTAGAAAGAGACCACCTTTATTAGCTCAAATGGTTTTT	2580
35	2581	CAATGTTTACTTGCCATTCTCTAGATTAGTAGTACAGTTTGGGTTGTATATTTTTCTCTG	2640
	2641	TTCAAACCTGAAGGCTAGTTTGCTTCAAGTTTTTATTCAAGAAACAAATGTTGCCTTGAA	2700
	2701	GTGACTTAAGATATATATGGAGACATTACGTAACCTGTATGAAGACCGAGGTCTGAGAAG	2760
40	2761	GCTCTGTAATCTTGCGCTATTGCTCCCATCGGAGCCGTTACACACTTTTTATTCTTTGT	2820
	2821	ATTCATGCCCTTCTGTACTTTGTTTCTGACATTTATCACCATCAAGTTGAGGCTTAC	2880
45	2881	AGAGACACGGTTTTATTTTTAAAAAGCCTCTGGACCATTTGGAGCTGGAGCATTGCTATC	2940
	2941	AGGATGTCGGTGTCTGCACTGACTGTTTGAGTTGATATCATTAGGTTTCAGCAGAAATATCA	3000
	3001	GCCATGCTGCTGCAGTAGTAAATACAAAGGTTAATCAGTGTGGCGTAAAGTGGTGGATAA	3060
50	3061	GAATTATAACTGTGTCTTGTAAGTCCCTGACATTTAAGCTAACATGCGTACACTCAAAGAG	3120
	3121	GCAGGCCACACTTCTCCCAATGCCTAACATGAAGCACCTCACGGACGTGTCTGGCAACTT	3180
55	3181	GTGTAGAAGCTCTGCAGATGCCAGCCTGCGCCACCTAAGAGGCAGAAACAAATAGCAGTA	3240
	3241	GTGGAGTAGATGGCTGGAATGTTTCATGTTATCTCAAACAGTGAAGCAAAGTAAAAATC	3300
	3301	TGGAGGTTGTGTCAATGTGGAGAGTATTGCGAAATCTGCAATGATCCCAGATTTTCATTAG	3360
60	3361	TTTAAAAAAAAGAGAAAATAAGAAGAAGAAGAAAATCCACTTAAAAGTGTAAATCCTGAA	3420

		
	3421 TTTTATTATCGTTCAGATCTGCAGATGTCTCTGGGTTTTCTGCAGGTCTGAACTGCTG	3480
		
5	3481 CTGCCACGTTTATTTTATTTTCCCCGGTCAACAGGTGGCGCAGTCTGTACCTGGCATGC	3540
		
	3541 CTGTAAGGTGCTCGTGTGGTTTTGTTTTCTTTTTTCAGTCATGTGGATCAGCGATACT	3600
		
	3601 GCGTTCCTTCATTACATACTATGTCGCCACCTTTCACATTGTAACCTTGATCTGTGA	3660
		
10	3661 ATGCCTCTCGTAGCTAACAACCTGGTTTCATGCTGTTTAACATCTGTATGAACTGAAACAT	3720
		
	3721 ACGTCACGTATTTAGTGCCATATCTTCTTGATTTGCTTTTTCTTTTGTACTGTGTGTGT	3780
		
15	3781 GAATGTACACTTGTGTGATTTGAGTGTTTTGTTGTTCTTTTTATTTCTCTGTCTTAA	3840
		
	3841 TTTCTTTGACTGAAGATTTAAGTTTTAATGCTATTTTTTTAATAGCTTTTAAACTTCA	3900
		
	3901 GTCATTTTTTTAGGATTAATTGTCAAAATTGGATGGTAAATTATCAAATGTCCATCTGTC	3960
		
20	3961 CCCTTTGTTATGTTGTTTGTGTTTTGATTTACGCTCGGTCTTCATTTAATAACAAGCATT	4020
		
	4021 TCACCATGGTTTGTTAAGCTCATAATTTTTTCCCAGATTTCTCTGAATGTTTCCAATGAA	4080
		
25	4081 ACTGAACATGTTGACCACACAGTACCCTCAATCTTTAGGTTTTTTTTGTTTTGTCTTTTA	4140
		
	4141 AGAGGGGATGTTACTACACAGGAGGCCATTATTCCCGTTTTTTTTTTTTGTTTGTGTTTT	4200
		
	4201 TTAAATCATGTAATTGAACAACAGAAAATCGGATCCTGGTAAGATTCTGCACCAGCCCC	4260
		
30	4261 CCACCACCACCACCCACGTGCACACCTACAGCCTCCAAGCAGACGACTGTAAATGTACAA	4320
		
	4321 AAATCACCTGTACCTAGAGAAAAATGTATATATTTATTCCTCAAGGAGATGGCCACCTCT	4380
		
35	4381 TGGTCAATTTGGTTGTATGGTGCAATTATTATTATAATTATTATATATTTCTCCAGAATT	4440
		
	4441 ACCTGCTAGCCACTCCTGTTTTTAGTACAATGTGGTTTGTGGCCTGAACTCCCCTCTCTG	4500
		
	4501 TGTGCCTAAAATTAGCCAAGAAATGAGTATGGCAACCTAAGTAAGTAAAATGGTGGTTAT	4560
		
40	4561 TAATGTAAATATGGGAACTAATGATAAACTATTTATTAAAGGTTTATTGTACAATGAAA	4620
		
	4621 CGTTTCGGGTTGCCTCTGTGTTTCTGGTGGGTAACACAGGTGAAATCATGTTACTGTA	4680
		
45	4681 GCAGTGAGTGAGCATCTGAGCAGCGATAATCATTTGGTCGTTGCATTTACGGCGATGATC	4740
		
	4741 CTATAGTTAATGGCTGCTAAATCCCAGTAAGTCTCACTATAAACTGGTAGCATTCTGTT	4800
		
	4801 GGGCTTTACTTGCTGTTATATTACTGCACCCCCATTTTTTTTTTAATGTAATGCTCTGAC	4860
		
50	4861 TTTGCTGGCTGTTGGTTTTGTAAACCTGCCCTTTGAAGCTTAATGTTACCGCTAATGCCT	4920
		
	4921 CCTCCACCTACACAGTGTATATAGTCGTGCATTGACCTGAGCTCATTTATGGGCGGTGGA	4980
		
55	4981 TTTGTAATTAAATCCACATGGAGGCAGTAGTTACATCTGGCAGGAACTTAAAGAGTCTT	5040
		
	5041 CTCCCTGAATAACAGTGAACGCCAAAGTGGGAGATGTCACAAAATGTGATATTTATCCAAA	5100
		
	5101 ATAAAGAATACGATAAAGTGGCCAGAACAAATTTATTTTTGTTATTAATGTAGTGTAGGGG	5160
		
60	5161 AATTTAATGTCTTATAATTAGCAGCTAATAACTTGCCCATCATTTTGTGTAATTTCTGTG	5220

5221		5280
	TGAATGATGAAGTTTTACTGGGTCAATGCTCAAATCTTAAGGTGATTAATGAGTATTTGC	
5281		5281
	A	
5	.	

SEQ ID NOs 76 and 78 (Hiat1a mutant allele- 17nt deletion)

LENGTH: 5281bp and 234aa

TYPE: cDNA (SEQ ID NO: 76) and Protein (SEQ ID NO: 78)

10 ORGANISM: Nile tilapia

1	TTCTGCTTCGCCCTTGTATTAGACAGCCAATCGCTGGACGTCCTCCGCCAGAAGGGGTG	60
61	GGTTGACGTAGTACAGGAAGCCAGGCGAGGTGAGGTGGGAGGAGAGATCACAAAATTGT	120
121	TAGCTCGCTGCTAGCTGCCTCCTCCGATTTGCCCGAAGTGCATGAGCCAGGAGGCGAA	180
181	ATTTGTGGGGTTTTTTGGTTTTGATTGGCGCGACGATGACCCTCTGACCCTAAGAATGGA	240
20	241 CATAAGTTAATGATGACGGGGGAGAAGAAGAAGAAGCGGCTGAACCGCAGCATTCTT	300
	-M--M--T--G--E--K--K--K--K--R--L--N--R--S--I--L-	17
301	CTTGCAAAGAAAATTATAATAAAAGATGGAGGAAGTCCTCAGGGAATCGGGGAGCCAGT	360
25	18 -L--A--K--K--I--I--I--K--D--G--G--S--P--Q--G--I--G--E--P--S-	37
361	GTTTACCATGCTGTGGTGGTCATCTTCCTGGAGTTTTTTGCATGGGGTCTGCTCACTACC	420
	38 -V--Y--H--A--V--V--V--I--F--L--E--F--F--A--W--G--L--L--T--T-	57
421	CCGATGCTCACGGTATTACACCAGACATCCCCCAACACACATTCTGATGAATGGGCTC	480
30	58 -P--M--L--T--V--L--H--Q--T--F--P--Q--H--T--F--L--M--N--G--L-	77
481	ATTCATGGTGTGAAGGGCCTGTTATCATTTCTCAGTGCTCCGCTAATTGGAGCGTTGTCA	540
	78 -I--H--G--V--K--G--L--L--S--F--L--S--A--P--L--I--G--A--L--S-	97
541	GACGTATGGGACGCAAGTCCTTCTGCTGCTAACGGTCTTCTTCACTTGCGCGCCATT	600
35	98 -D--V--W--G--R--K--S--F--L--L--L--T--V--F--F--T--C--A--P--I-	117
601	CCGCTGATGAAGATCAGTCCATGGTGGTACTTTGCAGTCATCTCGATGTCCGGTGTTTTT	660
40	118 -P--L--M--K--I--S--P--W--W--Y--F--A--V--I--S--M--S--G--V--F-	137
661	GCCGTACCTTCTCTGTGATCTTTGCCTATGTGGCAGACATCACACAAGAGCATGAGAGG	720
	138 -A--V--T--F--S--V--I--F--A--Y--V--A--D--I--T--Q--E--H--E--R-	157
721	AGCACAGCTTATGGTTTGGTATCAGCTACCTTTGCAGCAAGCCTGGTTACCAGCCCAGCC	780
45	158 -S--T--A--Y--G--L--V--S--A--T--F--A--A--S--L--V--T--S--P--A-	177
781	ATTGGAGCCTACCTGTCTGAGGCTTACAGTGACACCTTGGTGTGATCCTGGCCACAGCC	840
	178 -I--G--A--Y--L--S--E--A--Y--S--D--T--L--V--V--I--L--A--T--A-	197
841	ATCGCACTGCTCGACATCTGCTTCATCCTGGTGGCTGTACCAGAGTCGCTGCCGAGAAAG	900
50	198 -I--A--L--L--D--I--C--F--I--L--V--A--V--P--E--S--L--P--E--K-	217
901	ATGAGCGCCCATCTCCTGGGAACAGGCAGACCCCTTCGCTTCTCTGCGTAAAGTGGGCCA	960
55	218 -M--S--A--H--L--L--G--T--G--R--P--L--R--C--V--S--A--*-	234

SEQ ID NOs 79 and 81 (wild-type Smap2)

LENGTH: 4207bp and 429aa

TYPE: cDNA (SEQ ID NO: 79) and Protein (SEQ ID NO: 81)

5 ORGANISM: Nile tilapia

	1	ATGACGGGCAAATCTGTGAAAGACGTTGACAGATACCAGGCTGTCCTCAACTCTTTACTG	60
	1	-M--T--G--K--S--V--K--D--V--D--R--Y--Q--A--V--L--N--S--L--L-	20
10	61	GCGCTGGAGGAGAACAAATACTGCGCTGACTGTGAATCGAAAGGTCCACGATGGGCATCC	120
	21	-A--L--E--E--N--K--Y--C--A--D--C--E--S--K--G--P--R--W--A--S-	40
	121	TGGAATTTGGGCATCTTCATCTGTATCCGCTGTGCTGGTATCCATCGAAACCTGGGGGTT	180
15	41	-W--N--L--G--I--F--I--C--I--R--C--A--G--I--H--R--N--L--G--V-	60
	181	CACATCTCCAAGGTCAAGTCTGTCAACCTGGATCAGTGGACGCAGGAGCAAGTCCAGTGT	240
	61	-H--I--S--K--V--K--S--V--N--L--D--Q--W--T--Q--E--Q--V--Q--C-	80
20	241	GTTCAAGAGATGGGAAATGCCAAGGCCAAACGGCTCTACGAGGCTTTTTTACCCGAGTGC	300
	81	-V--Q--E--M--G--N--A--K--A--K--R--L--Y--E--A--F--L--P--E--C-	100
	301	TTCCAGCGTCCCAGACAGACCAGGCTGCCGAGATCTTCATTAGGGACAAATACGAAAAG	360
	101	-F--Q--R--P--E--T--D--Q--A--A--E--I--F--I--R--D--K--Y--E--K-	120
25	361	AAGAAATACATGGATAAAGTTATTGACATCCAGATGCTCAGGAAAGAAAAGAGTTGTGAC	420
	121	-K--K--Y--M--D--K--V--I--D--I--Q--M--L--R--K--E--K--S--C--D-	140
	421	AACATCCCAAAGGAGCCAGTTGTATTTGAGAAGATGAAATTGGTAGTTAAAAAGGAGAAC	480
30	141	-N--I--P--K--E--P--V--V--F--E--K--M--K--L--V--V--K--K--E--N-	160
	481	ACTAAGAAAAAGACGTCAGCCCAAAGACAGATTCCCAGTCTGTACAGACCTGCTCGGA	540
	161	-T--K--K--K--D--V--S--P--K--T--D--S--Q--S--V--T--D--L--L--G-	180
35	541	CTAGAAGTCTTTTATGTTGCAAGTCTGCACCTAAAAAGCAAATAAACACGTCAGACTCT	600
	181	-L--E--L--L--L--C--C--K--S--A--P--K--K--Q--I--N--T--S--D--S-	200
	601	GCCCTGGATCTCTTCAGCTCCCTCGCAGCCCCCTCCCCTGCTTCCTCTACAAAAAGCACG	660
	201	-A--L--D--L--F--S--S--L--A--A--P--S--P--A--S--S--T--K--S--T-	220
40	661	GTAGTAGACACCATGCCTCAGAGCAGAGTGACTGCCTCAGTGCCTGAGAATCTGAGCTTG	720
	221	-V--V--D--T--M--P--Q--S--R--V--T--A--S--V--P--E--N--L--S--L-	240
	721	TTCTTAGGCCCAGCACCCAAAGCAGAGGAGGGCACAGTCAAGAACTATCCAAGGACTCC	780
45	241	-F--L--G--P--A--P--K--A--E--E--G--T--V--K--K--L--S--K--D--S-	260
	781	ATTCTTTCCCTGTACGCCTCCACTCCCTCGGTACATGCCAGCAGTATGGCCGCACATGGC	840
	261	-I--L--S--L--Y--A--S--T--P--S--V--H--A--S--S--M--A--A--H--G-	280
50	841	TTGTACATGAACCAAATGGGATATCCAACACACCCGTACGGTCCATACCATCTTTAGCC	900
	281	-L--Y--M--N--Q--M--G--Y--P--T--H--P--Y--G--P--Y--H--S--L--A-	300
	901	CAGGCAGGGGAATGGGAGGCACTATGATGACATCACAGATGGCCATGATGGGGCAGCAG	960
	301	-Q--A--G--G--M--G--G--T--M--M--T--S--Q--M--A--M--M--G--Q--Q-	320
55	961	CAGAGCGGGGTGATGGCGGTGCCACAAAACAGCATGATTGGAATTCAGCAGAACTGCATG	1020
	321	-Q--S--G--V--M--A--V--P--Q--N--S--M--I--G--I--Q--Q--N--C--M-	340

