

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

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
5 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
10 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
15 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
20 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
25 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.

30 [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
5 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-
10 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
15 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
20 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
25 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
30 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
15 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
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 of 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 *Elav/2* 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 *Elav/2^{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 *Cyp9a1a* 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 *Cyp9a1a* 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 Gsdf 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*, *Smcp2*, *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^{6/6}*) 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) of thru gem 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
5 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
10 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
15 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,
20 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)
25 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.

30 [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.

10 **[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*,
 15 *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
 20 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
 25 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 Resarch). One microgram of linearized template was used to synthesize capped RNA using the mMMESSAGE 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 place 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 ul 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 curves 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 $5 \cdot 10^6$ 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 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.

Table 1: Primers

full gene name	Tilapia homolog gene (alias)	Genotyping RT-PCR primers						Amplicon size (bp)
		Forward primer exon	SEQ ID NO	Forward primer sequence	Reverse primer exon	SEQ ID NO	Reverse primer sequence	
cytochrome P450, family 17, subfamily A, polypeptide 1	Cyp17	1	SEQ47	GAACCAAAACCCCTCTGTGTAAGT	1	SEQ48	GTAATTCACTCCGACAGGCTCAG	184
		1	SEQ47	GAACCAAAACCCCTCTGTGTAAGT	1	SEQ48	GTAATTCACTCCGACAGGCTCAG	184
cytochrome P450, family 19, subfamily A, polypeptide 1a gene	Cyp19a1a	1	SEQ49	ggagATGAATCTCTGTAG	1	SEQ50	ATGGCATTTGAGGTACACAGA	63
		9	SEQ5	TGATGGAGAGCTTCACTACGAA	9	SEQ6	GTTCAGGTTAAATTGATTG	186
Tight junction protein 1a	Tjp1a	15	SEQ51	GTTCAAGACGGAGAGAGT	15	SEQ52	AAAAATCCACACATGGTT	61
		17	SEQ53	tgcttggtcagtgTATC	17	SEQ54	AATGCGTTCGAAATGTAGAA	71
Casein Kinase II subunit alpha	Csnk2a2	5'UTR	SEQ11	glaillagaagcgggaagc	1	SEQ12	CAGTTTGGCACAATGAGCATCGTA	153
		1	SEQ13	ATGCTCATGTGCCAAACTG	2	SEQ14	gCTTCAGGATTTTCAACCACT	222
Hippocampus abundant transcript 1a	Hla1	Intron3-4	SEQ15	lactatcatcagpaggat	Intron4-5	SEQ16	cgagcagagccgagcagctatct	211
		8	SEQ55	CATCTGCTTCACTCTGTGTGGCTG	6	SEQ18	actatagaCGGAAGGGGTCT	110
small ArGAP2	Snap2	2	SEQ56	AATTGGGACATCTTCACTCTGTAT	2	SEQ57	GACAGACTTGACCTTGGAGATG	81
		9	SEQ21	AGAGGAGGCGACAGTCAAGAAAC	10	SEQ22	TTGGATATCCCATTTGGTTCT	228
Golgi-associated PDZ and coiled-coil motif containing gene	Gopc	1	SEQ58	ATGCTGCTTTCGACTGGATGC	1	SEQ59	gCCATCGAAACATGGACATACGTG	76
		Intron1-2	SEQ25	tgccctttaaaccacta	2	SEQ26	CTCAGCTTGGCCTTTCCTTGACAT	207
doublesex and mab-3 related transcription factor 1	Dmr1	5'UTR-1	SEQ27	tggcaggagccatAGAGCCAG	1	SEQ28	AGACAGTATCCGTGATTTCTAC	135
		Intron2-3	SEQ29	ctctcatctctctgtctc	3	SEQ30	GGGTTTCCAGCAGGAGGTCAGA	140
growth/differentiation factor 6-B-like	Gsdf	2	SEQ31	tttgttaggttcccaaggtg	2	SEQ32	TGGCTGTGTGAGAAAGATGCTG	158
		4	SEQ33	agaTCTGGGCGCTGGGACA	4	SEQ34	ggtaataaacCTGTGTGTTGG	145
Follicle stimulating hormone receptor	FSHR	Intron10-11	SEQ35	ttttctcgtctgtctgtg	11	SEQ36	AAAGAGCTGAATAGGAGGAAGTT	137
		15	SEQ37	CATCTTGGGCTTCTCTGTGT	15	SEQ38	CTTGAGGCGAGCTGAGATGGC	181
Vitellogenin 4a	Vtg4a	7	SEQ39	GCAATCGTTGATGCTCTTGAC	7	SEQ40	CTGAGACTCTATGCTGTGATA	163
		22	SEQ41	AGAAATCATCAACACATCAAG	22	SEQ42	GACTTGTGAGCAGTTGCATCAA	227
Vitellogenin Ab	VtgAb	5	SEQ43	tttgtgtagtcttggag	5	SEQ44	gctctacAGCTTCACAATCAT	183
		22	SEQ45	CTTCTGACCAAGTCAATTGAG	22	SEQ46	AGACTTGTGAGACTAGAG	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 *Elav/2* 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 translucid 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. *elav/2*-null progeny from heterozygous parents; see Example 11). Knockout strategies other than *Elav/2* 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**

[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*, *Smap2* [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.

[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.

[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 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 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- 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. 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-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.

[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.

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[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.

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[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.

Gene categories	Gene names	phenotype in F0 albino	Selected F1 genotypes (hemizygous)	Selected double hemizygous mutant	F2 homozygous
FEM	<i>Cyp17</i>	80% male (n=20)	<i>Cyp17</i> ^{$\Delta 18/+$}		<i>Cyp17</i> ^{$\Delta 18/\Delta 18$}
	<i>Cyp19a1a</i>	75% male (n=23)	<i>Cyp19</i> ^{$\Delta 7/+$} , <i>Cyp19</i> ^{$\Delta 10/+$}		<i>Cyp19</i> ^{$\Delta 10/\Delta 10$}
SMS	<i>Tjp1a</i>	Reduced fertility in males (70-90%)	<i>Tjp1a</i> ^{$\Delta 7/+$}	<i>Tjp1a</i> ^{$\Delta 7/+$} and <i>Cyp19</i> ^{$\Delta 10/+$}	<i>Tjp1a</i> ^{$\Delta 7/\Delta 7$}
	<i>Csnk2a2</i>		<i>Csnk2a2</i> ^{$\Delta 22/+$}	<i>Csnk2a2</i> ^{$\Delta 22/+$} and <i>Cyp17</i> ^{$\Delta 18/+$}	<i>Csnk2a2</i> ^{$\Delta 22/\Delta 22$}
	<i>Hiat1</i>		<i>Hiat1</i> ^{$\Delta 17/+$}	<i>Hiat1</i> ^{$\Delta 17/+$} and <i>Cyp17</i> ^{$\Delta 18/+$}	<i>Hiat1</i> ^{$\Delta 17/\Delta 17$}
	<i>Smap2</i>		<i>Smap2</i> ^{$\Delta 17/+$}	<i>Smap2</i> ^{$\Delta 17/+$} and <i>Cyp19</i> ^{$\Delta 10/+$}	<i>Smap2</i> ^{$\Delta 17/\Delta 17$}
	<i>Gopc</i>		<i>Gopc</i> ^{$\Delta 8/+$}		<i>Gopc</i> ^{$\Delta 8/\Delta 8$}
MA	<i>Dmrt1</i>	50% female (n=24)	<i>Dmrt1</i> ^{$\Delta 27/+$} , <i>Dmrt1</i> ^{$\Delta 13/+$}		
	<i>Gsdf</i>	95% female (n=20)	<i>Gsdf</i> ^{$\Delta 5/+$} , <i>Gsdf</i> ^{$\Delta 22/+$}		
FLS	<i>FSHR</i>	Sterile females (2 in 6 tested)	<i>FSHR</i> ^{$\Delta 2/+$}	<i>FSHR</i> ^{$\Delta 2/+$} and <i>Dmrt1</i> ^{$\Delta 27/+$}	
	<i>VtgAa</i>	Sterile females (3 in 5 tested)	<i>VtgAa</i> ^{$\Delta 5/+$} , <i>VtgAa</i> ^{$\Delta 25/+$}	<i>VtgAa</i> ^{$\Delta 25/+$} and <i>Gsdf</i> ^{$\Delta 22/+$}	<i>VtgAa</i> ^{$\Delta 25/\Delta 25$}
	<i>VtgAb</i>	Reduced fertility in females (70-90%)	<i>VtgAb</i> ^{$\Delta 8/+$}	<i>VtgAb</i> ^{$\Delta 8/+$} and <i>VtgAa</i> ^{$\Delta 27/+$}	<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.

[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
5 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
10 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
15 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.
20 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
25 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
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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.

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

[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
10 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.

[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 *Elav/2* 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

TGTAACGACGCGCCAGTtgaagtgctacataaaag

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

TAGGAGTGCAGCAAGCATgttctacatcatcacccttctc

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

TGTA AACGACGGCCAGTTGATGGAGAGCTTCATCTACGAA

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

TGTA AACGACGGCCAGTGAAAAAGGATGgtgagggatgac**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

TGTA AACGACGGCCAGTgtattagaaggcgggtgaaggtc25 **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

TAGGAGTGCAGCAAGCATATGCTCATGTGCCAACTG

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
- 15 **SEQ ID NO 20**
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
TGTAAAACGACGGCCAGTAGAGGAGGGCACAGTCAAGAAAC
- 30 **SEQ ID NO 22**
LENGTH: 22
TYPE: DNA
ORGANISM: Artificial Sequence
OTHER INFORMATION: Description of Artificial Sequence: Primer
35 SEQUENCE: 22
TTGGATATCCCATTTGGTTCAT
- SEQ ID NO 23**

LENGTH: 40

TYPE: DNA

ORGANISM: Artificial Sequence

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

5 SEQUENCE: 23

TAGGAGTGCAGCAAGCATttaacggtgtggcagagatt

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

TGTAAAACGACGGCCAGTtgccctttaaccaccta

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

TAGGAGTGCAGCAAGCATttgccaggacccATGAGCCAG

SEQ ID NO 28

LENGTH: 23

5 TYPE: DNA

ORGANISM: Artificial Sequence

OTHER INFORMATION: Description of Artificial Sequence: Primer

SEQUENCE: 28

AGACACGTATCCGTGATTTCTAC

10

SEQ ID NO 29

LENGTH: 41

TYPE: DNA

ORGANISM: Artificial Sequence

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

SEQUENCE: 29

TGTAAAACGACGGCCAGTctcttcacctctgtgtctcatc

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

20 tgtaaactatacCTGTGTGTTGG

SEQ ID NO 35

LENGTH: 38

TYPE: DNA

ORGANISM: Artificial Sequence

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

SEQUENCE: 35

TAGGAGTGCAGCAAGCATtttctccgcttgcttctgc

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 TGTAAAACGACGGCCAGTCATCTTGGCGTTCTTCTGTGT

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

TAGGAGTGCAGCAAGCATGCAATCCTTGATGCTCCTTGAC

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

TGTAAAACGACGGCCAGTAGAAGATCATCAAACACATCACG

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

TAGGAGTGCAGCAAGCATtttgtgatctagTCTGGAG**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

TGTAAACGACGGCCAGTAGAAGATCATCAAACACATCACG**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

20 GTAATTCACCTCCGCAGGCTCAG

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
5 GTTCAAGAAGGGAGAGAGT
SEQ ID NO 52
LENGTH: 18
TYPE: DNA
ORGANISM: Artificial Sequence
10 OTHER INFORMATION: Description of Artificial Sequence: Primer
SEQUENCE: 52
AAAAATTCCCACATCGTT
SEQ ID NO 53
15 LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
OTHER INFORMATION: Description of Artificial Sequence: Primer
SEQUENCE: 53
20 tgcttggcttcagTGTATC
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
10	241	ATGATGGGCTCCACAGTGTGATTGTCATCAACCAGCATGTGCACGCCAAAGAAGTCTTG	300
	81	-M--M--G--S--H--S--V--I--V--I--N--Q--H--V--H--A--K--E--V--L-	100
15	301	CTCAAGAAGGGAAGACGTTTGCAGGAAGACCTAGAACTGTAACCACAGATATTCTGACT	360
	101	-L--K--K--G--K--T--F--A--G--R--P--R--T--V--T--T--D--I--L--T-	120
20	361	AGAGATGGGAAGGACATTGCATTTGGAGACTACAGTGTACGTGGAAGTTCACAGGAAG	420
	121	-R--D--G--K--D--I--A--F--G--D--Y--S--A--T--W--K--F--H--R--K-	140
25	421	ATAGTCCATGGAGCCCTGTGCATGTTTGGAGAAGGTTCTGCCTCTATTGAGAAGACCATT	480
	141	-I--V--H--G--A--L--C--M--F--G--E--G--S--A--S--I--E--K--T--I-	160
30	481	TGTGCAGAGGCCCAGTCTCTGTGCTCCGTGCTGTCTGAGGCAGCAGATGTGGGACTGGCC	540
	161	-C--A--E--A--Q--S--L--C--S--V--L--S--E--A--A--D--V--G--L--A-	180
35	541	CTGGATCTTGCTCCTGAGCTGACTCGCGCTGTCAACACGTTATCTGTCTCTCTGCTTC	600
	181	-L--D--L--A--P--E--L--T--R--A--V--T--N--V--I--C--S--L--C--F-	200
40	601	AACTCGTCTACTGCGGAGGCGACTCAGAGTTTGAGACAATGCTGCAGTACAGCCAGGGC	660
	201	-N--S--S--Y--C--R--G--D--S--E--F--E--T--M--L--Q--Y--S--Q--G-	220
45	661	ATTGTGGACACTGTGGCTAAAGACAGCCTGGTAGACATTTCCCTGGCTTCAGATCTTT	720
	221	-I--V--D--T--V--A--K--D--S--L--V--D--I--F--P--W--L--Q--I--F-	240
50	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
55	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
60	841	GCTCTGCTAAGAGCCCAAGCGCAGTGGGAGAAACAACACATCAGAGATAAGTGCAGAG	900
	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	GTGGAAACCACTACCACTGTGCTCAAATGGGCCATAACGTACCTCATTATCACCAGAG	1020
	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
	1081	CTCACCGACAGAGGCAGTCTGCCTTATCTGGAGGCCACCATTAGGGAAGTATTGCGGATT	1140
	361	-L--T--D--R--G--S--L--P--Y--L--E--A--T--I--R--E--V--L--R--I-	380
	1141	CGCCCCGTGGCACCCTACTCATCCCCCATGTGGCTCTCTGTGACACCAGCATTTGAGAT	1200
	381	-R--P--V--A--P--L--L--I--P--H--V--A--L--C--D--T--S--I--G--D-	400
	1201	TTACAGTGAGAAAAGAACTCGAGTCATTATCAACCTGTGGGCTCTGCACCATGATGAG	1260
	401	-F--T--V--R--K--G--T--R--V--I--I--N--L--W--A--L--H--H--D--E-	420
	1261	AAGGAGTGGAAGAACCAGAGCGGTTTGACCCCTGGCCGGTTCTTGAAGTGAAGGCACA	1320
	421	-K--E--W--K--N--P--E--R--F--D--P--G--R--F--L--K--S--E--G--T-	440

