

TRANSGENIC BETTA

CROSS REFERENCE TO RELATED APPLICATIONS

This application is being filed on 13 November 2019, as a PCT International patent application, and claims priority to U.S. Patent Application No. 62/760,445, filed November 13, 2018 and entitled Transgenic Green Betta, U.S. Patent Application No. 62/760,464, filed November 13, 2018 and entitled Transgenic Orange Betta, U.S. Patent Application No. 62/760,480, filed November 13, 2018 and entitled Transgenic Red Betta, U.S. Patent Application No. 62/760,489, filed November 13, 2018 and entitled Transgenic Purple Betta, and U.S. Patent Application No. 62/760,498, filed November 13, 2018 and entitled Transgenic Blue Betta, the disclosures of which are hereby incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

This invention relates to transgenic fish. Specifically, the invention relates to orange transgenic betta. Specifically, the invention relates to purple transgenic betta. Specifically, the invention relates to blue transgenic betta. Specifically, the invention relates to green transgenic betta. Specifically, the invention relates to red transgenic betta. Specifically, the invention relates to pink transgenic betta.

INTRODUCTION

Transgenic technology involves the transfer of a foreign gene into a host organism enabling the host to acquire a new and inheritable trait. Transgenic technology has many potential applications. For example, it can be used to introduce a transgene into a fish in order to create new varieties of fish. There are many ways of introducing a foreign gene into fish, including: microinjection (*e.g.*, Zhu *et al.*, 1985; Du *et al.*, 1992), electroporation (Powers *et al.*, 1992), sperm-mediated gene transfer (Khoo *et al.*, 1992; Sin *et al.*, 1993), gene bombardment or gene gun (Zelenin *et al.*, 1991), liposome-mediated gene transfer (Szelei *et al.*, 1994), and the direct injection of DNA into muscle tissue (Xu *et al.*, 1999). The first transgenic fish

report was published by Zhu *et al.*, (1985) using a chimeric gene construct consisting of a mouse metallothionein gene promoter and a human growth hormone gene. Most of the early transgenic fish studies have concentrated on growth hormone gene transfer with an aim of generating fast growing fish. While a majority of early attempts used heterologous growth hormone genes and promoters and failed to produce these fish (e.g. Chourrout *et al.*, 1986; Penman *et al.*, 1990; Brem *et al.*, 1988; Gross *et al.*, 1992), enhanced growth of transgenic fish has been demonstrated in several fish species including Atlantic salmon, several species of Pacific salmon, and loach (e.g. Du *et al.*, 1992; Delvin *et al.*, 1994, 1995; Tsai *et al.*, 1995).

10 **[0001]** The Siamese fighting fish (*Betta splendens*), also sometimes colloquially known as the betta, is a species in the gourami family, which is popular as an aquarium fish. Species assigned to *Betta splendens* display a broad diversity of body shapes and coloration. The genus is well known among aquarists. Bettas can be territorial fish and are prone to high levels of aggression towards each other. Two males in proximity will almost always attack each other, if they do not have the ability to escape this will usually result in the death of one or both of the fish. Female bettas can also become territorial towards each other if they are housed in too small an aquarium.

20 **[0002]** Wild fish exhibit strong colors only when agitated. Breeders have been able to make this coloration permanent, and a wide variety of hues breed true. Colors available to the aquarist include red, orange, yellow, blue, steel blue, turquoise/green, black, pastel, white ("opaque" white, not to be confused with albino) and multi-colored fish.

25 **[0003]** Bettas are found in many different colors due to different layers of pigmentation in their skin. The layers (from furthest within to the outer layer) consists of red, yellow, black, iridescent (blue and green), and metallic (not a color of its own, but reacts with the other colors to change how they are perceived). Any combination of these layers can be present, leading to a wide variety of colors.

30 **[0004]** However, for the ornamental fish industry, the bold colors can be enhanced or novel colors can be introduced to provide a more efficient display of the various colors. The availability of such Bettas having modified pigmentation by

transgenesis with fluorescent proteins would result in better products for the ornamental fish industry due to better visualization of the various colors.

5 [0005] Many fluorescent proteins are known in the art and have been used to investigate various cellular processes, including fluorescent proteins exhibiting various green, red, pink, yellow, orange, blue, or purple colors. Although transgenic experiments involving fluorescent proteins have provided new markers and reporters for transgenesis, progress in the field of developing and producing Bettas that express such proteins has been limited.

TRANSGENIC BETTA

10 [0006] In certain embodiments, the present disclosure concerns making transgenic fluorescent fish and providing such fish to the ornamental fish industry.

[0007] In some embodiments, transgenic fish or methods of making transgenic fish are provided. In certain aspects, the transgenic fish are fertile, transgenic, fluorescent fish. In a particular embodiment, the fish for use with the disclosed constructs and methods is the Betta. Betta skin color is determined by pigment cells in the skin, which contain pigment granules called melanosomes (black or brown color), xanthosomes (yellow color), erythrosomes (orange or red color), or iridosomes (iridescent colors, including white color). The number, size, and density of the pigment granules per pigment cell influence the color of the fish skin.

20 [0008] In certain specific embodiments, there are provided transgenic Betta or progeny thereof comprising specific transgenic integration events, referred to herein as transformation events. These fish are of particular interest because, for example, they embody an aesthetically pleasing green color. Transgenic fish comprising these specific transgenic events may be homozygous or heterozygous (including, for example, hemizygous) for the transformation event. Homozygous fish bred with fish lacking a transformation event will in nearly all cases produce 100% heterozygous offspring. Germ cells, eggs, sperm, and embryos comprising these specific transgenic events are also included as part of the invention.

30 [0009] In one such embodiment regarding a specific transgenic integration event, a green transgenic Betta or progeny thereof is provided comprising chromosomally integrated transgenes, wherein the Betta comprises the "Green Betta

1 transformation event,” cryopreserved testes comprising the Green Betta 1
transformation event having been deposited at the ECACC. The chromosomally
integrated transgenes may be present on one integrated expression cassette or two or
more integrated expression cassettes. In certain aspects, such a transgenic Betta is a
5 fertile, transgenic Betta. Such a transgenic Betta may be homozygous or
heterozygous (including, for example, hemizygous) for the transgenes or integrated
expression cassette(s).