	1021	ATGGGGCAGCAGAATGGCTTAATGGGACAGCAACAAAGTGGGATGATAGGACAGCAGCAG	1080
	341	-M--G--Q--Q--N--G--L--M--G--Q--Q--Q--S--G--M--I--G--Q--Q--Q--	360
5	1081	CAGGTTGGGGGTTTGCCCGCATTACCCAGCAGCAGGCTTACGGAGTCCAGCAAGCCCAG	1140
	361	-Q--V--G--G--L--P--A--L--P--Q--Q--Q--A--Y--G--V--Q--Q--A--Q--	380
	1141	CAGCTACAGTGGAAACATCAGCCAGATGACTCAGCACATGGCCGGCGTGAATCTTTACAAC	1200
10	381	-Q--L--Q--W--N--I--S--Q--M--T--Q--H--M--A--G--V--N--L--Y--N--	400
	1201	ACCAGCGGTATGATGGGATACAGCGGTCAACAAATGGGAGGTTTACGACAGTCCAAGTTCG	1260
	401	-T--S--G--M--M--G--Y--S--G--Q--Q--M--G--G--S--A--A--P--S--S--	420
	1261	GCACACATGACAGCGCACGTGTGGAAATGAGCTTGTCTATCTGAGATTCGATGGAGTGCC	1320
15	421	-A--H--M--T--A--H--V--W--K--*--.....	429
	1321	AACGACCCACAAAAGGAGAAGAGAAACGCCGTGGATCAGACTCTCCATTAAACATTTTCT	1380
	1381	GATGCAAGGGAGGAGGAGGAGGAGAAGAAGAAGAAGGTTTGAGAAACCACTACTACC	1440
20	1441	TCTCTCTCTCCTCTCTGGCCGCGCTTCCTCTTGCCGTCTCATGCATAGCCATGTTCTGCA	1500
	1501	GATTTCCATGTTTGCCTTCAGGACCTTTTCATATGATGACTAAGACAAGGGGGTTCTGAG	1560
25	1561	GCCACTGGTTAGGACTCCAGAGCTTTCTTTCTGCCTAGCCTTTATGAGAGAGCGCTCGTG	1620
	1621	TGCAGAAACATTATGAGGGTATCAAGCAGCTGCAGAATTGCACTGTTTCTTATTTAATCA	1680
	1681	GATGGCACTGGGGTTGGCATTGGGGTTAGCCTAGCTTTAAAAGCTCAAATAGACCGAGAT	1740
30	1741	ATATAATCTGGTAACCTAAATAGGTGGCTCATACTTTAAATTCATTAGCCCTACATTACC	1800
	1801	AGTATTTACCCAAGTATGGAGCGACATTTAGTGATGATATGTACAGTGGCCCTGAGAGG	1860
35	1861	TCAAACACACTGCAGCCTAATAAAACACCAGCAAAAATGAAAAATGGTGCAAAAGCACAC	1920
	1921	AAAACATAATGGAAGGTCAATAAAACCCAAATGGAAATAGAAAGAAAAACACTGGAGAAGC	1980
	1981	TAGCAGAAAAAATCTCACAAAACACAACGAAATGTTTTTGGCTAAAATGTGACGGCTA	2040
40	2041	ACAGCTAACAGTAAACGGCTAACAGCAACCATGTACCTACAGTGTCCATTGTGTTTTGTC	2100
	2101	AGAATTTTTTTTTTCTATGTCCATTGTATTTTAATCAACTTCTGTGGTGCTTTTGCAAAAT	2160
45	2161	TTTTCTGTTTTGCTGGTGTTCCTACAGTTGCAGTGCATGTGACCTCTCAGGGCCACCGT	2220
	2221	AGACATAGCTACATTTTAACAGCAGCCATATTTGCAAAGTGTAGCAACTACAACCTTTATT	2280
	2281	CAGCCAATTTCAAGGTAGAGATTTAGAGCTTTTCAAAGTATATTTTCACATAAGTGAGA	2340
50	2341	TGAGCTGCTGCTAATTCACTTAATAATCATTAACAAATATAAAAGCTAGGCTAGCCTAAT	2400
	2401	AGTCCCTTCATGCTGCATGCAGAAGACAAATACACATAACCATTTTTTAGCAACATATATC	2460
55	2461	TAGAAATTTCTACTCATTTAACAATATTTAATTCAAGCAACAAAACCTACCTACACAGCC	2520
	2521	CGTAATATTGATGTCTTCATCTCAATTTCTAGAGGGCTTCTTTTAGAATCTTTAATCTTG	2580
60	2581	ACTTTTAAAGTGTCAAAAAGTCCAAAACCATATTTTGGGAGACCAAAGATCAACACTAGCTT	2640

	2641	TACTGTAAGTGGACAGTATTCCTGTATGCTTATTCCTGTTCAACCACTTAACTAGTGATT	2700
	2701		
	2701	AATAGAAAAAAAAAACAGCAATTCAGCAGTCCGGCATCACTGTCTTCACTGTGCTGTTCT	2760
	2761		
5	2761	TTCACCAAGGGTAGGACACTTAAAAAAAAAGAAAAAGAAAGAAATCATTTGCATGCA	2820
	2821		
	2821	GTGTCATCAGCGCCCGCACACCTCCAGTTAAGAATCTACCTGGTGCATTAGTGGCCTCAA	2880
	2881		
10	2881	ATAACGTTGAATGTCTGTAAATAGGAGGTGAACAGAGAAGTGGGAGTAGAGACGGAAAAAC	2940
	2941		
	2941	TTCAAGGTGAAGGTGAGCCGGGTTTCAGATGCTTCCACTGAATTGCATGAAAAGAATGTG	3000
	3001		
	3001	TATCTAGCTCTGATTGTATGTACTGTACTGTATGTTTGTAAAGATTTGCGAATGTGTCTC	3060
	3061		
15	3061	TCTGAATGTTTCTCCCTCTGACTCAGTCTTTGACAAAGACTGACAAAAAACTATAAAAA	3120
	3121		
	3121	AAAATAGGTAAAAACATATGTTCTGAATGTGATCTCGGTTGACTCGTTTGATCGCGCGCAA	3180
	3181		
20	3181	TTGTTCTTCGGTGTGTTTTGTTTTTATATATTCCTTGTCTAGAAACGTACACCTTGTG	3240
	3241		
	3241	TCTCTGGAATGTCTGTGCTCGATGGCATCCTGTGGGTTTCCAGTTTTGCTGTAACGGCCT	3300
	3301		
	3301	CACCTTTGCGTTGGGGGCAACAGTGAGCTGTTTTGTTTTTTTTTCTTTTGGAGAGGGG	3360
	3361		
25	3361	ATGGGAGTATTTAACAATCTGGCCAAACCACATCGTGAAGCATAAAGCGATTGTAAACC	3420
	3421		
	3421	ACAATCTTTCACGTCTGTTTAAGCTGATGCTTGTACGCTTCTCCACACAAACCATCTCT	3480
	3481		
30	3481	GTGCCCCGATTCTCTTAAAGTGTTGCTAAATCTGCCTTTTCTGATAAATGCTTATGGA	3540
	3541		
	3541	AATGCTGTGTTTCTCTTATTTAATTTTATTTGACACTTGTGTTAAGCTGGTAAGATGCTG	3600
	3601		
	3601	CTTTTAATGTGAGTGGCAGCAATATAGGAGGTGCCTATGTGCAGCATATAAGGTCTTATT	3660
	3661		
35	3661	TCACAACAGTGTGACAGCAGTCACCTTCTCCACTGAGAGCAACATTTATATAAGAGA	3720
	3721		
	3721	GAGCACATCCAGCACAGCAACAGCAAATCTGTCAGTCAACAAAAGTTTCTGGAAAGGCAG	3780
	3781		
40	3781	TGCAAGTCCACCTCTGTGGACGCTCAGGCCTCACCTGAGTTTTTCCATTTGTGATCAGGC	3840
	3841		
	3841	TACTTTTTTTTTTGGTCCGATATTTTTTCAATGAAACAAAACGAATAAAGGAATGTAAC	3900
	3901		
	3901	TTGTACGTACTTGTGATCAAGATACTGTATATTTTAATTCTTTATCAAATATCGCTGT	3960
	3961		
45	3961	ATATTATGTTTCTTAAACAACATGTTCTGTATATTAGTTTTTCTTTTCCACATGCTTTGC	4020
	4021		
	4021	CCCACTTTACACAATTTCAATAAAATTTAACAATGTATATGTGACATATGATAATTGTCC	4080
	4081		
50	4081	CTGTGAAAACATGCAAATAAATATTGTTTTGGTTAAATTTATGTTGTTTGTGTTGTTGT	4140
	4141		
	4141	GTTCAATTGCTGGGTGTCAGGAGTTTTCTGTATGCAACTCAGGTGAGATAAAACGCTC	4200
	4201		
	4201	AGACAGG	4207
			
55			

SEQ ID NOs 80 and 82 (Smap2 mutant allele- 17nt deletion)

LENGTH: 4207bp and 118aa

TYPE: cDNA (SEQ ID NO: 80) and Protein (SEQ ID NO: 82)

ORGANISM: Nile tilapia

5	1	ATGACGGGCAAATCTGTGAAAGACGTTGACAGATACCAGGCTGTCCTCAACTCTTTACTG	60
	1	-M--T--G--K--S--V--K--D--V--D--R--Y--Q--A--V--L--N--S--L--L--	20
	61	GCGCTGGAGGAGAACAAATACTGCGCTGACTGTGAATCGAAAGGTCCACGATGGGCATCC	120
	21	-A--L--E--E--N--K--Y--C--A--D--C--E--S--K--G--P--R--W--A--S--	40
10	121	TGGAATTTGGGCATCTTCATCTGTATCCGCTGTGCTGGGGGTTACATCTCCAAGGTCAA	180
	41	-W--N--L--G--I--F--I--C--I--R--C--A--G--G--S--H--L--Q--G--Q--	60
	181	GTCTGTCAACCTGGATCAGTGGACGCAGGAGCAAGTCCAGTGTGTTCAAGAGATGGGAAA	240
	61	-V--C--Q--P--G--S--V--D--A--G--A--S--P--V--C--S--R--D--G--K--	80
15	241	TGCCAAGGCCAAACGGCTCTACGAGGCTTTTTTACCCGAGTGCTTCCAGCGTCCCGAGAC	300
	81	-C--Q--G--Q--T--A--L--R--G--F--F--T--R--V--L--P--A--S--R--D--	100
20	301	AGACCAGGCTGCCGAGATCTTCATTAGGGACAAATACGAAAAGAAGAAATACATGGATAA	360
	101	-R--P--G--C--R--D--L--H--*--	118

SEQ ID NOs 83 and 85 (wild-type Csnk2a2)

25 LENGTH: 1053bp and 350aa

TYPE: cDNA (SEQ ID NO: 83) and Protein (SEQ ID NO: 85)

ORGANISM: Nile tilapia

30	1	ATGCCTGGCCCCACACCGACCATCAGCAAAGCTCGGGTTTACACCGACGTTAATACACAG	60
	1	-M--P--G--P--T--P--T--I--S--K--A--R--V--Y--T--D--V--N--T--Q--	20
	61	AAGAACAGAGAGTACTGGGACTACGATGCTCATGTGCCAAACTGGAGTAATCAAGACAAC	120
	21	-K--N--R--E--Y--W--D--Y--D--A--H--V--P--N--W--S--N--Q--D--N--	40
35	121	TATCAGCTGGTGCGTAAACTGGGCAGAGGGAAGTACAGTGAAGTGTGTTGAGGCCATAAAT	180
	41	-Y--Q--L--V--R--K--L--G--R--G--K--Y--S--E--V--F--E--A--I--N--	60
40	181	GTGACCAATAATGAGAAAGTGGTGGTGAATACTCTGAAGCCTGTCAAGAAGAAGAAGATC	240
	61	-V--T--N--N--E--K--V--V--V--K--I--L--K--P--V--K--K--K--K--I--	80
	241	AAACGCGAAATCAAAATTCTTGAAAACCTGCGAGGAGGAACCAACATCATCCGCCTGGTG	300
	81	-K--R--E--I--K--I--L--E--N--L--R--G--G--T--N--I--I--R--L--V--	100
45	301	GACACGGTCAAAGACCCGGTGTCCAGAACCAGCGCTAGTCTTTGAGTACATCAATAAC	360
	101	-D--T--V--K--D--P--V--S--R--T--P--A--L--V--F--E--Y--I--N--N--	120
	361	ACAGATTTTAAGGAGCTTTACCAGAAGCTGACAGACTACGATATCCGTTACTACATGTAT	420
	121	-T--D--F--K--E--L--Y--Q--K--L--T--D--Y--D--I--R--Y--Y--M--Y--	140
50	421	GAGCTTCTAAAGGCTCTGGACTTCTGTGACAGTATGGGGATCATGCACAGGGACGTGAAG	480
	141	-E--L--L--K--A--L--D--F--C--H--S--M--G--I--M--H--R--D--V--K--	160
55	481	CCGCACAATGTGATGATTGACCACCAGCTGAGGAAGCTGCGTCTTATAGATTGGGGTTTG	540
	161	-P--H--N--V--M--I--D--H--Q--L--R--K--L--R--L--I--D--W--G--L--	180
	541	GCTGAATTTTACCATCCCGCTCAGGAATATAATGTCAGGGTGGCCTCGCGCTATTTCAA	600

	181	-A--E--F--Y--H--P--A--Q--E--Y--N--V--R--V--A--S--R--Y--F--K-	200
	601	GGCCCCGAGCTGCTAGTGGACTATCAGATGTATGATTACAGTTTGGACATGTGGAGTCTC	660
5	201	-G--P--E--L--L--V--D--Y--Q--M--Y--D--Y--S--L--D--M--W--S--L-	220
	661	GGCTGCATGTTGGCCAGTATGATTTTCCTGAAGGAACCGTTTTTTCATGGCCAGGACAAC	720
	221	-G--C--M--L--A--S--M--I--F--L--K--E--P--F--F--H--G--Q--D--N-	240
10	721	TATGACCAGCTGGTCCGCATCGCTAAGGTTCTCGGCACCGATGAGCTCTTTGGCTACCTG	780
	241	-Y--D--Q--L--V--R--I--A--K--V--L--G--T--D--E--L--F--G--Y--L-	260
	781	CACAAATATCACATAGAACTGGACACTCGCTTCAAAGACATGCTGGGGCAGCAAACACGG	840
	261	-H--K--Y--H--I--E--L--D--T--R--F--K--D--M--L--G--Q--Q--T--R-	280
15	841	AAACGCTGGGAGCAGTTTCATCCAATCAGAGAACCAGCACCTGGTGAGTCCAGAGGCTCTG	900
	281	-K--R--W--E--Q--F--I--Q--S--E--N--Q--H--L--V--S--P--E--A--L-	300
	901	GACCTGCTGGACAAGCTGCTGCGCTATGACCACCAGCAGAGGCTGACGGCGGCCGAGGCC	960
20	301	-D--L--L--D--K--L--L--R--Y--D--H--Q--Q--R--L--T--A--A--E--A-	320
	961	ATGCAGCACCCGTACTTCTATCCTGTGGTGAAGGAACAAGCAAATGCCAACACAGATGGC	1020
	321	-M--Q--H--P--Y--F--Y--P--V--V--K--E--Q--A--N--A--N--T--D--G-	340
25	1021	TCAAAGGCAATAAGCAGCTCCAATGCAACATGA	1053
	341	-S--K--A--I--S--S--S--N--A--T--*-	350

SEQ ID NOs 84 and 86 (Csnk2a2 mutant allele- 22nt deletion)

LENGTH: 1053bp and 31aa

30 TYPE: cDNA (SEQ ID NO: 84) and Protein (SEQ ID NO: 86)

ORGANISM: Nile tilapia

	1	ATGCTCATGTGCCAAACTGGAGTAATCAAGACAACATATCAGCTGGTGCCTAACTGGGCA	60
35	1	-M--L--M--C--Q--T--G--V--I--K--T--T--I--S--W--C--V--N--W--A-	20
	61	GAGGGAAGTACAGTGAAGTGTGTTGAGGCCATAAATGTGACCAATAATGAGAAAGTGGTG	120
	21	-E--G--S--T--V--K--C--L--R--P--*-	31

40

SEQ ID NOs 87and 89 (wild-type Gope)

LENGTH: 1335bp and 444aa

TYPE: cDNA (SEQ ID NO: 87) and Protein (SEQ ID NO: 89)

ORGANISM: Nile tilapia

45	1	ATGTCTGCTTCGACTGGATGCTCCCCATCGGGCCAGCACTCGGGCCTTGTCCCCAGTATG	60
	1	-M--S--A--S--T--G--C--S--P--S--G--Q--H--S--G--L--V--P--S--M-	20
50	61	TCCATGTTTTCGATGGCTAGAAGTGTGAGAGAAGGAATTTGATAAGGCTTTCGTGGATGTG	120
	21	-S--M--F--R--W--L--E--V--L--E--K--E--F--D--K--A--F--V--D--V-	40
	121	GATCTGTTGCTTGGAGAAATAGATCCAGATCAAGTGGATATAACGTATGAGGGTCGGCAG	180