	1321	GGACTGACAATCCCATCACCCAGCTACCTGCCCTTTGGTGCTGGGCTGAGAGTATGTTTA	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	GGTGAGGCCTTGGCCAAGATGGAGCTCTTTCTCTCCTGTCTGGATCCTGCAGCGCTTC	1440
	461	-G--E--A--L--A--K--M--E--L--F--L--F--L--S--W--I--L--Q--R--F-	480
	1441	ACTCTGTCTGTCCCACCAGGCCACAGTCTGCCAGTCTGGAGGGAAAGTTTGGAGTGGTC	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	TTGGTTGCCTGGAGTCACACGGACAAGAAAATTGTGCCAGGTCTTCTTTCTGTTTGGGT	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	TTGGGCCCCACTTCTGTCATATCTGAGATTTATCTGGACTGGCATAGGCACAGCCAGCAAC	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	ATACTAAGCAGATCTTCAGCAGTGCACCATGTGCTGAAGAACGGAACTATACTTCACGT	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	AACAACGTAACCTCTGTGGAAGAAAGATACGCACCTATTTTGCTAAAGCTCTGACAGGCCCA	480
	140	-N--N--V--T--L--W--K--K--I--R--T--Y--F--A--K--A--L--T--G--P-	159
	481	AATTGTCAGCAGACGGCGGATGTTTGCGTCTCCTCCATACAGGCTCACCTGGACCACCTG	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	GACAGCCTGGGACACGTTGATGTCCTCAATTTGCTGCGCTGCACCGTCTGGACATCTCT	600
	180	-D--S--L--G--H--V--D--V--L--N--L--L--R--C--T--V--L--D--I--S-	199
	601	AACCGACTCTTCCTGGACGTACCTCTCAATGAGAAAGAGCTGATGCTGAAGATTCAAAG	660
15	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	ATTCAACACAGGCACAAGACAGCAACCCAGGAGTTACAAGATGCCATTAAACGTCTTGTA	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	GAGCTCATATTTGCACAAAACCCAGGTGAGCTGTCTGCTGAGAATGTGACGCAGTGCCTG	900
	280	-E--L--I--F--A--Q--N--H--G--E--L--S--A--E--N--V--T--Q--C--V-	299
	901	CTGGAGATGGTGATTGCAGCTCCGGACACTCTGTCCCTCAGTCTTCTTCATGCTTCTG	960
30	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	TACGAATGCTTGGCGTTCACCCAGTGGTGGACTTCACCATGCGTCGAGCCCTGTCTGAT	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	GACATCATAGAAGGCTACAGGATCTCGAAGGGCACAACATCATTTCTGAACACAGGCCGA	1200
	380	-D--I--I--E--G--Y--R--I--S--K--G--T--N--I--I--L--N--T--G--R-	399
	1201	ATGCACCGCACCGAGTTTTCCTCAAAGCCAATCAATTTAACCTGGAACACTTTGAAAAC	1260
45	400	-M--H--R--T--E--F--F--L--K--A--N--Q--F--N--L--E--H--F--E--N-	419
	1261	AATGTTCCCTCGGCGCTACTTTTCAGCCGTTTCGGTTCAGGCCCTCGCGCATGCATTGGCAAG	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	CACATCGCCATGGTGATGATGAAATCCATTTTGGTGACACTGCTGTCTCAGTACTCTGTT	1380
	440	-H--I--A--M--V--M--M--K--S--I--L--V--T--L--L--S--Q--Y--S--V-	459
	1381	TGTACTCACGAGGGCCCGATCCTGGACTGCCTCCACAAACCAACCTTTCCAGCAG	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
	1501	AGAAGCAGCTGTCAAACCTCCGAGACCCGAACCTTTAGCTGTACCTGCACCTTTGTATA	1560
60	500	-R--S--S--C--Q--T--L--R--D--P--N--L--*-.....	511

5
 1561 CTTAATTTGTATAATCTTATAACGACACACAGCTAGCCTTTATATTTTGATAGACGCAAA 1620

 1621 GATTGTATTTGTACTCAAACGTATGCATGATGTGAAATGTACATTTAAACCTGCTAACA 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)

LENGTH: 1707bp and 11aa

20 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	GT CATATGGGAACAGCACACAGTTACCCTTCACAGGGCCCCAGGATTGGGTTTGGCATT	120
	21	-V--I--W--E--Q--H--T--V--T--L--H--R--A--P--G--F--G--F--G--I-	40
	121	GCCATCTCGGGTGGGCGAGACAACCTCATTTCCAGAGTGGTGAACATCTATTGTAATA	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	TCGGATGTGCTGAAAGGAGGTCCTGCAGAGGGTCTGCTACAAGAAAATGATCGAGTAGTA	240
	61	-S--D--V--L--K--G--G--P--A--E--G--L--L--Q--E--N--D--R--V--V-	80
20	241	ATGGTCAATGCAGTCTCTATGGACAATGTAGAGCATGCCTATGCTGTTCAACAACCTCGA	300
	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	TCACGGCACGGGGACAGGAGACGATGTCTGAGCACGAGGAGGAGACAGCGATGAGGCT	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
35	541	TCACAAGCCTCTTCTGCTCCACCCAGGCCCTCCAAGGTCACTCTTGTCAGTCCCGCAA	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
	201	-N--E--E--Y--G--L--R--L--A--S--H--I--F--V--K--D--I--S--P--E-	220
40	661	AGCCTTGACGCCAGAGATGGAACATTCAGGAGGGAGATGTTGTACTTAAGATTAACGGC	720
	221	-S--L--A--A--R--D--G--N--I--Q--E--G--D--V--V--L--K--I--N--G-	240
	721	ACAGTTACAGAGAACCTATCACTGACAGATGCCAAGAAGCTGATTGAGAGGTCAAAGGCG	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
50	841	GACGACAGCATCCCATCAGCCAATAACTCAGACAGAGATGACATTTAGAGATACATTCA	900
	281	-D--D--S--I--P--S--A--N--N--S--D--R--D--D--I--S--E--I--H--S-	300
	901	CTGACATCCGATCATTCCAATCGATCCCATGGGAGAGGAAGTCAATCCCATTGCGCTGAC	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	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
	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	GACGTGGACCTGCCTGTGAGCCCATCTGATGCCCTGTACCCAGTGCTGCACATGATGAC	1260
	401	-D--V--D--L--P--V--S--P--S--D--A--P--V--P--S--A--A--H--D--D-	420
	1261	AGCATTCTCAGACCAAGTATGAAGCTGGTCAAGTTCAGAAGGGAGAGAGTGTCTGGTCTG	1320
	421	-S--I--L--R--P--S--M--K--L--V--K--F--K--K--G--E--S--V--G--L-	440
15	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	GCTAACATCATCCGGAAGAGGCTGTGCTTTTTCTGCTCGATCTTCCAAAAGGAGATGAC	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	GGTGACTCCTTCTACATTCTGAACGCATTTTGAATATGAAAAGAGTCACCGTATGGGCTG	1620
	521	-G--D--S--F--Y--I--R--T--H--F--E--Y--E--K--E--S--P--Y--G--L-	540
30	1621	AGCTTTAACAAGGAGAGGTTTTCCGTGTGGTAGACACACTCTATAATGGCAAATTAGGC	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	TCCTGGCTCGCTATCCGTATCGGCAAGAACCACAGGAAGTGGAAAGAGGCATAATCCCC	1740
	561	-S--W--L--A--I--R--I--G--K--N--H--Q--E--V--E--R--G--I--I--P-	580
	1741	AACAAGAATAGAGCCGAGCAGCTATCCAGTGTGCAGTACACCCTTCCTAAAACGCCTGGG	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	GGCGACAGAGCTGACTTCTGGAGGTTTCTGAGGGCTGCGGAGTTCCAAGAGGAATTTGCGG	1860
	601	-G--D--R--A--D--F--W--R--F--R--G--L--R--S--S--K--R--N--L--R-	620
	1861	AAAAGCAGGGAGGACCTGTCGGCCAGCCTGTTTCTGAGACCAAGTTCCCTGCCTATGAGAGG	1920
	621	-K--S--R--E--D--L--S--A--Q--P--V--Q--T--K--F--P--A--Y--E--R-	640
45	1921	GTGGTGTCTGAGGGAAGCTGGGTTCTCTGAGGCCTGTGGTTATCTTTGGGCCGATTGCAGAC	1980
	641	-V--V--L--R--E--A--G--F--L--R--P--V--V--I--F--G--P--I--A--D-	660
	1981	GTGGCCCGAGAGAACTGGCCAGGGAGGTGCCCGAAGTGTGAGCTAGCCAAGAGTGAA	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	CAGATCATTGATCGAGACAAGCATGCAGTGCTGGATATAACCCCGAATGCAGTGGACCGA	2160
	701	-Q--I--I--D--R--D--K--H--A--V--L--D--I--T--P--N--A--V--D--R-	720
	2161	CTGAACACTACGCTCAGTGGTATCCAATTGTGGTGTCTCAACCCGGACACCAAGCAGGGC	2220
	721	-L--N--Y--A--Q--W--Y--P--I--V--V--F--L--N--P--D--T--K--Q--G-	740
60			

	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	AACAACATGAACGATGGTTGGTTGGAGCACTGAAAGAAATCATCCATCAGCAGCAGAAC	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	CAGCTGGTGTGGGTTTCAGAGGGCAAGGCTGATGGAGTTGGCGACGATGACCTGGACATC	2460
	801	-Q--L--V--W--V--S--E--G--K--A--D--G--V--G--D--D--D--L--D--I-	820
	2461	CACGACGACCGCCTTTCTACCTGTGCGCGCCAGGCAGTGAGTATTCATGTACAGCACC	2520
15	821	-H--D--D--R--L--S--Y--L--S--A--P--G--S--E--Y--S--M--Y--S--T-	840
	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
20	2581	CAGGAGCTGGATGAAACGCTGAACGATGACGTGGGTCCACCCACGGAGCCTGCCATCAG	2640
	861	-Q--E--L--D--E--T--L--N--D--D--V--G--P--P--T--E--P--A--I--T-	880
	2641	CGGTCCTCTGAGCCTGTCCGTGAGGACCCGCTGTCTATCCAAGAGCCCCCTGGCTATGTC	2700
	881	-R--S--S--E--P--V--R--E--D--P--P--V--I--Q--E--P--P--G--Y--V-	900
25	2701	AGTACCCGCACACAGTGACGCCGACCCCTGAACCGCATCGACCCGGCTGGTTTCAAG	2760
	901	-S--Y--P--H--T--V--Q--P--D--P--L--N--R--I--D--P--A--G--F--K-	920
	2761	GCACCAGCGCCGAGCAGATGTTTCAGAAGGATCCGTACAGCACAGACAACATAGGCAGA	2820
30	921	-A--P--A--P--Q--Q--M--F--Q--K--D--P--Y--S--T--D--N--I--G--R-	940
	2821	GGTGGTCAACGGCATGAAGCCTGTGACGTACAACCTCAGCAGGGGTATCACCCCGACGAG	2880
	941	-G--G--H--G--M--K--P--V--T--Y--N--P--Q--Q--G--Y--H--P--D--E-	960
35	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	GGCGGTGGCTACCAGGAGCCAAAGTACCGTAACATGAGAGCTATGAGAACAGCGTGCCT	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	GCCTACGATCCCCGTCTCTTACGGTGAGGGCCCCGACTCTCATTACACCCCTCCCCTG	3120
45	1021	-A--Y--D--P--R--P--P--Y--G--E--G--P--D--S--H--Y--T--P--P--L-	1040
	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
50	3181	ACAGTTTTCAGCACCTGTCCGTTACGATGATCTCCGCCTCCCCCTCAGCCGTCTGAATTG	3240
	1061	-T--V--S--A--P--V--R--Y--D--D--L--P--P--P--P--Q--P--S--E--L-	1080
	3241	CACTATGACCCAAATTCTCACCTGAGCACATACCCCTCAGCTGCCCGCTCACCAGAACCC	3300
	1081	-H--Y--D--P--N--S--H--L--S--T--Y--P--S--A--A--R--S--P--E--P-	1100
55	3301	GCTGCCCAGCGACCCGCTTATAACCAGGGACAGCATCGCAGCAGAAAGGTTACAAACCT	3360
	1101	-A--A--Q--R--P--A--Y--N--Q--G--P--A--S--Q--Q--K--G--Y--K--P-	1120
	3361	CAGCAGTACGATCTGCTCTGTGAACCTCTGAATCCAGCCCCAGCCTTCATAAAGTCGAG	3420
60	1121	-Q--Q--Y--D--P--A--P--V--N--S--E--S--S--P--S--L--H--K--V--E-	1140

	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	GAGGAGGATCCAGCCATGCGGCCTCAGTCAGTACTGACGAGGGTCAAAATGTTTGAGAAC	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	GCCGATTTGCCCCTGAAAGCTGGTGGAGTAATCCCTAAAGCAAATTCTCTGAGCAACCTG	3660
	1201	-A--D--L--P--L--K--A--G--G--V--I--P--K--A--N--S--L--S--N--L-	1220
	3661	GATCAAGAGAAGACCTTTAGCAGAGGGCCAGACCTCAGAAGCCTCAGTCCAAGGGATCC	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	GATGACATCGTGCCTCCAACCATTTATGACCCCTGATGAGGATGAGGACTACTACAGGAAA	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	TTTTCTTAAATGATGGACTCTGTGGAGAAACCAAGCCCACTGGAGAAAAATATGAACCA	3960
	1301	-F--S--L--M--M--D--S--V--E--K--P--S--P--L--E--K--K--Y--E--P-	1320
	3961	GTTCCTCAAGTGACACCAGCTGTGCCACCGGCCACGCTGCCCAAGCCCTCACCTGATGGT	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	AAAATTGACTGTAGTCAGGATTTTATCTCATCTCTTTGACTGATGTGCGTTGCTCTTCC	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	ACAGCCAAACCTCCTGCTCGAGAGGACACGGTCCAGACCAACTTTCTTCTCACAAGAGC	4140
	1361	-T--A--K--P--P--A--R--E--D--T--V--Q--T--N--F--L--P--H--K--S-	1380
	4141	TTCCCTGAGAAGTCTCCAGTCAATGGCACCAAGTGAACAGCCTCCAAAGACGGTCACTAGC	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	GCCAAGCCTTTTTCGCGCAAGTTCGACAGTCCCTAAATCAACCACAACCTCCTGTCCAAT	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	GACAAGCCTGAGAGTGTCCCAAGGGACGGAGCTCGAGTCGGTAAAGCCTCAGGTACCC	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	CCCCGATCCAATAACCAGCAGAACAACGTAAACGCCGTGCCCAAGGCCATCCCTGTGAGC	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	CCCAGTGCCTTAGAGGATGATGAAGATGAAGACGAAGGTCACACTGTGGTGGCAACAGCT	4560
	1501	-P--S--A--L--E--D--D--E--D--E--D--E--G--H--T--V--V--A--T--A-	1520
	4561	CGTGGCATCTTCAACTCTAACGGTGGCGTTCTGAGCTCCATCGAGACAGGTGTCAGCATC	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	ATTATCCACAGGGTGCCATCCCCGACGGCGTGGAGCAAGAGATTTACTTCAAGGTCTGT	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	CTGGTGATGTGTGGACCTCACGGCCTAAAGTTCTTGAAGCCTGTGGAGCTACGCTTACCT	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	CACTGTGCGTCAATGACCCCTGATGGTTGGTCTTTTGTCTAAAAATCCTCCGACTCCTCG	4860
	1601	-H--C--A--S--M--T--P--D--G--W--S--F--A--L--K--S--S--D--S--S-	1620
	4861	TCGGGTGATCCAAAAAGCTGGCAGAACAGTCTCTCACGGAGACCCCACTACCTGGTG	4920
	1621	-S--G--D--P--K--S--W--Q--N--K--S--L--T--G--D--P--N--Y--L--V-	1640
15	4921	GGAGCCAACTGTGTCTCTGTGCTCATTGACCACTTTTAAAGAAGAAGCAGCAGGTGTGAT	4980
	1641	-G--A--N--C--V--S--V--L--I--D--H--F--*-.	1652
20	4981	GTTACTGAATGTGGAAGAATGGCGGATGAAATGAAGACGATGGAACGCACGCACGCAAA	5040
			