[0010] Also disclosed are methods of providing a transgenic Betta
comprising the Green Betta 1 transformation event to the ornamental fish market. In
10 some embodiments, the method comprises obtaining a transgenic Betta or progeny
thereof comprising chromosomally integrated transgenes, wherein the Betta
comprises the “Green Betta 1 transformation event,” cryopreserved testes
comprising the Green Betta 1 transformation event being deposited at the ECACC,
and distributing the fish to the ornamental fish market. Such fish may be distributed
15 by a grower to a commercial distributor, or such fish may be distributed by a grower
or a commercial distributor to a retailer such as, for example, a multi-product retailer
having an ornamental fish department.

[0011] In some aspects, methods of producing a transgenic Betta are
provided comprising: (a) obtaining a Betta that exhibits fluorescence and comprises
20 one or more chromosomally integrated transgenes or expression cassettes, wherein
the Betta comprises the “Green Betta 1 transformation event,” cryopreserved testes
comprising the Green Betta 1 transformation event being deposited at the ECACC;
and (b) breeding the obtained Betta with a second Betta to provide a transgenic Betta
comprising the Green Betta 1 transformation event. The second Betta may be a
25 transgenic or non-transgenic Betta.

[0012] In further embodiments, also provided are methods of producing a
transgenic organism, the method comprising using cryopreserved testes comprising
the Green Betta 1 transformation, such cryopreserved testes deposited at the
ECACC, to produce transgenic offspring. Such offspring may be, for example, a
30 Betta, a species of the *Betta splendens* family, a fish species or genus related to
Betta, or another fish species or genus. In some aspects, the fish may be produced
using *in vitro* fertilization.

[0013] In another such embodiment regarding a specific transgenic integration event, a orange transgenic Betta or progeny thereof is provided comprising chromosomally integrated transgenes, wherein the Betta comprises the
5 “Orange Betta 1 transformation event,” cryopreserved testes comprising the Orange Betta 1 transformation event having been deposited at the ECACC. The chromosomally integrated transgenes may be present on one integrated expression cassette or two or more integrated expression cassettes. In certain aspects, such a transgenic Betta is a fertile, transgenic Betta. Such a transgenic Betta may be
10 homozygous or heterozygous (including, for example, hemizygous) for the transgenes or integrated expression cassette(s).

[0014] Also disclosed are methods of providing a transgenic Betta comprising the Orange Betta 1 transformation event to the ornamental fish market. In some embodiments, the method comprises obtaining a transgenic Betta or
15 progeny thereof comprising chromosomally integrated transgenes, wherein the Betta comprises the “Orange Betta 1 transformation event,” cryopreserved testes comprising the Orange Betta 1 transformation event being deposited at the ECACC, and distributing the fish to the ornamental fish market. Such fish may be distributed by a grower to a commercial distributor, or such fish may be distributed by a grower
20 or a commercial distributor to a retailer such as, for example, a multi-product retailer having an ornamental fish department.

[0015] In some aspects, methods of producing a transgenic Betta are provided comprising: (a) obtaining a Betta that exhibits fluorescence and comprises one or more chromosomally integrated transgenes or expression cassettes, wherein
25 the Betta comprises the “Orange Betta 1 transformation event,” cryopreserved testes comprising the Orange Betta 1 transformation event being deposited at the ECACC; and (b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Orange Betta 1 transformation event. The second Betta may be a transgenic or non-transgenic Betta.

30 [0016] In further embodiments, also provided are methods of producing a transgenic organism, the method comprising using cryopreserved testes comprising the Orange Betta 1 transformation, such cryopreserved testes deposited at the

ECACC, to produce transgenic offspring. Such offspring may be, for example, a Betta, a species of the *Betta splendens* family, a fish species or genus related to Betta, or another fish species or genus. In some aspects, the fish may be produced using *in vitro* fertilization.

5 **[0017]** In another such embodiment regarding a specific transgenic integration event, a red transgenic Betta or progeny thereof is provided comprising chromosomally integrated transgenes, wherein the Betta comprises the “Red Betta 1 transformation event,” cryopreserved testes comprising the Red Betta 1 transformation event having been deposited at the ECACC. The chromosomally
10 integrated transgenes may be present on one integrated expression cassette or two or more integrated expression cassettes. In certain aspects, such a transgenic Betta is a fertile, transgenic Betta. Such a transgenic Betta may be homozygous or heterozygous (including, for example, hemizygous) for the transgenes or integrated expression cassette(s).

15 **[0018]** Also disclosed are methods of providing a transgenic Betta comprising the Red Betta 1 transformation event to the ornamental fish market. In some embodiments, the method comprises obtaining a transgenic Betta or progeny thereof comprising chromosomally integrated transgenes, wherein the Betta comprises the “Red Betta 1 transformation event,” cryopreserved testes comprising
20 the Red Betta 1 transformation event having been deposited at the ECACC, and distributing the fish to the ornamental fish market. Such fish may be distributed by a grower to a commercial distributor, or such fish may be distributed by a grower or a commercial distributor to a retailer such as, for example, a multi-product retailer having an ornamental fish department.

25 **[0019]** In some aspects, methods of producing a transgenic Betta are provided comprising: (a) obtaining a Betta that exhibits fluorescence and comprises one or more chromosomally integrated transgenes or expression cassettes, wherein the Betta comprises the “Red Betta 1 transformation event,” cryopreserved testes comprising the Red Betta 1 transformation event having been deposited at the
30 ECACC; and (b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Red Betta 1 transformation event. The second Betta may be a transgenic or non-transgenic Betta.

[0020] In further embodiments, also provided are methods of producing a transgenic organism, the method comprising using cryopreserved testes comprising the Red Betta 1 transformation, such cryopreserved testes having been deposited at the ECACC, to produce transgenic offspring. Such offspring may be, for example, a
5 Betta, a species of the *Betta splendens* family, a fish species or genus related to Betta, or another fish species or genus. In some aspects, the fish may be produced using *in vitro* fertilization.

