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(54) **Title: COMPOSITIONS FOR MOSQUITO CONTROL AND USES OF SAME**

(57) **Abrégé/Abstract:**
A composition-of-matter for mosquito control is provided. The composition comprises a cell which comprises an exogenous naked dsRNA which specifically down- regulates expression of a gene being endogenous to a mosquito or which specifically down-regulated expression of a gene being endogenous to a mosquito pathogen. Further provided is a composition-of-matter for mosquito control, comprising a cell comprising a nucleic acid larvicide. Also provided are methods of producing and using the compositions.

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(57) Abstract: A composition-of-matter for mosquito control is provided. The composition comprises a cell which comprises an exogenous naked dsRNA which specifically down- regulates expression of a gene being endogenous to a mosquito or which specifically down-regulated expression of a gene being endogenous to a mosquito pathogen. Further provided is a composition-of-matter for mosquito control, comprising a cell comprising a nucleic acid larvicide. Also provided are methods of producing and using the compositions.

COMPOSITIONS FOR MOSQUITO CONTROL AND USES OF SAME

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions for mosquito control and uses of same.

Mosquitoes are the major vectors for a number of human and animal diseases, including malaria, yellow fever and dengue fever. Over 1 million people die from mosquito-borne diseases every year, and hundreds of millions more experience pain and suffering from illnesses transmitted by mosquitoes.

There is neither specific medication nor vaccine for Dengue. The only way currently to control the disease is to control the mosquito, *Aedes aegypti*, which spreads the disease. There is no cure for yellow fever but there is a vaccine; however it is expensive and not available to protect other parts of the world. There is no currently available drug regimen guarantees 100% protection against Malaria, and prevention of infection requires taking antimalarial medication as directed in addition to prevention of mosquito bites. Antimalarials do not actually prevent the disease but only act in the bloodstream to suppress clinical symptoms by inhibiting parasite development in red blood cells.

In order to prevent human disease caused by the viruses and parasites mentioned above, a systematic mosquito surveillance system is required. Nowadays, it is accepted that the success of such actions depends on the implementation of an integrated mosquito management program (IMM).

The aim of these programs is to optimize the control of mosquitoes in an economical and environmentally friendly way. Specifically, Integrated Mosquito Management is a comprehensive mosquito prevention/control strategy that utilizes all available mosquito control methods singly or in combination to exploit the known vulnerabilities of mosquitoes in order to reduce their numbers to tolerable levels while maintaining a quality environment. IMM does not emphasize mosquito elimination or eradication. Integrated mosquito management methods are specifically tailored to safely counter each stage of the mosquito life cycle. Prudent mosquito management practices for the control of immature mosquitoes (larvae and pupae) include such methods as the use of biological controls (native, noninvasive predators), source reduction (water or vegetation management or other compatible land management uses), water sanitation practices as well as the use of registered larvicides. When source elimination or larval control measures are not feasible or are clearly inadequate, or when faced with imminent mosquito-borne disease, application of registered adulticides may be needed. However, larvicides/adulticides efficacy is now threatened by the rise of resistance in target populations. Such phenomenon is occurring worldwide in all major disease vector mosquito species and spreads at a rapid rate [Harris et al. (2010) Am. J. Trop. Med. Hyg. 83, 277e284; Marcombe et al. (2009a) Am. J. Trop. Med. Hyg. 80, 745e751; Marcombe et al. (2009b) BMC Genomics 10, 494; Ranson et al. (2009) Malar. J. 8, 299].

Larviciding is an ecologically safe preventive method used to interrupt the development of larvae or pupa into adult mosquitoes. Larviciding is also a general term for killing immature mosquitoes by applying agents, collectively called larvicides, to control mosquito larvae and/or pupae. Larvicides may be grouped into two broad categories: biorational pesticides (biopesticides) and conventional, broad-spectrum chemical pesticides.

Biochemical agents such as Insect Growth Regulators (IGRS) controls insects by interrupting their life cycle, rather than through direct toxicity. Based on this mode of action, the U.S. Environmental Protection Agency (EPA) considers it to be a biochemical pesticide. The IGRS mimics naturally occurring insect biochemicals that are responsible for insect development. Through the mimicry, IGRS keeps the mosquito larvae from developing into adults that would emerge from the pupae. It is able to exert this effect at very small concentrations. The first IGRS, which contained several methoprene isomers, was registered in 1975 [Henrick, (2007) Methoprene. In: Floore, T.G. (Ed.). Biorational Control of Mosquitoes. Bulletin of the American Mosquito Control Association No. 7. St Louis, MO: Allen Press]. Methoprene products currently are the only IGRS registered for use in the USA. Methoprene is a juvenile hormone (JH) analog, which mimicries the natural hormone from insects. JH is involved in the regulation of physiological processes in insects including mating and metamorphosis. Therefore, these chemicals interfere with normal insect growth and maturation and induce abnormal larval growth patterns.

Resistance has been defined as ‘the developed ability in a strain of insects to tolerate doses of toxicants that would prove lethal to the majority of individuals in a normal population of the same species' [Clark & Yamaguchi, (2002) Scope and Status of Pesticide Resistance. In Agrochemical Resistance: Extent, Mechanism and Detection, eds. J.M Clark & I. Yamaguchi, pp 1-22. Washington, DC: American Chemical Society]. In a susceptible population, individuals with resistant genes to a given insecticide are rare, and usually range between 10^{-5} and 10^{-8} in number, but widespread use of a toxicant favors the prevalence of the resistant individuals. These individuals multiply fast in the absence of intraspecific competition and, over a number of generations, quickly become the dominant proportion of the population. Hence, the insecticide is no longer effective and the insects are considered to be resistant.

In addition to pesticides and insecticides, chemicals commonly used in agriculture also include fertilizers, herbicides, fungicides and various adjuvants that increase their efficiency. Although these compounds are usually non-toxic to insects, their presence in breeding sites has been shown to affect tolerance to insecticides via the modulation of their detoxification system. For instance, Chironomus tentans larvae exposed to the herbicide alachlor respond by enhanced GST activities [Li et al. (2009) Insect Biochem. Mol. Biol., 39, 745e754]. *Ae. albopictus* larvae exposed for 48 h to the fungicides triadimefon, diniconazole and pentachlorophenol showed an increased tolerance to carbaryl [Suwanchaichinda and Brattsten, (2001) Pestic. Biochem. Physiol., 70, 63e73]. The strong effect observed with pentachlorophenol was further linked to a strong induction of P450s. Poupardin et al. [(2008) Insect Biochem. Mol. Biol. 38, 540e551; (2010) Insect Mol. Biol., 19, 185e193] demonstrated that exposing *Ae. aegypti* larvae to a sub-lethal dose of copper sulphate, frequently used in agriculture as a fungicide, enhance their tolerance to the pyrethroid permethrin. This effect was correlated to an elevation of P450 activities and the induction of CYP genes preferentially transcribed in detoxification tissues and showing high homology to known pyrethroid metabolizers. Similarly, exposing *Ae. Aegypti* larvae to the herbicide glyphosate, the active molecule of Roundup, led to a significant increase of their tolerance to permethrin together with the induction of multiple detoxification genes [(Riaz et al. (2009) Aquat. Toxicol., 93, 61e69].

Mosquito resistance has also been described against biolarvicides. Specifically, the development of resistance in *Culex quinquefasciatus* to the Biopesticide *Bacillus* *sphaericus* (B.s.) has been noted by Rodcharoen et al., *Journal of Economic Entomology*, Vol. 87, No. 5, 1994, pp. 1133-1140. In addition, resistance to methoprene was soon demonstrated in several species [Dyte, (1972) Nature, 238(5358):48-9; Cerf & Georghiou, (1972) Nature, 239(5372):401-2].

One method of introducing dsRNA to the larvae is by dehydration. Specifically, larvae are dehydrated in a NaCl solution and then rehydrated in water containing double-stranded RNA. This process is suggested to induce gene silencing in mosquito larvae.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a composition-of-matter for mosquito control, comprising a cell comprising an exogenous naked dsRNA which specifically down-regulates expression of a gene being endogenous to a mosquito or which specifically down-regulated expression of a gene being endogenous to a mosquito pathogen.

According to an aspect of some embodiments of the present invention there is provided a composition-of-matter for mosquito control, comprising a cell comprising a nucleic acid larvicide.

According to an aspect of some embodiments of the present invention there is provided a composition-of-matter for mosquito control, comprising a cell comprising a nucleic acid larvicide affecting fertility or fecundity of a female mosquito.

According to an aspect of some embodiments of the present invention there is provided a composition-of-matter for mosquito control comprising a nucleic acid larvicide that targets a piRNA pathway gene and/or a sterility gene.

According to an aspect of some embodiments of the present invention there is provided a composition-of-matter for mosquito control comprising a nucleic acid larvicide that targets a gene comprising Aub (AAEL007698) and Argonaute-3 (AAEL007823).

According to some embodiments of the invention, the nucleic acid larvicide comprises at least one dsRNA.

According to some embodiments of the invention, the composition-of-matter comprises a dsRNA which comprises SEQ ID NO: 1858 and a dsRNA which comprises SEQ ID NO: 1823.

According to an aspect of some embodiments of the present invention there is provided a method of producing a larvicidal composition, the method comprising introducing into a cell a nucleic acid larvicide, thereby producing the larvicide.

According to an aspect of some embodiments of the present invention there is provided a method of producing a larvicidal composition, the method comprising introducing into a cell a nucleic acid larvicide affecting fertility or fecundity of a female mosquito, thereby producing the larvicide.

According to some embodiments of the invention, the introducing is effected by electroporation.

According to some embodiments of the invention, the introducing is effected by particle bombardment.

According to some embodiments of the invention, the introducing is effected by chemical-based transfection.

According to some embodiments of the invention, the nucleic acid larvicide down-regulates a target gene selected from the group consisting of:

(i) affecting larval survival;

(ii) interfering with metamorphosis of larval stage to adulthood;

(iii) affecting susceptibility of mosquito larvae to a larvicide;

(iv) affecting susceptibility of an adult mosquito to an adulticide/insecticide; and

(v) affecting fertility or fecundity of a male or female mosquito.

According to some embodiments of the invention, the target gene is selected from the group consisting of 1-427, 430-1813, 1826-1832.

According to some embodiments of the invention, the target gene is selected from the group consisting of P-glycoprotein (AAEL010379), Argonaute-3 (AAEL007823), Cytochrome p450 (CYP9J26), Sodium channel (AAEL008297), Aub (AAEL007698), AeSCP-2 (AF510492.1), AeAct-4 (AY531222.2), AAEL002000, AAEL005747, AAEL005656, AAEL017015, AAEL005212, AAEL005922, AAEL000903 and AAEL005049.

According to some embodiments of the invention, the target gene comprises Aub (AAEL007698) and Argonaute-3 (AAEL007823).

According to some embodiments of the invention, the nucleic acid larvicide which down-regulates the target gene is a dsRNA.

According to some embodiments of the invention, the dsRNA comprises SEQ ID NOs: 1858 and 1823.

According to some embodiments of the invention, the cell is an algal cell.

According to some embodiments of the invention, the cell is a microbial cell.

According to some embodiments of the invention, the cell is a bacterial cell.

According to some embodiments of the invention, the composition further comprises a food-bait.

According to some embodiments of the invention, the composition is formulated in a formulation selected from the group consisting of technical powder, wettable powder, dust, pellet, briquette, tablet and granule.

According to some embodiments of the invention, the granule is selected from the group consisting of an impregnated granule, dry flowable, wettable granule and water dispersible granule.

According to some embodiments of the invention, the composition is formulated as a non-aqueous or aqueous suspension concentrate.

According to some embodiments of the invention, the composition is formulated as a semi-solid form.

According to some embodiments of the invention, the semi-solid form comprises an agarose.

According to some embodiments of the invention, the cell is lyophilized.

According to some embodiments of the invention, the cell is non-transgenic.

According to some embodiments of the invention, the composition-of-matter or method further comprises an RNA-binding protein.

According to some embodiments of the invention, the nucleic acid larvicide comprises a dsRNA.

According to some embodiments of the invention, the dsRNA is a naked dsRNA.

According to some embodiments of the invention, the dsRNA comprises a carrier.

According to some embodiments of the invention, the carrier comprises a polyethyleneimine (PEI).

According to some embodiments of the invention, the dsRNA is effected at a dose of 0.001-1 µg/µL for soaking or at a dose of 1 pg to 10 µg/larvae for feeding.

According to some embodiments of the invention, the dsRNA is selected from the group consisting of SEQ ID NOs: 1822-1825 and 1857-1868.

According to some embodiments of the invention, the dsRNA is selected from the group consisting of siRNA, shRNA and miRNA.

According to some embodiments of the invention, the cell is devoid of a heterologous promoter for driving expression of the dsRNA in the plant.

According to some embodiments of the invention, the nucleic acid larvicide is greater than 15 base pairs in length.

According to some embodiments of the invention, the nucleic acid larvicide is 19 to 25 base pairs in length.

According to some embodiments of the invention, the nucleic acid larvicide is 30-100 base pairs in length.

According to some embodiments of the invention, the nucleic acid larvicide is 100-800 base pairs in length.

According to some embodiments of the invention, the composition further comprises at least one of a surface-active agent, an inert carrier vehicle, a preservative, a humectant, a feeding stimulant, an attractant, an encapsulating agent, a binder, an emulsifier, a dye, an ultra-violet protector, a buffer, a flow agent or fertilizer, micronutrient donors, or other preparations that influence the growth of the plant.

According to some embodiments of the invention, the composition of matter has an inferior impact on an adult mosquito as compared to the larvae.

According to some embodiments of the invention, the composition further comprises a chemical larvicide or a biochemical larvicide or a combination of same.

According to some embodiments of the invention, the larvicide is selected from the group consisting of Temephos, Diflubenzuron, methoprene, Bacillus sphaericus, and Bacillus thuringiensis israelensis.

According to some embodiments of the invention, the larvicide comprises an adulticide.

According to some embodiments of the invention, the adulticide is selected from the group consisting of deltamethrin, malathion, naled, chlorpyrifos, permethrin, resmethrin and sumithrin.

According to an aspect of some embodiments of the present invention there is provided a method of controlling or exterminating mosquitoes, the method comprising feeding larvae of the mosquitoes with an effective amount of the composition-of-matter of some embodiments of the invention, thereby controlling or exterminating the mosquitoes.

According to some embodiments of the invention, the mosquitoes comprise female mosquitoes capable of transmitting a disease to a mammalian organism.