	5041	CACACACATATACCACTACACACACACACACTGACAGACGCACTCCAAGCAAACCAAC	5100
			
	5101	ACACAGCATAGAGTATGAAGAAGACCCAGACAGTGTGGACGAAGGAGAGACACCAATGA	5160
			
25	5161	TCGTTACGAGCTGTTCTTTAACTCAATTTCAAAGTTTTGATGTAAATGATGCATGCCC	5220
			
	5221	AACGTCACGTGACGATTGACACTTATATATAAAGCAATGTTTAAATGTAATTTTCTTTTTT	5280
			
30	5281	CTTTTTTTACAAAAGTATAGATGGATGTATGGCTTTTGAGGCAGCATACATGCTTGAAAA	5340
			
	5341	ATCTGTGTCAATGTATTTATGCTATATATAGCCTACAGTATATATAGAAGAATAGAGAAGA	5400
			
	5401	AATGGACTCGAATTCGATCGCCAGTCAACATCTTGTTGTTTTTTCAGTTCAGGGGACTG	5460
			
35	5461	GATTTTTTGTGTTTGTGTTGTTGTTGTTTCCCTTCCACATTGAAGGAATCTTACTGA	5520
			
	5521	AGGTTTGATACAGTTGGTTTAAGGAGGTGGCAAGACATGAGCTGGACATGAACCCAAGCA	5580
			
40	5581	GCAGCAACAGCACACTTTTAGAGACGTTCTTCCTACACTTCTCACTTGTTCCTCCTTT	5640
			
	5641	CTTACCTTTGTAGCTTCCTCTCTTACTGAGCACACCTCTCTCCTTCCAGCCTGAGGGA	5700
			
	5701	GATCTATGCATGTTCTTTACTCAGGTCCAGTAGCCTCCTCGGTTCTTCTCTCACATCTAC	5760
45	5761	TTAATATCTTTCTTTCTGTGCACTCTTTGCACTCACACAAATAAGCAGTGATGCCTT	5820
			
	5821	ATCTGCAGATTATTCACCTTTTCATTAAGAAAAAAGTAAGTTATGATAAATTATGGTAT	5880
			
50	5881	AATGTCATTTGTTTGGCATTTTTTTGTGAACCCTCTGTATAAATAAAGTTGGGTTTAGC	5940
			
	5941	ACAGCGAGAAACAGTCGATAAAAGATAACAAAGGTATGCTCTCTTTTATCTGCTATGCA	6000
			
	6001	TTGCTTAAAAACAAAAACCATCAGAGAAGAAGTGGCTGTAAATAAAGCTAGCATATTGC	6060
			
55	6061	CTTGTTTCTTTTTTGTGTAATGAACCTTTTGTATGTCTTTCTTTTGTATAAACTT	6120
			
	6121	AGAGAAAACATGTTTTAAAAAGCAGAAGGAAGTGAAAGTGGTTATCTTTGTATTATGGG	6180
			
60	6181	CATACCTTCAAGCCTTTGAATTGTAACCTAACATAATACATCACACCTTTTCTACCGAT	6240
			

	6241	ATGTTGCCGCGCTATTTACCGTCTCAAAAAGGTCGCTCTTTTTTATTTTATTTCTAT	6300
	6301	TTTTATTACTTTGTCCACGTAGGGTTAAGGAAAGTATTGCGGCTCAGATGCATTTAAAA	6360
5	6361	CATCTTCATTTGGAAGGGTGTGCTCTCAAAGTGTCCCTCTCACTGATTCTGATACTGGA	6420
	6421	TGCTATATTGTGTGCCCTTAAATGTATTTTGTACTAATAGACAATATATTACGTATGGC	6480
10	6481	ACACCAGTACTGTTTTACTTTTAGATATAACACGGCTTTATTGGATATTAGCTCTTCACT	6540
	6541	TGTGGCTGACTTTTTTTTTTTTCCCTCTGCAACACAATTTTAAGTATACCACTGTATTA	6600
	6601	ATAAATAAAATCATTTTTTAAATTAAAGAGTGTGTAAGGATTTTTTATTTTTTTTACCCT	6660
15	6661	ACAGGGTTTGTAT	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	AAAGAGGAAAAACAATGCATCATATAACTTTATAAGTAAGAGTGCGGCGATGGAGGAAACC	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	GTCATATGGGAACAGCACACAGTTACCTTCACAGGGCCCCAGGATTGGGTTTGGCATT	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	GCCATCTCGGGTGGGCGAGACAACCCTCATTTCCAGAGTGGTGAACATCTATTGTAATA	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	ATGGTCAATGCAGTCTCTATGGACAATGTAGAGCATGCCTATGCTGTTCAACAACCTCGA	300
	81	-M--V--N--A--V--S--M--D--N--V--E--H--A--Y--A--V--Q--Q--L--R-	100
	301	AAGAGTGGCAAAAATGCAAAGATAACTATTGCGAGAAAAAGGAAAGTACAAATCCCAGCG	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	GGGAGGCGGGATCACAGTGCCTCACAGGAGAGGAGCATCTACCACGCTCCGATCGCCGA	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	TCACAAGCCTCTTCTGCTCCACCCAGGCCCTCCAAGGTCACTCTTGTCAAGTCCCGCAA	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	AGCCTTGCAGCCAGAGATGGAACATTCAGGAGGGAGATGTTGTACTTAAGATTAACGGC	720

5	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
	241	-T--V--T--E--N--L--S--L--T--D--A--K--K--L--I--E--R--S--K--G-	260
10	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	GACGACAGCATCCCATCAGCCAATAACTCAGACAGAGATGACATTTGAGAGATACATTCA	900
15	281	-D--D--S--I--P--S--A--N--N--S--D--R--D--D--I--S--E--I--H--S-	300
	901	CTGACATCCGATCATTTCCAATCGATCCCATGGGAGAGGAAGTCAATCCCATTGCGCTGAC	960
	301	-L--T--S--D--H--S--N--R--S--H--G--R--G--S--Q--S--H--S--P--D-	320
20	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
25	341	-G--F--L--W--E--R--A--E--E--L--I--K--Q--E--W--V--V--K--Q--E-	360
	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
30	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
	1201	GACGTGGACCTGCCTGTGAGCCCATCTGATGCCCTGTACCCAGTGCTGCACATGATGAC	1260
35	401	-D--V--D--L--P--V--S--P--S--D--A--P--V--P--S--A--A--H--D--D-	420
	1261	AGCATTCTCAGACCAAGTATGAAGCTGGTCAAGTTCAGAAGGGAGAGAGTGTGCGTTAG	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	TTCTGCTTCGCCCTTGTATTAGACAGCCAATCGCTGGACGCTCACTCCGCCAGAAGGGGTG	60
	61	GGTTGACGTAGTACAGGAAGCCAGGCGAGGTGAGGTGGGGAGGAGAGATCACAAAATTGT	120
	121	TAGCTCGCTGCTAGCTGCCTCCTCCGATTTGCCCGAAGTGCGATGAGCCAGGAGGCGAA	180
45	181	ATTTGTGGGGTTTTTTGGTTTTTGATTGGCGGACGATGACCTCTGACCCTAAGAATGGA	240
	241	CATAAGTTAATGATGACGGGGGAGAAGAAGAAGAAGCGGCTGAACCGCAGCATTCCTT	300
	17	M--M--T--G--E--K--K--K--K--K--R--L--N--R--S--I--L-	17
50	301	CTTGCAAAGAAAATTATAATAAAAGATGGAGGAAGTCTCAGGGAATCGGGGAGCCCACT	360
	18	-L--A--K--K--I--I--I--K--D--G--G--S--P--Q--G--I--G--E--P--S-	37
	361	GTTTACCATGCTGTGGTGGTCATCTTCTGGAGTTTTTTGCATGGGGTCTGCTCACTACC	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
	481	ATTCATGGTGTGAAGGGCTGTTATCATTTCTCAGTGCTCCGCTAATTGGAGCGTTGTCA	540
5	78	-I--H--G--V--K--G--L--L--S--F--L--S--A--P--L--I--G--A--L--S-	97
	541	GACGTATGGGGACGCAAGTCCTTCCTGCTGCTAACGGTCTTCTTCACTTGCGCGCCCATT	600
	98	-D--V--W--G--R--K--S--F--L--L--L--T--V--F--F--T--C--A--P--I-	117
	601	CCGCTGATGAAGATCAGTCCATGGTGGTACTTTGCAGTCATCTCGATGTCCGGTGTTTT	660
10	118	-P--L--M--K--I--S--P--W--Y--F--A--V--I--S--M--S--G--V--F-	137
	661	GCCGTCACCTTCTCTGTGATCTTTGCCTATGTGGCAGACATCACACAAGAGCATGAGAGG	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
	901	ATGAGGCCAGCGTCATGGGGAGCGCCCATCTCCTGGGAACAGGCAGACCCCTTCGCTTCT	960
25	218	-M--R--P--A--S--W--G--A--P--I--S--W--E--Q--A--D--P--F--A--S-	237
	961	CTGCGTAAAGTGGGCCAGGACTCTACGGTGCTCCTCATCTGTATCACAGTGTCTCTCTCC	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
	1201	GGCCTCGGCTTTTCTAGATCCTCCAGCTGGCCTGGTATGGCTTTGGATCTCAGCCCTGGATG	1260
40	318	-G--L--G--F--Q--I--L--Q--L--A--W--Y--G--F--G--S--Q--P--W--M-	337
	1261	ATGTGGGCAGCTGGAGCCGTTGCTGCCATGTCCAGCATCACGTTCCCCGCCATCAGCGCC	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	ATTGTGTCCCGTAATGCGGATCCTGACCAGCAAGGTGTTGTTGAGGCGATGATCACTGGA	1380
	358	-I--V--S--R--N--A--D--P--D--Q--Q--G--V--V--Q--G--M--I--T--G-	377
	1381	ATTCGAGGCCCTCTGTAACGGTTTGGGTCCTGCTCTTTACGGCTTCGCTTCTACTTATTC	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
	1501	CCCACTGACGAGAGTGCCATCATCCAGGTCCTCCCTTCTTGGTGCATGCTCAGTG	1560
55	418	-P--T--D--E--S--A--I--I--P--G--P--P--F--L--F--G--A--C--S--V-	437
	1561	CTGCTGTCTCTGCTGGTGGCGCTGTTTCATCCCGGAGCACACTGGGCCCGGTATGAGGCC	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	GGCTCCTACAAGAAGCACAGCAACGGGGCACAGAGTCACTCCACAGCCCGCAAGGCAGC	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	ATCACAAGGACACACACGCCCTCAGGCACACGTCACACTCGAGTGCCGCAAAGAGATGT	1860
10	1861	TTGTCTGTTTTGCTGTCCACAGCACAAAGTTGGGCGCTCCTTCCCTTAGCAACCTTTTCT	1920
	1921	TTATAATAGCTGGGTTATTTGTGAGGACTTTCTAAAGACCCTGTGTGAAGAAAGTGTGTCG	1980
	1981	AGCATCATCAGGGCTGCAGTGGAGACCCTGTATGTGTGTGTGTGTGTGTGTGTGTGTGT	2040
15	2041	GTGTGTGTGGCTGAGCTGAGCTGAGCTGGACTCCAATCTTTGGTTTGTCTGAAGTTGTAA	2100
	2101	CAGTGGAGCACACAACAGCTTGTCCCCCTCCTGGCGCGAAACAGGACTGAAGTGACTTTG	2160
20	2161	GTTAATGTGCGAGTGGGATATATCTCTGATACGTTACTAAATACCTGTGTGACTCTTG	2220
	2221	ATTATTCCTCTTTAGTTAGCCAAGTGGCACCTTCGTTTGTGAGGAGAGCGTGACGAAC	2280
	2281	GCCCTCTCACATGCTAATACTTCTGTTCTGATGCTTGTCTTTATGACTACAGCTCTGTTT	2340
25	2341	AGGCGTCCAAGAAGGAAACATAGTTCTTCTCTGTGTGGACAACAGGGGAGCGCAGCAGC	2400
	2401	TGTAAACCTGTGAAAGGAGCCTGCAAACCAGTATTGGAGAGGCGCTGCCTAATTGCAGT	2460
30	2461	CAGGGTTGGCAACCAGTTCAGATACAAAAGCTTTGTTAGGACCAGGTTTTGTTCAAATA	2520
	2521	TCAAACCTCTTACAGAGAGATGACTAGAAGAGACCACTTTATTAGCTCAAAATGGTTTTT	2580
	2581	CAATGTTTACTTGCCATTCTCTAGATTAGTAGTACAGTTTGGGTTGTATATTTTCTCTG	2640
35	2641	TTCAAACGAAGGCTAGTTGTGCTTCAAGTTTTTATTCAAGAAACAAATGTTGCCTTGAA	2700
	2701	GTGACTTAAGATATATATGGAGACATTACGTAACCTGTATGAAGACCGAGGTCTGAGAAG	2760
40	2761	GCTCTGTAATCTTGCGCTATTGCTCCCATCGGAGCCGTTACACACTTTTTATTCCCTTGT	2820
	2821	ATTCATGCCCTTCCTGTTACTTTGTTTCTGACATTTATCACCATCAAGTTGAGGCTTAC	2880
	2881	AGAGACACGGTTTTATTTTTTAAAAAGCCTCTGGACCATTTGGAGCTGGAGCATTGCTATC	2940
45	2941	AGGATGTCGGTGTCTGCACTGACTGTTTGAGTTGATATCATTAGGTTGAGCAGAATATCA	3000
	3001	GCCATGCTGCTGCAGTAGTAAATACAAAGGTTAATCAGTGTGGCGTAAAGTGGTGGATAA	3060
50	3061	GAATTATAACTGTGTCTTGTAGTCCCTGACATTTAAGCTAACATGCGTACACTCAAAGAG	3120
	3121	GCAGGCCACACTTCTCCCAATGCCTAACATGAAGCACCTCACGGACGTGTCTGGCAACTT	3180
	3181	GTGTAGAAGCTCTGCAGATGCCAGCCTGCGCCACCTAAGAGGCAGAAACAAATAGCAGTA	3240
55	3241	GTGGAGTAGATGGCTGGAAATGTTTCATGTTATCCTCAAACAGTGAAGCAAAGTAAAAATC	3300
	3301	TGGAGGTTGTGTCATGTGGAGAGTATTGCGAAATCTGCAATGATCCCAGATTTATTAG	3360
60	3361	TTTAAAAAAAAGAGAAAAATAAGAAGAAGAAAAATCCACTTAAAAGTGTAATCCTGAA	3420

		
3421	TTTTTATTATCGTTCAGATCTGCAGATGTCTCTGGGTTTTTCTGCAGGTCTGAACTGCTG	3480
		
5	3481 CTGCCACGTTTATTTTATTTTCCCCGGTCAACAGGTGGCGCAGTCTGTACCTGGCATGC	3540
		
3541	CTGTAAGGTGCTCGTGTGGTTTTTGTCTTTTCTTTTTCAGTCATGTGGATCAGCGATACT	3600
		
3601	GCGTTCCTTCATTACATACTATGTCGCCACCTTTCCACATGTAACTTTGATCTGTGA	3660
		
10	3661 ATGCTCTCGTAGCTAACAACTGGTTTCATGCTGTTTAAACATCTGTATGAACTGAAACAT	3720
		
3721	ACGTCACGTATTAGTGCCATATCTTCTTGATTGCTTTTTCTTTGTACTGTGTGTGT	3780
		
3781	GAATGTACACTTGTGTGATTTGAGTGTTTTGTGTCTTTTATTTTCTCTTGTCTTAA	3840
15		
3841	TTTCTTTGACTGAAGATTAAAGTTTAAATGCTATTTTAAATAGCTTTTAAACTTCA	3900
		
3901	GTCAATTTTTTAGGATTAATTGTCAAAATTGGATGGTAAATATCAAATGTCCATCTGTC	3960
		
20	3961 CCCTTTGTATGTTGTTTGTGTTTGTGATTTCAGCCTCGGTCTTCATTTAATAACAAGCATT	4020
		
4021	TCACCATGGTTTGTAAAGCTCATAATTTTTTCCCAGATTTCTCTGAATGTTTCCAATGAA	4080
		
4081	ACTGAACATGTTGACCACACAGTACCCCTCAATCTTTAGGTTTTTTTTGTTTTGTCTTTTA	4140
25		
4141	AGAGGGGATGTTACTACACAGGAGGCCATTATCCCGTTTTTTTTTTTTGTTTTGTTTTT	4200
		