[0021] In another embodiment regarding a specific transgenic integration event, a purple transgenic Betta or progeny thereof is provided comprising
10 chromosomally integrated transgenes, wherein the Betta comprises the "Purple Betta 1 transformation event," cryopreserved testes comprising the Purple Betta 1 transformation event having been deposited at the ECACC. The chromosomally integrated transgenes may be present on one integrated expression cassette or two or more integrated expression cassettes. In certain aspects, such a transgenic Betta is a
15 fertile, transgenic Betta. Such a transgenic Betta may be homozygous or heterozygous (including, for example, hemizygous) for the transgenes or integrated expression cassette(s).

[0022] Also disclosed are methods of providing a transgenic Betta comprising the Purple Betta 1 transformation event to the ornamental fish market. In
20 some embodiments, the method comprises obtaining a transgenic Betta or progeny thereof comprising chromosomally integrated transgenes, wherein the Betta comprises the "Purple Betta 1 transformation event," cryopreserved testes comprising the Purple Betta 1 transformation event being deposited at the ECACC, and distributing the fish to the ornamental fish market. Such fish may be distributed
25 by a grower to a commercial distributor, or such fish may be distributed by a grower or a commercial distributor to a retailer such as, for example, a multi-product retailer having an ornamental fish department.

[0023] In some aspects, methods of producing a transgenic Betta are provided comprising: (a) obtaining a Betta that exhibits fluorescence and comprises
30 one or more chromosomally integrated transgenes or expression cassettes, wherein the Betta comprises the "Purple Betta 1 transformation event," cryopreserved testes comprising the Purple Betta 1 transformation event being deposited at the ECACC;

and (b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Purple Betta 1 transformation event. The second Betta may be a transgenic or non-transgenic Betta.

[0024] In further embodiments, also provided are methods of producing a transgenic organism, the method comprising using cryopreserved testes comprising the Purple Betta 1 transformation, such cryopreserved testes deposited at the ECACC, to produce transgenic offspring. Such offspring may be, for example, a Betta, a species of the *Betta splendens* family, a fish species or genus related to Betta, or another fish species or genus. In some aspects, the fish may be produced using *in vitro* fertilization.

[0025] In another embodiment regarding a specific transgenic integration event, a blue transgenic Betta or progeny thereof is provided comprising chromosomally integrated transgenes, wherein the Betta comprises the “Blue Betta 1 transformation event,” cryopreserved testes comprising the Blue Betta 1 transformation event having been deposited at the ECACC. The chromosomally integrated transgenes may be present on one integrated expression cassette or two or more integrated expression cassettes. In certain aspects, such a transgenic Betta is a fertile, transgenic Betta. Such a transgenic Betta may be homozygous or heterozygous (including, for example, hemizygous) for the transgenes or integrated expression cassette(s).

[0026] Also disclosed are methods of providing a transgenic Betta comprising the Blue Betta 1 transformation event to the ornamental fish market. In some embodiments, the method comprises obtaining a transgenic Betta or progeny thereof comprising chromosomally integrated transgenes, wherein the Betta comprises the “Blue Betta 1 transformation event,” cryopreserved testes comprising the Blue Betta 1 transformation event being deposited at the ECACC, and distributing the fish to the ornamental fish market. Such fish may be distributed by a grower to a commercial distributor, or such fish may be distributed by a grower or a commercial distributor to a retailer such as, for example, a multi-product retailer having an ornamental fish department.

[0027] In some aspects, methods of producing a transgenic Betta are provided comprising: (a) obtaining a Betta that exhibits fluorescence and comprises

one or more chromosomally integrated transgenes or expression cassettes, wherein the Betta comprises the “Blue Betta 1 transformation event,” cryopreserved testes comprising the Blue Betta 1 transformation event being deposited at the ECACC; and (b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Blue Betta 1 transformation event. The second Betta may be a transgenic or non-transgenic Betta.

[0028] In further embodiments, also provided are methods of producing a transgenic organism, the method comprising using cryopreserved testes comprising the Blue Betta 1 transformation, such cryopreserved testes deposited at the ECACC, to produce transgenic offspring. Such offspring may be, for example, a Betta, a species of the *Betta splendens* family, a fish species or genus related to Betta, or another fish species or genus. In some aspects, the fish may be produced using *in vitro* fertilization.

[0029] As used in this specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one.

[0030] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

[0031] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0032] Any embodiment of any of the present methods, kits, and compositions may consist of or consist essentially of—rather than comprise/include/contain/have—the described features and/or steps. Thus, in any of the claims, the term “consisting of” or “consisting essentially of” may be substituted for any of the open-ended linking verbs recited above, in order to change the scope of a given claim from what it would otherwise be using the open-ended linking verb.

[0033] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since
5 various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION

Transgenic Fish

[0034] In some aspects, the present disclosure regards transgenic fish.
10 Methods of making transgenic fish are described in, for example, U.S. Patent Nos. 7,135,613; 7,700,825; 7,834,239, each of which is incorporated by reference in its entirety. For example, a transgenic green Betta may be generated using an expression cassette encoding green fluorescent protein (GFP), such as ZsGreen1. In another example, a transgenic orange Betta may be generated using an expression
15 cassette encoding orange fluorescent protein (YFP), such as ZsYellow1. In another example, a transgenic red Betta may be generated using an expression cassette encoding red fluorescent protein (RFP), such as DsRed2. In another example, a transgenic purple Betta may be generated using an expression cassette encoding purple fluorescent protein (PFP), such as FP635. In another example, a transgenic
20 blue Betta may be generated using an expression cassette encoding blue fluorescent protein (BFP), such as TagBFP, or TagBFP in combination with aeCP597.

[0035] It is preferred that fish belonging to species and varieties of fish of commercial value, particularly commercial value within the ornamental fish industry, be used. Such fish include but are not limited to bettas, catfish, zebrafish
25 and other danios, medaka, carp, tilapia, goldfish, tetras, barbs, sharks (family cyprinidae, such as rainbow shark), angelfish, loach, koi, glassfish, discus, eel, goby, gourami, guppy, Xiphophorus, hatchet fish, Molly fish, or pangasius. A particular fish for use in the context of the present disclosure is a Betta. Bettas are increasingly popular ornamental animals and would be of added commercial value in various
30 colors. Betta embryos are easily accessible and nearly transparent. Betta skin color is determined by pigment cells in the skin, which contain pigment granules called

melanosomes. The number, size, and density of the melanosomes per pigment cell influence the color of the fish skin.