According to some embodiments of the invention, the mosquitoes are of a species selected from the group consisting of *Aedes aegypti* and *Anopheles gambiae*.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is a flowchart illustration depicting introduction of dsRNA into mosquito larvae via soaking with “naked” dsRNA. In short, third instar larvae were treated (in groups of 100 larvae) in a final volume of 3 mL of dsRNA solution in autoclaved water with 0.5 μg/μL dsRNA. The control group was kept in 3 ml sterile water only. Larvae were soaked in the dsRNA solutions for 24 hr at 27 °C, and then transferred into new containers (300 larvae/1500 mL of chlorine-free tap water), which were also maintained at 27 °C, and were provided with lab dog/cat diet (Purina Mills) suspended in water as a source of food on a daily basis. As pupae developed, they were transferred to individual vials to await eclosion and sex sorting. For bioassays purpose only females up to five days old were used. Then, mosquitoes were subjected to pyrethroid adulticide assay.

FIG. 2 is a flowchart illustration depicting introduction of dsRNA into mosquito larvae via soaking with "naked" dsRNA plus additional larvae feeding with food-containing dsRNA. After soaking in the dsRNA solutions for 24 hr at 27 °C (as indicated in Figure 1 above), the larvae were transferred into new containers (300 larvae/1500 mL of chlorine-free tap water), and were provided agarose cubes containing 300 μg of dsRNA once a day for a total of four days. The larvae were reared until adult stage. For bioassays purpose only females up to five days old are used. Then, mosquitoes were subjected to pyrethroid adulticide assay.

FIG. 3 is a flowchart illustration depicting introduction of dsRNA into mosquito larvae via feeding with food-containing dsRNA only. Third instar larvae were fed (in groups of 300 larvae) in a final volume of 1500 mL of chlorine-free tap water with agarose cubes containing 300 μg of dsRNA once a day for a total of four days. The larvae were reared until adult stage. For bioassays purpose only females up to five days old are used. Then, mosquitoes were subjected to pyrethroid adulticide assay.

FIG. 4 is a flowchart illustration depicting dsRNA production.

FIGs. 5A-C are graphs illustrating the dose-response curves for 3- to 5-day-old *Aedes aegypti* female mosquitoes on insecticide-susceptible Rockefeller strain (Figure 5A) and on insecticide-resistant Rio de Janeiro strain (Figure 5B). Mosquitoes were exposed to different concentrations of deltamethrin in 250-mL glass bottles for up to 24 hours and the percentage of mortality for each time point is shown. Figure 5C, comparison of the mortality rates of female mosquitoes from Rockefeller (Rock) and Rio de Janeiro (RJ) strains exposed to 2 μg/mL of deltamethrin for different time-points. Data represent mean values of three replicates with standard deviation.

FIGs. 6A-B are photographs illustrating allele specific PCR for genotyping *kdr* mutations in the *Aedes aegypti* Rio de Janeiro strain. Figures 6A-B represent reactions for the 1016 and 1534 mutation sites, respectively. Amplicons were resolved in a 10 % polyacrylamide gel electrophoresis and stained with Gel Red. Figure 6A, amplicons of approximately 80 and 100 bp correspond to alleles 1016 Val^{+} and 1016 Ile^{kdr}, respectively. Figure 6B, amplicons of 90 and 110 bp correspond to alleles 1534 Phe^{+} and 1534 Cys^{kdr}, respectively. Rockefeller *Ae. aegypti* mosquito strain was used as positive homozygous dominant control for both mutation sites. C-: negative control.

FIGs. 7A-C are graphs illustrating that sodium channel gene silencing on *Ae. aegypti* mosquitoes (RJ strain) results in increased susceptibility to Pyrethroid adulticide. Figure 7A, larvae from *Ae. aegypti* RJ strain (3^{rd} instar) were soaked for 24 hours in 0.5 μg/μL of sodium channel dsRNA or only in water, and then reared until adult stage. Adult females were exposed to deltamethrin (0.5 μg/bottle) for different time-points, as indicated, and mortality rates for each time point is shown. Data show the mean ± standard deviation of four replicates, and is representative of 3 independent experiments. Figure 7B, adult mosquitoes (males and females) previously soaked with sodium channel dsRNA or only water were collected before the treatment with deltamethrin and analyzed for sodium channel mRNA expression using qPCR method. Figure 7C, live and immediately dead female mosquitoes were collected after exposure to deltamethrin and the mRNA expression of sodium channel was determined by qPCR analysis. ***p<0.0001; ****p<0.00001.

FIG. 8 is a graph illustrating that sodium channel gene silencing on *A. aegypti* mosquitoes (RJ strain) results in increased susceptibility to Pyrethroid adulticide. Larvae from *Ae. aegypti* RJ strain (3^{rd} instar) were soaked for 24 hours in 0.5 μg/μL of sodium channel dsRNA or only in water, and then were fed 4 times with food plus agarose 2% containing dsRNA until they reach pupa stage. After emergence, adult females were exposed to deltamethrin (0.5 μg/bottle) for different time-points, as indicated, and mortality rates for each time point is shown. Data show the mean ± standard deviation of four replicates, and is representative of 3 independent experiments. *p<0.01; ***p<0.0001.

FIG. 9 is a graph illustrating that feeding CYP9J29 dsRNA to larvae affects the susceptibility of adult *Ae. aegypti* mosquitoes to Pyrethroid adulticide. Larvae from *A.*

aegypti RJ strain (3rd instar) were soaked for 24 hours in 0.1 µg/µL of target #3 (CYP9J26) dsRNA or only in water; and then were fed 4 times with food plus agarose 2% containing dsRNA until they reach pupa stage. Adult females were exposed to deltamethrin (0.5 µg/bottle) for different time-points, as indicated, and then percentage of mortality for each time point is shown. Data represent the mean ± standard deviation of four replicates. $^{**}p<0.001$.

FIGs. 10A-C are graphs illustrating gene silencing in *A. aegypti* larvae. 3rd instar larvae from *Ae. aegypti* were soaked for 24 hours in 0.5 µg/mL of (Figure 10A) P-glycoprotein (PgP); (Figure 10B) Ago-3 or (Figure 10C) sodium channel dsRNA. Larvae soaked only in water were used as control. At 6, 24 and 48 hours after the end of dsRNA treatment, larvae were collected and analysed for PgP, Ago-3 and Sodium channel mRNA expression by qPCR. Data represent the mean ± standard deviation of four replicates. $^{*}p<0.01$ $^{**}p<0.001$; $^{***}p<0.0001$; $^{****}p<0.00001$.

FIGs. 11A-B are graphs illustrating P-glycoprotein and Ago-3 expression in *Ae. aegypti* adult mosquitoes soaked with dsRNA. Third instar larvae from *Ae. aegypti* were soaked for 24 hours in 0.5 µg/mL of (Figure 11A) P-glycoprotein (PgP) and (Figure 11B) Ago-3, and then reared until adult stage. Adult mosquitoes (males and females) previously soaked with the indicated dsRNA or only water were collected and analyzed for PgP and Ago-3 mRNA expression using qPCR method. Data represent the mean ± standard deviation of five replicates. $^{**}p<0.001$.

FIG. 12 is a flowchart illustration depicting introduction of dsRNA into mosquito larvae via soaking with different doses of "naked" dsRNA plus additional larvae feeding with food-containing dsRNA. Step a) 100 larvae from *A. aegypti* Rockefeller strain (3rd instar) were soaked for 24 hours with the respective dsRNAs (concentration range from 0.02-0.5 µg/µL) or only in water and were then fed 2 times with food plus agarose 2% containing dsRNA until they reach adult stage (Step b). Step c) The adults arising were allowed to copulate for 3-5 days. Step d) mosquitoes were fed with defibrinated sheep blood. Step e) after blood feeding 15 fully-engorged females were transferred into 3 small cages to be assayed for oviposition. Step f) the total number of laid eggs and the percentage of hatched eggs were counted. FIGs. 13A-B are graphs illustrating larvae from *Ae. aegypti* Rockefeller strain (3rd instar) soaked for 24 hours in 0.5 µg/µL of Aubergine (Aub) or Argonaute-3 (Ago) dsRNAs or water only. After soaking, larvae were separated in 3 different cages (containing 100 larvae each) and were treated twice with agarose plug containing dsRNA. The adults arising were allowed to copulate for 3-5 days and then fed with defibrinated sheep blood. After blood feeding 15 fully-engorged females were transferred into small cages to be assayed for oviposition. (Figure 13A) The total number of laid eggs and the percentage of hatched eggs were counted (Figure 13B).

FIGs. 14A-B are graphs illustrating larvae from *Ae. aegypti* Rockefeller strain (3^{rd} instar) soaked for 24 hours in 0.02 μg/μL of AeAct-4 dsRNA or water only. After soaking, larvae were separated in 3 different cages (containing 100 larvae each) and were treated twice with agarose plug containing dsRNA. The adults arising were allowed to copulate for 3-5 days and then fed with defibrinated sheep blood. After blood feeding 15 fully-engorged females were transferred into small cages to be assayed for oviposition. (Figure 14A) The total number of laid eggs and the percentage of hatched eggs were counted (Figure 14B).

FIGs. 15A-B are graphs illustrating Larvae from *A. aegypti* Rockefeller strain (3^{rd} instar) soaked for 24 hours in 0.05 μg/μL of AAEL005922 dsRNA or 0.06 μg/μL of AAEL000903 dsRNA or water only. After soaking, larvae were separated in 3 different cages (containing 100 larvae each) and were treated twice with agarose plug containing dsRNA. The adults arising were allowed to copulate for 3-5 days and then fed with defibrinated sheep blood. After blood feeding 15 fully-engorged females were transferred into small cages to be assayed for oviposition. (Figure 15A) The total number of laid eggs and the percentage of hatched eggs were counted (Figure 15B).

FIGs. 16A-B are graphs illustrating larvae from *Ae. aegypti* Rockefeller strain (3^{rd} instar) soaked for 24 hours in 0.06 μg/μL of AAEL017015 dsRNA, or 0.06 μg/μL of AAEL005212 dsRNA, 0.5 μg/μL of Aubergine (Aub) + Argonaute-3 (Ago) dsRNA or water only. After soaking, larvae were separated in 3 different cages (containing 100 larvae each) and treated twice with agarose plug containing dsRNA. The adults arising were allowed to copulate for 3-5 days and then fed with defibrinated sheep blood. After blood feeding 15 fully-engorged females were transferred into small cages to be assayed for oviposition. (Figure 16A) The total number of laid eggs and the percentage of hatched eggs were counted (Figure 16B).

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions for mosquito control and uses of same.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

It is understood that any Sequence Identification Number (SEQ ID NO) disclosed in the instant application can refer to either a DNA sequence or a RNA sequence, depending on the context where that SEQ ID NO is mentioned, even if that SEQ ID NO is expressed only in a DNA sequence format or a RNA sequence format. For example, SEQ ID NO: 1822 is expressed in a DNA sequence format (*e.g.*, reciting T for thymine), but it can refer to either a DNA sequence that corresponds to an endo 1,4 beta gluconase nucleic acid sequence, or the RNA sequence of an RNA molecule nucleic acid sequence. Similarly, though some sequences are expressed in a RNA sequence format (*e.g.*, reciting U for uracil), depending on the actual type of molecule being described, it can refer to either the sequence of a RNA molecule comprising a dsRNA, or the sequence of a DNA molecule that corresponds to the RNA sequence shown. In any event, both DNA and RNA molecules having the sequences disclosed with any substitutes are envisioned.

While reducing the present invention to practice, the present inventors have uncovered that feeding dsRNA to mosquito larvae is an effective method for silencing gene expression in adult mosquitoes.

Specifically, the present inventors have shown that feeding mosquito larvae with dsRNA targeting specific genes for two to four days (via agarose cubes, until they reach pupa stage) with or without previous soaking with dsRNA for 24 hours (e.g. sodium channel, PgP, ago-3 and Cytochrome p450) efficiently decreases gene expression (Figures 10A-C) and results in higher susceptibility (Figures 8, 9) in adult mosquitoes. Importantly, female mosquitoes showed a decreased expression in the mRNA level for sodium channel before deltamethrin treatment (Figure 7B) and dead female mosquitoes previously treated with dsRNA showed a striking decrease in mRNA expression level for sodium channel (Figure 7C). Furthermore, it was illustrated that feeding mosquito larvae with dsRNA significantly reduced the number of hatchings of eggs of adult female mosquitoes (Figures 13A-B, 14A-B, 15A-B and 16A-B).

According to an aspect of the invention there is provided a composition-of-matter for mosquito control, comprising a cell comprising an exogenous naked dsRNA which specifically down-regulates expression of a gene being endogenous to a mosquito or which specifically down-regulated expression of a gene being endogenous to a mosquito pathogen.

As used herein the term “exogenous” refers to an externally added nucleic acid molecule which is not naturally occurring in the cell.

According to an aspect of the invention there is provided a composition-of-matter for mosquito control, comprising a cell which comprises a nucleic acid larvicide.

According to another aspect of the invention there is provided a composition-of-matter for mosquito control, comprising a cell comprising a nucleic acid larvicide affecting fertility or fecundity of a female mosquito.

The term "mosquito" or “mosquitoes” as used herein refers to an insect of the family Culicidae. The mosquito of the invention may include an adult mosquito, a mosquito larva, a pupa or an egg thereof.

An adult mosquito is defined as any of slender, long-legged insect that has long proboscis and scales on most parts of the body. The adult females of many species of mosquitoes are blood-eating pests. In feeding on blood, adult female mosquitoes transmit harmful diseases to humans and other mammals.

A mosquito larvae is defined as any of an aquatic insect which does not comprise legs, comprises a distinct head bearing mouth brushes and antennae, a bulbous thorax that is wider than the head and abdomen, a posterior anal papillae and either a pair of respiratory openings (in the subfamily Anophelinae) or an elongate siphon (in the subfamily Culicinae) borne near the end of the abdomen.

Typically, a mosquito’s life cycle includes four separate and distinct stages: egg, larva, pupa, and adult. Thus, a mosquito’s life cycle begins when eggs are laid on a water surface (e.g. *Culex, Culiseta,* and *Anopheles* species) or on damp soil that is flooded by water (e.g. *Aedes* species). Most eggs hatch into larvae within 48 hours. The larvae live in the water feeding on microorganisms and organic matter and come to the surface to breathe. They shed their skin four times growing larger after each molting and on the fourth molt the larva changes into a pupa. The pupal stage is a resting, non-feeding stage of about two days. At this time the mosquito turns into an adult. When development is complete, the pupal skin splits and the mosquito emerges as an adult.