4201	TTTAAATCATGTAATTGAACAACAGAAAATCGGATCCTGGTAAGATTCTGCACCAGCCCC	4260
		
30	4261 CCACCACCACCACCCACGTGCACACCTACAGCCTCCAAGCAGACGACTGTAATGTACAA	4320
		
4321	AAATCACCTGTACCTAGAGAAAAATGTATATATTTATTCCTCAAGGAGATGGCCACCTCT	4380
		
4381	TGGTCAATTTGGTTGTATGGTGCATTTATTATATAATTATTATATATTTCTCCAGAATT	4440
35		
4441	ACCTGCTAGCCACTCCTGTTTTTAGTACAATGTGGTTTGTGGCCTGAACTCCCCTCTCTG	4500
		
4501	TGTGCCTAAAATTAGCCAAGAAATGAGTATGGCAACCTAAGTAAGTAAATGGTGGTTAT	4560
		
40	4561 TAATGTAAATATGGGAACTAATGATAAACTATTTATTAAAGGTTTATTGTACAATGAAA	4620
		
4621	CGTTTCGGGTGCGCTCTGTGGTTTCTGGGTGGGTAACACAGGTGAAATCATGTTACTGTA	4680
		
4681	GCAGTGAGTGAGCATCTGAGCAGCGATAATCATTTGGTCGTTGCATTTACGGCGATGATC	4740
45		
4741	CTATAGTTAATGGCTGCTAAATCCCAGTAAGTCTCACTATAAACTGGTAGCATTCCTGTT	4800
		
4801	GGGCTTTACTTGCTGTTATATTACTGCACCCCATTTTTTTTTTAATGTAATGCTCTGAC	4860
		
50	4861 TTTGCTGGCTGTTGGTTTTGTAAACCTGCCCTTTGAAGCTTAATGTTACCGCTAATGCCT	4920
		
4921	CCTCCACCTACACAGTGTATATAGTCGTGCATTGACCTGAGCTCATTTATGGCGGTGGA	4980
		
55	4981 TTTGTAATTAAATCCACATGGAGGCAGTAGTTACATCTGGCAGGAACTTAAAGAGTCTT	5040
		
5041	CTCCCTGAATAACAGTGAACGCAAAGTGGGAGATGTCACAAAATGTGATATTTATCCAAA	5100
		
5101	ATAAAGAATACGATAAAGTGGCCAGAACAATTTATTTTGTATTAAATGTAGTGTAGGGG	5160
		
60	5161 AATTTAATGTCTTATAATTAGCAGCTAATAACTTGCCCATCATTTTGTGAATTTCTGTG	5220

5221 TGAATGATGAAGTTTACTGGGTCAATGCTCAAATCTTAAGGTGATTAATGAGTATTTC 5280
5281 A 5281
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 TTCTGCTTCGCCCTTGTATTAGACAGCCAATCGCTGGACGTCACTCCGCCAGAAGGGGTG 60
61 GGTTGACGTAGTACAGGAAGCCAGGCGAGGTGAGGTGGGGAGGAGATCACAAAATTGT 120
121 TAGCTCGCTGCTAGCTGCCTCCTCCGATTTGCCCGAAGTGCATGAGCCCAGGAGGCGAA 180
181 ATTTGTGGGGTTTTTTGGTTTTTGATTGGCGCGACGATGACCTCTGACCTAAGAATGGA 240
241 CATAAGTTAATGATGACGGGGGAGAAGAAGAAGAAGCGGCTGAACCGCAGCATTCTT 300
.....-M--M--T--G--E--K--K--K--K--R--L--N--R--S--I--L- 17
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 GTTTACCATGCTGTGGTGGTCATCTTCTGGAGTTTTTTGCATGGGGTCTGCTCACTACC 420
38 -V--Y--H--A--V--V--V--I--F--L--E--F--F--A--W--G--L--L--T--T- 57
421 CCGATGCTCACGGTATTACACCAGACATTCCTCCCAACACACATTCCTGATGAATGGGCTC 480
58 -P--M--L--T--V--L--H--Q--T--F--P--Q--H--T--F--L--M--N--G--L- 77
481 ATTCATGGTGTGAAGGGCCTGTTATCATTTCTCAGTGCCTCCGCTAATTGGAGCGTTGTCA 540
78 -I--H--G--V--K--G--L--L--S--F--L--S--A--P--L--I--G--A--L--S- 97
541 GACGTATGGGGACGCAAGTCCTTCTCTGCTGCTAACGGTCTTCTTCACTTGCGCGCCCATT 600
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
118 -P--L--M--K--I--S--P--W--W--Y--F--A--V--I--S--M--S--G--V--F- 137
661 GCCGTCACCTTCTCTGTGATCTTTGCCTATGTGGCAGACATCACACAAGAGCATGAGAGG 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
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
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
901 ATGAGCGCCCATCTCCTGGGAACAGGCAGACCCCTTCGCTTCTCTGCGTAAAGTGGGCCA 960
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	ATGACGGGCAAACTCTGTGAAAGACGTTGACAGATACCAGGCTGTCTCAACTCTTTACTG	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	CACATCTCCAAGGTCAAGTCTGTCAACCTGGATCAGTGGACGAGGAGCAAGTCCAGTGT	240
	61	-H--I--S--K--V--K--S--V--N--L--D--Q--W--T--Q--E--Q--V--Q--C-	80
	241	GTTCAAGAGATGGGAAATGCCAAGGCCAAACGGCTCTACGAGGCTTTTTTACCCGAGTGC	300
20	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	ACTAAGAAAAAGACGTCAGCCCAAAGACAGATTCCAGTCTGTACAGACCTGCTCGGA	540
	161	-T--K--K--K--D--V--S--P--K--T--D--S--Q--S--V--T--D--L--L--G-	180
	541	CTAGAAGTCTTTTATGTGCAAGTCTGCACCTAAAAAGCAAATAAACACGTCAGACTCT	600
35	181	-L--E--L--L--L--C--C--K--S--A--P--K--K--Q--I--N--T--S--D--S-	200
	601	GCCCTGGATCTCTTCAGTCCCTCGCAGCCCCCTCCCCTGCTTCTCTACAAAAGCACG	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	TTCTTAGGCCAGCACCCAAAGCAGAGGAGGGCACAGTCAAGAACTATCCAAGGACTCC	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
	841	TTGTACATGAACCAAATGGGATATCCAACACACCCGTACGGTCCATACCATTTCTTAGCC	900
50	281	-L--Y--M--N--Q--M--G--Y--P--T--H--P--Y--G--P--Y--H--S--L--A-	300
	901	CAGGCAGGGGGAATGGGAGGCACTATGATGACATCACAGATGGCCATGATGGGGCAGCAG	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	CAGGTTGGGGGTTTGGCCGCATTACCCAGCAGCAGGCTTACGGAGTCCAGCAAGCCCAG	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	ACCAGCGGTATGATGGGATACAGCGGTCAACAAATGGGAGGTTTCAGCAGCTCCAAGTTCG	1260
	401	-T--S--G--M--M--G--Y--S--G--Q--Q--M--G--G--S--A--A--P--S--S--	420
15	1261	GCACACATGACAGCGCACGTGTGGAATGAGCTTGTCTATCTGAGATTCGATGGAGTGCC	1320
	421	-A--H--M--T--A--H--V--W--K--*--.....	429
	1321	AACGACCCACAAAAGGAGAAGAGAAACGCCGTGGATCAGACTCTCCATTAAACATTTTCT	1380
	1381	GATGCAAGGGAGGAGGAGGAGGAGAAGAAGAAGAGTTTGAGAAACCACTACTACC	1440
20	1441	TCTCTCTCTCCTCTCTGCGCGCTTCTCTTGCCGTCTCATGCATAGCCATGTTCTGCA	1500
	1501	GATTTCCATGTTTGCCTTCAGGACCTTTTCATATGATGACTAAGACAAGGGGGTTCTGAG	1560
25	1561	GCCACTGGTTAGGACTCCAGAGCTTTCTTTCTGCCTAGCCTTTATGAGAGAGCGCTCGTG	1620
	1621	TGCAGAAACATTATGAGGTATCAAGCAGCTGCAGAATTGCACTGTTCTTATTTAATCA	1680
	1681	GATGGCACTGGGGTTGGCATTGGGGTTAGCCTAGCTTTAAAGCTCAAATAGACCGAGAT	1740
30	1741	ATATAATCTGGTAACCTAAATAGGTGGCTCATACTTTAAATTATTAGCCCTACATTACC	1800
	1801	AGTATTTACCCAACCTGATGGAGCGACATTTAGTGATGATATGTACAGTGGCCCTGAGAGG	1860
35	1861	TCAAACACACTGCAGCCTAATAAAACACCAGCAAAAATGAAAATGGTGCAAAGCACAC	1920
	1921	AAAACATAATGGAGGTCAATAAAACCCAATGGAAATAGAAAGAAAAACACTGGAGAAGC	1980
	1981	TAGCAGAAAAAATCTCAGAAAACACACAGAAATGTTTTGGCTAAAATGTGACGGCTA	2040
40	2041	ACAGCTAACAGTAAACGGCTAACAGCAACCATGTACCTACAGTGTCATTGTGTTTTGTC	2100
	2101	AGAATTTTTTTTTCTATGTCCATTGTATTTTAATCAACTTCTGTGGTGCTTTTGCAAAT	2160
45	2161	TTTTCTGTTTTGCTGGTGTTCCTACAGTTGCAGTGCATGTGACCTCTCAGGGCCACCGT	2220
	2221	AGACATAGCTACATTTTAACAGCAGCCATATTTGCAAAGTGTAGCAACTACAACTTTATT	2280
	2281	CAGCCAATTTCAAGGTAGAGATTTAGAGCTTTTCAAAAGTATATTTTACATAAGTGAGA	2340
50	2341	TGAGCTGCTGCTAATCACTTAATAATCATTAACAAATATAAAAGCTAGGCTAGCCTAAT	2400
	2401	AGTCCCTTCATGCTGCATGCAGAAGACAAATACACATAACCATTTTGTAGCAACATATATC	2460
55	2461	TAGAAATTTCTACTCATTTAACAATATTTAATCAAGCAACAAAACCTACCTACACAGCC	2520
	2521	CGTAATATTGATGTCTTCATCTCAATTTCTAGAGGGCTTCTTTTAGAATCTTTAATCTTG	2580
60	2581	ACTTTAAAGTGTCAAAGTCCAAAACCATATTTTGGGAGACCAAAGATCAACACTAGCTT	2640

	2641	TACTGTAAGTGGACAGTATTCTGTATGCTTATTCTGTTCACCACTTAAGTAGTGATT	2700
			
	2701	AATAGAAAAAAAAACAGCAATTCAGCAGTCCGGCATCACTGTCTTCACTGTGCTGTTCT	2760
			
5	2761	TTCACCAAGGGTAGGACACTTAAAAAAAAAGAAAAGAAAGAAATCATTTTGCATGCA	2820
			
	2821	GTGTCATCAGCGCCCGCACACCTCCAGTTAAGAATCTACCTGGTGCATTAGTGGCCTCAA	2880
			
10	2881	ATAACGTTGAATGTCTGTAAATAGGAGGTGAACAGAGAAGTGGGAGTAGAGACGGAAAAAC	2940
			
	2941	TTCAGGTGAAGTCTCAGCGGGTTTCAGATGCTTCCACTGAATGCATGAAAAGAATGTG	3000
			
	3001	TATCTAGCTCTGATTGTATGTACTGTACTGTATGTTTGTAAAGATTTGCGAATGTGTCTC	3060
			
15	3061	TCTGAATGTTTCTCCCTCTGACTCAGTCTTTGACAAAGACTGACAAAAAACTATAAAAA	3120
			
	3121	AAAATAGGTAAACATATGTTCTGAATGTGATCTCGGTTGACTCGTTTGATCGCGCGCAA	3180
			
20	3181	TTGTTCTTCGGTGTGTTTTTGTTTTTATATATTCCTTGTCTAGAAACGTACACCTTGTG	3240
			
	3241	TCTCTGGAATGTCTGTGCTCGATGGCATCCTGTGGGTTTCCAGTTTTGCTGTAACGGCCT	3300
			
	3301	CACCTTTGCGTTGGGGGCAAACAGTGAGCTGTTTTGTTTTTTTTCTTTTTGAGAGGGG	3360
			
25	3361	ATGGGAGTATTTAACAATCTGGCCAAACCACATCGTGAAGCATAAAGCGATTGTAAACC	3420
			
	3421	ACAATCTTTCACGTCTGTTTAAGCTGATGCTTGTACGCTTCTCCACACAAACCATCTCT	3480
			
30	3481	GTGCCCCGATTTCTCTTAAAAGTGTGCTAAATCTGCCTTTTCTGATAAATGCTTATGGA	3540
			
	3541	AATGCTGTGTTTCTCTTATTTAATTTTATTTTGACACTTGTGTTAAGCTGGTAAGATGCTG	3600
			
	3601	CTTTTAATGTGAGTGGCAGCAATATAGGAGGTGCCTATGTGCAGCATATAAGGTCTTATT	3660
			
35	3661	TCACAACAGTGTGACAGCAGCAGTCACCTTCTCCACTGAGAGCAACATTTATATAAGAGA	3720
			
	3721	GAGCACATCCAGCACAGCAACAGCAAATCTGTCAAGTCAACAAAAGTTTCTGGAAAGGCAG	3780
			
40	3781	TGCAAGTCCACCTCTGTGGACGCTCAGGCCTCACCTGAGTTTTTCCATTTGTGATCAGGC	3840
			
	3841	TACTTTTTTTTTGGTCCGATATTTTTTCAATGAAACAAAAACGAATAAAGGAATGTAAC	3900
			
	3901	TTGTACGTACTTGTGATCAAGATACTGTATATTTAATTCTTATCAAAATATCGCTGT	3960
			
45	3961	ATATTATGTTTCTTAAACAACATGTTCTGTATATTAGTTTTTCTTTTCCACATGCTTTGC	4020
			
	4021	CCCACTTTACACAATTTCAATAAAATTTAACAATGTATATGTGACATATGATAATTGTCC	4080
			
50	4081	CTGTGAAAACATGCAATAAATATTGTTTGGTTAAATTTTATGTTGTTTGTGTTGTG	4140
			
	4141	GTTCAATTGCTGGGTGTCAGGAGTTTTCTGTTATGCAACTCAGGTGAGAATAAACGCTC	4200
			
	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

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5      1 ATGACGGGCAAATCTGTGAAAGACGTTGACAGATACCAGGCTGTCTCAACTCTTTACTG      60
      1 -M--T--G--K--S--V--K--D--V--D--R--Y--Q--A--V--L--N--S--L--L--      20

      61 GCGCTGGAGGAGAACTACTGCGCTGACTGTGAATCGAAAGGTCCACGATGGGCATCC      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 TGGAAATTTGGGCATCTTCATCTGTATCCGCTGTGCTGGGGGTTACATCTCCAAGGTCAA      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--P--G--F--F--T--P--V--L--P--A--S--R--D--      100

      301 AGACCAGGCTGCCGAGATCTTCATTAGGGACAAATACGAAAAGAAGAAATACATGGATAA      360
20     101 -R--P--G--C--R--D--L--H--*      118

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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

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30     1 ATGCCTGGCCCCACACCGACCATCAGCAAGCTCGGGTTTACACCGACGTTAATACACAG      60
      1 -M--P--G--P--T--P--T--I--S--K--A--R--V--Y--T--D--V--N--T--Q--      20