5 **[0036]** In commercial aquaculture, green Bettas are spawned naturally pairwise. A mature male is placed into a 1-3 gallon tanks and left overnight. If the male builds a bubble nest, a female is added and left overnight. After the spawning, the female is removed and the male is left in the tank to take care of the developing eggs. After three days, the male is removed and the fry are fed freshly hatched Artemia nauplii for 3-4 weeks, until the fry are large enough to be safely moved to a grow-out vat. It takes about three months for bettas to become mature.

10 Line Regeneration from cryopreserved testes

[0037] Gonad freezing methods are well-known in the art; *see, e.g.*, Lee et al. (2013), and Seki et al. (2017), both of which are incorporated herein by reference in their entireties. To obtain the transgenic fish disclosed herein, germ cells obtained from dissociated frozen betta gonads may be used.

15 **[0038]** In an example embodiment, young adult male green bettas are euthanized using an overdose of sedative, such as Tricaine. The fish are decapitated, blotted dry, sprayed with 70% ethanol, dissected and their testes are removed and transferred into ice-cold L-15 medium. When the desired amount of tissue was obtained, the testes were transferred into ice-cold vitrification solution (L-15
20 medium containing 30% (v/v) of ethylene glycol, 21% (w/v) Ficoll PM-70, and 0.35M sucrose). After 20 minutes of incubation, testes were blotted to remove the vitrification solution and submerged into liquid nitrogen. The samples are kept in liquid nitrogen for long term storage.

[0039] To recover green betta line, the cryopreserved testes are thawed in
25 0.2M sucrose solution in L-15 medium at 25°C for 5 seconds and incubated in the same solution for 5 minutes at 0°C. The thawed testes are transferred into L-15 medium containing 10% Fetal Bovine Serum (FBS), minced, transferred into L-15 medium containing 0.2% collagenase H, 0.15% dispase II, and 900 U/ml of DNase I and incubated for 1 hour at 25°C with constant gentle agitation to dissociate the
30 tissue into cells. The cell suspension is rinsed once with L-15 containing 10% FBS, and filtered through 60-micron mesh. The cell suspension is then injected into 7 day

old betta fry anesthetized with Tricaine. The injected fry are reared to adulthood and spawned using regular protocol to obtain green betta progeny.

[0040] The present disclosure further encompasses progeny of a transgenic fish containing the Green Betta 1 transformation event, as well as such transgenic fish derived from a transgenic fish egg, sperm cell, embryo, or other cell containing a genomically integrated transgenic construct. "Progeny," as the term is used herein, can result from breeding two transgenic fish of the invention, or from breeding a first transgenic fish of the invention to a second fish that is not a transgenic fish of the invention. In the latter case, the second fish can, for example, be a wild-type fish, a specialized strain of fish, a mutant fish, or another transgenic fish. The second fish may be of the same species, or may be of a different species or genus. The hybrid progeny of these matings have the benefits of the transgene for fluorescence combined with the benefits derived from these other lineages.

[0041] The simplest way to identify fish containing the Green Betta 1 transformation event is by visual inspection, as the fish in question would be green colored and immediately distinguishable from non-transgenic fish.

[0042] In an example embodiment, young adult male orange bettas are euthanized using an overdose of sedative, such as Tricaine. The fish are decapitated, blotted dry, sprayed with 70% ethanol, dissected and their testes are removed and transferred into ice-cold L-15 medium. When the desired amount of tissue was obtained, the testes were transferred into ice-cold vitrification solution (L-15 medium containing 30% (v/v) of ethylene glycol, 21% (w/v) Ficoll PM-70, and 0.35M sucrose). After 20 minutes of incubation, testes were blotted to remove the vitrification solution and submerged into liquid nitrogen. The samples are kept in liquid nitrogen for long term storage.

[0043] To recover orange betta line, the cryopreserved testes are thawed in 0.2M sucrose solution in L-15 medium at 25°C for 5 seconds and incubated in the same solution for 5 minutes at 0°C. The thawed testes are transferred into L-15 medium containing 10% Fetal Bovine Serum (FBS), minced, transferred into L-15 medium containing 0.2% collagenase H, 0.15% dispase II, and 900 U/ml of DNase I and incubated for 1 hour at 25°C with constant gentle agitation to dissociate the tissue into cells. The cell suspension is rinsed once with L-15 containing 10% FBS,

and filtered through 60-micron mesh. The cell suspension is then injected into 7 day old betta fry anesthetized with Tricaine. The injected fry are reared to adulthood and spawned using regular protocol to obtain orange betta progeny.

5 **[0044]** The present disclosure further encompasses progeny of a transgenic fish containing the Orange Betta 1 transformation event, as well as such transgenic fish derived from a transgenic fish egg, sperm cell, embryo, or other cell containing a genomically integrated transgenic construct. "Progeny," as the term is used herein, can result from breeding two transgenic fish of the invention, or from breeding a first transgenic fish of the invention to a second fish that is not a transgenic fish of
10 the invention. In the latter case, the second fish can, for example, be a wild-type fish, a specialized strain of fish, a mutant fish, or another transgenic fish. The second fish may be of the same species, or may be of a different species or genus. The hybrid progeny of these matings have the benefits of the transgene for fluorescence combined with the benefits derived from these other lineages.

15 **[0045]** The simplest way to identify fish containing the Orange Betta 1 transformation event is by visual inspection, as the fish in question would be orange colored and immediately distinguishable from non-transgenic fish.

[0046] In an example embodiment, young adult male red bettas are euthanized using an overdose of sedative, such as Tricaine. The fish are decapitated,
20 blotted dry, sprayed with 70% ethanol, dissected and their testes are removed and transferred into ice-cold L-15 medium. When the desired amount of tissue was obtained, the testes were transferred into ice-cold vitrification solution (L-15 medium containing 30% (v/v) of ethylene glycol, 21% (w/v) Ficoll PM-70, and 0.35M sucrose). After 20 minutes of incubation, testes were blotted to remove the
25 vitrification solution and submerged into liquid nitrogen. The samples are kept in liquid nitrogen for long term storage.