According to one embodiment, the mosquitoes are of the sub-families Anophelinae and Culicinae. According to one embodiment, the mosquitoes are of the genus *Culex, Culiseta, Anopheles* and *Aedes*. Exemplary mosquitoes include, but are not limited to, Aedes species e.g. *Aedes aegypti*, *Aedes albopictus, Aedes polynesiensis*, *Aedes australis*, *Aedes cantator*, *Aedes cinereus*, *Aedes rusticus*, *Aedes vexans*; Anopheles species e.g. *Anopheles gambiae, Anopheles freeborni,Anopheles arabiensis*, *Anopheles funestus*, *Anopheles gambiae Anopheles moucheti, Anopheles balabacensis*, *Anopheles baimaii*, *Anopheles culicifacies*, *Anopheles dirus*, *Anopheles latens*, *Anopheles leucosphyrus*, *Anopheles maculatus*, *Anopheles minimus*, *Anopheles fluviatilis s.l.*, *Anopheles sundaicus Anopheles superpictus*, *Anopheles farauti*, *Anopheles punctulatus*, *Anopheles sergentii*, *Anopheles stephensi*, *Anopheles sinensis*, *Anopheles atroparvus*, *Anopheles pseudopunctipennis, Anopheles bellator* and *Anopheles cruzii*; Culex species e.g. *C. annulirostris, C. antennatus*, *C. jenseni*, *C. pipiens*, *C. pusillus*, *C. quinquefasciatus*, *C. rajah*, *C. restuans, C. salinarius*, *C. tarsalis*, *C. territans*, *C. theileri* and *C. tritaeniorhynchus;* and Culiseta species e.g. *Culiseta incidens*, *Culiseta impatiens*, *Culiseta inornata* and *Culiseta particeps*.

According to one embodiment, the mosquitoes are capable of transmitting disease-causing pathogens. The pathogens transmitted by mosquitoes include viruses, protozoa, worms and bacteria.

Non-limiting examples of viral pathogens which may be transmitted by mosquitoes include the arbovirus pathogens such as Alphaviruses pathogens (e.g. Eastern Equine encephalitis virus, Western Equine encephalitis virus, Venezuelan Equine encephalitis virus, Ross River virus, Sindbis Virus and Chikungunya virus), Flavivirus pathogens (e.g. Japanese Encephalitis virus, Murray Valley Encephalitis virus, West Nile Fever virus, Yellow Fever virus, Dengue Fever virus, St. Louis encephalitis virus, and Tick-borne encephalitis virus), Bunyavirus pathogens (e.g. La Crosse Encephalitis virus, Rift Valley Fever virus, and Colorado Tick Fever virus) and Orbivirus (e.g. Bluetongue disease virus).

Non-limiting examples of worm pathogens which may be transmitted by mosquitoes include nematodes e.g. filarial nematodes such as Wuchereria bancrofti, Brugia malayi, Brugia pahangi, Brugia timori and heartworm (Dirofilaria immitis)).

Non-limiting examples of bacterial pathogens which may be transmitted by mosquitoes include gram negative and gram positive bacteria including Yersinia pestis, Borellia spp, Rickettsia spp, and Erwinia carotovora.

Non-limiting examples of protozoa pathogens which may be transmitted by mosquitoes include the Malaria parasite of the genus Plasmodium e.g. *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium berghei, Plasmodium gallinaceum,* and *Plasmodium knowlesi.*

According to one embodiment, the mosquito comprises a female mosquito being capable of transmitting a disease to a mammalian organism.

Non-limiting examples of mosquitoes and the pathogens which they transmit include species of the genus *Anopheles* (e.g. *Anopheles gambiae*) which transmit malaria parasites as well as microfilariae, arboviruses (including encephalitis viruses) and some species also transmit *Wuchereria bancrofti*; species of the genus *Culex* (e.g. *C. pipiens*) which transmit West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis and avian malaria; species of the genus *Aedes* (e.g. *Aedes aegypti*, *Aedes albopictus* and *Aedes polynesiensis*) which transmit nematode worm pathogens (e.g. heartworm (Dirofilaria immitis)), arbovirus pathogens such as Alphaviruses pathogens that cause diseases such as Eastern Equine encephalitis, Western Equine encephalitis, Venezuelan equine encephalitis and Chikungunya disease; Flavivirus pathogens that cause diseases such as Japanese encephalitis, Murray Valley Encephalitis, West Nile fever, Yellow fever, Dengue fever, and Bunyavirus pathogens that cause diseases such as LaCrosse encephalitis, Rift Valley Fever, and Colorado tick fever.

According to one embodiment, pathogens that may be transmitted by *Aedes aegypti* are Dengue virus, Yellow fever virus, Chikungunya virus and heartworm (Dirofilaria immitis).

According to one embodiment, pathogens that may be transmitted by *Aedes albopictus* include West Nile Virus, Yellow Fever virus, St. Louis Encephalitis virus, Dengue virus, and Chikungunya fever virus.

According to one embodiment, pathogens that may be transmitted by *Anopheles gambiae* include malaria parasites of the genus Plasmodium such as, but not limited to, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium berghei*, *Plasmodium gallinaceum*, and *Plasmodium knowlesi*.

As used herein the phrase “mosquito control” refers to managing the population of mosquitoes to reduce their damage to human health, economies, and enjoyment. According to some embodiments of the invention, mosquito management is typically effected using larvicidally effective compositions and compositions having mosquito “aversion activity” which causes a mosquito to avoid deleterious behavior such as a mosquito biting.

As used herein, the term "larvicidal" or “larvicidal activity” refers to the ability of interfering with a mosquito life cycle resulting in an overall reduction in the mosquito population. The larvicidal composition acts (down-regulates gene expression) at the larval stage. The activity of the larvicidal composition may be manifested immediately (e.g., by affecting larval survival) or only at later stages, as described below. For example, the term larvicidal includes inhibition of a mosquito from progressing from one form to a more mature form, e.g., transition between various larval instars or transition from larva to pupa or pupa to adult. Alternatively or additionally, the term larvicidal affects mosquito fertility or fecundity. Hence the down-regulation of the target gene may induce male or female sterility. Further, the term "larvicidal" is intended to encompass, for example, anti-mosquito activity during all phases of a mosquito life cycle; thus, for example, the term includes larvacidal, ovicidal, and adulticidal activity. According to a specific embodiment all of which stem from the activity at the larval stage. Alternatively or additionally, larvicide encompasses both “larva-specific” larvicides, and non-specific larvicides.”

According to one embodiment the larvicide may affect fertility or fecundity of a female mosquito. Affecting the fertility or fecundity of a mosquito typically does not kill the mosquito but affects the amount or quality of eggs the mosquito lays, as well as the ability to produce viable and/or fertile progeny. Thus, fertility refers to the ability of a population of female mosquitoes to yield eggs. Fecundity refers to a reduction in the number of progeny produced from the eggs.

Thus, fertility refers to the “ability” of a male and a female to reproduce a viable offspring.

The female mosquito may lay a reduced amount of eggs as compared to a female mosquito not affected by the larvicide composition of the invention. Alternatively, the quality of the eggs laid by the female mosquito may be damaged, e.g. the eggs may not hatch or may hatch at a reduced amount (e.g. 10 %, 20%, 30 %, 40 %, 50 %, 60%, 70 %, 80 %, 90 % or 100 % reduction in hatching as compared to eggs of a female mosquito not affected by the larvicide composition of the invention).

A population of female mosquitoes receiving the larvicide composition of the invention is considered to have sufficiently decreased fertility or fecundity if at least 30 %, 40 %, 50 %, 60%, 70 %, 80 %, 90 % or 100 % of the females in the population are infertile, e.g., unable to produce viable eggs.

Thus, the larvicide of the invention may generate a biased population of adult mosquitoes.

In addition the term may refer to rendering a mosquito at any stage, including adulthood, more susceptible to a pesticide as compared to the susceptibility of a mosquito of the same species and developmental stage which hasn’t been treated with the nucleic acid larvicide.

As used herein, the term "larvicidally effective" is used to indicate an amount or concentration of the nucleic acid larvicide which is sufficient to reduce the number of mosquitoes in a geographic locus as compared to a corresponding geographic locus in the absence of the amount or concentration of the composition.

Thus the nucleic acid larvicide of some embodiments of the invention down-regulates a target gene selected from the group consisting of:

(i) affecting larval survival;

(ii) interfering with metamorphosis of larval stage to adulthood;

(iii) affecting susceptibility of mosquito larvae to a larvicide;

(iv) affecting susceptibility of an adult mosquito to an adulticide/insecticide; and

(v) affecting fertility or fecundity of a male or female mosquito.

As used herein the term “affecting” or “interfering” refers to a gene which plays a role in the above mentioned biological activity. According to a specific embodiment, the target gene is a non-redundant gene, that is, its activity is not compensated by another gene in a pathway. When needed, down-regulation of a plurality of genes (e.g., in a pathway) participating in at least one of the above-mentioned activities is contemplated (as further described hereinbelow). Alternatively, according to a specific embodiment, the plurality of target genes are from groups (i) and (ii), (i) and (iii), (i) and (iv), (i) and (v), (ii) and (iii), (ii) and (iv), (ii) and (v), (iii) and (v) and (iv) and (v) and more.

The target gene may comprise a nucleic acid sequence which is transcribed to an mRNA which codes for a polypeptide.

Alternatively, the target gene can be a non-coding gene such as a miRNA or a siRNA.

According to a specific embodiment, the target gene is endogenous to the larvae.

According to a specific embodiment, the target gene is endogenous to the pathogen.

As used herein “endogenous” refers to a gene which expression (mRNA or protein) takes place in the larvae or the pathogen. Typically, the endogenous gene is naturally expressed in the larvae or the pathogen.

Below provided are exemplary genes. Orthologs and homologs are also contemplated according to the present teachings.

Homologous sequences include both orthologous and paralogous sequences. The term “paralogous” relates to gene-duplications within the genome of a species leading to paralogous genes. The term “orthologous” relates to homologous genes in different organisms due to ancestral relationship. Thus, orthologs are evolutionary counterparts derived from a single ancestral gene in the last common ancestor of given two species (Koonin EV and Galperin MY (Sequence - Evolution - Function: Computational Approaches in Comparative Genomics. Boston: Kluwer Academic; 2003. Chapter 2, Evolutionary Concept in Genetics and Genomics. Available from: ncbi (dot) nlm (dot) nih (dot) gov/books/NBK20255) and therefore have great likelihood of having the same function.

The term "ortholog" (also called orthologous genes) refers to genes in different species derived from a common ancestry (due to speciation).

According to a specific embodiment, the homolog sequences are at least 60 %, 65 %, 70 %, 75 %, 80%, 85 %, 90 %, 95 % or even identical to the sequences (nucleic acid or amino acid sequences) provided hereinbelow.

The nucleic acid agent will be selected according to the target larvae and hence target genes. Exemplary target genes of the invention include adulticide/larvicide targets and fertility/fecundity targets.

Exemplary target genes of the invention are listed in Tables 1-5 below.

Table 1

Seq ID	Gene Symbol
302	AAEL001340
303	AAEL001606
304	AAEL002425
305	AAEL002792
306	AAEL003660
307	AAEL004696
308	AAEL004974
309	AAEL006254
310	AAEL006488
311	AAEL006492
312	AAEL008042
313	AAEL008587
314	AAEL008844
315	AAEL008924
316	AAEL008958
317	AAEL009114
318	AAEL009174
319	AAEL009340
320	AAEL009969
321	AAEL010565
322	AAEL010789
323	AAEL010792
324	AAEL011474
325	AAEL011478
326	AAEL011663
327	AAEL011757
328	AAEL011921
329	AAEL014330
330	AGAP000460
331	AGAP000460

332	AGAP000460
333	AGAP000471
334	AGAP000471
335	AGAP000471
336	AGAP000662
337	AGAP000662
338	AGAP000662
339	AGAP001177
340	AGAP001177
341	AGAP001177
342	AGAP001179
343	AGAP001179
344	AGAP001179
345	AGAP001271
346	AGAP001271
347	AGAP001271
348	AGAP001278
349	AGAP001278
350	AGAP001278
351	AGAP001293
352	AGAP001293
353	AGAP001293
354	AGAP001335
355	AGAP001335
356	AGAP001335
357	AGAP001337
358	AGAP001337
359	AGAP001337
360	AGAP001339
361	AGAP001339
362	AGAP001339
363	AGAP001367
364	AGAP001367
365	AGAP001367
366	AGAP001388
367	AGAP001388
368	AGAP001388
369	AGAP001463
370	AGAP001463
371	AGAP001463
372	AGAP001478
373	AGAP001478
374	AGAP001478

375	AGAP001481
376	AGAP001481
377	AGAP001481
378	AGAP001498
379	AGAP001498
380	AGAP001498
381	AGAP002471
382	AGAP002471
383	AGAP002471
384	AGAP002801
385	AGAP004050
386	AGAP004416
387	AGAP004416
388	AGAP004416
389	AGAP004645
390	AGAP004930
391	AGAP006887
392	AGAP006887
393	AGAP006887
394	AGAP007963
395	AGAP008806
396	CPIJ001185
397	CPIJ001186
398	CPIJ001187
399	CPIJ001560
400	CPIJ003158
401	CPIJ003766
402	CPIJ004057
403	CPIJ004058
404	CPIJ004318
405	CPIJ005975
406	CPIJ005976
407	CPIJ007071
408	CPIJ007072
409	CPIJ007101
410	CPIJ007172
411	CPIJ007789
412	CPIJ008481
413	CPIJ008673
414	CPIJ009011
415	CPIJ009270
416	CPIJ011557
417	CPIJ011558

418	CPIJ011708
419	CPIJ012810
420	CPIJ013126
421	CPIJ015620
422	CPIJ015622
423	CPIJ017065
424	CPIJ017887
425	CPIJ019248
426	CPIJ019249
427	FBgn0127180

Table 1, cont.