	41	-D--L--L--L--G--E--I--D--P--D--Q--V--D--I--T--Y--E--G--R--Q-	60
5	181	AAGATGACCAGCCTCAGCTCCTGTTTCGCTCAGCTCTGTCATAAAACCCAGACTGTCTTC	240
	61	-K--M--T--S--L--S--S--C--F--A--Q--L--C--H--K--T--Q--T--V--F-	80
	241	CAGCTCAACCATAAACTAGAGGCTCAGCTGGTGGACCTGCGCTCAGAGTTGACCGAAGCT	300
10	81	-Q--L--N--H--K--L--E--A--Q--L--V--D--L--R--S--E--L--T--E--A-	100
	301	AAAGCTGCACGGGTGGTGGCAGAAAGGGAGGTCCACGACTTGCTCCTGCAGCTTCATGCT	360
	101	-K--A--A--R--V--V--A--E--R--E--V--H--D--L--L--L--Q--L--H--A-	120
15	361	CTCCAACCTGCAGCTTCATGTCAAGCAAGGCCAAGCTGAGGAGTCAGATACCATCAAAGAT	420
	121	-L--Q--L--Q--L--H--V--K--Q--G--Q--A--E--E--S--D--T--I--K--D-	140
	421	AAACTGCCTACACCAACCTTAGAAGAGCTGGAACAGGAGCTCGAGGCCAGTAAGAAGGAG	480
20	141	-K--L--P--T--P--T--L--E--E--L--E--Q--E--L--E--A--S--K--K--E-	160
	481	AAATTAGCAGAGGCCAAAAATGGAGGCAGAAACAGACTATATAAGAAAGAAAACGAGGCC	540
	161	-K--L--A--E--A--K--M--E--A--E--T--R--L--Y--K--K--E--N--E--A-	180
25	541	CTTCGCAGGCACATGGCAGTACTGCAGGCCGAAGTCTACGGAGCCAGACTGGCTGCTAAA	600
	181	-L--R--R--H--M--A--V--L--Q--A--E--V--Y--G--A--R--L--A--A--K-	200
	601	TACTTGGACAAGGAACTGGCTGGCAGGGTGCAGCAGATACAGTTACTGGGTCGTGACATG	660
30	201	-Y--L--D--K--E--L--A--G--R--V--Q--Q--I--Q--L--L--G--R--D--M-	220
	661	AAAGGGCCAGCACATGACAAGCTCTGGAATCAACTGGAGGCAGAAATTCACCTTCACCGC	720
	221	-K--G--P--A--H--D--K--L--W--N--Q--L--E--A--E--I--H--L--H--R-	240
35	721	CATAAACTGTGATCCGAGCATGTAGAGGTGCAAGTGACCCTAAGAGACCTCTTCCCTCT	780
	241	-H--K--T--V--I--R--A--C--R--G--R--S--D--P--K--R--P--L--P--S-	260
	781	CCTGTGGGACATGATCCAGACATGCTGAAGAAAACCCAGGGAGTTGGCCCTATCCGAAAG	840
40	261	-P--V--G--H--D--P--D--M--L--K--K--T--Q--G--V--G--P--I--R--K-	280
	841	GTTGTGCTGGTCAAAGAGGATCATGAGGGTCTAGGAATTTCCATTACAGGTGGGAAGGAG	900
	281	-V--V--L--V--K--E--D--H--E--G--L--G--I--S--I--T--G--G--K--E-	300
45	901	CACGGCGTTCCCATTTTTAATTTTCAGAGATCCATCCCAGTCAGCCCGCAGACAGATGTGGA	960
	301	-H--G--V--P--I--L--I--S--E--I--H--P--S--Q--P--A--D--R--C--G-	320
	961	GGGCTGCATGTTGGAGATGCCATCCTTGCTGTCAACAGCATCAATTTGCGAGATGCCAAA	1020
50	321	-G--L--H--V--G--D--A--I--L--A--V--N--S--I--N--L--R--D--A--K-	340
	1021	CATAAGGAAGCTGTCAACCATCTCTCTCAGCAGCGAGGACAGATAGAGTTTGAGGTCGTG	1080
	341	-H--K--E--A--V--T--I--L--S--Q--Q--R--G--Q--I--E--F--E--V--V-	360
55	1081	TACGTGGCTCCTGAAGTGGACAGCGATGATGAGAATGTGGAGTACGAGGATGACAGCGGT	1140
	361	-Y--V--A--P--E--V--D--S--D--D--E--N--V--E--Y--E--D--D--S--G-	380
	1141	CATCGCTACAGACTCTACCTGGATGAACTGGATGACAGCATCACAGCACCTAGCAAC	1200
60	381	-H--R--Y--R--L--Y--L--D--E--L--D--D--S--I--T--A--P--P--S--N-	400
	1201	AGTTCAGCATCACTTCAAGCACTGGAGAAGTTGTCACTGAGCAATGGAGCAGAGTCTGGA	1260
	401	-S--S--A--S--L--Q--A--L--E--K--L--S--L--S--N--G--A--E--S--G-	420
	1261	GATACTGGGATGTCCAGTGAGACACCTTCAGGGGAAACCCCTTCAAAGCCACCAGAAACT	1320
	421	-D--T--G--M--S--S--E--T--P--S--G--E--T--P--S--K--P--P--E--T-	440
	1321	GACTGCTCTTCCTAG	1335

441 -D--C--S--S--*-

444

SEQ ID NOs 88and 90 (Gope mutant allele- 8nt deletion)

LENGTH: 1335bp and 30aa

5 TYPE: cDNA (SEQ ID NO: 88) and Protein (SEQ ID NO: 90)

ORGANISM: Nile tilapia

10
1 ATGTCTGCTTCGACTGGATGCTCCCCAGCACTCGGGCCTTGTCCTCCAGTATGTCCATGTT 60
1 -M--S--A--S--T--G--C--S--P--A--L--G--P--C--P--Q--Y--V--H--V-- 20
61 TCGATGGCTAGAAGTGCTGGAGAAGGAATTGATAAGGCTTTCGTGGATGTGGATCTGTC 120
21 -S--M--A--R--S--A--G--E--G--I--*- 30
15

SEQ ID NOs 91 and 94 (wild-type DMRT-1)

LENGTH: 882bp and 293aa

TYPE: cDNA (SEQ ID NO: 91) and Protein (SEQ ID NO: 94)

20 ORGANISM: Nile tilapia

25
1 ATGAGCCAGGACAAACAGAGTAAGCAGGTACCGGATTGCAGCGGACCGATGTCCCCGACC 60
1 -M--S--Q--D--K--Q--S--K--Q--V--P--D--C--S--G--P--M--S--P--T-- 20
61 AAAGCCCAGAAATCCCCCAGGATGCCCAAGTGCTCTCGCTGTAGAAATCACGGATACGTG 120
21 -K--A--Q--K--S--P--R--M--P--K--C--S--R--C--R--N--H--G--Y--V-- 40
121 TCTCCACTGAAGGGACACAAGCGCTTTTGCAACTGGAGGGACTGCCAGTGTCCCAAATGC 180
41 -S--P--L--K--G--H--K--R--F--C--N--W--R--D--C--Q--C--P--K--C-- 60
181 AAATTGATCGCGGAGAGGCAGAGAGTCATGGCGGCCAGGTTGCTCTGAGGAGGCAGCAG 240
61 -K--L--I--A--E--R--Q--R--V--M--A--A--Q--V--A--L--R--R--Q--Q-- 80
241 GCCCAAGAAGAAGAGCTTGGGATTTGTAGTCCTGTGTCTCTGTCCGGTCCGAGATGATG 300
81 -A--Q--E--E--E--L--G--I--C--S--P--V--S--L--S--G--S--E--M--M-- 100
301 GTCAAGAATGAAGTTGGAGCAGACTGCCTGTCTCTGTGGAGGGACGGTCCCCGACACCT 360
101 -V--K--N--E--V--G--A--D--C--L--F--S--V--E--G--R--S--P--T--P-- 120
40
361 ACCAGCCACGCCACCTCTGCTGTCTACAGGGACCCGCTCGGCATCGTCCCCAGCCCATCT 420
121 -T--S--H--A--T--S--A--V--T--G--T--R--S--A--S--S--P--S--P--S-- 140
421 GCTGCTGCCAGGGCTCATACCGAGGGACCGTCTGACCTCCTGCTGGAAACCCCTATTAC 480
141 -A--A--A--R--A--H--T--E--G--P--S--D--L--L--L--E--T--P--Y--Y-- 160
481 AATTTCTACCAGCCTTCGCGCTACCCACCTACTATGGAAACCTTTACAACCTACTCGCAG 540
161 -N--F--Y--Q--P--S--R--Y--P--T--Y--Y--G--N--L--Y--N--Y--S--Q-- 180
50
541 TACCAGCAGATGCCTCATGGTGATGGCCGCTGCCAGCCACAGCGTGTCTGCTCAGTAC 600
181 -Y--Q--Q--M--P--H--G--D--G--R--L--P--S--H--S--V--S--S--Q--Y-- 200
601 CGCATGCACTCCTACTACCCAGCAGCCACCTACCTGACTCAGGGCCTGGGCTCCACCAGC 660

	201	-R--M--H--S--Y--Y--P--A--A--T--Y--L--T--Q--G--L--G--S--T--S-	220
	661	TGTGTGCCACCCCTTCTTTAGCCTGGATGACAACAATAACAGCTGCTCTGAGACCATGGCA	720
5	221	-C--V--P--P--F--F--S--L--D--D--N--N--N--S--C--S--E--T--M--A-	240
	721	GCCTCCTTCTCACCCGGCAGCATCTCCGCTGGTCACGACTCCACCATGGTCTGCCGCTCC	780
	241	-A--S--F--S--P--G--S--I--S--A--G--H--D--S--T--M--V--C--R--S-	260
10	781	ATCAGCTCCCTGGTTAACGGCGACGCCAAGGCTGAATGCGAGGCCAGCAGCCAGGCAGCC	840
	261	-I--S--S--L--V--N--G--D--A--K--A--E--C--E--A--S--S--Q--A--A-	280
	841	GGCTTCACCGTCGACGCCATCGAAGGCGGCCACCAAATAA	882
	281	-G--F--T--V--D--A--I--E--G--G--A--T--K--*-	293

15 **SEQ ID NOs 92 and 95 (DMRT-1 mutant allele- 7nt deletion)**

LENGTH: 882bp and 40aa

TYPE: cDNA (SEQ ID NO: 92) and Protein (SEQ ID NO: 95)

ORGANISM: Nile tilapia

20	1	ATGAGCCAGGACAAACAGAGTAAGCAGGTACCGGATTGCAGCGGACCCCGACCAAAGCCC	60
	1	-M--S--Q--D--K--Q--S--K--Q--V--P--D--C--S--G--P--R--P--K--P-	20
	61	AGAAATCCCCCAGGATGCCCAAGTGCTCTCGCTGTAGAAATCACGGATACGTGTCTCCAC	120
25	21	-R--N--P--P--G--C--P--S--A--L--A--V--E--I--T--D--T--C--L--H-	40
	121	TGAAGGGACACAAGCGCTTTTGCAACTGGAGGGACTGCCAGTGTCCCAAATGCAAATTGA	180
	41	-*-	40

30 **SEQ ID NOs 93 and 96 (DMRT-1 mutant allele- 13nt deletion)**

LENGTH: 882bp and 38aa

TYPE: cDNA (SEQ ID NO: 93) and Protein (SEQ ID NO: 96)

ORGANISM: Nile tilapia

35	1	ATGAGCCAGGACAAACAGAGTAAGCAGGTACCGGATTGCAGCGGACCAAAGCCCAGAAAT	60
	1	-M--S--Q--D--K--Q--S--K--Q--V--P--D--C--S--G--P--K--P--R--N-	20
	61	CCCCCAGGATGCCCAAGTGCTCTCGCTGTAGAAATCACGGATACGTGTCTCCACTGAAGG	120
40	21	-P--P--G--C--P--S--A--L--A--V--E--I--T--D--T--C--L--H--*-	38

SEQ ID NOs 97 and 100 (wild-type GSDF)

LENGTH: 840bp and 213aa

45 TYPE: cDNA (SEQ ID NO: 97) and Protein (SEQ ID NO: 100)

ORGANISM: Nile tilapia

	1	AACAGGGGAAAAGTCTACAGTGTTAACTATGTCAAGGCCACCTTGGGGTACAAGCAGATA	60
--	---	--	----

5	61	AAAACCGTGGTTCTCAGACCCTGACAAACAATACCTAGGGCAGCATCCCAGTTTTGTGCGC	120
	121	TACTATCTCCTCCTCCGACCAGACGTTTCGGGACCAACCGCAGCTTTTGTCTGCAGCCAGT	180
	181	CTTACGTGTTTCATCCACCATGGCCTTTCCATTTCATTGTCATGACATTACTTTTGGGCTCT	240
10		-M--A--F--P--F--I--V--M--T--L--L--L--G--S-	14
	241	TCCATGATGATGGCATTGTCTTGGATCCATCCAGGAAAGAACCCGAAGCTGCCGTCTTA	300
	15	-S--M--M--M--A--F--V--L--D--P--S--R--K--E--P--E--A--A--V--L-	34
15	301	GGTGACAGGTGCCAAGGTGAGTCATGGCAGTCCATCAGAAAGAACCTCCTTAGGGTTCTG	360
	35	-G--D--R--C--Q--G--E--S--W--Q--S--I--R--K--N--L--L--R--V--L-	54
	361	AACTTGCAGACTGAGCCGAGCTACCTGCCGGTGCAGTGGACAGTGTGAGAGAGCAGTGG	420
20	55	-N--L--Q--T--E--P--Q--L--P--A--G--A--L--D--S--V--R--E--Q--W-	74
	421	AACCGAACCTTCAGCATCGTTTCTCACACAGCCAAGCATACTGCAACCCAGCAGTCCCA	480
	75	-N--R--T--F--S--I--V--S--H--T--A--K--H--T--A--T--P--A--V--P-	94
25	481	GGCTACTCTGCATCAGCTGATAATGGAACAGTGCGAGCCTGAAGTGTGTTCCATTGCC	540
	95	-G--Y--S--A--S--A--D--N--G--N--S--A--S--L--K--C--C--S--I--A-	114
	541	TCAGAGATCTTCATGAAAGATCTGGGCTGGGACAGCTGGGTGATCCACCCGTTGAGTCTT	600
30	115	-S--E--I--F--M--K--D--L--G--W--D--S--W--V--I--H--P--L--S--L-	134
	601	ACCTATGTTTCAGTGCAGCAACCTGCAACTCTGCCATGACCACTGTTCAATGTCCATCATCC	660
	135	-T--Y--V--Q--C--A--T--C--N--S--A--M--T--T--V--Q--C--P--S--S-	154
35	661	CAAGTAAATGTCCAGGATGCCAACACACAGGACCAGGTGCCATGCTGTGCGGCCACCTCC	720
	155	-Q--V--N--V--Q--D--A--N--T--Q--D--Q--V--P--C--C--R--P--T--S-	174
	721	CAAGAAGAGGTGCCCATAGTCTATATGGATGGATCCAGCGCCATTGTCATGTCTCCATG	780
	175	-Q--E--E--V--P--I--V--Y--M--D--G--S--S--A--I--V--M--S--S--M-	194
	781	CAGCTGACCCGAGTTGTGGCTGTGAGCTGGGCAACTCTGAGGATCGTGGCAAGGAGTAG	840
	195	-Q--L--T--R--S--C--G--C--E--L--G--N--S--E--D--R--G--K--E--*-	213

SEQ ID NOs 98 and 101 (GSDF mutant allele- 5nt deletion)

40 LENGTH: 840bp and 56aa

TYPE: cDNA (SEQ ID NO: 98) and Protein (SEQ ID NO: 101)

ORGANISM: Nile tilapia

45	1	AACAGGGGAAAAGTCTACAGTGTTAACTATGTCAAGGCCACCTTGGGGTACAAGCAGATA	60
	61	AAAACCGTGGTTCTCAGACCCTGACAAACAATACCTAGGGCAGCATCCCAGTTTTGTGCGC	120
	121	TACTATCTCCTCCTCCGACCAGACGTTTCGGGACCAACCGCAGCTTTTGTCTGCAGCCAGT	180
50	181	CTTACGTGTTTCATCCACCATGGCCTTTCCATTTCATTGTCATGACATTACTTTTGGGCTCT	240
		-M--A--F--P--F--I--V--M--T--L--L--L--G--S-	14
	241	TCCATGATGATGGCATTGTCTTGGATCCATCCAGGAAAGAACCCGAAGCTGCCGTCTTA	300
55	15	-S--M--M--M--A--F--V--L--D--P--S--R--K--E--P--E--A--A--V--L-	34
	301	GGTGACAGGTGCCAAGGTGAGTCATGGCAGTCCATCAGAAAGAACCTCCGTTCTGAACTT	360

35	-G--D--R--C--Q--G--E--S--W--Q--S--I--R--K--N--L--L--R--S--E-	54
361	GCAGACTGAGCCGCAGCTACCTGCCGGTGCAGTGTGACAGAGCAGTGGAAACCG	420
55	-L--A--*-	56