[0047] To recover red betta line, the cryopreserved testes are thawed in 0.2M sucrose solution in L-15 medium at 25°C for 5 seconds and incubated in the same solution for 5 minutes at 0°C. The thawed testes are transferred into L-15 medium
30 containing 10% Fetal Bovine Serum (FBS), minced, transferred into L-15 medium containing 0.2% collagenase H, 0.15% dispase II, and 900 U/ml of DNase I and incubated for 1 hour at 25°C with constant gentle agitation to dissociate the tissue

into cells. The cell suspension is rinsed once with L-15 containing 10% FBS, and filtered through 60-micron mesh. The cell suspension is then injected into 7 day old betta fry anesthetized with Tricaine. The injected fry are reared to adulthood and spawned using regular protocol to obtain red betta progeny.

- 5 **[0048]** The present disclosure further encompasses progeny of a transgenic fish containing the Red Betta 1 transformation event, as well as such transgenic fish derived from a transgenic fish egg, sperm cell, embryo, or other cell containing a genomically integrated transgenic construct. "Progeny," as the term is used herein, can result from breeding two transgenic fish of the invention, or from breeding a
- 10 first transgenic fish of the invention to a second fish that is not a transgenic fish of the invention. In the latter case, the second fish can, for example, be a wild-type fish, a specialized strain of fish, a mutant fish, or another transgenic fish. The second fish may be of the same species, or may be of a different species or genus. The hybrid progeny of these matings have the benefits of the transgene for fluorescence
- 15 combined with the benefits derived from these other lineages.

- [0049]** The simplest way to identify fish containing the Red Betta 1 transformation event is by visual inspection, as the fish in question would be red colored and immediately distinguishable from non-transgenic fish. It should also be appreciated that depending on the specific RFP used and/or the insertion location of
- 20 the expression cassette, the transgenic red Betta may have a color that fades over the course of the transgenic red Betta's life. For example, the red transgenic Betta may change in color from red to pale red, or from red to pink. In addition, depending on the specific RFP used and/or the insertion location of the expression cassette, the transgenic red Betta may have a color that fades over generations. For example, the
- 25 red transgenic Betta may change in color from one generation to the next, such that an older generation may exhibit the red color, but a younger generation may appear pale red, or pink.

- [0050]** In an example embodiment, young adult male purple bettas are euthanized using an overdose of sedative, such as Tricaine. The fish are decapitated,
- 30 blotted dry, sprayed with 70% ethanol, dissected and their testes are removed and transferred into ice-cold L-15 medium. When the desired amount of tissue was obtained, the testes were transferred into ice-cold vitrification solution (L-15

medium containing 30% (v/v) of ethylene glycol, 21% (w/v) Ficoll PM-70, and 0.35M sucrose). After 20 minutes of incubation, testes were blotted to remove the vitrification solution and submerged into liquid nitrogen. The samples are kept in liquid nitrogen for long term storage.

5 **[0051]** To recover purple betta line, the cryopreserved testes are thawed in 0.2M sucrose solution in L-15 medium at 25°C for 5 seconds and incubated in the same solution for 5 minutes at 0°C. The thawed testes are transferred into L-15 medium containing 10% Fetal Bovine Serum (FBS), minced, transferred into L-15 medium containing 0.2% collagenase H, 0.15% dispase II, and 900 U/ml of DNase I
10 and incubated for 1 hour at 25°C with constant gentle agitation to dissociate the tissue into cells. The cell suspension is rinsed once with L-15 containing 10% FBS, and filtered through 60-micron mesh. The cell suspension is then injected into 7 day old betta fry anesthetized with Tricaine. The injected fry are reared to adulthood and spawned using regular protocol to obtain purple betta progeny.

15 **[0052]** The present disclosure further encompasses progeny of a transgenic fish containing the Purple Betta 1 transformation event, as well as such transgenic fish derived from a transgenic fish egg, sperm cell, embryo, or other cell containing a genomically integrated transgenic construct. “Progeny,” as the term is used herein, can result from breeding two transgenic fish of the invention, or from breeding a
20 first transgenic fish of the invention to a second fish that is not a transgenic fish of the invention. In the latter case, the second fish can, for example, be a wild-type fish, a specialized strain of fish, a mutant fish, or another transgenic fish. The second fish may be of the same species, or may be of a different species or genus. The hybrid progeny of these matings have the benefits of the transgene for fluorescence
25 combined with the benefits derived from these other lineages.

[0053] The simplest way to identify fish containing the Purple Betta 1 transformation event is by visual inspection, as the fish in question would be purple colored and immediately distinguishable from non-transgenic fish.

[0054] In an example embodiment, young adult male blue bettas are
30 euthanized using an overdose of sedative, such as Tricaine. The fish are decapitated, blotted dry, sprayed with 70% ethanol, dissected and their testes are removed and transferred into ice-cold L-15 medium. When the desired amount of tissue was

obtained, the testes were transferred into ice-cold vitrification solution (L-15 medium containing 30% (v/v) of ethylene glycol, 21% (w/v) Ficoll PM-70, and 0.35M sucrose). After 20 minutes of incubation, testes were blotted to remove the vitrification solution and submerged into liquid nitrogen. The samples are kept in liquid nitrogen for long term storage.

5 [0055] To recover blue betta line, the cryopreserved testes are thawed in 0.2M sucrose solution in L-15 medium at 25°C for 5 seconds and incubated in the same solution for 5 minutes at 0°C. The thawed testes are transferred into L-15 medium containing 10% Fetal Bovine Serum (FBS), minced, transferred into L-15 medium containing 0.2% collagenase H, 0.15% dispase II, and 900 U/ml of DNase I and incubated for 1 hour at 25°C with constant gentle agitation to dissociate the tissue into cells. The cell suspension is rinsed once with L-15 containing 10% FBS, and filtered through 60-micron mesh. The cell suspension is then injected into 7 day old betta fry anesthetized with Tricaine. The injected fry are reared to adulthood and spawned using regular protocol to obtain blue betta progeny.