Table 2A

	Seq ID	Gene Symbol	Annotation
Enzymes	55	AAEL012664	prolylcarboxypeptidase, putative
	56	AAEL002909	lysosomal acid lipase, putative
	57	AAEL005127	ribonuclease UK114, putative
	58	AAEL012636	cytochrome b5, putative
	59	AAEL010276	aminomethyltransferase
	60	AAEL013640	lung carbonyl reductase
	61	AAEL005416	oxidase/peroxidase
	62	AAEL013499	prophenoloxidase
	63	AAEL003716	ribonuclease UK114, putative
	64	AAEL012579	aspartate aminotransferase
	65	AAEL002600	serine protease
	66	AAEL005610	mitochondrial ATP synthase b chain
	67	AAEL006446	trehalose-6-phosphate synthase
	68	AAEL008770	proteasome subunit beta type
	69	AAEL001427	short-chain dehydrogenase
	70	AAEL013279	peptidyl-prolyl cis-trans isomerase (cyclophilin)
	71	AAEL009875	alanine aminotransferase
	72	AAEL005793	AMP dependent ligase
	73	AAEL007868	ubiquinol-cytochrome c reductase complex 14 kd protein
	74	AAEL008072	NADH-plastoquinone oxidoreductase
	75	AAEL009324	hydroxyacyl dehydrogenase
	76	AAEL008217	serine-type enodpeptidase,
	77	AAEL014944	cytochrome c oxidase polypeptide
	78	AAEL010819	vacuolar ATP synthase subunit H
	79	AAEL010500	glutathione-s-transferase theta, gst

Transport	80	AAEL005929	ATP-binding cassette transporter
	81	AAEL008381	oligopeptide transporter
	82	AAEL001626	zinc/iron transporter
	83	AAEL012702	ATP-binding cassette sub-family A member 3, putative
others	84	AAEL015515	antibacterial peptide, putative
	85	AAEL002295	leucine-rich transmembrane protein
	86	AAEL009556	Niemann-Pick Type C-2, putative
	87	AAEL005159	latent nuclear antigen, putative
	88	AAEL007325	Mob3B protein, putative
	89	AAEL000679	NEDD8, putative
	90	AAEL009209	galactose-specific C-type lectin, putative
	91	AAEL001826	odorant-binding protein 56a, putative
	92	AAEL002961	Osiris, putative
	93	AAEL006830	yellow protein precursor
	94	AAEL005772	odorant-binding protein 99c, putative
	95	AAEL002813	coupling factor, putative
	96	AAEL011090	complement component
	97	AAEL012230	flagellar protein, putative
Hypothetical proteins	98	AAEL011252	conserved hypothetical protein
	99	AAEL014506	conserved hypothetical protein
	100	AAEL003216	conserved hypothetical protein
	101	AAEL003241	conserved hypothetical protein
	102	AAEL007507	conserved hypothetical protein
	103	AAEL003064	conserved hypothetical protein
	104	AAEL010678	conserved hypothetical protein
	105	AAEL000269	conserved hypothetical protein
	106	AAEL006053	conserved hypothetical protein
	107	AAEL008750	conserved hypothetical protein
	108	AAEL010128	conserved hypothetical protein
	109	AAEL002898	conserved hypothetical protein
	110	AAEL007631	conserved hypothetical protein
	111	AAEL003479	conserved hypothetical protein
	112	AAEL013777	conserved hypothetical protein
	113	AAEL003428	conserved hypothetical protein
	114	AAEL014529	conserved hypothetical protein
	115	AAEL012645	conserved hypothetical protein
	116	AAEL004809	conserved hypothetical protein
	117	AAEL004343	conserved hypothetical protein
	118	AAEL003160	conserved hypothetical protein

	119	AAEL012357	conserved hypothetical protein
	120	AAEL009009	conserved hypothetical protein
	121	AAEL013793	conserved hypothetical protein
	122	AAEL002623	conserved hypothetical protein
	123	AAEL010163	conserved hypothetical protein
	124	AAEL002449	conserved hypothetical protein
	125	AAEL002302	conserved hypothetical protein
	126	AAEL008039	conserved hypothetical protein
	127	AAEL008073	conserved hypothetical protein
	128	AAEL007444	conserved hypothetical protein
	129	AAEL005171	conserved hypothetical protein
	130	AAEL006771	conserved hypothetical protein
	131	AAEL015140	conserved hypothetical protein
	132	AAEL001851	conserved hypothetical protein
	133	AAEL005558	conserved hypothetical protein
	134	AAEL002933	conserved hypothetical protein
	135	AAEL003225	conserved hypothetical protein
	136	AAEL001692	conserved hypothetical protein
	137	AAEL007592	conserved hypothetical protein
	138	AAEL005457	conserved hypothetical protein
	139	AAEL006494	conserved hypothetical protein
	140	AAEL013780	conserved hypothetical protein
	141	AAEL009257	conserved hypothetical protein
	142	AAEL000445	conserved hypothetical protein
	143	AAEL002955	conserved hypothetical protein
	144	AAEL002875	conserved hypothetical protein
	145	AAEL000304	conserved hypothetical protein
	146	AAEL000792	conserved hypothetical protein
	147	AAEL003936	conserved hypothetical protein
	148	AAEL006686	conserved hypothetical protein
	149	AAEL001677	conserved hypothetical protein
	150	AAEL000419	conserved hypothetical protein
	151	AAEL007648	conserved hypothetical protein
	152	AAEL006270	conserved hypothetical protein
	153	AAEL013377	conserved hypothetical protein
	154	AAEL002619	conserved hypothetical protein
	155	AAEL012866	conserved hypothetical protein
	156	AAEL014445	conserved hypothetical protein
	157	AAEL001065	conserved hypothetical protein
	158	AAEL011333	conserved hypothetical protein
	159	AAEL011078	conserved hypothetical protein
	160	AAEL010315	conserved hypothetical protein
	161	AAEL005270	conserved hypothetical protein

	162	AAEL004449	conserved hypothetical protein
	163	AAEL000896	conserved hypothetical protein
	164	AAEL010724	conserved hypothetical protein
	165	AAEL008802	conserved hypothetical protein

Table 2A, cont.

Table 2B

	Gene symbol	Gene Name
430	AAEL000043	gustatory receptor 64e, putative
431	AAEL000020	conserved hypothetical protein
432	AAEL000005	hypothetical protein
433	AAEL000049	three prime repair exonuclease 1, putative
434	AAEL000053	myotubularin
435	AAEL000033	conserved hypothetical protein
436	AAEL000046	conserved hypothetical protein
437	AAEL000054	mixed-lineage leukemia protein, mll
438	AAEL000011	conserved hypothetical protein
439	AAEL000055	conserved hypothetical protein
440	AAEL000543	C-Type Lectin (CTL) - mannose binding.
441	AAEL000559	glycosyl transferase
442	AAEL000562	conserved hypothetical protein
443	AAEL000554	fasciclin, putative
444	AAEL014092	conserved hypothetical protein
445	AAEL014105	hypothetical protein
446	AAEL014110	sulfite reductase
447	AAEL003935	conserved hypothetical protein
448	AAEL003940	hypothetical protein
449	AAEL014125	nk homeobox protein
450	AAEL014128	hypothetical protein
451	AAEL003971	conserved hypothetical protein
452	AAEL003959	short-chain dehydrogenase
453	AAEL014161	amino acids transporter
454	AAEL014163	conserved hypothetical protein
455	AAEL014183	hypothetical protein
456	AAEL003983	adenylate cyclase

457	AAEL003979	deadenylation factor EDEN-BP, putative
458	AAEL014196	conserved hypothetical protein
459	AAEL003988	hypothetical protein
460	AAEL003986	conserved hypothetical protein
461	AAEL014222	low-density lipoprotein receptor (ldl)
462	AAEL014229	conserved hypothetical protein
463	AAEL014237	conserved hypothetical protein
464	AAEL014250	insect replication protein a
465	AAEL004030	conserved hypothetical protein
466	AAEL004018	conserved hypothetical protein
467	AAEL014257	hypothetical protein
468	AAEL014268	hypothetical protein
469	AAEL014271	conserved hypothetical protein
470	AAEL014272	molybdopterin cofactor sulfurase (mosc)
471	AAEL014276	conserved hypothetical protein
472	AAEL014283	conserved hypothetical protein
473	AAEL014291	hypothetical protein
474	AAEL004055	conserved hypothetical protein
475	AAEL004076	Ubiquitin-like modifier-activating enzyme 5 (Ubiquitin-activating enzyme 5)
476	AAEL004068	hypothetical protein
477	AAEL014302	conserved hypothetical protein
478	AAEL014306	hypothetical protein
479	AAEL014304	hypothetical protein
480	AAEL014318	conserved hypothetical protein
481	AAEL014314	DNA primase
482	AAEL014317	conserved hypothetical protein
483	AAEL014316	conserved hypothetical protein
484	AAEL014319	hypothetical protein

485	AAEL004084	conserved hypothetical protein
486	AAEL004094	pou domain
487	AAEL004104	hypothetical protein
488	AAEL014324	conserved hypothetical protein
489	AAEL004130	conserved hypothetical protein
490	AAEL004129	conserved hypothetical protein
491	AAEL014341	metallocarboxypeptidase, putative
492	AAEL014342	hypothetical protein
493	AAEL004190	hypothetical protein
494	AAEL004178	ribose-phosphate pyrophosphokinase 1,
495	AAEL004172	tubulin alpha chain
496	AAEL004176	microtubule binding protein, putative
497	AAEL004150	fibrinogen and fibronectin
498	AAEL004188	conserved hypothetical protein
499	AAEL004191	selenocysteine-specific elongation factor
500	AAEL014367	hypothetical protein
501	AAEL014373	GPCR Dopamine Family
502	AAEL014389	conserved hypothetical protein
503	AAEL000584	sex-determining region y protein, sry
504	AAEL000589	serine/threonine protein kinase
505	AAEL000585	hypothetical protein
506	AAEL017305	odorant receptor
507	AAEL014402	conserved hypothetical protein
508	AAEL014411	cytochrome P450
509	AAEL004234	conserved hypothetical protein
510	AAEL014428	ABC transporter
511	AAEL014431	conserved hypothetical protein
512	AAEL014441	conserved hypothetical protein

513	AAEL004253	hypothetical protein
514	AAEL004300	conserved hypothetical protein
515	AAEL004302	conserved hypothetical protein
516	AAEL004296	conserved hypothetical protein
517	AAEL004317	hypothetical protein
518	AAEL004315	hypothetical protein
519	AAEL004348	NF-180, putative
520	AAEL004328	origin recognition complex subunit
521	AAEL014524	DNA replication licensing factor MCM4
522	AAEL014533	conserved hypothetical protein
523	AAEL014536	embryonic ectoderm development protein
524	AAEL014548	Thioredoxin Peroxidase.
525	AAEL014557	homeobox protein cdx
526	AAEL004386	heme peroxidase
527	AAEL004390	heme peroxidase
528	AAEL004399	GPCR Glycoprotein Hormone Family
529	AAEL017431	hypothetical protein
530	AAEL004388	heme peroxidase
531	AAEL004396	GPCR Octopamine/Tyramine Family
532	AAEL014572	tetraspanin 97e
533	AAEL017559	hypothetical protein
534	AAEL014577	conserved hypothetical protein
535	AAEL014589	reticulocalbin
536	AAEL004416	histone deacetylase
537	AAEL004443	nitrilase, putative
538	AAEL004437	dual-specificity protein phosphatase, putative
539	AAEL004429	conserved hypothetical protein
540	AAEL014627	short-chain dehydrogenase

541	AAEL014633	conserved hypothetical protein
542	AAEL000604	hypothetical protein
543	AAEL000601	hypothetical protein
544	AAEL004482	conserved hypothetical protein
545	AAEL004462	hypothetical protein
546	AAEL004468	hypothetical protein
547	AAEL004479	organic cation transporter
548	AAEL004486	valacyclovir hydrolase
549	AAEL004510	hypothetical protein
550	AAEL004493	ribosome biogenesis protein tsr1 (20S rRNA accumulation protein 1)
551	AAEL017243	hypothetical protein
552	AAEL004518	Clip-Domain Serine Protease family C
553	AAEL004537	hypothetical protein
554	AAEL004539	hypothetical protein
555	AAEL014693	conserved hypothetical protein
556	AAEL004559	synaptosomal associated protein
557	AAEL004562	DNA polymerase eta
558	AAEL004592	tyrosine-protein kinase src64b
559	AAEL004598	hypothetical protein
560	AAEL014744	conserved hypothetical protein
561	AAEL004627	hypothetical protein
562	AAEL004635	hypothetical protein
563	AAEL004638	conserved hypothetical protein
564	AAEL004645	hypothetical protein
565	AAEL004650	hypothetical protein
566	AAEL017478	hypothetical protein
567	AAEL004623	band 4.1-like protein 5, putative
568	AAEL004642	hypothetical protein

569	AAEL014756	hypothetical protein
570	AAEL004680	nuclear lamin L1 alpha, putative
571	AAEL004698	DNA primase large subunit
572	AAEL004697	synoviolin
573	AAEL014791	hypothetical protein
574	AAEL014800	conserved hypothetical protein
575	AAEL014814	conserved hypothetical protein
576	AAEL017122	hypothetical protein
577	AAEL017102	hypothetical protein
578	AAEL014823	conserved hypothetical protein
579	AAEL014825	conserved hypothetical protein
580	AAEL014835	conserved hypothetical protein
581	AAEL004777	Glycoprotein Hormone Family
582	AAEL017453	hypothetical protein
583	AAEL004748	pupal cuticle protein, putative
584	AAEL004771	pupal cuticle protein, putative
585	AAEL004782	pupal cuticle protein, putative
586	AAEL014844	conserved hypothetical protein
587	AAEL000666	pmp22 peroxisomal membrane protein, putative
588	AAEL000672	cyclin a
589	AAEL000664	hypothetical protein
590	AAEL000662	conserved hypothetical protein
591	AAEL000649	conserved hypothetical protein
592	AAEL000658	conserved hypothetical protein
593	AAEL000675	hypothetical protein
594	AAEL004796	hypothetical protein
595	AAEL004789	hypothetical protein
596	AAEL004818	conserved hypothetical protein

597	AAEL004805	potassium-dependent sodium-calcium exchanger, putative
598	AAEL004821	potassium-dependent sodium-calcium exchanger, putative
599	AAEL004842	conserved hypothetical protein
600	AAEL004837	hypothetical protein
601	AAEL004850	hypothetical protein
602	AAEL004835	conserved hypothetical protein
603	AAEL004858	conserved hypothetical protein
604	AAEL004841	conserved hypothetical protein
605	AAEL004882	conserved hypothetical protein
606	AAEL004892	conserved hypothetical protein
607	AAEL004864	hypothetical protein
608	AAEL014904	DEAD box ATP-dependent RNA helicase
609	AAEL014908	conserved hypothetical protein
610	AAEL014911	synaptic vesicle protein
611	AAEL017811	RNase MRP
612	AAEL014918	lysosomal acid lipase, putative
613	AAEL014924	cytochrome P450
614	AAEL014925	conserved hypothetical protein
615	AAEL004914	conserved hypothetical protein
616	AAEL004903	conserved hypothetical protein
617	AAEL014927	sodium/chloride dependent transporter
618	AAEL016998	hypothetical protein
619	AAEL014946	protease U48 caax prenyl protease rce1
620	AAEL014956	internalin A, putative
621	AAEL004955	hypothetical protein
622	AAEL004949	elongase, putative
623	AAEL004976	conserved hypothetical protein
624	AAEL004970	conserved hypothetical protein