      61 AAGAACAGAGAGTACTGGGACTACGATGCTCATGTGCCAACTGGAGTAATCAAGACAAC      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 TATCAGCTGGTGCCTAACTGGGCAGAGGGAAGTACAGTGAAGTGTGAGGCCATAAAT      180
      41 -Y--Q--L--V--R--K--L--G--R--G--K--Y--S--E--V--F--E--A--I--N--      60

      181 GTGACCAATAATGAGAAAGTGGTGGTGAAAATCCTGAAGCCTGTCAAGAAGAAGAAGATC      240
      61 -V--T--N--N--E--K--V--V--V--K--I--L--K--P--V--K--K--K--K--I--      80

40     241 AAACGCGAAATCAAAATCTTGAAAACCTGCGAGGAGGAACCAACATCATCCGCTGGTG      300
      81 -K--R--E--I--K--I--L--E--N--L--R--G--G--T--N--I--I--R--L--V--      100

      301 GACACGGTCAAAGACCCGGTGTCCAGAACACCGCGCTAGTCTTTGAGTACATCAATAAC      360
45     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 GAGCTTCTAAAGGCTCTGGACTTCTGTACAGTATGGGGATCATGCACAGGGACGTGAAG      480
      141 -E--L--L--K--A--L--D--F--C--H--S--M--G--I--M--H--R--D--V--K--      160

      481 CCGCACAATGTGATGATTGACCACCAGCTGAGGAAGCTGCGTCTTATAGATTGGGGTTTG      540
55     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

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5	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
	201	-G--P--E--L--L--V--D--Y--Q--M--Y--D--Y--S--L--D--M--W--S--L-	220
10	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
	721	TATGACCAGCTGGTCCGCATCGCTAAGGTTCTCGGCACCGATGAGCTCTTTGGCTACCTG	780
15	241	-Y--D--Q--L--V--R--I--A--K--V--L--G--T--D--E--L--F--G--Y--L-	260
	781	CACAAATATCACATAGAAGTGGACACTCGCTTCAAAGACATGCTGGGGCAGCAAACACGG	840
	261	-H--K--Y--H--I--E--L--D--T--R--F--K--D--M--L--G--Q--Q--T--R-	280
20	841	AAACGCTGGGAGCAGTTCATCCAATCAGAGAACCAGCACCTGGTGAGTCCAGAGGCTCTG	900
	281	-K--R--W--E--Q--F--I--Q--S--E--N--Q--H--L--V--S--P--E--A--L-	300
	901	GACCTGCTGGACAAGCTGCTGCGCTATGACCACAGCAGAGGCTGACGGCGGCCGAGGCC	960
25	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
30	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

35	1	ATGCTCATGTGCCAACTGGAGTAATCAAGACAACATATCAGCTGGTGCGTAACTGGGCA	60
	1	M-L-M-C-Q-T-G-V-I-R-T-T-I-S-W-C-V-N-W-A-	20
	61	GAGGGAAGTACAGTGAAGTGTGTTGAGGCCATAAATGTGACCAATAATGAGAAAGTGGTG	120
40	21	E-G-S-T-V-K-C-L-R-P--*	31

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	ATGTCTGCTTCGACTGGATGCTCCCCATCGGGCCAGCACTCGGGCCTTGTCCTCCAGTATG	60
	1	-M--S--A--S--T--G--C--S--P--S--G--Q--H--S--G--L--V--P--S--M-	20
	61	TCCATGTTTCGATGGCTAGAGTGCTGGAGAAGGAATTTGATAAGGCTTTCGTGGATGTG	120
50	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
	81	-Q--L--N--H--K--L--E--A--Q--L--V--D--L--R--S--E--L--T--E--A-	100
10	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
	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
15	421	AAACTGCCTACACCAACCTTAGAAGAGCTGGAACAGGAGCTCGAGGCCAGTAAGAAGGAG	480
	141	-K--L--P--T--P--T--L--E--E--L--E--Q--E--L--E--A--S--K--K--E-	160
	481	AAATTAGCAGAGGCCAAAAATGGAGGCAGAAACCAGACTATATAAGAAAGAAAACGAGGCC	540
20	161	-K--L--A--E--A--K--M--E--A--E--T--R--L--Y--K--K--E--N--E--A-	180
	541	CTTCGAGGCACATGGCAGTACTGCAGGCCAAGTCTACGGAGCCAGACTGGCTGCTAAA	600
	181	-L--R--R--H--M--A--V--L--Q--A--E--V--Y--G--A--R--L--A--A--K-	200
25	601	TACTTGGACAAGGAACTGGCTGGCAGGGTGCAGCAGATACAGTTACTGGGTCGTGACATG	660
	201	-Y--L--D--K--E--L--A--G--R--V--Q--Q--I--Q--L--L--G--R--D--M-	220
	661	AAAGGGCCAGCAGCATGACAAGCTCTGGAATCAACTGGAGGCAGAAATTCACCTTCACCGC	720
	221	-K--G--P--A--H--D--K--L--W--N--Q--L--E--A--E--I--H--L--H--R-	240
30	721	CATAAACTGTGATCCGAGCATGTAGAGGTGGAAGTGACCCTAAGAGACCTCTTCCCTCT	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
35	261	-P--V--G--H--D--P--D--M--L--K--K--T--Q--G--V--G--P--I--R--K-	280
	841	GTGTGCTGGTCAAAGAGGATCATGAGGGTCTAGGAATTTCCATTACAGGTGGGAAGGAG	900
	281	-V--V--L--V--K--E--D--H--E--G--L--G--I--S--I--T--G--G--K--E-	300
40	901	CACGGCGTTCCCATTTTAAATTTAGAGATCCATCCAGTCAGCCCGCAGACAGATGTGGA	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
	321	-G--L--H--V--G--D--A--I--L--A--V--N--S--I--N--L--R--D--A--K-	340
45	1021	CATAAGGAAGCTGTCACCATTTCTCTCTCAGCAGCGAGGACAGATAGAGTTTGAGGTCGTG	1080
	341	-H--K--E--A--V--T--I--L--S--Q--Q--R--G--Q--I--E--F--E--V--V-	360
	1081	TACGTGGCTCCTGAAGTGGACAGCGATGATGAGAATGTGGAGTACGAGGATGACAGCGGT	1140
50	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
	381	-H--R--Y--R--L--Y--L--D--E--L--D--D--S--I--T--A--P--P--S--N-	400
55	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
60	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

1 ATGTCTGCTTCGACTGGATGCTCCCCAGCACTCGGGCCTTGTCCCCAGTATGTCCATGTT 60
1 -M--S--A--S--T--G--C--S--P--A--L--G--P--C--P--Q--Y--V--H--V-- 20
10 61 TCGATGGCTAGAAAGTGTGGAGAAGGAATTGATAAGGCTTTCGTGGATGTGGATCTGTCT 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

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
25 61 AAAGCCCAGAAATCCCCCAGGATGCCCCAAGTGCTCTCGCTGTAGAAATCACGGATACGTG 120
21 -K--A--Q--K--S--P--R--M--P--K--C--S--R--C--R--N--H--G--Y--V-- 40
121 TCTCCACTGAAGGGACACAAGCGCTTTTGAACCTGGAGGGACTGCCAGTGTCCCAAATGC 180
30 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 GCCCAAGAAGAAGAGCTTGGGATTTGTAGTCTGTGTCTGTCTGTCGGTTCCGAGATGATG 300
35 81 -A--Q--E--E--E--L--G--I--C--S--P--V--S--L--S--G--S--E--M--M-- 100
301 GTCAAGAATGAAGTTGGAGCAGACTGCCTGTTCTCTGTGGAGGGACGGTCCCCGACACCT 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 ACCAGCCACGCCACCTCTGCTGTACAGGGACCCGCTCGGCATCGTCCCCAGCCCATCT 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
45 141 -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
541 TACCAGCAGATGCCTCATGGTGATGGCCGCCTGCCAGCCACAGCGTGTCTGTCTCAGTAC 600
50 181 -Y--Q--Q--M--P--H--G--D--G--R--L--P--S--H--S--V--S--S--Q--Y-- 200
601 CGCATGCACTCTACTACCCAGCAGCCACCTACCTGACTCAGGGCCTGGGCTCCACCAGC 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
221 -C--V--P--P--F--F--S--L--D--D--N--N--N--S--C--S--E--T--M--A- 240
5 721 GCCTCCTTCTCACCCGGCAGCATCTCCGCTGGTCACGACTCCACCATGGTCTGCCGCTCC 780
241 -A--S--F--S--P--G--S--I--S--A--G--H--D--S--T--M--V--C--R--S- 260
781 ATCAGCTCCCTGGTTAACGGCGACGCCAAGGCTGAATGCGAGGCCAGCAGCCAGGCAGCC 840
10 261 -I--S--S--L--V--N--G--D--A--K--A--E--C--E--A--S--S--Q--A--A- 280
841 GGCTTCACCGTCGACGCCATCGAAGGCGGCGCCACCAAATAA 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 ATGAGCCAGGACAAACAGAGTAAGCAGGTACCGGATTGCAGCGGACCCGACCAAAGCCC 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 P 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--P--N- 20
61 CCCCAGGATGCCCAAGTGCTCTCGCTGTAGAAATCACGGATACGTGTCTCCACTGAAGG 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

	61	AAAACCGTGGTTCTCAGACCCTGACAAACAATACCTAGGGCAGCATCCCAGTTTGTTCGC	120
5	121	TACTATCTCCTCCTCCGACCAGACGTTCTGGGACCAACCGCAGCTTTTGTCTGCAGCCAGT	180
	181	CTTACGTGTTTCATCCACCATGGCCTTTCCATTATTGTCATGACATTACTTTTGGGCTCT	240
		-M--A--F--P--F--I--V--M--T--L--L--L--G--S-	14
10	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	GGTGACAGGTGCCAAGGTGAGTCATGGCAGTCCATCAGAAAGAACCTCCTTAGGGTTCTG	360
	35	-G--D--R--C--Q--G--E--S--W--Q--S--I--R--K--N--L--L--R--V--L-	54
15	361	AACCTGACAGACTGAGCCGACGTACCTGCCGGTGCAGTGGACAGTGTGAGAGAGCAGTGG	420
	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
20	75	-N--R--T--F--S--I--V--S--H--T--A--K--H--T--A--T--P--A--V--P-	94
	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
25	541	TCAGAGATCTTCATGAAAGATCTGGGCTGGGACAGCTGGGTGATCCACCCGTTGAGTCTT	600
	115	-S--E--I--F--M--K--D--L--G--W--D--S--W--V--I--H--P--L--S--L-	134
	601	ACCTATGTTTCAGTGCGCAACCTGCAACTCTGCCATGACCACTGTTCAATGTCCATCATCC	660
	135	-T--Y--V--Q--C--A--T--C--N--S--A--M--T--T--V--Q--C--P--S--S-	154
30	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	CAAGAAGAGGTGCCCATAGTCTATATGGATGGATCCAGCGCCATTGTGTCATGTCTCCATG	780
35	175	-Q--E--E--V--P--I--V--Y--M--D--G--S--S--A--I--V--M--S--S--M-	194
	781	CAGCTGACCCGACGTTGTGGCTGTGAGCTGGGCAACTCTGAGGATCGTGGCAAGGAGTAG	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	AACAGGGGAAAAGTCTACAGTGTAACTATGTCAAGGCCACCTTGGGGTACAAGCAGATA	60
	61	AAAACCGTGGTTCTCAGACCCTGACAAACAATACCTAGGGCAGCATCCCAGTTTGTTCGC	120
	121	TACTATCTCCTCCTCCGACCAGACGTTCTGGGACCAACCGCAGCTTTTGTCTGCAGCCAGT	180
50	181	CTTACGTGTTTCATCCACCATGGCCTTTCCATTATTGTCATGACATTACTTTTGGGCTCT	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 GCAGACTGAGCCGCAGCTACCTGCCGGTGCAGTGGACAGTGTGAGAGCAGTGGAAACCG 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

 15 61 AAAACCGTGGTTCTCAGACCCTGACAAACAATACCTAGGGCAGCATCCCAGTTTGTCTGC 120

 121 TACTATCTCCTCCTCCGACCAGACGTTCCGGACCAACCGCAGCTTTTGTCTGCAGCCAGT 180

 181 CTTACGTGTTTCATCCACCATGGCCTTTCCATTGTCATGACATTACTTTTGGGCTCT 240
-M--A--F--P--F--I--V--M--T--L--L--L--G--S-- 14
 20 241 TCCATGATGATGGCATTGTCTTGGATCCATCCAGGAAAGAACCCGAAGCTGCCGCTCTTA 300
 15 -S--M--M--M--A--F--V--L--D--P--S--R--K--E--P--E--A--A--V--L-- 34

 25 301 GGTGACAGGTGCCAAGGTGAGTCATGGCAGTCCATCTGAACTGTCAGACTGAGCCGCAGC 360
 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

1 GCATTCACTACTGCATGACAGAAAACACCAAAACACCTCACATTTCTCTAGCTGACCT 60

 35 61 GGCGCCGAACCCCTCGAGCGGACAGACAGGCAAAGGCGTTCATATCAAATGTGGAGTGTGG 120
-M--W--S--V-- 4
 40 121 ACCAGAGACAATATCAGAGTAAATACACAAAAGAAGACAACTAGAAAAGTGAAACCAC 180
 5 D--Q--R--Q--Y--Q--S--K--I--H--K--R--R--Q--T--R--K--V--K--P-- 24

 181 TCTGTGGACCCAGGCAGACTGAAATGATGCTGGTGATGTTTGGAGTCACGGCGTTTCCCT 240
 25 L--C--G--P--R--Q--T--E--M--M--L--V--M--F--G--V--T--A--F--P-- 44

 45 241 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 TGGAGAGTATCGGTGCTTTTGCCTTTTCTGGCCTCCCTCACCTCACCAAAATCTTAATAT 420
 85 L--E--S--I--G--A--F--A--F--S--G--L--P--H--L--T--K--I--L--I-- 104