15 [0056] The present disclosure further encompasses progeny of a transgenic fish containing the Blue Betta 1 transformation event, as well as such transgenic fish derived from a transgenic fish egg, sperm cell, embryo, or other cell containing a genomically integrated transgenic construct. "Progeny," as the term is used herein, can result from breeding two transgenic fish of the invention, or from breeding a first transgenic fish of the invention to a second fish that is not a transgenic fish of the invention. In the latter case, the second fish can, for example, be a wild-type fish, a specialized strain of fish, a mutant fish, or another transgenic fish. The second fish may be of the same species, or may be of a different species or genus. The hybrid progeny of these matings have the benefits of the transgene for fluorescence combined with the benefits derived from these other lineages.

25 [0057] The simplest way to identify fish containing the Blue Betta 1 transformation event is by visual inspection, as the fish in question would be blue colored and immediately distinguishable from non-transgenic fish.

30 [0058] EXAMPLES

[0059] Certain embodiments of the invention are further described with reference to the following examples. These examples are intended to be merely

illustrative of the invention and are not intended to limit or restrict the scope of the present invention in any way and should not be construed as providing conditions, parameters, reagents, or starting materials that must be utilized exclusively in order to practice the art of the present invention.

5 Example 1 - Green transgenic Betta

[0060] Transgenic fish exhibiting a green color are provided. The specific transgenic events embodied in these fish are designated the “Green Betta 1 transformation event”. Germ cells from these fish may be implanted into Betta fry and thereby breed transgenic Betta that comprise these specific transgenic
10 integration events. Cryopreserved testes from this line were deposited at the European Collection of Cell Cultures (ECACC), Public Health England, CRYOSTORES, Bld. 17, Porton Down, Salisbury, SP4 OJG, United Kingdom, under the provisions of the Budapest Treaty as “Green Betta 1”.

Example 2 - Orange transgenic Betta

15 [0061] Transgenic fish exhibiting an orange color are provided. The specific transgenic events embodied in these fish are designated the “Orange Betta 1 transformation event”. Germ cells from these fish may be implanted into Betta fry and thereby breed transgenic Betta that comprise these specific transgenic
20 integration events. Cryopreserved testes from this line were deposited at the European Collection of Cell Cultures (ECACC), Public Health England, CRYOSTORES, Bld. 17, Porton Down, Salisbury, SP4 OJG, United Kingdom, under the provisions of the Budapest Treaty as “Orange Betta 1”.

Example 3 – Red and/or Pink transgenic Betta

[0062] Transgenic fish exhibiting a red color are provided. The specific
25 transgenic events embodied in these fish are designated the “Red Betta 1 transformation event”. Germ cells from these fish may be implanted into Betta fry and thereby breed transgenic Betta that comprise these specific transgenic
integration events. Cryopreserved testes from this line were deposited at the European Collection of Cell Cultures (ECACC), Public Health England,
30 CRYOSTORES, Bld. 17, Porton Down, Salisbury, SP4 OJG, United Kingdom, under the provisions of the Budapest Treaty as “Red Betta 1”.

Example 4 - Purple transgenic Betta

[0063] Transgenic fish exhibiting a purple color are provided. The specific transgenic events embodied in these fish are designated the “Purple Betta 1 transformation event”. Germ cells from these fish may be implanted into Betta fry and thereby breed transgenic Betta that comprise these specific transgenic integration events. Cryopreserved testes from this line were deposited at the European Collection of Cell Cultures (ECACC), Public Health England, CRYOSTORES, Bld. 17, Porton Down, Salisbury, SP4 OJG, United Kingdom, under the provisions of the Budapest Treaty as “Purple Betta 1”.

10 Example 5 - Blue transgenic Betta

[0064] Transgenic fish exhibiting a blue color are provided. The specific transgenic events embodied in these fish are designated the “Blue Betta 1 transformation event”. Germ cells from these fish may be implanted into Betta fry and thereby breed transgenic Betta that comprise these specific transgenic integration events. Cryopreserved testes from this line were deposited at the European Collection of Cell Cultures (ECACC), Public Health England, CRYOSTORES, Bld. 17, Porton Down, Salisbury, SP4 OJG, United Kingdom, under the provisions of the Budapest Treaty as “Blue Betta 1”.

[0065] The fluorescent transgenic fish have use as ornamental fish in the market. Stably expressing transgenic lines can be developed by breeding a transgenic individual with a wild-type fish, mutant fish, or another transgenic fish. The desired transgenic fish can be distinguished from non-transgenic fish by observing the fish in white light, sunlight, ultraviolet light, blue light, or any other useful lighting condition that allows visualization of the green, orange, red, pink, purple or blue color of the transgenic fish.

[0066] The fluorescent transgenic fish should also be valuable in the market for scientific research tools because they can be used for embryonic studies such as tracing cell lineage and cell migration. Additionally, these fish can be used to mark cells in genetic mosaic experiments and in fish cancer models.

30 [0067] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been

- described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be
- 5 apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope, and concept of the invention as defined by the appended claims.
- 10 **[0068]** The above specification, examples and data provide a complete description of the manufacture and use of the composition of the invention. Since many embodiments of the invention can be made without departing from the spirit and scope of the invention, the invention resides in the claims hereinafter appended.

What is claimed is:

1. A transgenic Betta comprising a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the “Green Betta 1 transformation event,” cryopreserved testes comprising the Green
5 Betta 1 transformation event having been deposited at ECACC.
2. The transgenic Betta of claim 1, further defined as a fertile, transgenic Betta.
3. The transgenic Betta of claim 1, wherein the fish is homozygous for the integrated expression cassette.
- 10 4. The transgenic Betta of claim 1, wherein the fish is heterozygous for the integrated expression cassette.
5. A method of providing a transgenic Betta to the ornamental fish market, comprising obtaining a transgenic Betta in accordance with claim 1, and distributing the fish to the ornamental fish market.
- 15 6. The method of claim 5, wherein the fish are distributed by a grower to a commercial distributor.
7. The method of claim 5, wherein the fish are distributed by a grower or a commercial distributor to a retailer.
8. The method of claim 7, wherein the retailer is a multi-product retailer
20 having an ornamental fish department.
9. A method of producing a transgenic Betta comprising:
 - (a) obtaining a Betta that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the “Green Betta 1 transformation event,” cryopreserved testes comprising the Green Betta 1
25 transformation event having been deposited at ECACC; and
 - (b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Green Betta 1 transformation event.
10. The method of claim 9, wherein the second Betta is a non-transgenic Betta.
- 30 11. A progeny of a transgenic Betta of claim 1 that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta and progeny exhibit fluorescence and comprise the “Green Betta 1

transformation event,” cryopreserved testes comprising the Green Betta 1 transformation event having been deposited at ECACC.