625	AAEL005006	cytochrome P450
626	AAEL005000	conserved hypothetical protein
627	AAEL005007	hypothetical protein
628	AAEL005009	groucho protein (enhancer of split)
629	AAEL014984	adult cuticle protein, putative
630	AAEL014986	conserved hypothetical protein
631	AAEL014992	rab gdp/GTP exchange factor
632	AAEL014989	peptidoglycan recognition protein-1, putative
633	AAEL005040	conserved hypothetical protein
634	AAEL005033	conserved hypothetical protein
635	AAEL015011	hypothetical protein
636	AAEL000692	partner of sld5
637	AAEL005078	zinc finger protein
638	AAEL005049	heterogeneous nuclear ribonucleoprotein
639	AAEL005070	conserved hypothetical protein
640	AAEL005087	hypothetical protein
641	AAEL015018	toll
642	AAEL015035	transcription enhancer factor, putative
643	AAEL015038	palmitoyl-protein thioesterase
644	AAEL015047	hypothetical protein
645	AAEL005123	hypothetical protein
646	AAEL005110	conserved hypothetical protein
647	AAEL005115	hypothetical protein
648	AAEL005128	hypothetical protein
649	AAEL005103	conserved hypothetical protein
650	AAEL005145	conserved hypothetical protein
651	AAEL015071	gustatory receptor 64a, putative
652	AAEL005212	hypothetical protein

653	AAEL005197	conserved hypothetical protein
654	AAEL005175	lipin
655	AAEL005217	membrin
656	AAEL005215	conserved hypothetical protein
657	AAEL005235	conserved hypothetical protein
658	AAEL005238	mck1
659	AAEL015083	conserved hypothetical protein
660	AAEL015080	conserved hypothetical protein
661	AAEL005241	lateral signaling target protein 2
662	AAEL005259	conserved hypothetical protein
663	AAEL005261	conserved hypothetical protein
664	AAEL015107	conserved hypothetical protein
665	AAEL005286	hypothetical protein
666	AAEL015119	cuticle protein, putative
667	AAEL000749	conserved hypothetical protein
668	AAEL005326	conserved hypothetical protein
669	AAEL005312	conserved hypothetical protein
670	AAEL015136	Niemann-Pick Type C-2, putative
671	AAEL005348	hypothetical protein
672	AAEL005351	leucine-rich transmembrane protein
673	AAEL005362	hypothetical protein
674	AAEL005364	adaptin, alpha/gamma/epsilon
675	AAEL015161	conserved hypothetical protein
676	AAEL005383	Adenosine monophosphate-protein transferase FICD homolog (EC 2.7.7.n1)
677	AAEL005420	p15-2a protein, putative
678	AAEL017015	hypothetical protein
679	AAEL005430	hypothetical protein
680	AAEL005439	mical

681	AAEL005452	conserved hypothetical protein
682	AAEL005474	hypothetical protein
683	AAEL000773	kinesin heavy chain
684	AAEL000754	conserved hypothetical protein
685	AAEL000779	hypothetical protein
686	AAEL000776	conserved hypothetical protein
687	AAEL000790	conserved hypothetical protein
688	AAEL000793	venom allergen
689	AAEL000769	arginine/serine-rich splicing factor
690	AAEL005513	mothers against dpp protein
691	AAEL015222	adult cuticle protein, putative
692	AAEL015232	GTP-binding protein rit
693	AAEL005549	hypothetical protein
694	AAEL005588	conserved hypothetical protein
695	AAEL005584	hypothetical protein
696	AAEL015243	hypothetical protein
697	AAEL005638	conserved hypothetical protein
698	AAEL005637	vegetatible incompatibility protein HET-E-1, putative
699	AAEL017446	gustatory receptor Gr33a
700	AAEL005656	myosin heavy chain, nonmuscle or smooth muscle
701	AAEL015270	hypothetical protein
702	AAEL005668	conserved hypothetical protein
703	AAEL000816	carbonic anhydrase
704	AAEL000805	conserved hypothetical protein
705	AAEL000819	hypothetical protein
706	AAEL000800	microsomal dipeptidase
707	AAEL005707	gonadotropin inducible transcription factor
708	AAEL005684	chitinase

709	AAEL005724	conserved hypothetical protein
710	AAEL005716	conserved hypothetical protein
711	AAEL005721	conserved hypothetical protein
712	AAEL015293	zinc finger protein
713	AAEL005786	conserved hypothetical protein
714	AAEL005775	cytochrome P450
715	AAEL005796	eukaryotic translation initiation factor 4e type
716	AAEL005804	hypothetical protein
717	AAEL005805	alanyl aminopeptidase
718	AAEL005808	alanyl aminopeptidase
719	AAEL005853	amino acid transporter
720	AAEL005856	signal recognition particle receptor alpha subunit (sr-alpha)
721	AAEL005859	amino acid transporter
722	AAEL000903	Enhancer of yellow 2 transcription factor
723	AAEL000912	conserved hypothetical protein
724	AAEL000889	hypothetical protein
725	AAEL000884	eukaryotic translation initiation factor 2 alpha kinase 1 (heme-regulated eukaryotic initiation factor eif-2-alpha kinase)
726	AAEL000905	hypothetical protein
727	AAEL000923	conserved hypothetical protein
728	AAEL005938	hypothetical protein
729	AAEL005936	conserved hypothetical protein
730	AAEL005932	conserved hypothetical protein
731	AAEL005924	hypothetical protein
732	AAEL017009	odorant receptor
733	AAEL005922	hypothetical protein
734	AAEL005916	hypothetical protein
735	AAEL015327	conserved hypothetical protein
736	AAEL015330	hypothetical protein

737	AAEL015336	conserved hypothetical protein
738	AAEL005990	adrenodoxin reductase, putative
739	AAEL006010	conserved hypothetical protein
740	AAEL005998	rap GTPase-activating protein
741	AAEL006031	conserved hypothetical protein
742	AAEL006023	Vanin-like protein 1 precursor, putative
743	AAEL006030	hypothetical protein
744	AAEL006037	hypothetical protein
745	AAEL006045	reticulon/nogo receptor
746	AAEL006055	potassium channel interacting protein
747	AAEL006084	conserved hypothetical protein
748	AAEL006091	rab6
749	AAEL006098	conserved hypothetical protein
750	AAEL000938	conserved hypothetical protein
751	AAEL000945	conserved hypothetical protein
752	AAEL000971	smile protein
753	AAEL000973	conserved hypothetical protein
754	AAEL000932	conserved hypothetical protein
755	AAEL000953	conserved hypothetical protein
756	AAEL000964	regulatory factor X-associated ankyrin-containing protein, putative
757	AAEL000934	clathrin light chain
758	AAEL006111	hypothetical protein
759	AAEL006140	mitosis inhibitor protein kinase
760	AAEL006138	hypothetical protein
761	AAEL006130	hypothetical protein
762	AAEL006208	conserved hypothetical protein
763	AAEL006211	conserved hypothetical protein
764	AAEL006219	heparan sulphate 2-o-sulfotransferase

765	AAEL006239	glycerol kinase
766	AAEL006243	hypothetical protein
767	AAEL006241	sugar transporter
768	AAEL015376	conserved hypothetical protein
769	AAEL006258	pickpocket
770	AAEL015380	conserved hypothetical protein
771	AAEL006277	conserved hypothetical protein
772	AAEL006279	hypothetical protein
773	AAEL006262	mitochondrial carrier protein
774	AAEL006298	conserved hypothetical protein
775	AAEL006303	integral membrane protein, Tmp21-I (p23), putative
776	AAEL006286	conserved hypothetical protein
777	AAEL000113	conserved hypothetical protein
778	AAEL000128	P130
779	AAEL000141	immunophilin FKBP46, putative
780	AAEL000154	conserved hypothetical protein
781	AAEL000999	DNA replication licensing factor MCM7
782	AAEL017422	hypothetical protein
783	AAEL000974	zinc finger protein
784	AAEL000980	hypothetical protein
785	AAEL006308	px serine/threonine kinase (pxk)
786	AAEL006321	1-acylglycerol-3-phosphate acyltransferase
787	AAEL006309	conserved hypothetical protein
788	AAEL006341	conserved hypothetical protein
789	AAEL006326	deoxyribonuclease I, putative
790	AAEL006340	conserved hypothetical protein
791	AAEL006360	conserved hypothetical protein
792	AAEL006370	amsh

793	AAEL006371	oviductin
794	AAEL006392	hypothetical protein
795	AAEL006405	hypothetical protein
796	AAEL006450	integral membrane protein, putative
797	AAEL006447	GATA transcription factor (GATAb)
798	AAEL006460	par-6 gamma
799	AAEL006455	calcium-activated potassium channel
800	AAEL006498	long wavelength sensitive opsin
801	AAEL006523	crk
802	AAEL006538	peroxisomal membrane protein 2, pxmp2
803	AAEL006556	hypothetical protein
804	AAEL006564	mitochondrial RNA splicing protein
805	AAEL006581	juvenile hormone-inducible protein, putative
806	AAEL015414	hypothetical protein
807	AAEL006603	conserved hypothetical protein
808	AAEL006660	conserved hypothetical protein
809	AAEL006657	hypothetical protein
810	AAEL006656	conserved hypothetical protein
811	AAEL006654	conserved hypothetical protein
812	AAEL006662	hypothetical protein
813	AAEL006672	conserved hypothetical protein
814	AAEL006684	Putative oxidoreductase GLYR1 homolog (EC 1.-.-.-)(Glyoxylate reductase 1 homolog)(Nuclear protein NP60 homolog)
815	AAEL006704	fibrinogen and fibronectin
816	AAEL006694	hypothetical protein
817	AAEL017095	hypothetical protein
818	AAEL006706	conserved hypothetical protein
819	AAEL006713	U2 snrnp auxiliary factor, small subunit
820	AAEL006727	multisynthetase complex, auxiliary protein, p38, putative

821	AAEL001093	PHD finger protein
822	AAEL001053	hypothetical protein
823	AAEL001088	beta-1,3-galactosyltransferase
824	AAEL001067	hypothetical protein
825	AAEL006761	hypothetical protein
826	AAEL006766	hypothetical protein
827	AAEL006777	hypothetical protein
828	AAEL006759	hypothetical protein
829	AAEL006768	hypothetical protein
830	AAEL006801	conserved hypothetical protein
831	AAEL017112	hypothetical protein
832	AAEL006823	AMP dependent ligase
833	AAEL006847	conserved hypothetical protein
834	AAEL006876	igf2 mRNA binding protein, putative
835	AAEL006906	NBP2b protein, putative
836	AAEL006923	conserved hypothetical protein
837	AAEL006931	hypothetical protein
838	AAEL006934	Mediator of RNA polymerase II transcription subunit 19 (Mediator complex subunit 19)
839	AAEL006939	smaug protein
840	AAEL001148	homeobox protein
841	AAEL001137	hypothetical protein
842	AAEL001121	n-acetylgalactosaminyltransferase
843	AAEL001116	hypothetical protein
844	AAEL001125	p15-2b protein, putative
845	AAEL001113	inorganic phosphate cotransporter, putative
846	AAEL006972	hepatocellular carcinoma-associated antigen
847	AAEL006961	lipase
848	AAEL006982	lipase

849	AAEL006998	conserved hypothetical protein
850	AAEL006997	hypothetical protein
851	AAEL006986	conserved hypothetical protein
852	AAEL007007	DNA replication licensing factor MCM2
853	AAEL007053	hypothetical protein
854	AAEL007046	mitochondrial brown fat uncoupling protein
855	AAEL007056	btf
856	AAEL007073	hypothetical protein
857	AAEL007075	conserved hypothetical protein
858	AAEL007071	conserved hypothetical protein
859	AAEL017835	18S_rRNA
860	AAEL007095	adult cuticle protein, putative
861	AAEL007101	adult cuticle protein, putative
862	AAEL007091	single-stranded DNA-binding protein mssp-1
863	AAEL007093	conserved hypothetical protein
864	AAEL007097	4-nitrophenylphosphatase
865	AAEL007128	sugar transporter
866	AAEL017220	hypothetical protein
867	AAEL007134	hypothetical protein
868	AAEL001176	s-adenosylmethionine decarboxylase
869	AAEL017083	hypothetical protein
870	AAEL001180	hypothetical protein
871	AAEL001162	conserved hypothetical protein
872	AAEL001169	Ribosome biogenesis protein BOP1 homolog
873	AAEL017069	hypothetical protein
874	AAEL001158	fructose-1,6-bisphosphatase
875	AAEL001157	light protein
876	AAEL007171	protein phosphatase 2c

877	AAEL007152	hypothetical protein
878	AAEL007158	nnp-1 protein (novel nuclear protein 1) (nop52)
879	AAEL007162	autophagy related gene
880	AAEL007173	conserved hypothetical protein
881	AAEL007198	Osiris, putative
882	AAEL007221	brain-specific homeobox protein, putative
883	AAEL007229	conserved hypothetical protein
884	AAEL007262	hypothetical protein
885	AAEL007261	conserved hypothetical protein
886	AAEL007270	hypothetical protein
887	AAEL015464	histone H1, putative
888	AAEL007287	conserved hypothetical protein
889	AAEL007290	conserved hypothetical protein
890	AAEL007308	glycosyltransferase
891	AAEL007298	conserved hypothetical protein
892	AAEL007323	deoxyuridine 5'-triphosphate nucleotidohydrolase
893	AAEL007339	conserved hypothetical protein
894	AAEL007333	hypothetical protein
895	AAEL001201	hypothetical protein
896	AAEL007399	conserved hypothetical protein
897	AAEL007432	serine collagenase 1 precursor, putative
898	AAEL007427	zinc finger protein
899	AAEL007438	dipeptidyl-peptidase
900	AAEL007448	dipeptidyl-peptidase
901	AAEL017387	hypothetical protein
902	AAEL007445	conserved hypothetical protein
903	AAEL007441	translocon-associated protein, gamma subunit
904	AAEL007447	hypothetical protein