	421	CTAAAAATGCTGCTCTGAGGAATATCGGGGCTTTTGTCTTCTCCAACCTCCCTGAACTCA	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	GTGAGATAATCATAACAAAATCAAAACACCTGAGTTTCATCCACCCCGATGCATTCAGGA	540
	125	S--E--I--I--I--T--K--S--K--H--L--S--F--I--H--P--D--A--F--R--	144
10	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	TCTCCAAGATCCATTCCACCGCCTGCTTCTGCTGGATCTTCAGGACAACAGCCACATAA	660
	165	F--S--K--I--H--S--T--A--C--F--L--L--D--L--Q--D--N--S--H--I--	184
15	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
	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
25	841	CCAGTGAGTTGGTGGTACTAGACGTCTCCGAAACAGCCCTCACCTCTTTGCCAGACTCGA	900
	245	S--S--E--L--V--V--L--D--V--S--E--T--A--L--T--S--L--P--D--S--	264
	901	TCCTTGATGGCCTCAAGAGGCTGATTGCCGAGTCAGCCTTCAACCTGAAAGAAGTTCCTC	960
	265	I--L--D--G--L--K--R--L--I--A--E--S--A--F--N--L--K--E--L--P--	284
30	961	CTATTGAGCTCTTTACCAAAGTGCACAGGCAAGCTGACATACCCATCACACTGCTGCG	1020
	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	CTAAAAATAACCTGCACTTCTTCAAGGAATACTGCTCCAACCTCCACCAACATCACTTGCA	1140
	325	A--K--N--N--L--H--F--F--R--E--Y--C--S--N--S--T--N--I--T--C--	344
	1141	GCCCGGCCCTGTGACTTTAACCCCTGTGAAGATATCATGTCTGCTACCCCTTACGCA	1200
	345	S--P--A--P--D--D--F--N--P--C--E--D--I--M--S--A--T--P--L--R--	364
40	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
45	1261	TGTTAGGCAGCCGCTATAAGCTGACTGTTCTCGATTCTCATGTGCCACCTGGCCTTTG	1320
	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
55	1441	TCTTCACGGTGTTCGCCAGTGAGCTGTGAGTGTACCTTGACAGCAATCACCGTGGAGC	1500
	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
60	1561	GCATCATCATGACAATAGGTTGGATCTTCTCCTTGCTGGCTGCACTGCTGCCACAGTTG	1620
	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--N--I--L--A--F--F--C--V--C--	544
	1741	GCTGCTACCTCAGCATCTACCTCACCTTTCGCAAGCCTTCATCAGCGGCAGCCACGCCG	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	ACACCCGTGTGGCTCAACGCATGGCCGTCTCATCTTCACAGACTTCATCTGCATGGCTC	1860
	565	D--T--R--V--A--Q--R--M--A--V--L--I--F--T--D--F--I--C--M--A--	584
	1861	CGATCTCCTTCTTCGCCATCTCAGCTGCCCTCAAGCTCCCTCTCATCACCGTCTCAGACT	1920
15	585	P--I--S--F--F--A--I--S--A--A--L--K--L--P--L--I--T--V--S--D--	604
	1921	CCAAGCTACTGTTGGTGTATTCTACCCCATCAACTCGTGCTCCAACCCCTTCTTATATG	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	CCTTTTTACCCGTAACCTTCAGAAGGGATTCTTTCTCCTCGCAGCTCGCTTCGGGCTGT	2040
	625	A--F--F--T--R--N--F--R--R--D--F--F--L--L--A--A--R--F--G--L--	644
	2041	TTAAGACTCGAGCACAGATTTACCGGACAGAGGTTCCCTCGTGTGAGCAGCCAACATGGA	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	CCTCTCCAAAGAACAGCCGTGTTATCTTGTTATCTTGGTCAATACGTTAAGTCTAGATG	2160
	665	T--S--P--K--N--S--R--V--I--L--Y--S--L--V--N--T--L--S--L--D--	684
	2161	GAAAAACAAGAGTGCTGACTTTTACGCACATTTACAGGTACGGACTGTTTGCCTTGATTGC	2220
30	685	G--K--Q--E--C--*--.....	689
	2221	ATATTATATCCATACAAACAGGCTGCTAATTCCTTAAAATGATGCCTCAGATCATGTCTT	2280
	2281	TTGATCACTACCTGGGAAAATTTTCTATCTACTTAGACTAGAAAGAAAAAACACAAA	2340
35	2341	AGGCAACCAAGTGAAGGCAAAAGAGCTGAGAACTCTTTTTGACAATTTGACCCAGGAG	2400
	2401	TCTGCAAAACACAGTGATTGTTAAAATAAACAATGCTCTTGCTCTTGCTTCTGTTGTGC	2460
40	2461	TCCTAATCTGATGCTGTGTTTTTTGGGCTTGAGCCAGTGAAGGCTTCCACTGAAGACTGC	2520
	2521	TCTTCAGTCAATAAATAGCATCCAGAGACCCAGCTCTCAACAGAGGTGATGATCCTCTAT	2580
	2581	ATAAAGATGTTGGTCAGTTCAACAAAGAAGTTGATGCTTGTCTCTGTGCAAGTCTGAGAT	2640
45	2641	CTCTGTTAGGGATGTACATGTACAAGTGGTCAAGATTGGACTTCCAGGCCATGAGACCAG	2700
	2701	AGGTCTACAAGTCACAAAACCTTTTAAAGCTTTTATAAAATTATATATATCTATGTCGC	2760
50	2761	CACAATCTGAGCAGTTCAGACACTGATGATTCCAGACTGATCACTGACCCAAGAGAAAGC	2820
	2821	ATGCATACATGTTCCACCTGTCTTTAAGGTTACACATAAATCAACATGTTTCAATCAC	2880
	2881	AATAGTATCAGTTGACTATTAGCACAAGTACACACAGCGTTCAGTGGCATGTCTAAAC	2940
55	2941	CTGGTTACCTGAGCTATGCTCTGCAGCAATCCATGCAAACATGACCACAAAAGAACTAAT	3000
	3001	TATACACTCACTGGCCACTTTATTAGGTATACTTGTTGGCTGCTTGGTAATGCAAATAC	3060
60	3061	TTAATGAGCCAATCGCATGGTAGCAGCTCAGTGCATTTAGACATGTAATCTGGGGCATTT	3120

		
3121	TTAAGATTTTTTAAATGTGGTGGCAGGACGACAGACCAAGAACACAGTAGAGGGGGACATT	3180
		
5	3181 TAAATATTTGATTAGCAAAAAGATCAGAAAACAGACAGAAATATTGGGCATGATTTTTG	3240
		
3241	GTGTGCAACCTTATGTTTTATTACAAGTTATTGTGTGAAAAGTGGTGCTGCAGAATGCT	3300
		
3301	CTACATAGAATTTTGTGTGGACAATTGTTTTGCAACGTGGAAAAAGAAGTATTTAGACT	3360
		
10	3361 TAACCTAAGTAAAAGTTGTAATTGCACCTAAATAGCTTAATAGTTCACAAGTTATATAAT	3420
		
3421	CAAAATGTATTCAAAGTGCCATAAGTAAACACACTCTTTATATAGAATGGCCCTTTTTTT	3480
		
3481	CTCGTCTCTTTAATGAGGCAGCTGTTGATGAGTTGATTCCCTGATATATTGTTCAATAGA	3540
15		
3541	TTCATTTTAAAAAATACAATTAAATGTACAAAATAAGAAGAAGCTAAAATAATTTGGGGT	3600
		
3601	GGGCTAATGCCACTCCAAGCTCCTCCCCCTCCAAACATGCCTCTATGTAGACATAATCAA	3660
		
20	3661 GACAACCTTGCTAAAGTTCAAAATGAGCATCAGAATGGGAAAAAGGTGACTGAAGTGACTT	3720
		
3721	TGAAAGTTAATTTGTTGTTGGTGCCAGATGGTCCCACATGTCGCCACAATCTGCTGGCCG	3780
		
3781	GACTGCAAACTGATAGGAAAGCAACAGTAACTTAAATAACTTCTATACAATCAAGGTGTA	3840
25		
3841	CAAGTTACATAAGAAAACAGGGCATCACTTAGTCTGATAACTCTTGATTTCTATTCTGAC	3900
		
3901	ATTCTTATAGTAGGTTTCAAGTTTGAATTTAACTGAGCAAACTGAGTCACAAAGCTCAGAT	3960
		
30	3961 CATCTAAAACGTATTTCTTGAAAATGAAAAGGAGTTTACCACAGTCACCACATTTCAATC	4020
		
4021	CAGCAGAGCACATTTGGGATTTAGTCAAATGGGAGATTGCCATCACAGATGTGCAGCTGA	4080
		
4081	CAAACTTGCAAGCCTGTGTGATGCTATCACATCAATATGGACCACAATCTCTGAGGAAT	4140
35		
4141	GTTTCCTCCACTCAATTCAGTTGAATATATTTTAAAAATTAAGACAGTGTGAAGACAAA	4200
		
4201	GGGGTGTTTAACCTAGCAAAATGTACCAATAAAGTAGCTAGTGAAGTGTAGTTTGACTAA	4260
		
40	4261 ATCTGGGTGAGACAGCTCTTTTAGATACCCATGGGTTTCTTTTAACTCAAGTGAAGTGCC	4320
		
4321	AGATGGGTGGAGTTCTCAGCAACATAATTTAGAGGTAAAAGAAGAAAAGTGGAGGGGG	4380
		
4381	GAGAAAACATGACTTCATCTACTATGTAACAAACACCATCCGTCTGGCATCCCAAGAT	4440
45		
4441	AATCTAACAACTAAAATGCCTCAGAATGGTTTTTAAGCAGGTTGGATGCTTGGGATTTT	4500
		
4501	AGCATATGCACACTGCAAAAGAAACATATTCAATCAACATTGAGTGTGTGATTGAATGA	4560
		
50	4561 TATTCATTAAGAAGAACTGCAGGGACCTGCTGATTAAACAATCTCCTCATACCCCAGT	4620
		
4621	CTGCTGAACCTCTCAATGTCTACAATTTGCCACCAACTCCGTCTATTTTGTAGCCACAG	4680
		
4681	ACCTGTAATTATCTTTGAAATGTAATTATGTTTACGTTTTCAAACAAACATCCAATTAAG	4740
55		
4741	TGTCACTTTTGAATCTGTTTTCTGAAGAATATTTCAATGTGCTGTTTTTACTACTATTT	4800
		
4801	TATAAAGTGTTTTATTATATCTCTCAGCTTGAATAGATTTTGTATGATGAATGTGAGCG	4860
		
60	4861 TTTGAAGAGGCGTGACAAACAGAAAACTCTCTCACACACACATATGCAATAATTGAG	4920

5	4921	CTGTCTTTATCTAGCAATGCTGTCCTTCAGAGCATCCAAAGCTTTCAGGACAAAGTGAC	4980
	4981	CCTCCCAACCTCTGCTCTGTGCAGCAAAGTGGGTGGGTGGGCGTAGGAGGAGAGGTACGC	5040
	5041	AGCTGCTCTTTCTGCTTATTACGGGGGATGGATATGGCAGCTAGATAAGCTGTGTGT	5100
10	5101	GCGCGCACACACACACACACACACAATAGCAACCCACACTCTCAAGGCTGCAGC	5160
	5161	TGCAAGAAGGAATCCAAGACCATCTCATGATATGGATACACTGCCTCCTACATGCCAAC	5220
	5221	ATTCAAAGTTAGGGTGCAATTATATACTTTCACCACCAGGTGATGCTACTGGGGCTAGAT	5280
15	5281	TTCTGGTGAGTTTACCTCCATCTGTTTGCACAAAAGTCCAAACAAATTCACAGTCTCAG	5340
	5341	TAGATCCTACAAATTTTGCTCGATGTTGTCTTATGAGAAAAATAAATAAATAATATTTT	5400
	5401	TTTCCTAAATTTGCTTTTTTTTAAAAATAACTTTTATTTCTACATAATTTTCATAAAGA	5460
20	5461	TTATATCAATTCCTGCATGAGGATTAATGCTCATCAGACAGTTACCTGTCCCCTACATAC	5520
	5521	ACTGTATTCTTCTTCATTTTTATATCATATCATATAGTTTTCCAAGTAAAAGATAAATC	5580
	5581	ACTCTAATGCATTTGCACTCAAATTTATGTGCACAAAAAAGTGAGTGTGCAATACAG	5640
25	5641	AAAGACATGCCGTTATGCTCTCTGACATCTTCTCTAGACAGCACTGGAGATGGTATAACA	5700
	5701	AAACACCCTCAGTATAAAGCCTTCAAGTTCATGACTAATCGTTGGCAGCTAAACAATGCC	5760
	5761	CTCTGGTGGTCGTCGTCGTCATAATAAATATACAAGTTAAAGTGTTAAAGTTGTATTCCACT	5820
30	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	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
50	181	TCTGTGGACCCAGGCAGACTGAAATGATGCTGGTGATGTTTGGAGTCACGGCGTTTCCCT	240
	25	L--C--G--P--R--Q--T--E--M--M--L--V--M--F--G--V--T--A--F--P--	44
55	241	CCAACATCTCCAACGCCAGTGCCTGGAAGTTAAGCAGACGCAGATCAGAGAGATTGAGC	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	AGGGCGCCCTCTCCAGCCTCCAGCATCTAATGGAAGTACCATTCTGAGAACGACCTGC	360
	65	Q--G--A--L--S--S--L--Q--H--L--M--E--L--T--I--S--E--N--D--L--	84

	361	TGGAGAGTATCGGTGCTTTTGCCTTTTCTGGCCTCCCTCACCTCACCAAAATCTTAATAT	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
10	481	GTGAGATAATCATAACAAAATCAAAACACCTGAGTTTCATCCACCCCGATGCATTCAGGA	540
	125	S--E--I--I--I--T--K--S--K--H--L--S--F--I--H--P--D--A--F--R--	144
	541	ACATGGCAAGACTACGGTCTTGACTATCTCCAACACCGGGCTGAGGATTTTCCAGACT	600
	145	N--M--A--R--L--R--F--L--T--I--S--N--T--G--L--R--I--F--P--D--	164
15	601	TCTCCAAGATCCATTCACCGCCTGCTTTCTGCTGGATCTTCAGGACAACAGCCACATAA	660
	165	F--S--K--I--H--S--T--A--C--F--L--L--D--L--Q--D--N--S--H--I--	184
	661	AGAGAGTCCCTGCCAATGCCTTCAGAGGCTCTGCACTCAAACCTTCGCAGAGATACGGC	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	TCACCAGAAATGGCATCAAGGAGGTGGCAAGTACGCGCTTCAACGGAAACAAAGATGCACA	780
	205	L--T--R--N--G--I--K--E--V--A--S--D--A--F--N--G--T--K--M--H--	224
25	781	GACTGTTCTAGGAGGCAACCGACAGCTTACTCACATCAGTCCAATGCCTTTGTGGGTT	840
	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
30	901	GATGGCCTCAAGAGGCTGATTGCCGAGTCAGCCTTCAACCTGAAAGAACTTCCTCCTATT	960
	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