5

SEQ ID NOs 99 and 102 (GSDF mutant allele- 22nt deletion)

LENGTH: 840bp and 46aa

TYPE: cDNA (SEQ ID NO: 99) and Protein (SEQ ID NO: 102)

10 ORGANISM: Nile tilapia

1	AACAGGGGAAAAGTCTACAGTGTAACTATGTCAAGGCCACCTTGGGGTACAAGCAGATA	60
61	AAAACCGTGGTTCTCAGACCCTGACAAACAATACCTAGGGCAGCATCCCAGTTTGTGCGC	120
121	TACTATCTCCTCCTCCGACCAGACGTTTCGGGACCAACCGCAGCTTTTGTCTGCAGCCAGT	180
181	CTTACGTGTTTCATCCACCATGGCCTTTCCATTCATTGTCATGACATTACTTTTGGGCTCT	240
20	-M--A--F--P--F--I--V--M--T--L--L--L--G--S-	14
241	TCCATGATGATGGCATTGTCTTGGATCCATCCAGGAAAGAACCCGAAGCTGCCGTCTTA	300
15	-S--M--M--M--A--F--V--L--D--P--S--R--K--E--P--E--A--A--V--L-	34
301	GGTGACAGGTGCCAAGGTGAGTCATGGCAGTCCATCTGAACCTGCAGACTGAGCCGCAGC	360
25	35 -G--D--R--C--Q--G--E--S--W--Q--S--I--*-	46

SEQ ID NOs 103 and 105 (wild-type FSHR)

30 LENGTH: 5853bp and 689aa

TYPE: cDNA (SEQ ID NO: 103) and Protein (SEQ ID NO: 105)

ORGANISM: Nile tilapia

35	1 GCATTCACTACTGCATGACAGAAAACACCAAAACACCTCACATTTCTCTCTAGCTGACCT	60
		
61	GGCGCCGAACCCCTCGAGCGGACAGACAGGCAAAGGCGTTCATATCAAATGTGGAGTGTGG	120
	-M--W--S--V--	4
40	121 ACCAGAGACAATATCAGAGTAAAATACACAAAAGAAGACAACTAGAAAAGTGAAACCAC	180
	5 D--Q--R--Q--Y--Q--S--K--I--H--K--R--R--Q--T--R--K--V--K--P--	24
181	TCTGTGGACCCAGGCAGACTGAAATGATGCTGGTGTGTTTGGAGTCACGCGTTTTCCCT	240
45	25 L--C--G--P--R--Q--T--E--M--M--L--V--M--F--G--V--T--A--F--P--	44
241	CCAACATCTCCAACGCCAGTGCCTGGAAGTTAAGCAGACGCAGATCAGAGAGATTTCAGC	300
45	S--N--I--S--N--A--Q--C--L--E--V--K--Q--T--Q--I--R--E--I--Q--	64
301	AGGGCGCCCTCTCCAGCCTCCAGCATCTAATGGAAGTACCATTCTGAGAACGACCTGC	360
50	65 Q--G--A--L--S--S--L--Q--H--L--M--E--L--T--I--S--E--N--D--L--	84
361	TGGAGAGTATCGGTGCTTTTGCCTTTTCTGGCCTCCCTCACCTACCAAAATCTTAATAT	420
85	L--E--S--I--G--A--F--A--F--S--G--L--P--H--L--T--K--I--L--I--	104

	421	CTAAAAATGCTGCTCTGAGGAATATCGGGGCTTTTGTGTTTCTCCAACCTCCCTGAACTCA	480
	105	S--K--N--A--A--L--R--N--I--G--A--F--V--F--S--N--L--P--E--L--	124
5	481	GTGAGATAATCATAACAAAATCAAAACACCTGAGTTTCATCCACCCCGATGCATTGAGGA	540
	125	S--E--I--I--I--T--K--S--K--H--L--S--F--I--H--P--D--A--F--R--	144
	541	ACATGGCAAGACTACGGTTCTTGACTATCTCCAACACCGGGCTGAGGATTTTCCAGACT	600
10	145	N--M--A--R--L--R--F--L--T--I--S--N--T--G--L--R--I--F--P--D--	164
	601	TCTCCAAGATCCATTCCACCGCTGCTTTCTGCTGGATCTTCAGGACAACAGCCACATAA	660
	165	F--S--K--I--H--S--T--A--C--F--L--L--D--L--Q--D--N--S--H--I--	184
	661	AGAGAGTCCCTGCCAATGCCTTCAGAGGCCTCTGCACTCAAACCTTCGCAGAGATACGGC	720
15	185	K--R--V--P--A--N--A--F--R--G--L--C--T--Q--T--F--A--E--I--R--	204
	721	TCACCAGAAATGGCATCAAGGAGGTGGCAAGTGACGCCTTCAACGGAACAAAGATGCACA	780
	205	L--T--R--N--G--I--K--E--V--A--S--D--A--F--N--G--T--K--M--H--	224
20	781	GACTGTTCTAGGAGGCAACCGACAGCTTACTCACATCAGTCCCAATGCCTTTGTGGGTT	840
	225	R--L--F--L--G--G--N--R--Q--L--T--H--I--S--P--N--A--F--V--G--	244
	841	CCAGTGAGTTGGTGGTACTAGACGTCTCCGAAACAGCCCTCACCTCTTTGCCAGACTCGA	900
25	245	S--S--E--L--V--V--L--D--V--S--E--T--A--L--T--S--L--P--D--S--	264
	901	TCCTTGATGGCCTCAAGAGGCTGATTGCCGAGTCAGCCTTCAACCTGAAAGAACTTCCTC	960
	265	I--L--D--G--L--K--R--L--I--A--E--S--A--F--N--L--K--E--L--P--	284
	961	CTATTCAGCTCTTTACCAAACCTGCACCAGGCAAAGCTGACATACCCATCACACTGCTGCG	1020
30	285	P--I--Q--L--F--T--K--L--H--Q--A--K--L--T--Y--P--S--H--C--C--	304
	1021	CTTTCCTGAACATGCACAGAAACAGATCGAGATGGCACTCACTGTGTGACAACCCCGAGG	1080
	305	A--F--L--N--M--H--R--N--R--S--R--W--H--S--L--C--D--N--P--E--	324
35	1081	CTAAAAATAACCTGCACCTTCTTCAGGGAATACTGCTCCAACTCCACCAACATCACTTGCA	1140
	325	A--K--N--N--L--H--F--F--R--E--Y--C--S--N--S--T--N--I--T--C--	344
	1141	GCCCGGCCCTGATGACTTTAACCCCTGTGAAGATATCATGTCTGCTACCCCTTACGCA	1200
40	345	S--P--A--P--D--D--F--N--P--C--E--D--I--M--S--A--T--P--L--R--	364
	1201	TCCTCATCTGGATCATCTCTGTCTCGCCCTGCTGGGCAACGCAGTAGTTCTCCTTGAT	1260
	365	I--L--I--W--I--I--S--V--L--A--L--L--G--N--A--V--V--L--L--V--	384
	1261	TGTTAGGCAGCCGCTATAAGCTGACTGTTTCCTCGATTCTCATGTGCCACCTGGCCTTTG	1320
45	385	L--L--G--S--R--Y--K--L--T--V--P--R--F--L--M--C--H--L--A--F--	404
	1321	CTGACCTCTGCATGGGCATCTACCTGGTAGTCATAGCAACCGTGGATATGCTCACACGTG	1380
	405	A--D--L--C--M--G--I--Y--L--V--V--I--A--T--V--D--M--L--T--R--	424
50	1381	GACGGTACTACAACATATGCTATAGACTGGCAGATGGGCTTGGGCTGCAATGCTGCAGGCT	1440
	425	G--R--Y--Y--N--Y--A--I--D--W--Q--M--G--L--G--C--N--A--A--G--	444
	1441	TCTTCACGGTGTTCGCCAGTGAGCTGTGCTGTTTACCTTGACAGCAATCACCGTGGAGC	1500
55	445	F--F--T--V--F--A--S--E--L--S--V--F--T--L--T--A--I--T--V--E--	464
	1501	GCTGGCACACCATCACGCATGCTCTGCGACTTGACCGCAAACCTTCGCCTGAGACACGCCT	1560
	465	R--W--H--T--I--T--H--A--L--R--L--D--R--K--L--R--L--R--H--A--	484
	1561	GCATCATCATGACAATAGGTTGGATCTTCTCCTTGCTGGCTGCACTGCTGCCCACAGTTG	1620
60	485	C--I--I--M--T--I--G--W--I--F--S--L--L--A--A--L--L--P--T--V--	504

	1621	GGATCAGCAGCTATGGCAAAGTGAGCATCTGCCTCCCCATGGATGTTGAGTCCCTAGTCT	1680
	505	G--I--S--S--Y--G--K--V--S--I--C--L--P--M--D--V--E--S--L--V--	524
5	1681	CCCAGTTCTACGTGGTCTGTCTTCTCCTCCTCAACATCTTGGCGTTCTTCTGTGTGTGCG	1740
	525	S--Q--F--Y--V--V--C--L--L--L--L--N--I--L--A--F--F--C--V--C--	544
	1741	GCTGCTACCTCAGCATCTACCTCACCTTTCGCAAGCCTTCATCAGCGGCAGCCCACGCCG	1800
10	545	G--C--Y--L--S--I--Y--L--T--F--R--K--P--S--S--A--A--A--H--A--	564
	1801	ACACCCGTGTGGCTCAACGCATGGCCGTCTCATCTTACAGACTTCATCTGCATGGCTC	1860
	565	D--T--R--V--A--Q--R--M--A--V--L--I--F--T--D--F--I--C--M--A--	584
15	1861	CGATCTCCTTCTTCGCCATCTCAGCTGCCCTCAAGCTCCCTCTCATCACCGTCTCAGACT	1920
	585	P--I--S--F--F--A--I--S--A--A--L--K--L--P--L--I--T--V--S--D--	604
	1921	CCAAGCTACTGTTGGTGTCTATTCTACCCCATCAACTCGTGTCTCAACCCCTTCTTATATG	1980
	605	S--K--L--L--L--V--L--F--Y--P--I--N--S--C--S--N--P--F--L--Y--	624
20	1981	CCTTTTTTCAACCGTAACCTCAGAAGGGATTCTTTCTCCTCGCAGCTCGCTTCGGGCTGT	2040
	625	A--F--F--T--R--N--F--R--R--D--F--F--L--L--A--A--R--F--G--L--	644
	2041	TTAAGACTCGAGCACAGATTTACCGGACAGAGGGTTCCCTCGTGTCTCAGCAGCCAACATGGA	2100
25	645	F--K--T--R--A--Q--I--Y--R--T--E--G--S--S--C--Q--Q--P--T--W--	664
	2101	CCTCTCCAAAGAACAGCCGTGTTATCTTGTATTCTTGGTCAATACGTTAAGTCTAGATG	2160
	665	T--S--P--K--N--S--R--V--I--L--Y--S--L--V--N--T--L--S--L--D--	684
30	2161	GAAACAAGAGTGCTGACTTTTACGCACATTTACAGGTACGGACTGTTTGCCTTGATTGC	2220
	685	G--K--Q--E--C--*--.....	689
	2221	ATATTATATCCATACAAACAGGCTGCTAATTCCTTAAAATGATGCCTCAGATCATGTCTT	2280
	2281	TTGATCACTACCTGGGAAAATTTTTCTATCTACTTAGACTAGAAAGAAAAAACACAAA	2340
35	2341	AGGCAACCAAGTGGAAGGCAAAAGAGCTGAGAATCTTTTTTGACAATTTGACCCAGGAG	2400
	2401	TCTGCAAAACACAGTGATTGTTAAAATAAACAATGCTCTTGCTCTTGCTTCTGTTTGTGC	2460
40	2461	TCCTAATCTGATGCTGTGTTTTTTGGGCTTGAGCCAGTGAAGGCTTCCACTGAAGACTGC	2520
	2521	TCTTCAGTCAATAAATAGCATCCAGAGACCCAGCTCTCAACAGAGGTGATGATCCTCTAT	2580
	2581	ATAAAGATGTTGGTCAGTTCAACAAAGAAGTTGATGCTTGCTCTGTGCAAGTCTGAGAT	2640
45	2641	CTCTGTTAGGGATGTACATGTACAAGTGGTCAAGATTGGACTTCCAGGCCATGAGACCAG	2700
	2701	AGGTCTACAAGTCACAAAACCTTTTAAAGCTTTTATAAAATTATATATATCTATGTCGC	2760
50	2761	CACAATCTGAGCAGTTCAGACACTGATGATTCCAGACTGATCACTGACCCAAGAGAAAGC	2820
	2821	ATGCATACATGTTCCACCTGTCTTTTAAAGTTACACATAAATCAACATGTTTCAATCAC	2880
	2881	AATAGTATCAGTTGACTATTTCAGCACAAAGTACACACAGCGTTCAGTGGCATGTCTAAAC	2940
55	2941	CTGGTTACCTGAGCTATGCTCTGCAGCAATCCATGCAAACATGACCACAAAAGAACTAAT	3000
	3001	TATACACTCACTGGCCACTTTATTAGGTATACTTGTTTGGCTGCTTGGAATGCAAATAC	3060
60	3061	TTAATGAGCCAATCGCATGGTAGCAGCTCAGTGCATTTAGACATGTAATCTGGGGCATT	3120

	3121	 TTAAGATTTTTTAAATGTGGTGGCACGGCAGAGACCAAGAACACAGTAGAGGGGGACATT	3180
5	3181	 TAAATATTTGATTAGCAAAAAAGATCAGAAAACTGACAGAAATTATTGGGCATGATTTTTG	3240
	3241	 GTGTGCAACCTTATGTTTTATTACAAGTTTATTGTGTGAAAAGTGGTGCTGCAGAATGCT	3300
	3301	 CTACATAGAATTTTGTGTTGGACAATTGTTTTGCAACGTGAAAAAGAAGTATTTAGACT	3360
10	3361	 TAACCTAAGTAAAAGTTGTAATTGCACTTAAATAGCTTAATAGTTCACAAGTTATATAAT	3420
	3421	 CAAAATGTATTCAAAGTGCCTAAAGTAAACACACTCTTTATATAGAATGGCCCTTTTTTT	3480
	3481	 CTCGTCTCTTTAATGAGGCAGCTGTTGATGAGTTTGATTCTCTGATATATTGTTCAATAGA	3540
15	3541	 TTCATTTATAAAAAATACAATTAATGTACAAAATAAGAAGAAGCTAAAATAATTTGGGGT	3600
	3601	 GGGCTAATGCCACTCCAAGCTCCTCCCCCTCAAACATGCCTCTATGTAGACATAATCAA	3660
20	3661	 GACAACTTGCTAAAGTTCAAAATGAGCATCAGAATGGGAAAAAGGTGACTGAAGTGACTT	3720
	3721	 TGAAAGTTTAATTGTTGTTGGTGCCAGATGGTCCCACATGTCGCCACAATCTGCTGGCCG	3780
	3781	 GACTGCAAACTGATAGGAAAGCAACAGTAACTTAAATAACTTCTATACAATCAAGGTGTA	3840
25	3841	 CAAGTTACATAAGAAAACCTGGGCATCACTTAGTCTGATAACTCTTGATTCTATTCTGAC	3900
	3901	 ATTCTTATAGTAGGTTTCAGAGTTTGATTAACTGAGCAAACTGAGTCACAAAGCTCAGAT	3960
30	3961	 CATCTAAAAC TGATTTCTTGAAAATGAAAAGGAGTTCACCACAGTCACCACATTTCAATC	4020
	4021	 CAGCAGAGCACATTTGGGATTTAGTCAAATGGGAGATTGCCATCACAGATGTGCAGCTGA	4080
	4081	 CAAACCTTGCGACACCTGTGTGATGCTATCACATCAATATGGACCACAATCTCTGAGGAAT	4140
35	4141	 GTTTCCTCCACTCAATTCCAGTTGAATATATTTTAAAAATTAAGACAGTGTGAAGACAAA	4200
	4201	 GGGGTGTTTAACCTAGCAAAATGTACCCAATAAAGTAGCTAGTGAGTGTAGTTTGACTAA	4260
40	4261	 ATCTGGGTGACACAGCTCTTTTAGATACCCATGGGTTTCTTTTAACTCAAGTGAAGTGCC	4320
	4321	 AGATGGGTGGAGTTCTCAGCAACATAATTTAGAGGTAAAAGAAGAAAAGTGGAGGGGG	4380
	4381	 GAGAAAAC TAATGACTTCATCTACTATGTAACAAACACCATCCGTCTGGCATCCCAAGAT	4440
45	4441	 AATCTAACAAACTAAAATGCCTCAGAATGGTTTTTAAGCAGGTTGGATGCTTGGGATTTT	4500
	4501	 AGCATATGCACACTGCAAAAGAAACATATTCATTCAACATTCAGTGCTGTGATTGAATGA	4560
50	4561	 TATTCATTAAGAAGAACTGCAGGGACCTGCTGATTAACAATCTCCTCATAACCCAGT	4620
	4621	 CTGCTGAACCTCTCAATGTCTACAATTTGCCACCAACTCCGTCTATTTTGTAAAGCCACAG	4680
	4681	 ACCTGTAATTATCTTTGAAATGTAATTATGTTTACGTTTTCAAACAAACATCCAATTAAG	4740
55	4741	 TGTCACTTTTGAATCTGTTTTCCTGAAGAAATTTTCAATGTGCTGTTTTTACACTATTT	4800
	4801	 TATAAAGTGTTTTATTATATCCTCTCAGCTGAATAGATTTTGTATGATGAATGTGAGCG	4860
60	4861	 TTTGAAGAGGCGTGACAAACAGAAAACTCTCTCACACACACATATGCAATAATTGAG	4920