905	AAEL007457	insect origin recognition complex subunit
906	AAEL007456	zinc finger protein, putative
907	AAEL007464	hypothetical protein
908	AAEL007470	staufen
909	AAEL007458	amino acid transporter
910	AAEL007476	makorin
911	AAEL007484	protein transport protein sec23
912	AAEL001231	MIND-MELD/ADAM
913	AAEL001226	conserved hypothetical protein
914	AAEL007523	peroxisomal n1-acetyl-spermine/spermidine oxidase
915	AAEL007543	hypothetical protein
916	AAEL007554	conserved hypothetical protein
917	AAEL007563	Dual Oxidase: Peroxidase and NADPH-Oxidase domains.
918	AAEL007581	Rfc5p, putative
919	AAEL007584	conserved hypothetical protein
920	AAEL007604	odorant-binding protein 56a, putative
921	AAEL007612	hypothetical protein
922	AAEL007611	hypothetical protein
923	AAEL001240	GPCR Orphan/Putative Class B Family
924	AAEL007639	conserved hypothetical protein
925	AAEL007657	low-density lipoprotein receptor (ldl)
926	AAEL007656	receptor for activated C kinase, putative
927	AAEL007658	partitioning defective 3, par-3
928	AAEL007674	conserved hypothetical protein
929	AAEL007677	phospholysine phosphohistidine inorganic pyrophosphate phosphatase
930	AAEL007692	conserved hypothetical protein
931	AAEL007726	hypothetical protein
932	AAEL017004	hypothetical protein

933	AAEL007737	hypothetical protein
934	AAEL007757	conserved hypothetical protein
935	AAEL007761	chloride intracellular channel
936	AAEL007769	hypothetical protein
937	AAEL007768	TOLL pathway signaling.
938	AAEL007767	Protein kintoun
939	AAEL001246	Thymidylate kinase, putative
940	AAEL007813	hypothetical protein
941	AAEL017497	hypothetical protein
942	AAEL007810	conserved hypothetical protein
943	AAEL007819	hypothetical protein
944	AAEL017434	hypothetical protein
945	AAEL007835	serine/threonine protein kinase
946	AAEL007828	palmitoyl-protein thioesterase
947	AAEL015517	conserved hypothetical protein
948	AAEL007859	conserved hypothetical protein
949	AAEL007862	conserved hypothetical protein
950	AAEL007855	hypothetical protein
951	AAEL007867	hypothetical protein
952	AAEL007870	hypothetical protein
953	AAEL007873	hypothetical protein
954	AAEL007875	hypothetical protein
955	AAEL007907	serine/threonine protein kinase
956	AAEL007899	spermatogenesis associated factor
957	AAEL007896	hypothetical protein
958	AAEL007912	conserved hypothetical protein
959	AAEL007926	retinoid-inducible serine carboxypeptidase (serine carboxypeptidase
960	AAEL007922	conserved hypothetical protein

961	AAEL007921	zinc finger protein
962	AAEL001321	transcription factor dp
963	AAEL001296	hypothetical protein
964	AAEL007932	hypothetical protein
965	AAEL007939	conserved hypothetical protein
966	AAEL007959	conserved hypothetical protein
967	AAEL007977	hypothetical protein
968	AAEL007991	conserved hypothetical protein
969	AAEL007987	SEC63 protein, putative
970	AAEL007997	conserved hypothetical protein
971	AAEL008001	conserved hypothetical protein
972	AAEL008020	sorting nexin
973	AAEL017171	hypothetical protein
974	AAEL008043	PNR-like nuclear receptor
975	AAEL008041	bleomycin hydrolase
976	AAEL008050	hypothetical protein
977	AAEL008074	hypothetical protein
978	AAEL008057	myosin light chain kinase
979	AAEL008065	hypothetical protein
980	AAEL000208	copii-coated vesicle membrane protein P24
981	AAEL000186	conserved hypothetical protein
982	AAEL000193	histone-lysine n-methyltransferase
983	AAEL000185	eukaryotic translation initiation factor
984	AAEL001327	conserved hypothetical protein
985	AAEL001348	conserved hypothetical protein
986	AAEL001357	hypothetical protein
987	AAEL017348	hypothetical protein
988	AAEL008088	conserved hypothetical protein

989	AAEL017518	hypothetical protein
990	AAEL008101	hypothetical protein
991	AAEL008099	procollagen-lysine,2-oxoglutarate 5-dioxygenase
992	AAEL008126	GPCR Latrophilin Family
993	AAEL008137	hypothetical protein
994	AAEL008150	hypothetical protein
995	AAEL008182	conserved hypothetical protein
996	AAEL008184	conserved hypothetical protein
997	AAEL008183	t complex protein
998	AAEL008185	conserved hypothetical protein
999	AAEL008189	conserved hypothetical protein
1000	AAEL008220	conserved hypothetical protein
1001	AAEL008233	conserved hypothetical protein
1002	AAEL008236	sidestep protein
1003	AAEL008224	hypothetical protein
1004	AAEL008257	heterogeneous nuclear ribonucleoprotein 27c
1005	AAEL008256	cyclin A3, putative
1006	AAEL001372	sentrin/sumo-specific protease senp7
1007	AAEL008261	hypothetical protein
1008	AAEL008320	conserved hypothetical protein
1009	AAEL008322	GPCR Frizzled/Smoothened Family
1010	AAEL008351	POSSIBLE INTEGRAL MEMBRANE EFFLUX PROTEIN EFPA, putative
1011	AAEL008356	hypothetical protein
1012	AAEL008359	hypothetical protein
1013	AAEL015551	conserved hypothetical protein
1014	AAEL001399	conserved hypothetical protein
1015	AAEL001437	conserved hypothetical protein
1016	AAEL001439	mitochondrial ribosomal protein, L22, putative

1017	AAEL008379	P38 mapk
1018	AAEL008387	atrial natriuretic peptide receptor
1019	AAEL008406	cationic amino acid transporter
1020	AAEL008421	cadherin
1021	AAEL017350	hypothetical protein
1022	AAEL008444	conserved hypothetical protein
1023	AAEL008461	surfeit locus protein
1024	AAEL008476	conserved hypothetical protein
1025	AAEL008500	DEAD box ATP-dependent RNA helicase
1026	AAEL008503	hypothetical protein
1027	AAEL001451	DNA repair protein Rad62, putative
1028	AAEL001464	conserved hypothetical protein
1029	AAEL008510	sphingosine kinase a, b
1030	AAEL008511	hypothetical protein
1031	AAEL008555	conserved hypothetical protein
1032	AAEL008522	conserved hypothetical protein
1033	AAEL008544	zinc finger protein, putative
1034	AAEL008537	transcription factor grauzone, putative
1035	AAEL008535	conserved hypothetical protein
1036	AAEL008521	conserved hypothetical protein
1037	AAEL017089	hypothetical protein
1038	AAEL008533	conserved hypothetical protein
1039	AAEL008547	conserved hypothetical protein
1040	AAEL008526	conserved hypothetical protein
1041	AAEL008570	glycoprotein, putative
1042	AAEL008557	conserved hypothetical protein
1043	AAEL008583	conserved hypothetical protein
1044	AAEL008580	conserved hypothetical protein

1045	AAEL008579	zinc finger protein
1046	AAEL015565	hypothetical protein
1047	AAEL008602	conserved hypothetical protein
1048	AAEL008593	NAD dependent epimerase/dehydratase
1049	AAEL008623	conserved hypothetical protein
1050	AAEL008638	cytochrome P450
1051	AAEL001490	acylphosphatase, putative
1052	AAEL001505	conserved hypothetical protein
1053	AAEL001506	U3 small nucleolar ribonucleoprotein protein mpp10
1054	AAEL001481	hypothetical protein
1055	AAEL015571	conserved hypothetical protein
1056	AAEL008678	conserved hypothetical protein
1057	AAEL008675	hypothetical protein
1058	AAEL008722	hypothetical protein

1059	AAEL008724	conserved hypothetical protein
1060	AAEL008729	hypothetical protein
1061	AAEL008748	hypothetical protein
1062	AAEL008759	hypothetical protein
1063	AAEL008777	proto-oncogene tyrosine-protein kinase abl1
1064	AAEL008781	serine-type enodpeptidase,
1065	AAEL008794	conserved hypothetical protein
1066	AAEL008817	hexamerin 2 beta
1067	AAEL008822	conserved hypothetical protein
1068	AAEL008809	pickpocket, putative
1069	AAEL008797	hypothetical protein
1070	AAEL001525	conserved hypothetical protein
1071	AAEL008839	hypothetical protein
1072	AAEL008842	hypothetical protein
1073	AAEL008863	protein regulator of cytokinesis 1 prc1
1074	AAEL008868	conserved hypothetical protein
1075	AAEL008875	conserved hypothetical protein
1076	AAEL008886	conserved hypothetical protein
1077	AAEL008884	hypothetical protein
1078	AAEL008900	p15-2a protein, putative
1079	AAEL008894	conserved hypothetical protein
1080	AAEL008915	sodium-and chloride-activated ATP-sensitive potassium channel
1081	AAEL008923	ring finger protein
1082	AAEL001545	conserved hypothetical protein
1083	AAEL001585	predicted protein
1084	AAEL001595	conserved hypothetical protein
1085	AAEL001569	conserved hypothetical protein
1086	AAEL001576	conserved hypothetical protein

1087	AAEL001588	glutamate carboxypeptidase
1088	AAEL001581	conserved hypothetical protein
1089	AAEL001559	conserved hypothetical protein
1090	AAEL008942	conserved hypothetical protein
1091	AAEL008939	conserved hypothetical protein
1092	AAEL008983	adult cuticle protein, putative
1093	AAEL009022	adenylate cyclase type
1094	AAEL009021	peptidylprolyl isomerase
1095	AAEL009023	conserved hypothetical protein
1096	AAEL009057	cyclin e
1097	AAEL009084	slender lobes, putative
1098	AAEL009080	importin 7,
1099	AAEL001614	conserved hypothetical protein
1100	AAEL001622	dual specificity mitogen-activated protein kinase kinase MAPKK
1101	AAEL001609	hypothetical protein
1102	AAEL009147	conserved hypothetical protein
1103	AAEL009153	M-type 9 protein, putative
1104	AAEL009160	skp1
1105	AAEL009163	conserved hypothetical protein
1106	AAEL009167	bone morphogenetic protein 5/7, bmp5/7
1107	AAEL009187	conserved hypothetical protein
1108	AAEL009189	encore protein
1109	AAEL001663	hypothetical protein
1110	AAEL001666	conserved hypothetical protein
1111	AAEL001650	conserved hypothetical protein
1112	AAEL009241	translation initiation factor if-2
1113	AAEL009233	zinc metalloprotease
1114	AAEL009248	conserved hypothetical protein

1115	AAEL009260	conserved hypothetical protein
1116	AAEL009261	hypothetical protein
1117	AAEL009263	conserved hypothetical protein
1118	AAEL009262	hypothetical protein
1119	AAEL009277	hypothetical protein
1120	AAEL009290	hypothetical protein
1121	AAEL009296	histone H3, putative
1122	AAEL009309	lipid depleted protein
1123	AAEL009322	hypothetical protein
1124	AAEL001684	boule protein, putative
1125	AAEL001700	hypothetical protein
1126	AAEL001697	adenylate cyclase, putative
1127	AAEL001703	serine-type enodpeptidase,
1128	AAEL001691	adenylate cyclase
1129	AAEL001693	serine-type enodpeptidase,
1130	AAEL009335	adhesion regulating molecule 1 (110 kda cell membrane glycoprotein)
1131	AAEL009348	conserved hypothetical protein
1132	AAEL009393	conserved hypothetical protein
1133	AAEL009411	DNA-binding protein smubp-2
1134	AAEL009425	hypothetical protein
1135	AAEL009442	hypothetical protein
1136	AAEL015633	conserved hypothetical protein
1137	AAEL009452	hypothetical protein
1138	AAEL009456	hypothetical protein
1139	AAEL009454	conserved hypothetical protein
1140	AAEL009470	conserved hypothetical protein
1141	AAEL009465	replication factor c / DNA polymerase iii gamma-tau subunit
1142	AAEL009463	hypothetical protein

1143	AAEL009484	conserved hypothetical protein
1144	AAEL000224	serine protease
1145	AAEL000223	alpha-glucosidase
1146	AAEL000235	hypothetical protein
1147	AAEL001711	activin receptor type I, putative
1148	AAEL001727	hypothetical protein
1149	AAEL009510	glucosamine-fructose-6-phosphate aminotransferase
1150	AAEL009500	conserved hypothetical protein
1151	AAEL009508	zinc finger protein
1152	AAEL017066	hypothetical protein
1153	AAEL009518	timeout/timeless-2
1154	AAEL009522	hypothetical protein
1155	AAEL009533	conserved hypothetical protein
1156	AAEL009551	Toll-like receptor
1157	AAEL009576	conserved hypothetical protein
1158	AAEL015640	transcription factor IIIA, putative
1159	AAEL001745	candidate tumor suppressor protein
1160	AAEL001734	bric-a-brac
1161	AAEL009586	hypothetical protein
1162	AAEL009600	Ecdysone receptor isoform A Nuclear receptor
1163	AAEL009602	conserved hypothetical protein
1164	AAEL009646	conserved hypothetical protein
1165	AAEL009667	conserved hypothetical protein
1166	AAEL017306	hypothetical protein
1167	AAEL001781	origin recognition complex subunit
1168	AAEL001785	origin recognition complex subunit
1169	AAEL001788	hypothetical protein
1170	AAEL009710	adhesion regulating molecule 1 (110 kda cell membrane glycoprotein)

1171	AAEL009709	hypothetical protein
1172	AAEL009727	conserved hypothetical protein
1173	AAEL009716	hypothetical protein
1174	AAEL009729	conserved hypothetical protein
1175	AAEL009739	cbl-d
1176	AAEL009742	Homeobox protein abdominal-A homolog
1177	AAEL009755	conserved hypothetical protein
1178	AAEL009753	sodium-dependent phosphate transporter
1179	AAEL009773	geminin, putative
1180	AAEL009772	conserved hypothetical protein
1181	AAEL009770	ubiquitin-conjugating enzyme E2 i
1182	AAEL009798	transcription factor IIIA, putative
1183	AAEL009799	hypothetical protein
1184	AAEL001809	conserved hypothetical protein
1185	AAEL017094	hypothetical protein
1186	AAEL001795	orfY, putative
1187	AAEL009854	conserved hypothetical protein
1188	AAEL009834	hypothetical protein
1189	AAEL009842	galectin
1190	AAEL009845	galectin
1191	AAEL009836	conserved hypothetical protein
1192	AAEL009861	conserved hypothetical protein
1193	AAEL009896	hypothetical protein
1194	AAEL009894	leucine-rich immune protein (Coil-less)
1195	AAEL009886	kidney-specific Na-K-Cl cotransport protein splice isoform A, putative
1196	AAEL009918	conserved hypothetical protein
1197	AAEL009912	conserved hypothetical protein
1198	AAEL009935	conserved hypothetical protein