40	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
45	121	GGCCTGCTCGAGAAAGTTTCAGCGAGAGCTGGACTAAATATCAGCAGCAAAGTTAGCATC	180
	41	-G--L--L--E--K--G--S--A--R--A--G--L--N--I--S--S--K--V--S--I--	60
50	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
	81	-S--G--I--W--P--E--D--P--F--I--P--A--T--E--L--T--S--A--L--Q--	100
55	301	GCTGAGCTCACGACTCCCATTAAGTTTGAATATGTCAATGGTGCTGTTGGAAAAGTCTTC	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
	121	-A--P--E--T--V--S--T--T--V--L--N--I--Y--R--G--I--L--N--V--F-	140
5	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
	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
	601	ACAGAGAAATGTGGAAAGTGCCAGGAGGACACTAAAAACCTGAAAGGAGTTTCATCATAC	660
15	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	CTGATCCAGTTCTCACCTTTCACTGAGCAGCATGGAGCCGCCATATGGAGACCAAGCAA	780
	241	-L--I--Q--F--S--P--F--S--E--Q--H--G--A--A--H--M--E--T--K--Q-	260
	781	TCCTTGATGCTCCTTGACGTTTGAAGACCCCTTATGCACCCACTACACCACCACCCAG	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	GCTGAGTATTCACACCGTGGAAATCTCACATATCAGTTCTCCACTGAGCTTCTTCAGTTA	900
	281	-A--E--Y--S--H--R--G--N--L--T--Y--Q--F--S--T--E--L--L--Q--L-	300
	901	CCCATTCTGCTCCTCAATATCAACGACATAGAGTCTCAGCTCGAGGACACTCTGGTCAAA	960
30	301	-P--I--L--L--L--N--I--N--D--I--E--S--Q--L--E--D--T--L--V--K-	320
	961	CAGGCTGTAGAAAGAGTTCATGAAGATGCACCTCTGGAATTTTGAAGTTTGTCAACTC	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	GCCCACAGAAAATGGATCATGGACGCCATCCCTGCTGTGGGAAATCCTGATGCTCTGAGA	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
	1201	ATTACTTCATTTACATGGTGACAGCAACCACTGAGGCCATTGAGGTCATCGAGAGCCTA	1260
45	401	-I--T--S--F--H--M--V--T--A--T--T--E--A--I--E--V--I--E--S--L-	420
	1261	ACAAAGGAAAGCAAAATAGTGAGAAACCCAGTTCTGCGTCAGATTGTATTCTTGGCTAC	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	GGTACCATGATTTACAAACACTGCTATGAGAGGACTTCCTGTCTGCTGAGCTCATAACAG	1380
	441	-G--T--M--I--Y--K--H--C--Y--E--R--T--S--C--P--A--E--L--I--Q-	460
	1381	CCCATTCAAGACCTTCTTGCAGGCACTGAAAGATGGAAACACAGAGGACATCATCCTG	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	TTTGTGAAGGCTTTGGGAAATGCTGCGCATCCTTCTAGCCTCAAGAAAATCACAAGATG	1500
	481	-F--V--K--A--L--G--N--A--A--H--P--S--S--L--K--K--I--T--K--M-	500
	1501	CTGCCCTTACATAGTAAATTAGGTTTCATCACTGCCAGTGAGAGTTTCATGCTGAAGCCATG	1560
60	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	GAGACAAAACCTTCAATGAGTTTGGTGTGAGCTGTTGTTCATATTGTGAAGACAGATACA	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	AATTTGCAAGTAGTAAGCTTCACCTATTTCCACATGAAGTCCCTGACTAGGAGCACCAGC	1800
	581	-N--L--Q--V--V--S--F--T--Y--S--H--M--K--S--L--T--R--S--T--S-	600
	1801	GTTATTTATGCCTCAGTTGCTGCAGCATGCAAAGCTGCCCTGAGAATGTTGGGCCCCAAC	1860
	601	-V--I--Y--A--S--V--A--A--A--C--K--A--A--L--R--M--L--G--P--N-	620
15	1861	CTGGACAAACTGAGCTCACGTTTCAGCAAGCCATCCATGTCGACGTCTATAGCAGTCCC	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	TTTATGCTTGGTGTGCTGCGACTGCTTACTACATCAATGATGCTGCCACCATCATGCCC	1980
	641	-F--M--L--G--A--A--A--T--A--Y--Y--I--N--D--A--A--T--I--M--P-	660
	1981	AAATCTATTACGACTAGGATCAAGGCTTCTTTGCTGGAGCTGCTGCTGACATTCTGGAG	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	GTTGGAGTAAGAAGTCTGAGGGACTACAGGAGGCTTTTCTGAAAAACCCAGCAGTTTGTGAT	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
	701	-S--A--D--R--V--T--R--M--K--H--V--I--K--A--L--S--H--W--K--S-	720
30	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	AATGCTGACACGTTTGGTAGAGATGTTTCAAGGCTCTGCAGTCTGGTCTCTACTTTGCGC	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
	801	-P--M--E--L--G--L--Y--T--A--A--V--A--A--V--P--G--Q--I--K--V-	820
45	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
	2521	CACATTAGTACCAAGGTACACCAAGTGTGCTGTGAACACATTTGCTGTGTTGGGATA	2580
	841	-H--I--S--T--K--V--T--P--S--V--A--V--N--T--F--A--V--F--G--I-	860
50	2581	AACACTGCCATACTCCAGGCTGTGATGGTATCCAGAGCCAAACTCTACTCCATCACACCA	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	GCCAAAACGAAGTCACATTTAACATCAATGAGGGCTACTTGAATTTACAGCTCTTCCT	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
	901	-V--S--V--P--E--N--I--T--A--V--E--V--E--T--F--A--V--V--R--N-	920
60			

	2761	CCTGCTTCGGGAGAAAGAATCACTCCTGTGATCCCTGCCAACCAAGACAGATTCTTATA	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	GCTCAGACTGTTAACGCTGGACTCAAGGCCCTGTCTCAAGATTGCCACTGCTTACACGGGG	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	AAGATCATCAAACACATCAGCTTAGAGAAGAAGAAATCCCAGAGGAAACACCACTCTTA	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	TCCAGTTCAGGAGCTCTCGCTTTCATGTCAGATCCTCTTCTCCAATTCAGCTCTTCA	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	TCTGTCACTACTTCCAAGTCCAGCTTTGCATCGAGCTTTGCATCACTCTTCAGTCTTAGT	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	TCAAGCTCTTCTCACTACAGTGCAGCACCACAGAAAGCATCCTGCGAGTCGCCACAAACCC	3480
	1141	-S--S--S--S--H--Y--S--A--H--H--R--K--H--P--A--S--R--H--K--P-	1160
	3481	AAGGAGAAACACAAGCATCCACCTCTAAAGCCACATCGTCACAGGTTTCAAAGCAGA	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	TTCAGTCTTACATGGACAAAACACCAGCAGACTTCAAATCATTCTAGATGACATTGTT	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	CCTGATAACAACCTGGAGGCTCTGTGCTGATGGAGCCGTGTTGAGCATGCACAAAGTCAAA	3780
	1241	-P--D--N--N--W--R--L--C--A--D--G--A--V--L--S--M--H--K--V--K-	1260
	3781	GCTAAAATGAAGTGGGAGCAGAATGCAACCAATATGACACCACGATTACAACAGAACT	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	GGTCTTGTGCGTGGAAACCTGCAGCTCGGCTGAAGGTGGACTGGAATCGGCTACCGTCT	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	TTGATTCAAGAAAAGGACAGAAACAGCGACAAGCAGCTCTCGTTGACTGTGGCTGTAGTA	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	TCTGACAAGATCATCGACCTGATTGGAAAACACCGAGAAGCACTGTTTCATAAGCGGGCT	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	GTCTCTGGAAAAGTCAAGCACTTGTAGCTGCGGCTGGCGCAGCTGAATGTAGCTTCACC	4200
	1381	-V--S--G--K--V--K--H--L--L--A--A--A--G--A--A--E--C--S--F--T-	1400
	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
15	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	CCATATCGTTATCCCAAGCTGACATCCAGATCAAACAAAATGGCGAAGGCATCTCTGTG	4500
	1481	-P--Y--R--Y--P--Q--A--D--I--Q--I--K--Q--N--G--E--G--I--S--V-	1500
	4501	TATGCAGCTAGCTATGGTCTTCATGAAGTCTACTTTGACAAGAAGTCATGGAAGATTAAA	4560
30	1501	-Y--A--A--S--Y--G--L--H--E--V--Y--F--D--K--K--S--W--K--I--K-	1520
	4561	GTTGTGGACTGGATGAAGGGGAAGACTTGTGGGCTCTGTGGAAGGCTGACGGGGAGACC	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	TCTTGGATTCTGCCAGCTGAGAGCTGCAGAGACGCCACTGAGTGCCGTATGAGGCATGAA	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
	4801	GACCTGTGCTGCGCTGCATGGCTGGGTGCTTCCCTGTGCGCACCACCAACGTCAGTGTT	4860
45	1601	-D--P--V--L--R--C--M--A--G--C--F--P--V--R--T--T--N--V--T--V-	1620
	4861	GGCTTCCACTGCCTTCCAGCTGGTTCCAGCCCCCTCCAGCATGTATACGAGCGTGGACCTG	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	ATGGAACACTACGGAGAGTCACCTCGCCTGCACCTGCACTGCTCAGTGTGCTTAA	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
10	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
	61	-N--A--I--D--Q--N--T--Y--F--I--K--L--E--E--P--E--L--Q--E--Y-	80
15	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	GCTGAGCTCAGACTCCCATTAAGTTTGAATATGTCAATGGTGCTGTTGGAAAAGTCTTC	360
	101	-A--E--L--T--T--P--I--K--F--E--Y--V--N--G--A--V--G--K--V--F-	120
20	361	GGCCCTGAAACCGTCTCAACAACAGTGCTTAACATCTACAGAGGTATCCTGAATGTCTTT	420
	121	-A--P--E--T--V--S--T--T--V--L--N--I--Y--R--G--I--L--N--V--F-	140
25	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
	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
30	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
	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
40	721	CTGATCCAGTTCTCACCTTTTCAGTGAGCAGCATGGAGCCGCCCATATGGAGACCAAGCAA	780
	241	-L--I--Q--F--S--P--F--S--E--Q--H--G--A--A--H--M--E--T--K--Q-	260
	781	TCCTTGATGCTCCTTGACGTTTGAAGACCCCTTATGCACCCACTACACCACCAGGCTGA	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

	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
55	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	GCTGAGCTCACGACTCCCATTAAGTTTGAATATGTCAATGGTGTGTTGGAAAAGTCTTC	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
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	AAGACCAGGGACATGAGCCACTGTCAAGAAAGAATAACTAAAGACATGGGGTTAGCATAC	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	ACAGAGAAATGTGGAAGTGCCAGGAGGACACTAAAAACCTGAAAGGAGTTTTCATCATAC	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	CTGATCCAGTTCTCACCTTTTCAGTGAGCAGCATGGAGCCGCCCATATGGAGACCAAGCAA	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	TCCTTGATGCTCCTTGACGTTTCAAGACACCCAGGCTGAGTATTACACCGTGGAAATC	840
	261	-S--L--M--L--L--D--V--R--R--H--P--P--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	ACGCCTCTGCCTGCAGATCCTCACATCCACCAGCCATGAGGGTGCTTGTACTAGCTCTTG	120
		-M--R--V--L--V--L--A--L--	8
55	121	CTGTGGCTCTCGCAGTGGGGGACCAGTCCAACCTGGCCCCAGGATTCGCCTCTGTTAAGA	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
5	241 GAGCTGGGGTTAAATCCGGGGCAAAGTTTGTGATCAGTGCAACAAGTGCCAACGACTACA	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 TTGAGTATACAAACGGCGTTGTGGGAGACTGGCTGCACCTCCTGGGGTCTCCACAACAG	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 AGGAGAAAATCATGAAGGCCATCGGCTTGGAACACGTAGAGAAATGCCATGATTGTGAAG	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 GTGGTTCTCTGATTATGGAGGCTGTGCTAGAGAGGTTCAGAGTTTTCACCTTTCAACA	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 TTTTGAATGGCGTCTCAGATGGAGTCTAAGCAAATCTGACCTTCTGGATATTGAGA	900
	249 I--L--N--G--A--A--Q--M--E--S--K--Q--I--L--T--F--L--D--I--E--	268
	901 ACACCCCTGTGGATCATGCCAGATACACCTATGTTACCGCGGATCCCTGCAGTATGAGC	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
45	1021 AGATTGTCTAGCACTCTGAACCACCTGGTAGCCTCCAACGTGGCCAAGGTCCATGAAGATG	1080
	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 TCCCCACATTCGCACTCAGCTGCGCTTAAGTTCCTCATTGAGAAGCTCCTTGCTAATG	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 AGTTAAGTGAAGCTGCTATGGCTCTCTTGAATGTCTGCACTCTGTGACAGCTG	1320
	389 E--L--S--E--T--E--A--A--M--A--L--L--E--C--L--H--S--V--T--A--	408
	1321 ACCAGAAAACCATTGAACCTGTGAGAAGCCTGGCTGAGAACCACAGAGTGAACGTAACG	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	CGCAGCCATCTTGCTCATCTGATCTTGTGACACCTGTACATAGACAAGTTGCAGAGGCTG	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	GAGTCCCACTCAAAGTTCAGGTTGACGCTGTTCTAGCCCTGAGGAGAATTGCAAAGAGGG	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	CTTACATGAAGGCCATGGCCAAGACCACATCCCCTGACCACGTTTCTGTTGCTGCAGCAT	1920
	589	S--Y--M--K--A--M--A--K--T--T--S--P--D--H--V--S--V--A--A--A--	608
	1921	GTAATGTTGCCTTGAGGTTCTCAACCCCAAATTAGGCAGACTGAACTCCGCTACAGCC	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
35	2041	TCTTAATTAACGACGCTGCAACCGTGTTACCAAGAATGATTATGGCCAAAGCCCGTACTT	2100
	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	AGGCTCTTTTGAAGACGACATGAAAATTCTGAGAATGCAGACAGGATCACCAAGATTA	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	CTATGTATGTGAAGGCTCTTCGGACAAGAAATTGCATTTGCCAACATTGACAAATCCAAGG	2340
	729	S--M--Y--V--K--V--F--G--Q--E--I--A--F--A--N--I--D--K--S--K--	748
50	2341	TTGACCAGCTTATCCAGTTTGCCAGTGGACCTTTGAGAAACGTATTGAGAGATGCTGTGA	2400
	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	TGACTGCTGCATCTGTTGACGTCCAAGCCACTGTGTCAACACCTCTGCCTGTCAACTACC	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	GAGTTTCCCAGCTTCTGGAGTCCGATATCCAACCTGAGGGCTACAGTTGCTCCAAGTCTTG	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	CAAAAGCCAAAGTTTACACAGCTGTTCTGTCACAGATAAAAGCAAGGATTGACATTGTTA	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	AGGGCAACTTGAAGGTTGAGTTCCTGTCACTCCAGGGCATTACACAATTGCATCTGCAC	2820
	889	K--G--N--L--K--V--E--F--L--S--L--Q--G--I--N--T--I--A--S--A--	908
	2821	ATGCGGAGACGGTTGCCATTGCAAGAAATGTGGAAGACCTCCAGCCGCAAGAAGCACAC	2880
	909	H--A--E--T--V--A--I--A--R--N--V--E--D--L--P--A--A--R--S--T--	928
15	2881	CACTGATCTCATCTGAACTGCATCACAACTTTCAAAGGCCTCTCTCAACTCAAAGATCT	2940
	929	P--L--I--S--S--E--T--A--S--Q--L--S--K--A--S--L--N--S--K--I--	948
	2941	CCAGGATGGCATCTCTGTGACTGGTGGCATGTCTGCGTCATCTGAAATCATTCCTGCTG	3000
20	949	S--R--M--A--S--S--V--T--G--G--M--S--A--S--S--E--I--I--P--A--	968
	3001	ACCTGCCAAGTAAGATTGGGAGGAAATGAACTCCCTAAAACCTACAGGAAGAAAATCC	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	GTGCTTCAAGCAGAATGCTAGGATTCAAGGCCTACGCTGAGATTAAATCTCACAATGCCG	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	TTGCTCCAGCTTCTGGACCAGTCATTGAGAAGATTGAAGTTGAGATTCAAGTCGGAGATA	3240
	1029	I--A--P--A--S--G--P--V--I--E--K--I--E--V--E--I--Q--V--G--D--	1048
	3241	AAGCAGCAGAAAATATGATTAAGCGATTGACATGAGCGAAGAGGAGGAAAGCTCTTGAGG	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	ATAAGAATGTCTCTTGAAAATCAAGAAAATACTGGCACCTGGTCTCAAGAACACCACAT	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	CATCTTCCTCCAGCTCCTCCAGCTCCTCTTCATCCAGCTCTAGCTCCAACAAGTCTTCTT	3420
	1089	S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--S--N--K--S--S--	1108
	3421	CATCCAGTTCCCCTCCAGCAGCTCCCAGTCATCCAGCTCTCGTTCCCATAGGTCTCGCT	3480
45	1109	S--S--S--S--R--S--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
	3541	CTTCTCTCTTTCATCATCCAGATCTTCTTCCAGGTCACTTCCAGATCATCTTCCAGAT	3600
50	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	GCACTGCTTCATACTTGCTCAGCTCATCTAAGTCATCAAGCTCTAGATCCTCTCGGCGCA	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	GTGCTCAGTCTAAGCAACAAGTCTGCCTTGAAGTTCAGAAAGAACCACGTCCACAGGC	3840
	1229	S--A--Q--S--K--Q--L--L--A--L--K--F--R--K--N--H--V--H--R--	1248
5	3841	ATGCCATCTCCACACAGCGCGGAGCAGTCACAGCAGTGCCCGCAGCTTCGATTCCATCT	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	CCAATAGACTGCAGCTGATTTTTGCCAGAGTCGCTGAGAAGGACAACTGGAGAATCTGTG	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	CCGACATTGTGCAGCTGAGTTTCGCACAAGATGATGGCCAAGATTGCCTGGGGTGCTGAAT	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	GCAAGCAATACTCCACCATGATTGTAGCTGAACTGGTCTTTTGGGTCATGAGCCCGCAG	4200
	1349	C--K--Q--Y--S--T--M--I--V--A--E--T--G--L--L--G--H--E--P--A--	1368
	4201	CCCGCTTGAAGCTGACCTGGGACAACTGCCAGGAAGCATAAAGCACTACGCAAAGAGGG	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	CGTTGAAATCCATTGTCCCTATTGCTCAAGAATATGGAGTAACTACGCAAAGGCCAAGA	4320
	1389	A--L--K--S--I--V--P--I--A--Q--E--Y--G--V--N--Y--A--K--A--K--	1408
	4321	ATCCTCGTAATCAAATCAAAGTACTGTAGCTGTTGCTACTGAGACAAGCATGAATATTG	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	TGCTGAACACACCAAAGCAATCATTTACAAGCGTGGGGTGTGTCTACCTGTGCTTTAC	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	CAATTGGAACACTGCTGCCGAGCTGCAAGCGACCCGGGACAACTGGGCTGACAAGATGT	4500
	1449	P--I--G--N--T--A--A--E--L--Q--A--T--R--D--N--W--A--D--K--M--	1468
	4501	CCTATTTGGTTACCAAAGCTAACGCAGTTGAATGCTCCCTCATCAACAACACTGACCA	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	AGGATTGCACACCAGAAGTCAAATTCATGGTTCTGCTGAAGAAAGACCAAATACAGGATC	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	AGAAGCAGATCAATGTTAAGATTCAGACATCGATGTGGACATGTATCGGAAGAACAACG	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	TGAAAGGAAAGACTTGTGGTGCCTGTGGAACGGCAAGCGGAAATGTCGGAGACGAGTACC	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 CTTCAAACACCTGCCGTGATCCCTCCGAGTGTTCATCAAGCAGGAATCTGTGAAGCTGG	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 AGTGCCTGCCCGGCTGTATCCCAGTGAGAACCCTACCGTCAACGTTGGCTTTCCTACTGCC	5220
	1689 Q--C--L--P--G--C--I--P--V--R--T--T--T--V--N--V--G--F--H--C--	1708
	5221 TGCCCACTGACACAACCTGTGGACCGTTCTGGTCTGAGCAGCTTCTTTGAGAAGAGCATCG	5280
	1709 L--P--S--D--T--T--V--D--R--S--G--L--S--S--F--F--E--K--S--I--	1728
15	5281 ACCTGAGGGATACTGCAGAAGCCCACCTGGCCTGTCGCTGCACTCCTCAGTGTGCTTAA	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 ACGCCTCTGCCTGCAGATCCTCAGCATCCAGCCATGAGGGTGCTTGTACTAGCTCTTG	120
	-M--R--V--L--V--L--A--L--	8
30	121 CTGTGGCTCTCGCAGTGGGGGACCACTCAACTTGGCCCCAGGATTGCGCTCTGTTAAGA	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 GAGCTGGGGTTAAATCCGGGGCAAAGTTTGTGATCAGTGCAACAAGTGCCAACGACTACA	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 TTCTGAAGCTTGTAACCTCAGTTGCTGGAGTACAGTGGCATCTGGCCCAAGATCCTT	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
55	601 GGGAGGGCCGAACGCATTATCTGACCAAGACCAAGGACCTGAACCACTGCCAGGAGAAA	660
	169 R--E--G--R--T--H--S--S--D--Q--D--Q--G--P--E--P--L--P--G--E--	188
	661 ATCATGAAGGCCATCGGCTTGGAAACACGTAGAGAAATGCCATGATTGTGAAGCTAGAGGA	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