	4921	CTGTCTTTATCTAGCAATGCTGTCCTTCAGAGCATCCAAAGCTTTCAAGGACAAAGTGAC	4980
5	4981	CCTCCCAACCTCTGCTCTGTGCAGCAAAGTGGGTGGGTGGGCGTAGGAGGAGAGGTACGC	5040
	5041	AGCTGCTCTTCTGCTTATTACGGGGGGATGGATATGGCAGCTAGATAAGCTGTGTGTGT	5100
	5101	GCGCGCACACACACACACACACACAATAGCAACCCACACTCTCAAGGCTGCAGC	5160
10	5161	TGCAAGAAGGAATCCAAGACCATCTCATTGATATGGATACACTGCCTCCTACATGCCAAC	5220
	5221	ATTCAAAGTTAGGGTGC AATTATATACTTTCACCACCAGGTGATGCTACTGGGGCTAGAT	5280
15	5281	TTCTGGTGAGTTTACCTCCATCTGTTTGACAAAAAGTCCAAACAAATTACCAGTCTCAG	5340
	5341	TAGATCCTACAAATTTTGCTCGATGTTGTCTTATGAGAAAAATAAATAAATAAATATTTT	5400
	5401	TTTCCTAAATTTGCTTTTTTTTAAATAACTTTTTATTCTACATAATTTTCATAAAAGA	5460
20	5461	TTATATCAATTCCTGCATGAGGATTAATGCTCATCAGACAGTTACCTGTCCCCTACATAC	5520
	5521	ACTGTATTTCTTCTTCATTTTATATCATATCATATAGTTTCCAAGTAAAAGATAAATC	5580
25	5581	ACTCTAATGCATTTGCACTCAAATTTATGTGCACAAAAAAGTGAGTGTGCAATACAG	5640
	5641	AAAGACATGCCGTTATGCTCTCTGACATCTCTCTAGACAGCACTGGAGATGGTATAACA	5700
	5701	AAACACCCTCAGTATAAAGCCTTCAAGTTCATGACTAATCGTTGGCAGCTAAACAATGCC	5760
30	5761	CTCTGGTGGTCGTCGTCGTCATAATAAATATACAAGTTAAAGTGTTAAAGTTGTATTCCACT	5820
	5821	CAAAATCTGTAATTTGGTTTGGGGTCAGTGTCC	5853

35 SEQ ID NOs 104 and 106 (FSHR mutant allele- 5nt deletion)

LENGTH: 5853bp and 264aa

TYPE: cDNA (SEQ ID NO: 104) and Protein (SEQ ID NO: 106)

ORGANISM: Nile tilapia

40	1	GCATTCACTACTGCATGACAGAAAACACCAAAACACCTCACATTTCTCTCTAGCTGACCT	60
	61	GGCGCCGAACCTCGAGCGGACAGACAGGCAAAGGCGTTCATATCAAATGTGGAGTGTGG	120
45		-M--W--S--V--	4
	121	ACCAGAGACAATATCAGAGTAAAATACACAAAAGAAGACAACTAGAAAAGTGAAACCAC	180
50	5	D--Q--R--Q--Y--Q--S--K--I--H--K--R--R--Q--T--R--K--V--K--P--	24
	181	TCTGTGGACCCAGGCAGACTGAAATGATGCTGGTGATGTTGGAGTCACGGCGTTTCCCT	240
55	25	L--C--G--P--R--Q--T--E--M--M--L--V--M--F--G--V--T--A--F--P--	44
	241	CCAACATCTCCAACGCCAGTGCCTGGAAGTTAAGCAGACGCAGATCAGAGAGATTCAGC	300
	45	S--N--I--S--N--A--Q--C--L--E--V--K--Q--T--Q--I--R--E--I--Q--	64
55	301	AGGGCGCCCTCTCCAGCCTCCAGCATCTAATGGAAGTACCATTTCTGAGAACGACCTGC	360
	65	Q--G--A--L--S--S--L--Q--H--L--M--E--L--T--I--S--E--N--D--L--	84

	361	TGGAGAGTATCGGTGCTTTTGCCTTTTCTGGCCTCCCTCACCTACCAAAATCTTAATAT	420
	85	L--E--S--I--G--A--F--A--F--S--G--L--P--H--L--T--K--I--L--I--	104
5	421	CTAAAAATGCTGCTCTGAGGAATATCGGGGCTTTTGTCTTCTCCAACCTCCCTGAACCTCA	480
	105	S--K--N--A--A--L--R--N--I--G--A--F--V--F--S--N--L--P--E--L--	124
	481	GTGAGATAATCATAACAAAATCAAAACACCTGAGTTTCATCCACCCCGATGCATTTCAGGA	540
10	125	S--E--I--I--I--T--K--S--K--H--L--S--F--I--H--P--D--A--F--R--	144
	541	ACATGGCAAGACTACGGTTCTTGACTATCTCCAACACCGGGCTGAGGATTTTTCAGACT	600
	145	N--M--A--R--L--R--F--L--T--I--S--N--T--G--L--R--I--F--P--D--	164
	601	TCTCCAAGATCCATTCCACCGCCTGCTTTCTGCTGGATCTTCAGGACAACAGCCACATAA	660
15	165	F--S--K--I--H--S--T--A--C--F--L--L--D--L--Q--D--N--S--H--I--	184
	661	AGAGAGTCCCTGCCAATGCCTTCAGAGGCCTCTGCACTCAAACCTTCGCAGAGATACGGC	720
	185	K--R--V--P--A--N--A--F--R--G--L--C--T--Q--T--F--A--E--I--R--	204
20	721	TCACCAGAAATGGCATCAAGGAGGTGGCAAGTGACGCCTTCAACGGAACAAAGATGCACA	780
	205	L--T--R--N--G--I--K--E--V--A--S--D--A--F--N--G--T--K--M--H--	224
	781	GACTGTTCTCAGGAGGCAACCGACAGCTTACTCACATCAGTCCCAATGCCTTTGTGGGTT	840
25	225	R--L--F--L--G--G--N--R--Q--L--T--H--I--S--P--N--A--F--V--G--	244
	841	CCAGTGAGTTGGTGGTACTAGACGTCTCCGAAACAGCCCTCTTTGCCAGACTCGATCCTT	900
	245	S--S--E--L--V--V--L--D--V--S--E--T--A--L--F--A--R--L--D--P--	264
	901	GATGGCCTCAAGAGGCTGATTGCCGAGTCAGCCTTCAACCTGAAAGAAGTTTCCTCTATT	960
30	265	*-	264

SEQ ID NOs 107 and 110 (wild-type VtgAa)

35 LENGTH: 4974bp and 1657aa

TYPE: cDNA (SEQ ID NO: 107) and Protein (SEQ ID NO: 110)

ORGANISM: Nile tilapia

	1	ATGAGAGCGCTCGTGCTCGCCCTGATTCTGGCCTTTGTGGCTGGTGATCTTCAACATCAA	60
40	1	-M--R--A--L--V--L--A--L--I--L--A--F--V--A--G--D--L--Q--H--Q-	20
	61	GATCCTGTTTTTGAAGCTGATAAAACCTATGTGTACAAGTATGAGGCGCTGCTCCTGGCG	120
	21	-D--P--V--F--E--A--D--K--T--Y--V--Y--K--Y--E--A--L--L--L--A-	40
45	121	GGCCTGCTCGAGAAAGGTTTCAGCGAGAGCTGGACTAAATATCAGCAGCAAAGTTAGCATC	180
	41	-G--L--L--E--K--G--S--A--R--A--G--L--N--I--S--S--K--V--S--I-	60
	181	AATGCTATAGACCAGAACACATACTTCATTAAGCTTGAGGAACCTGAGCTCCAGGAGTAT	240
50	61	-N--A--I--D--Q--N--T--Y--F--I--K--L--E--E--P--E--L--Q--E--Y-	80
	241	AGTGAATTTGGCCTGAGGATCCTTTTATCCCAGCAACTGAGCTGACTTCAGCCCTCCAA	300
	81	-S--G--I--W--P--E--D--P--F--I--P--A--T--E--L--T--S--A--L--Q-	100
	301	GCTGAGCTCAGACTCCCATTAAAGTTTGAATATGTCAATGGTGCTGTTGGAAAAGTCTTC	360
55	101	-A--E--L--T--T--P--I--K--F--E--Y--V--N--G--A--V--G--K--V--F-	120

	361	CCCCCTGAAACCGTCTCAACAACAGTGCTTAACATCTACAGAGGTATCCTGAATGTCTTT	420
	121	-A--P--E--T--V--S--T--T--V--L--N--I--Y--R--G--I--L--N--V--F-	140
5	421	CAGCTCAACGTCAAAAAGACACTAAATGTCTACGAGTTGCAGGAGGCTGGAACTCAGGGT	480
	141	-Q--L--N--V--K--K--T--L--N--V--Y--E--L--Q--E--A--G--T--Q--G-	160
	481	GTGTGCAAGACACTTTACTCCATCACTGAGGACACAGAGGCTGAACGTGTCTATCTGAGA	540
	161	-V--C--K--T--L--Y--S--I--T--E--D--T--E--A--E--R--V--Y--L--R-	180
10	541	AAGACCAGGGACATGAGCCACTGTCAAGAAAGAATAACTAAAGACATGGGGTTAGCATAC	600
	181	-K--T--R--D--M--S--H--C--Q--E--R--I--T--K--D--M--G--L--A--Y-	200
15	601	ACAGAGAAATGTGGAAAGTGCCAGGAGGACACTAAAAACCTGAAAGGAGTTTCATCATAC	660
	201	-T--E--K--C--G--K--C--Q--E--D--T--K--N--L--K--G--V--S--S--Y-	220
	661	AGTTACATCATGAAACCACTCGATAATGGCATCCAGATCAAGGAGGCATCGGTCCATGAG	720
	221	-S--Y--I--M--K--P--L--D--N--G--I--Q--I--K--E--A--S--V--H--E-	240
20	721	CTGATCCAGTTCTCACCTTTCAGTGAGCAGCATGGAGCCGCCATATGGAGACCAAGCAA	780
	241	-L--I--Q--F--S--P--F--S--E--Q--H--G--A--A--H--M--E--T--K--Q-	260
	781	TCCTTGATGCTCCTTGACGTTGCAAGACCCCTTATGCACCCACTACACCACCACCCAG	840
	261	-S--L--M--L--L--D--V--R--R--P--P--Y--A--P--T--T--P--P--P--Q-	280
25	841	GCTGAGTATTACACCGTGGAATCTCACATATCAGTTCTCCACTGAGCTTCTTCAGTTA	900
	281	-A--E--Y--S--H--R--G--N--L--T--Y--Q--F--S--T--E--L--L--Q--L-	300
30	901	CCCATTCTGCTCCTCAATATCAACGACATAGAGTCTCAGCTCGAGGACACTCTGGTCAAA	960
	301	-P--I--L--L--L--N--I--N--D--I--E--S--Q--L--E--D--T--L--V--K-	320
	961	CAGGCTGTAGAAAGAGTTCATGAAGATGCACCTCTGGAATTTTGAAGTTGTTCAACTC	1020
	321	-Q--A--V--E--R--V--H--E--D--A--P--L--E--F--L--K--F--V--Q--L-	340
35	1021	CTCCGTGCAGCCTCCAATGAAACTCTGGAGAACCTCTGGAGCAAACACTCAGGGATTTCT	1080
	341	-L--R--A--A--S--N--E--T--L--E--N--L--W--S--K--H--S--G--I--S-	360
	1081	CCCCACAGAAAATGGATCATGGACGCCATCCCTGCTGTGGGAAATCCTGATGCTCTGAGA	1140
	361	-A--H--R--K--W--I--M--D--A--I--P--A--V--G--N--P--D--A--L--R-	380
40	1141	TTTATCAAAGAGAAATACCTAGCAGAAACCATAACTGTGTTTGAAGCCGTTTCAGGCTTTG	1200
	381	-F--I--K--E--K--Y--L--A--E--T--I--T--V--F--E--A--V--Q--A--L-	400
45	1201	ATTACTTCATTTACATGGTGACAGCAACCACTGAGGCCATTGAGGTCATCGAGAGCCTA	1260
	401	-I--T--S--F--H--M--V--T--A--T--T--E--A--I--E--V--I--E--S--L-	420
	1261	ACAAAGGAAAGCAAAATAGTGAGAAACCCAGTTCTGCGTCAGATTGTATTCCTTGGCTAC	1320
	421	-T--K--E--S--K--I--V--R--N--P--V--L--R--Q--I--V--F--L--G--Y-	440
50	1321	GGTACCATGATTTACAAACACTGCTATGAGAGGACTTCCTGTCCTGCTGAGCTCATACAG	1380
	441	-G--T--M--I--Y--K--H--C--Y--E--R--T--S--C--P--A--E--L--I--Q-	460
	1381	CCCATTCAAGACCTTCTTGCGCAGGCACCTGAAAGATGGAAACACAGAGGACATCATCCTG	1440
	461	-P--I--Q--D--L--L--A--Q--A--L--K--D--G--N--T--E--D--I--I--L-	480
55	1441	TTTGTGAAGGCTTTGGGAAATGCTGCGCATCCTTCTAGCCTCAAGAAAATCACAAAGATG	1500
	481	-F--V--K--A--L--G--N--A--A--H--P--S--S--L--K--K--I--T--K--M-	500
60	1501	CTGCCCCACATAGTAAATTAGGTTTCATCACTGCCAGTGAGAGTTCATGCTGAAGCCATG	1560
	501	-L--P--L--H--S--K--L--G--S--S--L--P--V--R--V--H--A--E--A--M-	520