1199	AAEL001839	zinc carboxypeptidase
1200	AAEL001842	zinc carboxypeptidase
1201	AAEL017186	hypothetical protein
1202	AAEL001877	fucosyltransferase 11 (fut11)
1203	AAEL001858	hypothetical protein
1204	AAEL001867	sodium-dependent phosphate transporter
1205	AAEL001856	adenosine kinase
1206	AAEL009972	hypothetical protein
1207	AAEL009962	hypothetical protein
1208	AAEL009984	alanyl-tRNA synthetase
1209	AAEL009983	GPCR Frizzled/Smoothened Family
1210	AAEL009999	conserved hypothetical protein
1211	AAEL009998	conserved hypothetical protein
1212	AAEL010007	conserved hypothetical protein
1213	AAEL010011	conserved hypothetical protein
1214	AAEL010001	conserved hypothetical protein
1215	AAEL010014	hypothetical protein
1216	AAEL010033	DNA mismatch repair protein pms2
1217	AAEL001890	hypothetical protein
1218	AAEL001902	glutamate decarboxylase
1219	AAEL001908	hypothetical protein
1220	AAEL001904	arp2/3
1221	AAEL001900	lactosylceramide 4-alpha-galactosyltransferase (alpha- 1,4-galactosyltransferase)
1222	AAEL010060	signal recognition particle 68 kda protein
1223	AAEL010072	hypothetical protein
1224	AAEL010080	origin recognition complex subunit
1225	AAEL010094	cyclin b
1226	AAEL010081	conserved hypothetical protein

1227	AAEL010112	conserved hypothetical protein
1228	AAEL010097	conserved hypothetical protein
1229	AAEL010113	conserved hypothetical protein
1230	AAEL010109	conserved hypothetical protein
1231	AAEL010117	fibrinogen and fibronectin
1232	AAEL010118	kelch repeat protein
1233	AAEL010136	hypothetical protein
1234	AAEL010155	hypothetical protein
1235	AAEL010176	conserved hypothetical protein
1236	AAEL010189	Band 7 protein AAEL010189
1237	AAEL001927	hypothetical protein
1238	AAEL001917	ribosome biogenesis protein brix
1239	AAEL001939	hypothetical protein
1240	AAEL001933	membrane associated ring finger 1,8
1241	AAEL010194	hypothetical protein
1242	AAEL010242	conserved hypothetical protein
1243	AAEL010226	daughterless
1244	AAEL010229	hypothetical protein
1245	AAEL010253	conserved hypothetical protein
1246	AAEL010244	abrupt protein
1247	AAEL010246	conserved hypothetical protein
1248	AAEL010249	conserved hypothetical protein
1249	AAEL010290	short-chain dehydrogenase
1250	AAEL010289	beta nu integrin subunit
1251	AAEL010292	conserved hypothetical protein
1252	AAEL010294	membrane-associated guanylate kinase (maguk)
1253	AAEL015673	nucleolar complex protein
1254	AAEL002032	hypothetical protein

1255	AAEL002033	hypothetical protein
1256	AAEL002010	conserved hypothetical protein
1257	AAEL001972	TATA box binding protein (TBP)-associated factor,, putative
1258	AAEL002006	conserved hypothetical protein
1259	AAEL010311	conserved hypothetical protein
1260	AAEL010318	polyadenylate-binding protein
1261	AAEL010309	hypothetical protein
1262	AAEL010319	heat shock transcription factor (hsf)
1263	AAEL010343	aryl hydrocarbon receptor nuclear translocator (arnt protein) (hypoxia-inducible factor 1 beta)
1264	AAEL017368	hypothetical protein
1265	AAEL010378	conserved hypothetical protein
1266	AAEL010381	glucosyl/glucuronosyl transferases
1267	AAEL010370	aldehyde oxidase
1268	AAEL010420	hypothetical protein
1269	AAEL010422	replication-associated histone mRNA stem loop-binding protein, putative
1270	AAEL010417	conserved hypothetical protein
1271	AAEL010434	Vitellogenin-A1 Precursor (VG)(PVG1) [Contains Vitellin light chain(VL);Vitellin heavy chain(VH)]
1272	AAEL010437	heparan n-sulfatase
1273	AAEL010447	hypothetical protein
1274	AAEL010446	protein phosphatase 2c
1275	AAEL010454	hypothetical protein
1276	AAEL010473	NAD dependent epimerase/dehydratase
1277	AAEL002079	TATA binding protein, putative
1278	AAEL002054	hypothetical protein
1279	AAEL002058	hypothetical protein
1280	AAEL002063	cationic amino acid transporter
1281	AAEL010501	zinc finger protein

1282	AAEL010490	hypothetical protein
1283	AAEL010495	hypothetical protein
1284	AAEL010509	bridging integrator
1285	AAEL010503	hypothetical protein
1286	AAEL010507	hypothetical protein
1287	AAEL010510	conserved hypothetical protein
1288	AAEL010538	conserved hypothetical protein
1289	AAEL010546	heat shock factor binding protein, putative
1290	AAEL010562	hypothetical protein
1291	AAEL010587	conserved hypothetical protein
1292	AAEL010588	striatin, putative
1293	AAEL010578	mixed-lineage leukemia protein, mll
1294	AAEL002128	serine protease
1295	AAEL010631	conserved hypothetical protein
1296	AAEL010623	conserved hypothetical protein
1297	AAEL010627	conserved hypothetical protein
1298	AAEL010638	histone H1, putative
1299	AAEL010644	ribonucleoside-diphosphate reductase large chain
1300	AAEL010664	actin binding protein, putative
1301	AAEL010670	lethal(2)essential for life protein, l2efl
1302	AAEL010679	monocyte to macrophage differentiation protein
1303	AAEL010665	developmentally regulated RNA-binding protein
1304	AAEL010674	hypothetical protein
1305	AAEL010660	alpha-B-crystallin, putative
1306	AAEL017191	hypothetical protein
1307	AAEL010692	OCP-II protein, putative
1308	AAEL010708	hypothetical protein
1309	AAEL010709	hypothetical protein

1310	AAEL000289	conserved hypothetical protein
1311	AAEL000272	conserved hypothetical protein
1312	AAEL000260	conserved hypothetical protein
1313	AAEL002136	hypothetical protein
1314	AAEL017571	hypothetical protein
1315	AAEL010715	hypothetical protein
1316	AAEL017338	hypothetical protein
1317	AAEL010755	hypothetical protein
1318	AAEL010748	hypothetical protein
1319	AAEL010766	inositol triphosphate 3-kinase c
1320	AAEL010784	conserved hypothetical protein
1321	AAEL002184	F-actin capping protein beta subunit
1322	AAEL002181	cuticle protein, putative
1323	AAEL002219	zinc finger protein, putative
1324	AAEL010829	protein arginine n-methyltransferase
1325	AAEL010808	conserved hypothetical protein
1326	AAEL010827	programmed cell death protein 11 (pre-rRNA processing protein rrp5)
1327	AAEL010841	lupus la ribonucleoprotein
1328	AAEL010855	cdc6
1329	AAEL010877	conserved hypothetical protein
1330	AAEL010901	mannose binding lectin, putative
1331	AAEL010904	rothmund-thomson syndrome DNA helicase recq4
1332	AAEL010907	conserved hypothetical protein
1333	AAEL010908	hypothetical protein
1334	AAEL010912	dipeptidyl-peptidase
1335	AAEL002250	terminal deoxycytidyl transferase rev1
1336	AAEL010930	l-asparaginase
1337	AAEL010945	conserved hypothetical protein

1338	AAEL010965	cubulin
1339	AAEL002303	conserved hypothetical protein
1340	AAEL002319	hypothetical protein
1341	AAEL002320	hypothetical protein
1342	AAEL002307	leucine-rich transmembrane protein
1343	AAEL011003	hypothetical protein
1344	AAEL011013	single-minded
1345	AAEL011043	conserved hypothetical protein
1346	AAEL011062	hypothetical protein
1347	AAEL002332	hypothetical protein
1348	AAEL002354	heme peroxidase
1349	AAEL011085	conserved hypothetical protein
1350	AAEL011124	PHD finger protein
1351	AAEL011138	hypothetical protein
1352	AAEL011145	ribosomal protein S6 kinase, 90kD, polypeptide
1353	AAEL011161	conserved hypothetical protein
1354	AAEL011173	conserved hypothetical protein
1355	AAEL011172	conserved hypothetical protein
1356	AAEL011175	alkaline phosphatase
1357	AAEL011176	hypothetical protein
1358	AAEL011178	posterior sex combs protein
1359	AAEL002403	hypothetical protein
1360	AAEL002376	hypothetical protein
1361	AAEL002375	NBP2b protein, putative
1362	AAEL011199	conserved hypothetical protein
1363	AAEL011215	F-box and WD40 domain protein 7 (fbw7)
1364	AAEL002423	conserved hypothetical protein
1365	AAEL002417	troponin t, invertebrate

1366	AAEL002429	hypothetical protein
1367	AAEL011248	innexin
1368	AAEL011253	rho-GTPase-activating protein
1369	AAEL011264	phosphatidylethanolamine-binding protein
1370	AAEL011276	mitochondrial glutamate carrier protein
1371	AAEL011280	voltage-dependent p/q type calcium channel
1372	AAEL011291	protease m1 zinc metalloprotease
1373	AAEL011298	hypothetical protein
1374	AAEL011303	cell division protein ftsj
1375	AAEL011313	epoxide hydrolase
1376	AAEL011330	conserved hypothetical protein
1377	AAEL011326	conserved hypothetical protein
1378	AAEL002453	conserved hypothetical protein
1379	AAEL002442	conserved hypothetical protein
1380	AAEL002443	conserved hypothetical protein
1381	AAEL002454	conserved hypothetical protein
1382	AAEL011358	origin recognition complex subunit
1383	AAEL011357	maintenance of killer 16 (mak16) protein
1384	AAEL011362	hypothetical protein
1385	AAEL011400	conserved hypothetical protein
1386	AAEL011422	conserved hypothetical protein
1387	AAEL002473	hypothetical protein
1388	AAEL002462	hypothetical protein
1389	AAEL002477	hypothetical protein
1390	AAEL017575	hypothetical protein
1391	AAEL011473	chromatin regulatory protein sir2
1392	AAEL011498	copper-zinc (Cu-Zn) superoxide dismutase
1393	AAEL011496	chitinase

1394	AAEL016971	hypothetical protein
1395	AAEL011516	conserved hypothetical protein
1396	AAEL011515	hypothetical protein
1397	AAEL011520	sucrose transport protein
1398	AAEL011536	phosphoglucomutase
1399	AAEL011527	eukaryotic translation initiation factor
1400	AAEL011533	hypothetical protein
1401	AAEL011532	hypothetical protein
1402	AAEL011537	hypothetical protein
1403	AAEL002529	conserved hypothetical protein
1404	AAEL002503	yippee protein
1405	AAEL002522	adenosine deaminase acting on RNA (adar)-2
1406	AAEL002497	hypothetical protein
1407	AAEL011586	hypothetical protein
1408	AAEL011592	secreted mucin MUC17, putative
1409	AAEL011598	hypothetical protein
1410	AAEL011596	mitotic checkpoint serine/threonine-protein kinase bub1 and bubr1
1411	AAEL011597	conserved hypothetical protein
1412	AAEL011615	rab gdp/GTP exchange factor
1413	AAEL011633	fibrinogen and fibronectin
1414	AAEL011631	hypothetical protein
1415	AAEL011640	hypothetical protein
1416	AAEL011635	conserved hypothetical protein
1417	AAEL011648	cyclin d
1418	AAEL011655	aspartyl-tRNA synthetase
1419	AAEL011664	conserved hypothetical protein
1420	AAEL011653	thyroid hormone receptor interactor
1421	AAEL000322	hypothetical protein

1422	AAEL000352	hypothetical protein
1423	AAEL017203	hypothetical protein
1424	AAEL000356	cysteine-rich venom protein, putative
1425	AAEL000302	cysteine-rich venom protein, putative
1426	AAEL000375	cysteine-rich venom protein, putative
1427	AAEL000317	cysteine-rich venom protein, putative
1428	AAEL000348	conserved hypothetical protein
1429	AAEL000327	Ecdysone-induced protein 78C Nuclear receptor
1430	AAEL000324	tyrosine-protein kinase drl
1431	AAEL002557	cationic amino acid transporter
1432	AAEL002541	cystinosin
1433	AAEL002569	serine/threonine kinase
1434	AAEL011696	conserved hypothetical protein
1435	AAEL011686	starch branching enzyme ii
1436	AAEL011695	guanine nucleotide exchange factor
1437	AAEL011712	diacylglycerol kinase, alpha, beta, gamma
1438	AAEL011726	hypothetical protein
1439	AAEL011735	conserved hypothetical protein
1440	AAEL011743	hypothetical protein
1441	AAEL011754	conserved hypothetical protein
1442	AAEL011765	conserved hypothetical protein
1443	AAEL011767	hypothetical protein
1444	AAEL011761	cytochrome P450
1445	AAEL011769	cytochrome P450
1446	AAEL011780	DNA mismatch repair protein muts
1447	AAEL011809	glucose dehydrogenase
1448	AAEL011811	DNA replication licensing factor MCM3
1449	AAEL002652	hypothetical protein

1450	AAEL002635	conserved hypothetical protein
1451	AAEL011846	hypothetical protein
1452	AAEL011852	hypothetical protein
1453	AAEL011859	conserved hypothetical protein
1454	AAEL011862	conserved hypothetical protein
1455	AAEL011868	conserved hypothetical protein
1456	AAEL011870	rap55
1457	AAEL011875	conserved hypothetical protein
1458	AAEL011872	conserved hypothetical protein
1459	AAEL011892	receptor for activated C kinase, putative
1460	AAEL011895	odorant receptor
1461	AAEL017451	hypothetical protein
1462	AAEL017179	hypothetical protein
1463	AAEL017200	hypothetical protein
1464	AAEL011958	conserved hypothetical protein
1465	AAEL011979	calmodulin
1466	AAEL017253	hypothetical protein
1467	AAEL002690	beat protein
1468	AAEL002681	Vanin-like protein 1 precursor, putative
1469	AAEL011989	signal peptide peptidase
1470	AAEL012014	l-lactate dehydrogenase
1471	AAEL012020	conserved hypothetical protein
1472	AAEL012013	hypothetical protein
1473	AAEL012015	DEAD box ATP-dependent RNA helicase
1474	AAEL012012	conserved hypothetical protein
1475	AAEL012032	hypothetical protein
1476	AAEL012028	proacrosin, putative
1477	AAEL012030	preproacrosin, putative