10 TGTAACGACGGCCAGT**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.

WHAT IS CLAIMED IS:

1. 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.
2. 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.
3. The method of claim 2, wherein the fertility rescue comprises germline stem cell transplantation.
4. The method of claim 3, wherein the fertility rescue further comprises sex steroid alteration.

5. The method of claim 4, wherein the alteration of sex steroid is an alteration of estrogen, or an alteration of an aromatase inhibitor.
6. The method of any one of claims 3-5, wherein the germline stem cell transplantation comprises 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.
7. The method of claim 6, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or mollusk are homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.
8. The method of claim 6, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using ploidy manipulation.
9. The method of claim 6, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created by hybridization.
10. The method of claim 6, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using exposure to high levels of sex hormones.
11. The method of any one of claims 3-5, wherein the germline stem cell transplantation comprises 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.

12. The method of claim 11, wherein the germ cell-less fertile male fish, crustacean, or mollusk and the germ cell-less fertile female fish, crustacean, or mollusk are homozygous for the mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

13. The method of claim 11, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using ploidy manipulation.

14. The method of claim 11, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created by hybridization.

15. The method of claim 11, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using exposure to high levels of sex hormones.

16. The method of any one of claims 1-15, wherein the sterile sex-determined sterile fish, crustacean, or mollusk is a sterile male fish, crustacean, or mollusk.

17. The method of any one of claims 1-16, wherein the first mutation comprises a mutation in one or more genes that modulates the synthesis of androgen and/or estrogen.

18. The method of claim 17, wherein the first mutation comprises a mutation in one or more genes that modulate the expression of aromatase Cyp19a1a, Cyp17, or a combination thereof.

19. The method of claim 18, wherein the one or more genes that modulate the expression of aromatase Cyp19a1a is one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof.
20. The method of claim 17, wherein the one or more genes that modulate the expression of Cyp17 is *cyp17l* or an ortholog thereof.
21. The method of any one of claims 1-20, wherein the second mutation comprises a mutation in one or more genes that modulate spermiogenesis.
22. The method of claim 21, wherein the second mutation comprises a mutation in one or more genes that cause globozoospermia.
23. The method of claim 22, wherein the second mutation in one or more genes that cause globozoospermia causes sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof.
24. The method of claim 23, wherein the second mutation comprises a mutation in one or more genes selected from the group consisting of *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof.
25. The method of any one of claims 1-15, wherein the sterile sex-determined sterile fish, crustacean, or mollusk is a sterile female fish, crustacean, or mollusk.
26. The method of any one of claims 1-15 and 25, wherein the first mutation comprises a mutation in one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor.
27. The method of claim 26, wherein the one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor is one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.

28. The method of any one of claims 1-15 and 25-27, wherein the second mutation comprises a mutation in one or more genes that modulate oogenesis, folliculogenesis, or a combination.
29. The method of claim 28, wherein the one or more genes that modulate oogenesis modulates the synthesis of estrogen.
30. The method of claim 29, wherein the one or more genes that modulate the synthesis of estrogen is FSHR or an ortholog thereof.
31. The method of claim 28, wherein the one or more genes that modulate folliculogenesis modulates the expression of vitellogenins.
32. The method of claim 31, wherein the one or more genes that modulate the expression of vitellogenins is *vtgs* or an ortholog thereof.
33. The method of claim 31, wherein the one or more genes that modulate the expression of vitellogenins is 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.
34. 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 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.

35. The method of claim 34, wherein the mutation that directly or indirectly disrupts spermiogenesis is a mutation in *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, or an ortholog thereof.
36. The method of claim 34 or 35, wherein the mutation that directly disrupts vitellogenesis is 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.
37. The method of claim 34, 35, or 36, wherein the fertile female fish, crustacean, or mollusk and the fertile male fish, crustacean, or mollusk have a plurality of homozygous mutations that, in combination: directly or indirectly disrupt spermiogenesis; directly disrupt vitellogenesis; or both.
38. The method of any one of claims 34-37, wherein the fertility rescue comprises germline stem cell transplantation.
39. The method of claim 38, wherein the fertility rescue further comprises sex steroid alteration.
40. The method of claim 39, wherein the alteration of sex steroid is an alteration of estrogen, or an alteration of an aromatase inhibitor.
41. The method of any one of claims 38-40, wherein the germline stem cell transplantation comprises 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.

42. The method of claim 41, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or mollusk are homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.
43. The method of claim 41, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using ploidy manipulation.
44. The method of claim 41, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created by hybridization.
45. The method of claim 41, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using exposure to high levels of sex hormones.
46. The method of any one of claims 34-45, wherein the fertile female fish, crustacean, or mollusk and the fertile male fish, crustacean, or mollusk have an additional homozygous mutation that specifies sexual differentiation.
47. The method of claim 46, wherein the mutation that specifies sexual differentiation modulates the expression of aromatase Cyp19a1a, Cyp17, an inhibitor to aromatase Cyp19a1a, or a combination thereof.
48. The method of claim 47, wherein the mutation that modulates the expression of Cyp17 is a mutation in *cyp17l* or an ortholog thereof.
49. The method of claim 47 or 48, wherein the mutation that modulates the expression of aromatase Cyp19a1a inhibitor is a mutation in *Gsdf*, *dmrt1*, *Amh*, *Amhr*, or an ortholog thereof.

50. The method of any one of claims 34-45, wherein the breeding step comprises hybridization or hormonal manipulation and breeding strategies, to specify sexual differentiation.
51. The method of any one of claims 1-50, wherein the fish, crustacean, or mollusk is a fish.
52. 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.
53. The fertile homozygous mutated fish, crustacean, or mollusk of claim 52, wherein the fertility rescue comprises germline stem cell transplantation.
54. The fertile homozygous mutated fish, crustacean, or mollusk of claim 53, wherein the fertility rescue further comprises sex steroid alteration.
55. The fertile homozygous mutated fish, crustacean, or mollusk of claim 54, wherein the alteration of sex steroid is an alteration of estrogen, or an alteration of an aromatase inhibitor.
56. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 53-55, wherein the germline stem cell transplantation comprises 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.

57. The fertile homozygous mutated fish, crustacean, or mollusk of claim 56, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or mollusk are homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

58. The fertile homozygous mutated fish, crustacean, or mollusk of claim 56, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using ploidy manipulation.

59. The fertile homozygous mutated fish, crustacean, or mollusk of claim 56, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created by hybridization.

60. The fertile homozygous mutated fish, crustacean, or mollusk of claim 56, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using exposure to high levels of sex hormones.

61. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 53-55, wherein the germline stem cell transplantation comprises 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.

62. The fertile homozygous mutated fish, crustacean, or mollusk of claim 61, wherein the germ cell-less fertile male fish, crustacean, or mollusk and the germ cell-less fertile female fish, crustacean, or mollusk are homozygous for the mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

63. The fertile homozygous mutated fish, crustacean, or mollusk of claim 61, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using ploidy manipulation.
64. The fertile homozygous mutated fish, crustacean, or mollusk of claim 61, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created by hybridization.
65. The fertile homozygous mutated fish, crustacean, or mollusk of claim 61, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using exposure to high levels of sex hormones.
66. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 52-65, wherein the sterile sex-determined sterile fish, crustacean, or mollusk is a sterile male fish, crustacean, or mollusk.
67. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 52-66, wherein the first mutation comprises a mutation in one or more genes that modulates the synthesis of androgen and/or estrogen.
68. The fertile homozygous mutated fish, crustacean, or mollusk of claim 67, wherein the first mutation comprises a mutation in one or more genes that modulate the expression of aromatase Cyp19a1a, Cyp17, or a combination thereof.
69. The fertile homozygous mutated fish, crustacean, or mollusk of claim 68, wherein the one or more genes that modulate the expression of aromatase Cyp19a1a is one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof.
70. The fertile homozygous mutated fish, crustacean, or mollusk of claim 68, wherein the one or more genes that modulate the expression of Cyp17 is *cyp17l* or an ortholog thereof.

71. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 52-70, wherein the second mutation comprises a mutation in one or more genes that modulate spermiogenesis.

72. The fertile homozygous mutated fish, crustacean, or mollusk of claim 71, wherein the second mutation comprises a mutation in one or more genes that cause globozoospermia.

73. The fertile homozygous mutated fish, crustacean, or mollusk of claim 72, wherein the second mutation in one or more genes that cause globozoospermia causes sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof.

74. The fertile homozygous mutated fish, crustacean, or mollusk of claim 73, wherein the second mutation comprises a mutation in one or more genes selected from the group consisting of *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof.

75. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 52-65, wherein the sterile sex-determined sterile fish, crustacean, or mollusk is a sterile female fish, crustacean, or mollusk.

76. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 52-65 and 75, wherein the first mutation comprises a mutation in one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor.

77. The fertile homozygous mutated fish, crustacean, or mollusk of claim 76, wherein the one or more genes that modulate the expression of an aromatase Cyp19a1a inhibitor is one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.

78. The fertile homozygous mutated fish, crustacean, or mollusk of any one of claims 52-65 and 75-77, wherein the second mutation comprises a mutation in one or more genes that modulate oogenesis, folliculogenesis, or a combination.

79. The fertile homozygous mutated fish, crustacean, or mollusk of claim 78, wherein the one or more genes that modulate oogenesis modulates the synthesis of estrogen.
80. The fertile homozygous mutated fish, crustacean, or mollusk of claim 79, wherein the one or more genes that modulate the synthesis of estrogen is FSHR or an ortholog thereof.
81. The fertile homozygous mutated fish, crustacean, or mollusk of claim 80, wherein the one or more genes that modulate folliculogenesis modulates the expression of vitellogenins.
82. The fertile homozygous mutated fish, crustacean, or mollusk of claim 80, wherein the one or more genes that modulate the expression of vitellogenins is *vtgs* or an ortholog thereof.
83. The fertile homozygous mutated fish, crustacean, or mollusk of claim 82, wherein the one or more genes that modulate the expression of vitellogenins is 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.
84. 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.
85. The fertile fish, crustacean, or mollusk of claim 84, wherein the mutation that directly or indirectly disrupts spermiogenesis is a mutation in *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, or an ortholog thereof.
86. The fertile fish, crustacean, or mollusk of claim 84 or 85, wherein the mutation that directly disrupts vitellogenesis is 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.

87. The fertile fish, crustacean, or mollusk of claim 84, 85, or 86, wherein the fertile fish, crustacean, or mollusk has a plurality of homozygous mutations that, in combination: directly or indirectly disrupt spermiogenesis; directly disrupt vitellogenesis; or both.

88. The fertile fish, crustacean, or mollusk of any one of claims 84-87, wherein the fertility rescue comprises germline stem cell transplantation.

89. The fertile fish, crustacean, or mollusk of claim 88, wherein the fertility rescue further comprises sex steroid alteration.

90. The fertile fish, crustacean, or mollusk of claim 89, wherein the alteration of sex steroid is an alteration of estrogen, or an alteration of an aromatase inhibitor.

91. The fertile fish, crustacean, or mollusk of any one of claims 88-90, wherein the germline stem cell transplantation comprises 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.

92. The fertile fish, crustacean, or mollusk of claim 91, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish, crustacean, or mollusk are homozygous for a null mutation of the *dnd*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

93. The fertile fish, crustacean, or mollusk of claim 91, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using ploidy manipulation.

94. The fertile fish, crustacean, or mollusk of claim 91, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created by hybridization.
95. The fertile fish, crustacean, or mollusk of claim 91, wherein the germ cell-less recipient male fish, crustacean, or mollusk and the germ cell-less recipient female fish crustacean, or mollusk are created using exposure to high levels of sex hormones.
96. The fertile fish, crustacean, or mollusk of any one of claims 84-95, wherein the fertile fish, crustacean, or mollusk has an additional homozygous mutation that specifies sexual differentiation.
97. The fertile fish, crustacean, or mollusk of claim 96, wherein the mutation that specifies sexual differentiation modulates the expression of aromatase Cyp19a1a, Cyp17, an inhibitor to aromatase Cyp19a1a, or a combination thereof.
98. The fertile fish, crustacean, or mollusk of claim 97, wherein the one or more genes that modulate the expression of aromatase Cyp19a1a is one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof.
99. The fertile fish, crustacean, or mollusk of claim 97, wherein the one or more genes that modulate the expression of aromatase Cyp19a1a inhibitor is one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.
100. The fertile fish, crustacean, or mollusk of any one of claims 84-95, wherein producing a sterile sex-determined fish, crustacean, or mollusk comprises a breeding step comprising hybridization or hormonal manipulation and breeding strategies, to specify sexual differentiation.
101. The fertile fish, crustacean, or mollusk of any one of claims 52-100, wherein the fertile fish, crustacean, or mollusk is a fish.

102. 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.