	1561	ATGGCCTTGAAGAACATCGCCAAAAAGGAGCCTAAAACGGTCCAGTATTTAGCCTTTTCAG	1620
	521	-M--A--L--K--N--I--A--K--K--E--P--K--T--V--Q--Y--L--A--F--Q-	540
5	1621	CTCTACGGGGACAAGACTCTTCATTTCAGAGATCCGCATGCTTGCGTGCATGGTGCTCTTT	1680
	541	-L--Y--G--D--K--T--L--H--S--E--I--R--M--L--A--C--M--V--L--F-	560
	1681	GAGACAAAACCTTCAATGAGTTTGGTGTGAGCTGTTGTTTCATATTGTGAAGACAGATACA	1740
	561	-E--T--K--P--S--M--S--L--V--S--A--V--V--H--I--V--K--T--D--T-	580
10	1741	AATTTGCAAGTAGTAAGCTTCACCTATTCCCACATGAAGTCCCTGACTAGGAGCACCAGC	1800
	581	-N--L--Q--V--V--S--F--T--Y--S--H--M--K--S--L--T--R--S--T--S-	600
	1801	GTTATTTATGCCTCAGTTGCTGCAGCATGCAAAGCTGCCCTGAGAATGTTGGGCCCAAAC	1860
15	601	-V--I--Y--A--S--V--A--A--A--C--K--A--A--L--R--M--L--G--P--N-	620
	1861	CTGGACAAACTGAGCTCACGTTTCAGCAAAGCCATCCATGTCGACGTCTATAGCAGTCCC	1920
	621	-L--D--K--L--S--S--R--F--S--K--A--I--H--V--D--V--Y--S--S--P-	640
20	1921	TTTATGCTTGGTGTGCTGCGACTGCTTACTACATCAATGATGCTGCCACCATCATGCC	1980
	641	-F--M--L--G--A--A--A--T--A--Y--Y--I--N--D--A--A--T--I--M--P-	660
	1981	AAATCTATTACGACTAGGATCAAGGCTTTCTTTGCTGGAGCTGCTGCTGACATTCTGGAG	2040
	661	-K--S--I--T--T--R--I--K--A--F--F--A--G--A--A--A--D--I--L--E-	680
25	2041	GTTGGAGTAAGAACTGAGGGACTACAGGAGCCTTTTCTGAAAAACCCAGCAGTTTTTGAT	2100
	681	-V--G--V--R--T--E--G--L--Q--E--A--F--L--K--N--P--A--V--F--D-	700
	2101	AGTGCTGACAGGGTCACCAGGATGAAACATGTCATTAAGGCTCTCTCTCACTGGAAGTCT	2160
30	701	-S--A--D--R--V--T--R--M--K--H--V--I--K--A--L--S--H--W--K--S-	720
	2161	GCACCCAACAGCAAATCCCTGACTTCCATCTATGTCAAGTTCTTTGGACAAGAAGTTGCC	2220
	721	-A--P--N--S--K--S--L--T--S--I--Y--V--K--F--F--G--Q--E--V--A-	740
35	2221	TTTGTGACTTTTGACAAAATCTGGTTTGACAACATCTTTAATCTCATCTTTGCCAATAAC	2280
	741	-F--V--D--F--D--K--I--W--F--D--N--I--F--N--L--I--F--A--N--N-	760
	2281	AATGCTGACACGTTTGGTAGAGATGTTTTCAAGGCTCTGCAGTCTGGTCCTACTTTGCGC	2340
	761	-N--A--D--T--F--G--R--D--V--F--K--A--L--Q--S--G--P--T--L--R-	780
40	2341	TTTGTTAAGCCTCTGCTGGCTAATGAGGTGAGACGTATCATGCCTACTATAGCTGGTTTT	2400
	781	-F--V--K--P--L--L--A--N--E--V--R--R--I--M--P--T--I--A--G--F-	800
	2401	CCCATGGAGCTCGGTCTGTACACTGCTGCTGTGGCTGCTGTTCTGGTCAAATCAAAGTC	2460
45	801	-P--M--E--L--G--L--Y--T--A--A--V--A--A--V--P--G--Q--I--K--V-	820
	2461	ACCACGACTCCAGCTCTGCCAGAAGACTTTTATCTCAGATACCTTCTCAAGGCAGATATA	2520
	821	-T--T--T--P--A--L--P--E--D--F--Y--L--R--Y--L--L--K--A--D--I-	840
50	2521	CACATTAGTACCAAGGTCACACCAAGTGTCGCTGTGAACACATTTGCTGTGTTGGGATA	2580
	841	-H--I--S--T--K--V--T--P--S--V--A--V--N--T--F--A--V--F--G--I-	860
	2581	AACACTGCCATACTCCAGGCTGTGTCATGGTATCCAGAGCCAAACTCTACTCCATCACACCA	2640
	861	-N--T--A--I--L--Q--A--V--M--V--S--R--A--K--L--Y--S--I--T--P-	880
55	2641	GCCAAACTGAAGTCACATTTAATCAATGAGGGCTACTTGAATTTACAGCTCTTCCT	2700
	881	-A--K--T--E--V--T--F--N--I--N--E--G--Y--L--N--F--T--A--L--P-	900
	2701	GTTTCAGTGCCTGAAAACATTACAGCTGTGGAGGTTGAGACTTTTGCTGTGGTAAGAAAT	2760
60	901	-V--S--V--P--E--N--I--T--A--V--E--V--E--T--F--A--V--V--R--N-	920

	2761	CCTGCTTCGGGAGAAAGAATCACTCCTGTGATCCCTGCCAACCCAAGACAGATTCTTATA	2820
	921	-P--A--S--G--E--R--I--T--P--V--I--P--A--N--P--R--Q--I--L--I-	940
5	2821	TCCAGTAATACTTCTTCTGATGCTGTTAGTGAGTCAAGATCCGAAGAGTTCATTTCTCAG	2880
	941	-S--S--N--T--S--S--D--A--V--S--E--S--R--S--E--E--F--I--S--Q-	960
	2881	CGTCAGAAAGCTGGCATGCACATCAAATCTAAAATGGTGAAGAGTAAGAAGAAGTACTGC	2940
	961	-R--Q--K--A--G--M--H--I--K--S--K--M--V--K--S--K--K--K--Y--C-	980
10	2941	GCTCAGACTGTTAACGCTGGACTCAAGGCCTGTCTCAAGATTGCCACTGCTTACACGGGG	3000
	981	-A--Q--T--V--N--A--G--L--K--A--C--L--K--I--A--T--A--Y--T--G-	1000
	3001	GATGCTGCAGTGTATAAACTGGCTGGAAAGCACTCCGCTGCTTTTTCTGTACACCAATT	3060
15	1001	-D--A--A--V--Y--K--L--A--G--K--H--S--A--A--F--S--V--T--P--I-	1020
	3061	GAAGGTGAAGCTGCTGAGAGACTGGAATTAGAGGTTCAACTTGAAGTAAGGCTGCACAG	3120
	1021	-E--G--E--A--A--E--R--L--E--L--E--V--Q--L--G--S--K--A--A--Q-	1040
20	3121	AAGATCATCAAACACATCACGCTTAGAGAAGAAGAAATCCCAGAGGAAACACCAGTCTTA	3180
	1041	-K--I--I--K--H--I--T--L--R--E--E--E--I--P--E--E--T--P--V--L-	1060
	3181	ATGAAGCTCCACAAAATCCTGGCCTCTACCCAGAAGAATAGCACCATGTCCTCCTCATCC	3240
	1061	-M--K--L--H--K--I--L--A--S--T--Q--K--N--S--T--M--S--S--S--S-	1080
25	3241	TCCAGTTCAGGAGCTCTCGCTTTCATGTGATCCTCTTCTTCCAATTCAGCTCTTCA	3300
	1081	-S--S--S--R--S--S--R--F--H--V--R--S--S--S--S--N--S--S--S--S-	1100
	3301	TCCCATTCTAGCAGGAAGACCATTGATGCAACTGCTCAACAAGTCTTCAGCTTCTCCACC	3360
30	1101	-S--H--S--S--R--K--T--I--D--A--T--A--Q--Q--V--F--S--F--S--T-	1120
	3361	TCTGTGACTACTTCCAAGTCCAGCTTTGCATCGAGCTTTGCATCACTCTTCAGTCTTAGT	3420
	1121	-S--V--S--T--S--K--S--S--F--A--S--S--F--A--S--L--F--S--L--S-	1140
35	3421	TCAAGCTCTTCTCACTACAGTGCGCACCACAGAAAGCATCCTGCGAGTCGCCACAAACCC	3480
	1141	-S--S--S--S--H--Y--S--A--H--H--R--K--H--P--A--S--R--H--K--P-	1160
	3481	AAGGAGAAACACAAGCATCCACCTCTAAAGCCACATCGTCACAGGTTTTCAAAAGCAGA	3540
	1161	-K--E--K--H--K--H--P--T--S--K--A--T--S--S--Q--V--F--K--S--R-	1180
40	3541	AGCAGTGGCTCAAGCTTGGACGCTATCCAACATAAGAAGCGGTTCTTGACAGTCAAGCT	3600
	1181	-S--S--G--S--S--L--D--A--I--Q--H--K--K--R--F--L--D--S--Q--A-	1200
	3601	GCTATCTTTGGCATGATCTTCCGTGCTGTTAAAGCTGACACGAAGAAGCAGGGATACCAG	3660
45	1201	-A--I--F--G--M--I--F--R--A--V--K--A--D--T--K--K--Q--G--Y--Q-	1220
	3661	TTCAGTGTACATGGACAAAACCACAGCAGACTTCAAATCATTCTAGATGACATTGTT	3720
	1221	-F--T--A--Y--M--D--K--T--T--S--R--L--Q--I--I--L--D--D--I--V-	1240
50	3721	CCTGATAACAACTGGAGGCTCTGTGCTGATGGAGCCGTGTTGAGCATGCACAAAGTCAAA	3780
	1241	-P--D--N--N--W--R--L--C--A--D--G--A--V--L--S--M--H--K--V--K-	1260
	3781	GCTAAAATGAACTGGGGAGCAGAATGCAACCAATATGACACCACGATTACAACAGAAACT	3840
	1261	-A--K--M--N--W--G--A--E--C--N--Q--Y--D--T--T--I--T--T--E--T-	1280
55	3841	GGTCTTGTGCGGTCGAAACCCTGCAGCTCGGCTGAAGGTGGACTGGAATCGGCTACCGTCT	3900
	1281	-G--L--V--G--R--N--P--A--A--R--L--K--V--D--W--N--R--L--P--S-	1300
	3901	GATCTCAAGCACCATGCAAAGACGATGTATAAGTACATTTCTGCTCACATGCCTGCCGGC	3960
60	1301	-D--L--K--H--H--A--K--T--M--Y--K--Y--I--S--A--H--M--P--A--G-	1320

	3961	TTGATTTCAGGAAAAGGACAGAAACAGCGACAAGCAGCTCTCGTTGACTGTGGCTGTAGTA	4020
	1321	-L--I--Q--E--K--D--R--N--S--D--K--Q--L--S--L--T--V--A--V--V-	1340
5	4021	TCTGACAAGATCATCGACCTGATTTGGAAAAACCCGAGAAGCACTGTTTCATAAGCGGGCT	4080
	1341	-S--D--K--I--I--D--L--I--W--K--T--P--R--S--T--V--H--K--R--A-	1360
	4081	TTGCATCTTCCCATCACTCTGCCACGTAACGAGATCAAAGATCTTACTTCCTTCAGTGAC	4140
	1361	-L--H--L--P--I--T--L--P--R--N--E--I--K--D--L--T--S--F--S--D-	1380
10	4141	GTCTCTGGAAAAGTCAAGCACTTGTTAGCTGCGGCTGGCGCAGCTGAATGTAGCTTCACC	4200
	1381	-V--S--G--K--V--K--H--L--L--A--A--A--G--A--A--E--C--S--F--T-	1400
15	4201	GACAATACGCTGACCACATTCAACAACAAGAAATTAAAGAACGAGATGCCCTCAAACCTGC	4260
	1401	-D--N--T--L--T--T--F--N--N--K--K--L--K--N--E--M--P--S--N--C-	1420
	4261	TATCAGGTTCTGGCACAGGATGGCACAGACGAGCTGAAATTCATCGTTCTACTGAGGAAG	4320
	1421	-Y--Q--V--L--A--Q--D--G--T--D--E--L--K--F--I--V--L--L--R--K-	1440
20	4321	GATCGCACTGAACAGAAGCAGATCAGTGTGAAAATTGCTCATATAGACATTGACCTCTAT	4380
	1441	-D--R--T--E--Q--K--Q--I--S--V--K--I--A--H--I--D--I--D--L--Y-	1460
	4381	CAGAGGAGAACCAGTGTGACTGTGAATGTGAATGGGCTGGAAATACCCATGAGCAACCTG	4440
	1461	-Q--R--R--T--S--V--T--V--N--V--N--G--L--E--I--P--M--S--N--L-	1480
25	4441	CCATATCGTTATCCCCAAGCTGACATCCAGATCAAACAAAATGGCGAAGGCATCTCTGTG	4500
	1481	-P--Y--R--Y--P--Q--A--D--I--Q--I--K--Q--N--G--E--G--I--S--V-	1500
30	4501	TATGCAGCTAGCTATGGTCTTCATGAAGTCTACTTTGACAAGAAGTCATGGAAGATTTAA	4560
	1501	-Y--A--A--S--Y--G--L--H--E--V--Y--F--D--K--K--S--W--K--I--K-	1520
	4561	GTTGTGACTGGATGAAGGGGAAGACTTGTGGGCTCTGTGGAAAGGCTGACGGGGAGACC	4620
	1521	-V--V--D--W--M--K--G--K--T--C--G--L--C--G--K--A--D--G--E--T-	1540
35	4621	ATGCAGGAGTATCGCACACCCACTGGATGGATAGCCACGACAGCAGTGAGCTTTGCTCAT	4680
	1541	-M--Q--E--Y--R--T--P--T--G--W--I--A--T--T--A--V--S--F--A--H-	1560
	4681	TCTTGATTTCTGCCAGCTGAGAGCTGCAGAGACGCCACTGAGTGCCGTATGAGGCATGAA	4740
	1561	-S--W--I--L--P--A--E--S--C--R--D--A--T--E--C--R--M--R--H--E-	1580
40	4741	TCTGTGCAGCTGGAGAAACAGGAAAACGTGCAAGCTCAGAACTCCAAGTGCTACTCTGTC	4800
	1581	-S--V--Q--L--E--K--Q--E--N--V--Q--A--Q--N--S--K--C--Y--S--V-	1600
45	4801	GACCCTGTGCTGCGCTGCATGGCTGGGTGCTTCCCTGTGCGCACCACCAACGTCACCTGTT	4860
	1601	-D--P--V--L--R--C--M--A--G--C--F--P--V--R--T--T--N--V--T--V-	1620
	4861	GGCTTCCACTGCCTTCCAGCTGGTTCCAGCCCCTCCAGCATGTATACGAGCGTGACCTG	4920
	1621	-G--F--H--C--L--P--A--G--S--S--P--S--S--M--Y--T--S--V--D--L-	1640
50	4921	ATGGAAACTACGGAGAGTCACCTCGCCTGCACCTGCACTGCTCAGTGTGCTTAA	4974
	1641	-M--E--T--T--E--S--H--L--A--C--T--C--T--A--Q--C--A--*-	1657

SEQ ID NOs 108 and 111 (VtgAa mutant allele- 5nt deletion)

LENGTH: 4974bp and 279aa

55 TYPE: cDNA (SEQ ID NO: 108) and Protein (SEQ ID NO: 111)

ORGANISM: Nile tilapia

	1	ATGAGAGCGCTCGTGCTCGCCCTGATTCTGGCCTTTGTGGCTGGTGATCTTCAACATCAA	60
	1	-M--R--A--L--V--L--A--L--I--L--A--F--V--A--G--D--L--Q--H--Q-	20
5	61	GATCCTGTTTTTGAAGCTGATAAAACCTATGTGTACAAGTATGAGGCGCTGCTCCTGGCG	120
	21	-D--P--V--F--E--A--D--K--T--Y--V--Y--K--Y--E--A--L--L--L--A-	40
	121	GGCCTGCTCGAGAAAGGTTTCAGCGAGAGCTGGACTAAATATCAGCAGCAAAGTTAGCATC	180
10	41	-G--L--L--E--K--G--S--A--R--A--G--L--N--I--S--S--K--V--S--I-	60
	181	AATGCTATAGACCAGAACACATACTTCATTAAGCTTGAGGAACCTGAGCTCCAGGAGTAT	240
	61	-N--A--I--D--Q--N--T--Y--F--I--K--L--E--E--P--E--L--Q--E--Y-	80
	241	AGTGGAATTTGGCCTGAGGATCCTTTTATCCCAGCAACTGAGCTGACTTCAGCCCTCCAA	300
15	81	-S--G--I--W--P--E--D--P--F--I--P--A--T--E--L--T--S--A--L--Q-	100
	301	GCTGAGCTCAGACTCCCATTAAAGTTTGAATATGTCAATGGTGCTGTTGGAAAAGTCTTC	360
	101	-A--E--L--T--T--P--I--K--F--E--Y--V--N--G--A--V--G--K--V--F-	120
	361	GCCCTGAAACCGTCTCAACAACAGTGCTTAACATCTACAGAGGTATCCTGAATGTCTTT	420
20	121	-A--P--E--T--V--S--T--T--V--L--N--I--Y--R--G--I--L--N--V--F-	140
	421	CAGCTCAACGTCAAAAAGACACTAAATGTCTACGAGTTGCAGGAGGCTGGAACCTCAGGGT	480
25	141	-Q--L--N--V--K--K--T--L--N--V--Y--E--L--Q--E--A--G--T--Q--G-	160
	481	GTGTGCAAGACACTTTACTCCATCACTGAGGACACAGAGGCTGAACGTGTCTATCTGAGA	540
	161	-V--C--K--T--L--Y--S--I--T--E--D--T--E--A--E--R--V--Y--L--R-	180
	541	AAGACCAGGGACATGAGCCACTGTCAAGAAAGAATAACTAAAGACATGGGGTTAGCATAC	600
30	181	-K--T--R--D--M--S--H--C--Q--E--R--I--T--K--D--M--G--L--A--Y-	200
	601	ACAGAGAAATGTGGAAAGTGCCAGGAGGACACTAAAAACCTGAAAGGAGTTTCATCATAC	660
	201	-T--E--K--C--G--K--C--Q--E--D--T--K--N--L--K--G--V--S--S--Y-	220
35	661	AGTTACATCATGAAACCACTCGATAATGGCATCCAGATCAAGGAGGCATCGGTCCATGAG	720
	221	-S--Y--I--M--K--P--L--D--N--G--I--Q--I--K--E--A--S--V--H--E-	240
	721	CTGATCCAGTTCTCACCTTTCAGTGAGCAGCATGGAGCCGCCATATGGAGACCAAGCAA	780
40	241	-L--I--Q--F--S--P--F--S--E--Q--H--G--A--A--H--M--E--T--K--Q-	260
	781	TCCTTGATGCTCCTTGACGTTTCAAGACCCCTTATGCACCCACTACACCACAGGCTGA	840
	261	-S--L--M--L--L--D--V--R--R--P--P--Y--A--P--T--T--P--P--G--*-	279