12. The progeny fish of claim 11, further defined as a fertile, transgenic Betta.

5 13. The progeny fish of claim 11, wherein the fish is homozygous for the integrated expression cassette.

14. The progeny fish of claim 11, wherein the fish is heterozygous for the integrated expression cassette.

15. A method of providing a transgenic fish to the ornamental fish market, comprising obtaining a progeny fish in accordance with claim 11, and distributing the fish to the ornamental fish market.

16. The method of claim 15, wherein the fish are distributed by a grower to a commercial distributor.

17. The method of claim 16, wherein the fish are distributed by a grower or a commercial distributor to a retailer.

18. The method of claim 17, wherein the retailer is a multi-product retailer having an ornamental fish department.

19. A method of producing a transgenic fish comprising:

(a) obtaining a transgenic fish in accordance with claim 11; and

20 (b) breeding the obtained fish with a second fish to provide a transgenic fish comprising the Green Betta 1 transformation event.

20. The method of claim 19, wherein the second fish is a non-transgenic fish.

25 21. A transgenic Betta comprising a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the “Orange Betta 1 transformation event,” cryopreserved testes comprising the Orange Betta 1 transformation event having been deposited at ECACC.

30 22. The transgenic Betta of claim 21, further defined as a fertile, transgenic Betta.

23. The transgenic Betta of claim 21, wherein the fish is homozygous for the integrated expression cassette.
24. The transgenic Betta of claim 21, wherein the fish is heterozygous for the integrated expression cassette.
- 5 25. A method of providing a transgenic Betta to the ornamental fish market, comprising obtaining a transgenic Betta in accordance with claim 21, and distributing the fish to the ornamental fish market.
26. The method of claim 25, wherein the fish are distributed by a grower to a commercial distributor.
- 10 27. The method of claim 25, wherein the fish are distributed by a grower or a commercial distributor to a retailer.
28. The method of claim 27, wherein the retailer is a multi-product retailer having an ornamental fish department.
29. A method of producing a transgenic Betta comprising:
- 15 (a) obtaining a Betta that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the "Orange Betta 1 transformation event," cryopreserved testes comprising the Orange Betta 1 transformation event having been deposited at ECACC; and
- (b) breeding the obtained Betta with a second Betta to provide a transgenic
- 20 Betta comprising the Orange Betta 1 transformation event.
30. The method of claim 29, wherein the second Betta is a non-transgenic Betta.
31. A progeny of a transgenic Betta of claim 21 that comprises a chromosomally integrated expression cassette encoding a fluorescent protein,
- 25 wherein the Betta and progeny exhibit fluorescence and comprise the "Orange Betta 1 transformation event," cryopreserved testes comprising the Orange Betta 1 transformation event having been deposited at ECACC.
32. The progeny fish of claim 31, further defined as a fertile, transgenic Betta.
- 30 33. The progeny fish of claim 31, wherein the fish is homozygous for the integrated expression cassette.

34. The progeny fish of claim 31, wherein the fish is heterozygous for the integrated expression cassette.
35. A method of providing a transgenic fish to the ornamental fish market, comprising obtaining a progeny fish in accordance with claim 31, and
5 distributing the fish to the ornamental fish market.
36. The method of claim 35, wherein the fish are distributed by a grower to a commercial distributor.
37. The method of claim 36, wherein the fish are distributed by a grower or a commercial distributor to a retailer.
- 10 38. The method of claim 37, wherein the retailer is a multi-product retailer having an ornamental fish department.
39. A method of producing a transgenic fish comprising:
(a) obtaining a transgenic fish in accordance with claim 31; and
(b) breeding the obtained fish with a second fish to provide a
15 transgenic fish comprising the Orange Betta 1 transformation event.
40. The method of claim 39, wherein the second fish is a non-transgenic fish.
41. A transgenic Betta comprising a chromosomally integrated
20 expression cassette encoding a fluorescent protein, wherein the Betta comprises the "Red Betta 1 transformation event," cryopreserved testes comprising the Red Betta 1 transformation event having been deposited at ECACC.
42. The transgenic Betta of claim 41, further defined as a fertile,
25 transgenic Betta.
43. The transgenic Betta of claim 41, wherein the fish is homozygous for the integrated expression cassette.
44. The transgenic Betta of claim 41, wherein the fish is heterozygous for the integrated expression cassette.
- 30 45. A method of providing a transgenic Betta to the ornamental fish market, comprising obtaining a transgenic Betta in accordance with claim 41, and distributing the fish to the ornamental fish market.

46. The method of claim 45, wherein the fish are distributed by a grower to a commercial distributor.

47. The method of claim 45, wherein the fish are distributed by a grower or a commercial distributor to a retailer.

5 48. The method of claim 47, wherein the retailer is a multi-product retailer having an ornamental fish department.

49. A method of producing a transgenic Betta comprising:

(a) obtaining a Betta that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the “Red Betta
10 1 transformation event,” cryopreserved testes comprising the Red Betta 1 transformation event having been deposited at ECACC; and

(b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Red Betta 1 transformation event.

15 50. The method of claim 49, wherein the second Betta is a non-transgenic Betta.

51. A progeny of a transgenic Betta of claim 41 that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta and progeny exhibit fluorescence and comprise the “Red Betta 1
20 transformation event,” cryopreserved testes comprising the Red Betta 1 transformation event having been deposited at ECACC.