45

SEQ ID NOs 109 and 112 (VtgAa mutant allele- 25nt deletion)

LENGTH: 4974bp and 301aa

TYPE: cDNA (SEQ ID NO: 109) and Protein (SEQ ID NO: 112)

ORGANISM: Nile tilapia

50

55

	1	ATGAGAGCGCTCGTGCTCGCCCTGATTCTGGCCTTTGTGGCTGGTGATCTTCAACATCAA	60
	1	-M--R--A--L--V--L--A--L--I--L--A--F--V--A--G--D--L--Q--H--Q-	20
	61	GATCCTGTTTTTGAAGCTGATAAAACCTATGTGTACAAGTATGAGGCGCTGCTCCTGGCG	120
	21	-D--P--V--F--E--A--D--K--T--Y--V--Y--K--Y--E--A--L--L--L--A-	40

	121	GGCCTGCTCGAGAAAGGTTTCAGCGAGAGCTGGACTAAATATCAGCAGCAAAGTTAGCATC	180
	41	-G--L--L--E--K--G--S--A--R--A--G--L--N--I--S--S--K--V--S--I-	60
5	181	AATGCTATAGACCAGAACACATACTTCATTAAGCTTGAGGAACCTGAGCTCCAGGAGTAT	240
	61	-N--A--I--D--Q--N--T--Y--F--I--K--L--E--E--P--E--L--Q--E--Y-	80
	241	AGTGAATTTGGCCTGAGGATCCTTTTATCCCAGCAACTGAGCTGACTTCAGCCCTCCAA	300
	81	-S--G--I--W--P--E--D--P--F--I--P--A--T--E--L--T--S--A--L--Q-	100
10	301	GCTGAGCTCAGACTCCCATTAAAGTTTGAATATGTCAATGGTGCTGTTGGAAAAGTCTTC	360
	101	-A--E--L--T--T--P--I--K--F--E--Y--V--N--G--A--V--G--K--V--F-	120
	361	GCCCCTGAAACCGTCTCAACAACAGTGCTTAACATCTACAGAGGTATCCTGAATGTCTTT	420
15	121	-A--P--E--T--V--S--T--T--V--L--N--I--Y--R--G--I--L--N--V--F-	140
	421	CAGCTCAACGTCAAAAAGACACTAAATGTCTACGAGTTGCAGGAGGCTGGAACCTCAGGGT	480
	141	-Q--L--N--V--K--K--T--L--N--V--Y--E--L--Q--E--A--G--T--Q--G-	160
20	481	GTGTGCAAGACACTTTACTCCATCACTGAGGACACAGAGGCTGAACGTGTCTATCTGAGA	540
	161	-V--C--K--T--L--Y--S--I--T--E--D--T--E--A--E--R--V--Y--L--R-	180
	541	AAGACCAGGGACATGAGCCACTGTCAAGAAAGAATAACTAAAGACATGGGGTTAGCATAAC	600
	181	-K--T--R--D--M--S--H--C--Q--E--R--I--T--K--D--M--G--L--A--Y-	200
25	601	ACAGAGAAATGTGGAAAGTGCCAGGAGGACACTAAAAACCTGAAAGGAGTTTCATCATAAC	660
	201	-T--E--K--C--G--K--C--Q--E--D--T--K--N--L--K--G--V--S--S--Y-	220
	661	AGTTACATCATGAAACCACTCGATAATGGCATCCAGATCAAGGAGGCATCGGTCCATGAG	720
30	221	-S--Y--I--M--K--P--L--D--N--G--I--Q--I--K--E--A--S--V--H--E-	240
	721	CTGATCCAGTTCTCACCTTTCAGTGAGCAGCATGGAGCCGCCCATATGGAGACCAAGCAA	780
	241	-L--I--Q--F--S--P--F--S--E--Q--H--G--A--A--H--M--E--T--K--Q-	260
35	781	TCCTTGATGCTCCTTGACGTTTCAAGACACCCCAGGCTGAGTATTCACACCGTGGAATC	840
	261	-S--L--M--L--L--D--V--R--R--H--P--R--L--S--I--H--T--V--E--I-	280
	841	TCACATATCAGTTCTCCACTGAGCTTCTTCAGTTACCCATTCTGCTCCTCAATATCAACG	900
	281	S--H--I--S--S--P--L--S--F--F--S--Y--P--F--C--S--S--I--S--T-	300
40	901	ACATAGAGTCTCAGCTCGAGGACACTCTGGTCAAACAGGCTGTAGAAAGAGTTCATGAAG	960
	301	-T--*-	301

45 **SEQ ID NOs 113 and 115 (wild-type VtgAb)**

LENGTH: 5339bp and 1747aa

TYPE: cDNA (SEQ ID NO: 113) and Protein (SEQ ID NO: 115)

ORGANISM: Nile tilapia

50	1	CGCCATTTAGTTAATGATACATTTGATGGGCAACGTCAGCAAAAAATCTGCTTAAAAAGG	60
			
	61	ACGCCTCTGCCTGCAGATCTTCACATCCACCAGCCATGAGGGTGCTTGTACTAGCTCTTG	120
		-M--R--V--L--V--L--A--L--	8
55	121	CTGTGGCTCTCGCAGTGGGGGACCAGTCCAACCTTGGCCCCAGGATTCGCCTCTGTTAAGA	180
	9	A--V--A--L--A--V--G--D--Q--S--N--L--A--P--G--F--A--S--V--K--	28

	181	CCTACATGTACAAATATGAAGCGTTCTTATGGGCGGCCTGCCTGAAGAGGGCCTGGCTC	240
	29	T--Y--M--Y--K--Y--E--A--V--L--M--G--G--L--P--E--E--G--L--A--	48
5	241	GAGCTGGGGTTAAATCCGGGGCAAAGTTTGTATCAGTGCAACAAGTGCCAACGACTACA	300
	49	R--A--G--V--K--I--R--G--K--V--L--I--S--A--T--S--A--N--D--Y--	68
	301	TTCTGAAGCTTGTAGACCCTCAGTTGCTGGAGTACAGTGGCATCTGGCCCAAAGATCCTT	360
	69	I--L--K--L--V--D--P--Q--L--L--E--Y--S--G--I--W--P--K--D--P--	88
10	361	TCCATCCAGCCACCAAGCTCACCACAGCCCTGGCTACTCAGCTCTCGACACCGGTCAAGT	420
	89	F--H--P--A--T--K--L--T--T--A--L--A--T--Q--L--S--T--P--V--K--	108
	421	TTGAGTATACAAACGGCGTTGTTGGGAGACTGGCTGCACCTCCTGGGGTCTCCACAACAG	480
15	109	F--E--Y--T--N--G--V--V--G--R--L--A--A--P--P--G--V--S--T--T--	128
	481	TGCTGAATATCTACAGGGGCATCATCAACCTCCTGCAGCTGAATGTAAAGAAGACACAGA	540
	129	V--L--N--I--Y--R--G--I--I--N--L--L--Q--L--N--V--K--K--T--Q--	148
20	541	ATGTCTACGAGATGCAAGAGTCTGGAGCTCATGGTGTGTGCAAGACCAACTATGTGATCA	600
	149	N--V--Y--E--M--Q--E--S--G--A--H--G--V--C--K--T--N--Y--V--I--	168
	601	GGGAGGACGCGAGGGCCGAACGCATTTCATCTGACCAAGACCAAGGACCTGAACCACTGCC	660
	169	R--E--D--A--R--A--E--R--I--H--L--T--K--T--K--D--L--N--H--C--	188
25	661	AGGAGAAAATCATGAAGGCCATCGGCTTGGAAACAGTAGAGAAATGCCATGATTGTGAAG	720
	189	Q--E--K--I--M--K--A--I--G--L--E--H--V--E--K--C--H--D--C--E--	208
	721	CTAGAGGAAAGAGCCTGAAGGGAAGTCTTCTATAACTACATCATGAAGCCAGCACCCA	780
30	209	A--R--G--K--S--L--K--G--T--A--S--Y--N--Y--I--M--K--P--A--P--	228
	781	GTGGTTCTCTGATTATGGAGGCTGTGCTAGAGAGGTCATCGAGTTTTACCTTTCAACA	840
	229	S--G--S--L--I--M--E--A--V--A--R--E--V--I--E--F--S--P--F--N--	248
35	841	TTTTGAATGGCGCTGCTCAGATGGAGTCTAAGCAAATCTGACCTTCCTGGATATTGAGA	900
	249	I--L--N--G--A--A--Q--M--E--S--K--Q--I--L--T--F--L--D--I--E--	268
	901	ACACCCCTGTGGATCATGCCAGATACACCTATGTTTACC CGGATCCCTGCAGTATGAGC	960
	269	N--T--P--V--D--H--A--R--Y--T--Y--V--H--R--G--S--L--Q--Y--E--	288
40	961	ATGGCAGCGAGATTCTCCAGACACCCATCCATCTTCTGAGGGTCACCCATGCCGAGGCTC	1020
	289	H--G--S--E--I--L--Q--T--P--I--H--L--L--R--V--T--H--A--E--A--	308
	1021	AGATTGTGACACTCTGAACCACTGGTAGCCTCCAACGTGGCCAAGGTCCATGAAGATG	1080
45	309	Q--I--V--S--T--L--N--H--L--V--A--S--N--V--A--K--V--H--E--D--	328
	1081	CCCCTCTGAAGTTTGTGAGCTCATCCAGGTGATGCGTGTGGCCAGATTTGAGACTATTG	1140
	329	A--P--L--K--F--V--E--L--I--Q--V--M--R--V--A--R--F--E--T--I--	348
50	1141	AGTCCCTCTGGGCTCAGTTTAAATCTAGACCTGATCACAGGTACTGGTTACTGAATGCTG	1200
	349	E--S--L--W--A--Q--F--K--S--R--P--D--H--R--Y--W--L--L--N--A--	368
	1201	TCCCCACATTCGCACTCACGCTGCGCTTAAGTTCCTCATTGAGAAGCTCCTTGCTAATG	1260
	369	V--P--H--I--R--T--H--A--A--L--K--F--L--I--E--K--L--L--A--N--	388
55	1261	AGTTAAGTGAGACTGAAGCTGCTATGGCTCTCTTGGGAATGTCTGCACTCTGTGACAGCTG	1320
	389	E--L--S--E--T--E--A--A--M--A--L--L--E--C--L--H--S--V--T--A--	408
	1321	ACCAGAAAACCATTTGAACCTGTCAGAAGCCTGGCTGAGAACCACAGAGTGAAACGTAACG	1380
60	409	D--Q--K--T--I--E--L--V--R--S--L--A--E--N--H--R--V--K--R--N--	428

	1381	CTGTGCTCAACGAGATTGTGATGCTGGGCTGGGGCACTGTAATTTCCAGGTTCTGTAAAG	1440
	429	A--V--L--N--E--I--V--M--L--G--W--G--T--V--I--S--R--F--C--K--	448
5	1441	CGCAGCCATCTTGCTCATCTGATCTTGTGACACCTGTACATAGACAAGTTCAGAGGCTG	1500
	449	A--Q--P--S--C--S--S--D--L--V--T--P--V--H--R--Q--V--A--E--A--	468
	1501	TTGAAACTGGTGACATCGATCAGCTCACTGTCACTCTCAAATGCCTGGATAACGCTGGAC	1560
	469	V--E--T--G--D--I--D--Q--L--T--V--T--L--K--C--L--D--N--A--G--	488
10	1561	ATCCTGCTAGCCTTAAGACAATCATGAAGTTCTGCCTGGCTTTGGCAGTGCTGCTGCCC	1620
	489	H--P--A--S--L--K--T--I--M--K--F--L--P--G--F--G--S--A--A--A--	508
	1621	GAGTCCCCTCAAAAGTTCAGGTTGACGCTGTTCTAGCCCTGAGGAGAATTGCAAAGAGGG	1680
15	509	R--V--P--L--K--V--Q--V--D--A--V--L--A--L--R--R--I--A--K--R--	528
	1681	AACCCAAGATGGTCCAGGAAATAGCTGCTCAGTTGCTCATGGAAAAGCATCTCCATGCAG	1740
	529	E--P--K--M--V--Q--E--I--A--A--Q--L--L--M--E--K--H--L--H--A--	548
	1741	AACTGCGTATGGTTGCTGCCATGGTGCTCTTTGAGACTAAACTCCCCGTGGGTCTAGCAG	1800
20	549	E--L--R--M--V--A--A--M--V--L--F--E--T--K--L--P--V--G--L--A--	568
	1801	CTAGCATTTCCACAGCCTTGATCAAAGAAAAGAACCTGCAGGTCGTTAGCTTTGTCTACT	1860
	569	A--S--I--S--T--A--L--I--K--E--K--N--L--Q--V--V--S--F--V--Y--	588
25	1861	CTTACATGAAGCCATGGCCAAGACCACATCCCCTGACCACGTTTCTGTTGCTGCAGCAT	1920
	589	S--Y--M--K--A--M--A--K--T--T--S--P--D--H--V--S--V--A--A--A--	608
	1921	GTAATGTTGCCTTGAGGTTCTCAACCCCAAATTAGGCAGACTGAACTTCCGCTACAGCC	1980
30	609	C--N--V--A--L--R--F--L--N--P--K--L--G--R--L--N--F--R--Y--S--	628
	1981	GAGCCTTCCATGTGGATACCTATAACAATGCCTGGATGATGGGTGCTGCCGCCAGTGCCG	2040
	629	R--A--F--H--V--D--T--Y--N--N--A--W--M--M--G--A--A--A--S--A--	648
	2041	TCTTAATTAACGACGCTGCAACCGTGTTACCAAGAATGATTATGGCCAAAGCCCGTACTT	2100
35	649	V--L--I--N--D--A--A--T--V--L--P--R--M--I--M--A--K--A--R--T--	668
	2101	ACATGGCCGGAGCTTATGTTGATGCTTTTGAGGTTGGAGTGAGGACTGAGGGAATCCAGG	2160
	669	Y--M--A--G--A--Y--V--D--A--F--E--V--G--V--R--T--E--G--I--Q--	688
40	2161	AGGCTCTTTTGAAAAGACGACATGAAAATTCTGAGAATGCAGACAGGATCACCAAGATTA	2220
	689	E--A--L--L--K--R--R--H--E--N--S--E--N--A--D--R--I--T--K--I--	708
	2221	AACAAGCCATGAGAGCTCTTTCTGAGTGGAGGGCTAATCCTTCGAGCCAGGCCCTGGCCT	2280
45	709	K--Q--A--M--R--A--L--S--E--W--R--A--N--P--S--S--Q--A--L--A--	728
	2281	CTATGTATGTGAAGGTCTTCGGACAAGAAATTGCATTTGCCAACATTGACAAATCCAAGG	2340
	729	S--M--Y--V--K--V--F--G--Q--E--I--A--F--A--N--I--D--K--S--K--	748
	2341	TTGACCAGCTTATCCAGTTTGCCAGTGGACCTTTGAGAAACGTATTGAGAGATGCTGTGA	2400
50	749	V--D--Q--L--I--Q--F--A--S--G--P--L--R--N--V--F--R--D--A--V--	768
	2401	ATTCTGTGCTGTCTGGTTATGCAACACATTTTGCTAAACCAATGCTGCTCGGTGAGCTCC	2460
	769	N--S--V--L--S--G--Y--A--T--H--F--A--K--P--M--L--L--G--E--L--	788
55	2461	GTCTCATCCTTCCCACCACTGTTGGGTTGCCCATGGAGATCAGCCTCATTACATCCGCTG	2520
	789	R--L--I--L--P--T--T--V--G--L--P--M--E--I--S--L--I--T--S--A--	808
	2521	TGACTGCTGCATCTGTTGACGTCCAAGCCACTGTGTCAACCACTCTGCCTGTCAACTACC	2580
60	809	V--T--A--A--S--V--D--V--Q--A--T--V--S--P--P--L--P--V--N--Y--	828

	2581	GAGTTTCCCAGCTTCTGGAGTCCGATATCCAAGTGGGGCTACAGTTGCTCCAAGTCTTG	2640
	829	R--V--S--Q--L--L--E--S--D--I--Q--L--R--A--T--V--A--P--S--L--	848
5	2641	CCATGCAGACCTATGCATTCATGGGTGTGAACACCGCCTTAATCCAGGCTGCAGTGATGA	2700
	849	A--M--Q--T--Y--A--F--M--G--V--N--T--A--L--I--Q--A--A--V--M--	868
	2701	CAAAAGCCAAAGTTTACACAGCTGTTCTGCACAGATAAAAGCAAGGATTGACATTGTTA	2760
	869	T--K--A--K--V--Y--T--A--V--P--A--Q--I--K--A--R--I--D--I--V--	888
10	2761	AGGGCAACTTGAAGTTGAGTTCCTGTCACTCCAGGGCATTAAACACAATTGCATCTGCAC	2820
	889	K--G--N--L--K--V--E--F--L--S--L--Q--G--I--N--T--I--A--S--A--	908
	2821	ATGCGGAGACGGTTGCCATTGCAAGAAATGTGGAAGACCTCCCAGCCGCAAGAAGCACAC	2880
15	909	H--A--E--T--V--A--I--A--R--N--V--E--D--L--P--A--A--R--S--T--	928
	2881	CACTGATCTCATCTGAAACTGCATCACAACCTTTCAAAGGCCTCTCTCAACTCAAAGATCT	2940
	929	P--L--I--S--S--E--T--A--S--Q--L--S--K--A--S--L--N--S--K--I--	948
20	2941	CCAGGATGGCATCCTCTGTGACTGGTGGCATGTCTGCGTCATCTGAAATCATTCCTGCTG	3000
	949	S--R--M--A--S--S--V--T--G--G--M--S--A--S--S--E--I--I--P--A--	968
	3001	ACCTGCCAAGTAAGATTGGGAGGAAAATGAACTCCCTAAAACCTACAGGAAGAAAATCC	3060
	969	D--L--P--S--K--I--G--R--K--M--K--L--P--K--T--Y--R--K--K--I--	988
25	3061	GTGCTTCAAGCAGAATGCTAGGATTCAGGCTACGCTGAGATTAAATCTCACAATGCCG	3120
	989	R--A--S--S--R--M--L--G--F--K--A--Y--A--E--I--K--S--H--N--A--	1008
	3121	CCTACATCAGAGACTGCCCTCTCTACGCTCTGATCGGAAAGCATGCTGCTTCTGTTAGGA	3180
30	1009	A--Y--I--R--D--C--P--L--Y--A--L--I--G--K--H--A--A--S--V--R--	1028
	3181	TTGCTCCAGCTTCTGGACCAGTCATTGAGAAGATTGAAGTTGAGATTCAGGTCGGAGATA	3240
	1029	I--A--P--A--S--G--P--V--I--E--K--I--E--V--E--I--Q--V--G--D--	1048
	3241	AAGCAGCAGAAAATATGATTAAAGCGATTGACATGAGCGAAGAGGAGGAAGCTCTTGAGG	3300
35	1049	K--A--A--E--N--M--I--K--A--I--D--M--S--E--E--E--E--A--L--E--	1068
	3301	ATAAGAATGTCTCTTGA AAAATCAAGAAAATACTGGCACCTGGTCTCAAGAACACCACAT	3360
	1069	D--K--N--V--L--L--K--I--K--K--I--L--A--P--G--L--K--N--T--T--	1088
40	3361	CATCTTCTCCAGCTCCTCCAGCTCCTCTTCATCCAGCTCTAGCTCCAACAAGTCTTCTT	3420
	1089	S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--N--K--S--S--	1108
	3421	CATCCAGTTCCCGCTCCAGCAGCTCCCAGTCATCCAGCTCTCGTTCCCATAGGTCTCGCT	3480
45	1109	S--S--S--S--R--S--S--S--S--Q--S--S--S--S--R--S--H--R--S--R--	1128
	3481	CCAGAAAGTCCCAGTCTAGCAGCTCTCAGTCAAGCCGCTCTCCCTCAAGCTCTTCCTCCT	3540
	1129	S--R--K--S--Q--S--S--S--S--Q--S--S--R--S--P--S--S--S--S--S--	1148
50	3541	CTTCTCCTCTTCATCATCCAGATCTTCTTCCAGGTCATCTTCCAGATCATCTTCCAGAT	3600
	1149	S--S--S--S--S--S--S--R--S--S--S--R--S--S--S--R--S--S--S--R--	1168
	3601	CTTCTTCTAGGTCTCTCTCGCTCCAGAACTAAGATGGCTGACATTGTTGCTCCTATTA	3660
	1169	S--S--S--R--S--S--S--R--S--R--T--K--M--A--D--I--V--A--P--I--	1188
55	3661	TCACGACGTCCACCAGAGTGAGCAGTTCCTCCAGTCGATCAGCCTCTAACAGCTCCTCCA	3720
	1189	I--T--T--S--T--R--V--S--S--S--S--S--R--S--A--S--N--S--S--S--	1208
	3721	GCAGTGCTTCATACTTGCTCAGCTCATCTAAGTCATCAAGCTCTAGATCCTCTCGGCGCA	3780
60	1209	S--S--A--S--Y--L--L--S--S--S--K--S--S--S--S--R--S--S--R--R--	1228

	3781	GTGCTCAGTCTAAGCAACAACTGCTTGCCTTGAAGTTTCAGAAAGAACCACGTCCACAGGC	3840
	1229	S--A--Q--S--K--Q--Q--L--L--A--L--K--F--R--K--N--H--V--H--R--	1248
5	3841	ATGCCATCTCCACACAGCGCGGCAGCAGTCACAGCAGTGCCCGCAGCTTCGATTCCATCT	3900
	1249	H--A--I--S--T--Q--R--G--S--S--H--S--S--A--R--S--F--D--S--I--	1268
	3901	ACAATAAGGCCAAGTACCTCGCTAACACACTCACTCCTGCCATGTCCATTGCAATCCGTG	3960
	1269	Y--N--K--A--K--Y--L--A--N--T--L--T--P--A--M--S--I--A--I--R--	1288
10	3961	CCGTGAGAGTCGACCACAAGGTCCAGGGATACCAGCTAGCAGCTTACCTGGACAAACAGA	4020
	1289	A--V--R--V--D--H--K--V--Q--G--Y--Q--L--A--A--Y--L--D--K--Q--	1308
	4021	CCAATAGACTGCAGCTGATTTTTGCCAGAGTCGCTGAGAAGGACAACCTGGAGAATCTGTG	4080
15	1309	T--N--R--L--Q--L--I--F--A--R--V--A--E--K--D--N--W--R--I--C--	1328
	4081	CCGACATTGTGCAGCTGAGTTCGCACAAGATGATGGCCAAGATTGCCTGGGGTGCTGAAT	4140
	1329	A--D--I--V--Q--L--S--S--H--K--M--M--A--K--I--A--W--G--A--E--	1348
20	4141	GCAAGCAATACTCCACCATGATTGTAGCTGAAACTGGTCTTTTGGGTCATGAGCCCGCAG	4200
	1349	C--K--Q--Y--S--T--M--I--V--A--E--T--G--L--L--G--H--E--P--A--	1368
	4201	CCCGCTTGAAGCTGACCTGGGACAAACTGCCAGGAAGCATAAAGCACTACGCAAAGAGGG	4260
	1369	A--R--L--K--L--T--W--D--K--L--P--G--S--I--K--H--Y--A--K--R--	1388
25	4261	CGTTGAAATCCATTGTCCCTATTGCTCAAGAATATGGAGTAAACTACGCAAAGGCCAAGA	4320
	1389	A--L--K--S--I--V--P--I--A--Q--E--Y--G--V--N--Y--A--K--A--K--	1408
	4321	ATCCTCGTAATCAAATCAAACCTGACTGTAGCTGTTGCTACTGAGACAAGCATGAATATTG	4380
30	1409	N--P--R--N--Q--I--K--L--T--V--A--V--A--T--E--T--S--M--N--I--	1428
	4381	TGCTGAACACACCAAAGGCAATCATTTACAAGCGTGGGGTGTGTCTACCTGTTGCTTTAC	4440
	1429	V--L--N--T--P--K--A--I--I--Y--K--R--G--V--C--L--P--V--A--L--	1448
35	4441	CAATTGGAACACTGCTGCCGAGCTGCAAGCGACCCGGGACAACCTGGGCTGACAAGATGT	4500
	1449	P--I--G--N--T--A--A--E--L--Q--A--T--R--D--N--W--A--D--K--M--	1468
	4501	CCTATTTGGTTACCAAAGCTAACGCAGTTGAATGCTCCCTCATCAACAACACACTGACCA	4560
	1469	S--Y--L--V--T--K--A--N--A--V--E--C--S--L--I--N--N--T--L--T--	1488
40	4561	CATTCAACAACAGGAAAGCTAGAGATGAGCTGCCACACTCGTGCTACCAGGTCTTGGCTC	4620
	1489	T--F--N--N--R--K--A--R--D--E--L--P--H--S--C--Y--Q--V--L--A--	1508
	4621	AGGATTGCACACCAGAACTCAAATTCATGGTTCTGCTGAAGAAAGACCAAATACAGGATC	4680
45	1509	Q--D--C--T--P--E--L--K--F--M--V--L--L--K--K--D--Q--I--Q--D--	1528
	4681	AGAAGCAGATCAATGTTAAGATTTTCAGACATCGATGTGGACATGTATCGGAAGAACAACG	4740
	1529	Q--K--Q--I--N--V--K--I--S--D--I--D--V--D--M--Y--R--K--N--N--	1548
50	4741	CCATTGCGGTGATGGTTAACGGAGTTGAAATCCCTAACAGCAACCTGCCATACCTGCATC	4800
	1549	A--I--A--V--M--V--N--G--V--E--I--P--N--S--N--L--P--Y--L--H--	1568
	4801	CATCAGGTAACATACATATAAGACAGTCAAATGAAGGCATTACTCTCAATGCACCCAGCC	4860
	1569	P--S--G--N--I--H--I--R--Q--S--N--E--G--I--T--L--N--A--P--S--	1588
55	4861	ATGGTCTTCAGGAGGTCTTCCTTGGCTTCAACGAGCTGAGGGTTAAAGTTGCAGACTGGA	4920
	1589	H--G--L--Q--E--V--F--L--G--F--N--E--L--R--V--K--V--A--D--W--	1608
	4921	TGAAAGGAAAGACTTGTGGTGCCTGTGGAACGGCAAGCGGAAATGTCTGGAGACGAGTACC	4980
60	1609	M--K--G--K--T--C--G--A--C--G--T--A--S--G--N--V--G--D--E--Y--	1628

	4981 GCACACCCAGTGAACAGGTGACCAAGGATGCCATCAGCTACGCCCACTCCTGGGTTCTGT	5040
	1629 R--T--P--S--E--Q--V--T--K--D--A--I--S--Y--A--H--S--W--V--L--	1648
5	5041 CTTCAAACACCTGCCGTGATCCCTCCGAGTGTTCCATCAAGCAGGAATCTGTGAAGCTGG	5100
	1649 S--S--N--T--C--R--D--P--S--E--C--S--I--K--Q--E--S--V--K--L--	1668
	5101 AGAAGCGGGTGATCTTTGAAGGTGTGGAGTCCAAATGCTACTCTGTTGAGCCCGTGCTGC	5160
	1669 E--K--R--V--I--F--E--G--V--E--S--K--C--Y--S--V--E--P--V--L--	1688
10	5161 AGTGCCTGCCCGGCTGTATCCCAGTGAGAACCACTACCGTCAACGTTGGCTTTCCTACTGCC	5220
	1689 Q--C--L--P--G--C--I--P--V--R--T--T--T--V--N--V--G--F--H--C--	1708
	5221 TGCCCAGTGACACAACCTGTGGACCGTTCTGGTCTGAGCAGCTTCTTTGAGAAGAGCATCG	5280
15	1709 L--P--S--D--T--T--V--D--R--S--G--L--S--S--F--F--E--K--S--I--	1728
	5281 ACCTGAGGGATACTGCAGAAGCCACCTGGCCTGTCGCTGCACTCCTCAGTGTGCTTAA	5339
	1729 D--L--R--D--T--A--E--A--H--L--A--C--R--C--T--P--Q--C--A--*-	1747

SEQ ID NOs 114 and 116 (VtgAb mutant allele- 8nt deletion)

20 LENGTH: 5339bp and 202aa

TYPE: cDNA (SEQ ID NO: 114) and Protein (SEQ ID NO: 116)

ORGANISM: Nile tilapia

25	1 CGCCATTTAGTTAATGATACATTTGATGGGCAACGTCAGCAAAAAATCTGCTTAAAAAGG	60
		
	61 ACGCCTCTGCCTGCAGATCCTCACATCCACCAGCCATGAGGGTGCTTGTACTAGCTCTTG	120
	-M--R--V--L--V--L--A--L--	8
30	121 CTGTGGCTCTCGCAGTGGGGGACCAGTCCAACCTTGGCCCCAGGATTGCGCTCTGTTAAGA	180
	9 A--V--A--L--A--V--G--D--Q--S--N--L--A--P--G--F--A--S--V--K--	28
	181 CCTACATGTACAAATATGAAGCGGTTCTTATGGGCGGCCTGCCTGAAGAGGGCCTGGCTC	240
	29 T--Y--M--Y--K--Y--E--A--V--L--M--G--G--L--P--E--E--G--L--A--	48
35	241 GAGCTGGGGTTAAATCCGGGGCAAAGTTTGTATCAGTGCAACAAGTGCCAACGACTACA	300
	49 R--A--G--V--K--I--R--G--K--V--L--I--S--A--T--S--A--N--D--Y--	68
40	301 TTCTGAAGCTGTAGACCCTCAGTTGCTGGAGTACAGTGGCATCTGGCCCAAAGATCCTT	360
	69 I--L--K--L--V--D--P--Q--L--L--E--Y--S--G--I--W--P--K--D--P--	88
	361 TCCATCCAGCCACCAAGCTCACCACAGCCCTGGCTACTCAGCTCTCGACACCGGTCAAGT	420
	89 F--H--P--A--T--K--L--T--T--A--L--A--T--Q--L--S--T--P--V--K--	108
45	421 TTGAGTATACAAACGGCGTTGTTGGGAGACTGGCTGCACCTCCTGGGGTCTCCACAACAG	480
	109 F--E--Y--T--N--G--V--V--G--R--L--A--A--P--P--G--V--S--T--T--	128
	481 TGCTGAATATCTACAGGGGCATCATCAACCTCCTGCAGCTGAATGTAAAGAAGACACAGA	540
	129 V--L--N--I--Y--R--G--I--I--N--L--L--Q--L--N--V--K--K--T--Q--	148
50	541 ATGTCTACGAGATGCAAGAGTCTGGAGCTCATGGTGTGTGCAAGACCAACTATGTGATCA	600
	149 N--V--Y--E--M--Q--E--S--G--A--H--G--V--C--K--T--N--Y--V--I--	168
	601 GGGAGGGCCGAACGCATTCATCTGACCAAGACCAAGGACCTGAACCACTGCCAGGAGAAA	660
55	169 R--E--G--R--T--H--S--S--D--Q--D--Q--G--P--E--P--L--P--G--E--	188
	661 ATCATGAAGGCCATCGGCTTGAACACGTAGAGAAATGCCATGATTGTGAAGCTAGAGGA	720
	189 N--H--E--G--H--R--L--G--T--R--R--E--M--P--*-	202

SEQ ID NO 117

LENGTH: 18

5 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: 5' tailed primer extension sequence (FAM)

SEQUENCE: 1

TGTAAAACGACGGCCAGT

10

SEQ ID NO 118

LENGTH: 18

TYPE: DNA

ORGANISM: Artificial Sequence

15 OTHER INFORMATION: Description of Artificial Sequence: 5' tailed primer extension sequence (NED)

SEQUENCE: 3

TAGGAGTGCAGCAAGCAT

20 **[00195]** In the preceding description, for purposes of explanation, numerous details are set forth in order to provide a thorough understanding of the embodiments. However, it will be apparent to one skilled in the art that these specific details are not required.

[00196] The above-described embodiments are intended to be examples only. Alterations, modifications and variations can be effected to the particular embodiments by those of skill in the art. The scope of the claims should not be limited by the particular
25 embodiments set forth herein, but should be construed in a manner consistent with the specification as a whole.