52. The progeny fish of claim 51, further defined as a fertile, transgenic Betta.

53. The progeny fish of claim 51, wherein the fish is homozygous for the integrated expression cassette.

25 54. The progeny fish of claim 51, wherein the fish is heterozygous for the integrated expression cassette.

55. A method of providing a transgenic fish to the ornamental fish market, comprising obtaining a progeny fish in accordance with claim 51, and distributing the fish to the ornamental fish market.

30 56. The method of claim 55, wherein the fish are distributed by a grower to a commercial distributor.

57. The method of claim 56, wherein the fish are distributed by a grower or a commercial distributor to a retailer.

58. The method of claim 57, wherein the retailer is a multi-product retailer having an ornamental fish department.

5 59. A method of producing a transgenic fish comprising:

(a) obtaining a transgenic fish in accordance with claim 51; and

(b) breeding the obtained fish with a second fish to provide a transgenic fish comprising the Red Betta 1 transformation event.

10 60. The method of claim 59, wherein the second fish is a non-transgenic fish.

61. A transgenic Betta comprising a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the "Purple Betta 1 transformation event," cryopreserved testes comprising the Purple Betta 1 transformation event having been deposited at ECACC.

62. The transgenic Betta of claim 61, further defined as a fertile, transgenic Betta.

20 63. The transgenic Betta of claim 61, wherein the fish is homozygous for the integrated expression cassette.

64. The transgenic Betta of claim 61, wherein the fish is heterozygous for the integrated expression cassette.

25 65. A method of providing a transgenic Betta to the ornamental fish market, comprising obtaining a transgenic Betta in accordance with claim 61, and distributing the fish to the ornamental fish market.

66. The method of claim 65, wherein the fish are distributed by a grower to a commercial distributor.

67. The method of claim 65, wherein the fish are distributed by a grower or a commercial distributor to a retailer.

30 68. The method of claim 67, wherein the retailer is a multi-product retailer having an ornamental fish department.

69. A method of producing a transgenic Betta comprising:

(a) obtaining a Betta that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the “Purple Betta 1 transformation event,” cryopreserved testes comprising the Purple Betta 1 transformation event having been deposited at ECACC; and

5 (b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Purple Betta 1 transformation event.

70. The method of claim 69, wherein the second Betta is a non-transgenic Betta.

71. A progeny of a transgenic Betta of claim 61 that comprises a
10 chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta and progeny exhibit fluorescence and comprise the “Purple Betta 1 transformation event,” cryopreserved testes comprising the Purple Betta 1 transformation event having been deposited at ECACC.

72. The progeny fish of claim 71, further defined as a fertile, transgenic
15 Betta.

73. The progeny fish of claim 71, wherein the fish is homozygous for the integrated expression cassette.

74. The progeny fish of claim 71, wherein the fish is heterozygous for the integrated expression cassette.

20 75. A method of providing a transgenic fish to the ornamental fish market, comprising obtaining a progeny fish in accordance with claim 71, and distributing the fish to the ornamental fish market.

76. The method of claim 75, wherein the fish are distributed by a grower to a commercial distributor.

25 77. The method of claim 76, wherein the fish are distributed by a grower or a commercial distributor to a retailer.

78. The method of claim 77, wherein the retailer is a multi-product retailer having an ornamental fish department.

79. A method of producing a transgenic fish comprising:

30 (a) obtaining a transgenic fish in accordance with claim 71; and
(b) breeding the obtained fish with a second fish to provide a transgenic fish comprising the Purple Betta 1 transformation event.

80. The method of claim 79, wherein the second fish is a non-transgenic fish.

81. A transgenic Betta comprising a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the “Blue Betta 1 transformation event,” cryopreserved testes comprising the Blue Betta 1 transformation event having been deposited at ECACC.

82. The transgenic Betta of claim 81, further defined as a fertile, transgenic Betta.

83. The transgenic Betta of claim 81, wherein the fish is homozygous for the integrated expression cassette.

84. The transgenic Betta of claim 81, wherein the fish is heterozygous for the integrated expression cassette.

85. A method of providing a transgenic Betta to the ornamental fish market, comprising obtaining a transgenic Betta in accordance with claim 81, and distributing the fish to the ornamental fish market.

86. The method of claim 85, wherein the fish are distributed by a grower to a commercial distributor.

87. The method of claim 85, wherein the fish are distributed by a grower or a commercial distributor to a retailer.

88. The method of claim 87, wherein the retailer is a multi-product retailer having an ornamental fish department.

89. A method of producing a transgenic Betta comprising:

(a) obtaining a Betta that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta comprises the “Blue Betta 1 transformation event,” cryopreserved testes comprising the Blue Betta 1 transformation event having been deposited at ECACC; and

(b) breeding the obtained Betta with a second Betta to provide a transgenic Betta comprising the Blue Betta 1 transformation event.

90. The method of claim 89, wherein the second Betta is a non-transgenic Betta.

91. A progeny of a transgenic Betta of claim 81 that comprises a chromosomally integrated expression cassette encoding a fluorescent protein, wherein the Betta and progeny exhibit fluorescence and comprise the “Blue Betta 1 transformation event,” cryopreserved testes comprising the Blue Betta 1 transformation event having been deposited at ECACC.
92. The progeny fish of claim 91, further defined as a fertile, transgenic Betta.
93. The progeny fish of claim 91, wherein the fish is homozygous for the integrated expression cassette.
94. The progeny fish of claim 91, wherein the fish is heterozygous for the integrated expression cassette.
95. A method of providing a transgenic fish to the ornamental fish market, comprising obtaining a progeny fish in accordance with claim 91, and distributing the fish to the ornamental fish market.
96. The method of claim 95, wherein the fish are distributed by a grower to a commercial distributor.
97. The method of claim 96, wherein the fish are distributed by a grower or a commercial distributor to a retailer.
98. The method of claim 97, wherein the retailer is a multi-product retailer having an ornamental fish department.
99. A method of producing a transgenic fish comprising:
(a) obtaining a transgenic fish in accordance with claim 91; and
(b) breeding the obtained fish with a second fish to provide a transgenic fish comprising the Blue Betta 1 transformation event.
100. The method of claim 99, wherein the second fish is a non-transgenic fish.