1478	AAEL012041	sulphate transporter
1479	AAEL012055	dfg10 protein
1480	AAEL012057	enhancer of polycomb
1481	AAEL002719	conserved hypothetical protein
1482	AAEL002700	conserved hypothetical protein
1483	AAEL012130	ordml, arthropod
1484	AAEL012142	timeout/timeless-2
1485	AAEL012137	conserved hypothetical protein
1486	AAEL012141	odorant receptor
1487	AAEL012155	conserved hypothetical protein
1488	AAEL012164	spaetzle-like cytokine
1489	AAEL012209	ring finger protein
1490	AAEL017392	hypothetical protein
1491	AAEL012214	hypothetical protein
1492	AAEL012273	conserved hypothetical protein
1493	AAEL012279	Eukaryotic translation initiation factor 3 subunit J (eIF3j)
1494	AAEL012288	sugar transporter
1495	AAEL012295	conserved hypothetical protein
1496	AAEL012293	conserved hypothetical protein
1497	AAEL012300	conserved hypothetical protein
1498	AAEL012312	proliferation-associated 2g4 (pa2g4/ebp1)
1499	AAEL012314	conserved hypothetical protein
1500	AAEL002793	conserved hypothetical protein
1501	AAEL002785	DNA polymerase epsilon subunit b
1502	AAEL002811	conserved hypothetical protein
1503	AAEL002814	hypothetical protein
1504	AAEL002810	DNA replication licensing factor MCM5
1505	AAEL002774	slender lobes, putative

1506	AAEL012378	serine-type protease inhibitor
1507	AAEL012392	conserved hypothetical protein
1508	AAEL012388	predicted protein
1509	AAEL002843	conserved hypothetical protein
1510	AAEL002836	carbon catabolite repressor protein
1511	AAEL002848	tubulin beta chain
1512	AAEL002849	zinc finger protein, putative
1513	AAEL012418	deoxyribonuclease ii
1514	AAEL012427	conserved hypothetical protein
1515	AAEL012430	AMP dependent ligase
1516	AAEL012437	hypothetical protein
1517	AAEL012441	conserved hypothetical protein
1518	AAEL012458	hypothetical protein
1519	AAEL012461	monocarboxylate transporter
1520	AAEL012455	alcohol dehydrogenase
1521	AAEL000402	conserved hypothetical protein
1522	AAEL002867	phenylalanyl-tRNA synthetase alpha chain
1523	AAEL002856	conserved hypothetical protein
1524	AAEL002863	zinc finger protein
1525	AAEL002855	hypothetical protein
1526	AAEL002882	conserved hypothetical protein
1527	AAEL002872	cytochrome P450
1528	AAEL012473	vav1
1529	AAEL012480	sodium/calcium exchanger
1530	AAEL012499	histone H2A
1531	AAEL012504	hypothetical protein
1532	AAEL012526	hypothetical protein
1533	AAEL012527	conserved hypothetical protein

1534	AAEL012546	DNA replication licensing factor MCM6
1535	AAEL002889	hypothetical protein
1536	AAEL002884	hypothetical protein
1537	AAEL002905	conserved hypothetical protein
1538	AAEL012566	conserved hypothetical protein
1539	AAEL012586	conserved hypothetical protein
1540	AAEL012600	hypothetical protein
1541	AAEL012610	conserved hypothetical protein
1542	AAEL012618	conserved hypothetical protein
1543	AAEL012629	deoxyuridine 5'-triphosphate nucleotidohydrolase
1544	AAEL002932	conserved hypothetical protein
1545	AAEL002942	hypothetical protein
1546	AAEL002947	AMP dependent ligase
1547	AAEL012644	conserved hypothetical protein
1548	AAEL012647	conserved hypothetical protein
1549	AAEL012650	conserved hypothetical protein
1550	AAEL012658	rgs-gaip interacting protein gipc
1551	AAEL012684	conserved hypothetical protein
1552	AAEL012682	hypothetical protein
1553	AAEL012676	conserved hypothetical protein
1554	AAEL012708	conserved hypothetical protein
1555	AAEL012714	hypothetical protein
1556	AAEL017254	hypothetical protein
1557	AAEL002949	Osiris, putative
1558	AAEL002958	conserved hypothetical protein
1559	AAEL002991	hypothetical protein
1560	AAEL003003	glutamate-gated chloride channel
1561	AAEL002989	hypothetical protein

1562	AAEL012811	mitochondrial peptide chain release factor
1563	AAEL012810	conserved hypothetical protein
1564	AAEL012802	conserved hypothetical protein
1565	AAEL012812	exosome complex exonuclease RRP41, putative
1566	AAEL012830	anti-silencing protein
1567	AAEL012832	cytochrome B561
1568	AAEL012836	cytochrome B561
1569	AAEL012838	conserved hypothetical protein
1570	AAEL012876	conserved hypothetical protein
1571	AAEL012875	snare protein sec22
1572	AAEL003058	glucosyl/glucuronosyl transferases
1573	AAEL003051	conserved hypothetical protein
1574	AAEL012927	hypothetical protein
1575	AAEL012927	hypothetical protein
1576	AAEL012960	importin alpha
1577	AAEL012979	conserved hypothetical protein
1578	AAEL017272	GPCR Serotonin Family
1579	AAEL012996	rho guanine dissociation factor
1580	AAEL013004	conserved hypothetical protein
1581	AAEL013024	hypothetical protein
1582	AAEL003112	conserved hypothetical protein
1583	AAEL013036	conserved hypothetical protein
1584	AAEL013037	conserved hypothetical protein
1585	AAEL013038	hypothetical protein
1586	AAEL013051	conserved hypothetical protein
1587	AAEL013054	conserved hypothetical protein
1588	AAEL013045	exosome complex exonuclease RRP41, putative
1589	AAEL013078	glycosyltransferase

1590	AAEL013091	hypothetical protein
1591	AAEL017424	hypothetical protein
1592	AAEL003130	bcr-associated protein, bap
1593	AAEL013112	Peptidoglycan Recognition Protein (Long)
1594	AAEL013110	conserved hypothetical protein
1595	AAEL013149	conserved hypothetical protein
1596	AAEL013156	hypothetical protein
1597	AAEL013148	predicted protein
1598	AAEL013154	hypothetical protein
1599	AAEL013160	GPCR Frizzled/Smoothened Family
1600	AAEL013168	arrowhead
1601	AAEL000470	hypothetical protein
1602	AAEL000426	hypothetical protein
1603	AAEL003172	transcription factor IIIA, putative
1604	AAEL003158	conserved hypothetical protein
1605	AAEL003168	hypothetical protein
1606	AAEL013174	conserved hypothetical protein
1607	AAEL013179	8-oxoguanine DNA glycosylase
1608	AAEL013190	gustatory receptor Gr22
1609	AAEL013212	prefoldin, subunit, putative
1610	AAEL013216	conserved hypothetical protein
1611	AAEL013226	conserved hypothetical protein
1612	AAEL003186	hypothetical protein
1613	AAEL003207	hypothetical protein
1614	AAEL013240	conserved hypothetical protein
1615	AAEL013248	hypothetical protein
1616	AAEL013252	hypothetical protein
1617	AAEL013249	hypothetical protein

1618	AAEL013251	hypothetical protein
1619	AAEL013291	conserved hypothetical protein
1620	AAEL013288	conserved hypothetical protein
1621	AAEL013285	hypothetical protein
1622	AAEL003210	tetraspanin 29fa
1623	AAEL003214	salivary gland growth factor
1624	AAEL003267	hypothetical protein
1625	AAEL013311	hypothetical protein
1626	AAEL013312	dual-specificity protein phosphatase, putative
1627	AAEL013325	conserved hypothetical protein
1628	AAEL013344	lethal(2)essential for life protein, l2efl
1629	AAEL013338	lethal(2)essential for life protein, l2efl
1630	AAEL003312	hypothetical protein
1631	AAEL003321	hypothetical protein
1632	AAEL003285	translocation associated membrane protein
1633	AAEL013400	DEAD box ATP-dependent RNA helicase
1634	AAEL017652	18S_rRNA
1635	AAEL003355	conserved hypothetical protein
1636	AAEL003343	hypothetical protein
1637	AAEL003346	heparan sulphate 2-o-sulfotransferase
1638	AAEL003327	zinc finger protein
1639	AAEL013412	conserved hypothetical protein
1640	AAEL013453	sarcolemmal associated protein, putative
1641	AAEL013463	nucleolar protein 10
1642	AAEL017276	hypothetical protein
1643	AAEL003377	signal recognition particle
1644	AAEL003382	Ro ribonucleoprotein autoantigen, putative
1645	AAEL013465	conserved hypothetical protein

1646	AAEL013471	hypothetical protein
1647	AAEL013490	conserved hypothetical protein
1648	AAEL003435	conserved hypothetical protein
1649	AAEL003404	hypothetical protein
1650	AAEL013510	smaug protein
1651	AAEL013521	tryptophanyl-tRNA synthetase
1652	AAEL013539	SH2/SH3 adaptor protein
1653	AAEL013546	estrogen-related receptor (ERR)
1654	AAEL013562	zinc finger protein, putative
1655	AAEL003454	phocein protein, putative
1656	AAEL013564	conserved hypothetical protein
1657	AAEL013569	conserved hypothetical protein
1658	AAEL013593	hypothetical protein
1659	AAEL003494	goodpasture antigen-binding protein
1660	AAEL003493	GDI interacting protein, putative
1661	AAEL003502	hypothetical protein
1662	AAEL003507	Toll-like receptor
1663	AAEL013601	short-chain dehydrogenase
1664	AAEL013608	sugar transporter
1665	AAEL013616	hypothetical protein
1666	AAEL013635	conserved hypothetical protein
1667	AAEL003544	conserved hypothetical protein
1668	AAEL017367	hypothetical protein
1669	AAEL003542	conserved hypothetical protein
1670	AAEL003547	hypothetical protein
1671	AAEL013653	tata-box binding protein
1672	AAEL017342	hypothetical protein
1673	AAEL013690	DNA mismatch repair protein pms2

1674	AAEL000487	hypothetical protein
1675	AAEL000500	conserved hypothetical protein
1676	AAEL003554	leucine rich repeat protein
1677	AAEL013701	meiotic recombination protein spo11
1678	AAEL013724	conserved hypothetical protein
1679	AAEL013726	epsin 4/enthoprotin
1680	AAEL017141	hypothetical protein
1681	AAEL013733	Protein distal antenna
1682	AAEL013734	hypothetical protein
1683	AAEL013738	hypothetical protein
1684	AAEL003574	hypothetical protein
1685	AAEL003571	factor for adipocyte differentiation
1686	AAEL013761	ADP-ribosylation factor, arf
1687	AAEL013778	F-actin capping protein alpha
1688	AAEL013784	hypothetical protein
1689	AAEL017560	hypothetical protein
1690	AAEL003595	protein serine/threonine kinase, putative
1691	AAEL013789	conserved hypothetical protein
1692	AAEL013796	conserved hypothetical protein
1693	AAEL013799	hypothetical protein
1694	AAEL013805	conserved hypothetical protein
1695	AAEL013806	conserved hypothetical protein
1696	AAEL013809	conserved hypothetical protein
1697	AAEL013813	conserved hypothetical protein
1698	AAEL013830	bmp-induced factor
1699	AAEL013832	Homeobox protein abdominal-A homolog
1700	AAEL013838	hypothetical protein
1701	AAEL003646	conserved hypothetical protein

1702	AAEL003663	hypothetical protein
1703	AAEL003688	conserved hypothetical protein
1704	AAEL015684	hypothetical protein
1705	AAEL003657	zinc finger protein
1706	AAEL013852	conserved hypothetical protein
1707	AAEL013850	conserved hypothetical protein
1708	AAEL013860	hypothetical protein
1709	AAEL013872	hypothetical protein
1710	AAEL013896	smad4
1711	AAEL003767	hypothetical protein
1712	AAEL013940	chromatin assembly factor i P60 subunit
1713	AAEL013955	conserved hypothetical protein
1714	AAEL003797	hypothetical protein
1715	AAEL003804	conserved hypothetical protein
1716	AAEL003775	hypothetical protein
1717	AAEL003793	hypothetical protein
1718	AAEL003791	conserved hypothetical protein
1719	AAEL003792	conserved hypothetical protein
1720	AAEL003807	conserved hypothetical protein
1721	AAEL013958	NBP2b protein, putative
1722	AAEL013968	conserved hypothetical protein
1723	AAEL013965	conserved hypothetical protein
1724	AAEL013975	transcription factor IIIA, putative
1725	AAEL013989	protein translocation complex beta subunit, putative
1726	AAEL013998	conserved hypothetical protein
1727	AAEL014001	yellow protein precursor, putative
1728	AAEL003817	kappa b-ras
1729	AAEL003824	conserved hypothetical protein

1730	AAEL003861	bmp-induced factor
1731	AAEL014020	hypothetical protein
1732	AAEL014025	cell division cycle 20 (cdc20) (fizzy)
1733	AAEL014033	conserved hypothetical protein
1734	AAEL014036	hypothetical protein
1735	AAEL014047	hypothetical protein
1736	AAEL014055	thymidine kinase
1737	AAEL014583	60S acidic ribosomal protein P2
1738	AAEL005722	60S ribosomal protein L7a
1739	AAEL017931	U1 spliceosomal RNA
1740	AAEL017646	U1 spliceosomal RNA
1741	AAEL005266	40S ribosomal protein S14
1742	AAEL004175	40S ribosomal protein S17
1743	AAEL014562	60S ribosomal protein L12
1744	AAEL006785	60S ribosomal protein L18a
1745	AAEL007715	60S ribosomal protein L21
1746	AAEL007771	60S ribosomal protein L22
1747	AAEL005817	60S ribosomal protein L26
1748	AAEL006698	60S ribosomal protein L31
1749	AAEL003942	60S ribosomal protein L44 L41
1750	AAEL000987	60S ribosomal protein L8
1751	AAEL007699	60S ribosomal protein L9
1752	AAEL006511	anopheles stephensi ubiquitin
1753	AAEL007698	AUB
1754	AAEL005097	cold induced protein (BnC24A)
1755	AAEL004851	
1756	AAEL011424	histone H3
1757	AAEL000529	hypothetical protein

1758	AAEL004060	hypothetical protein
1759	AAEL004151	hypothetical protein
1760	AAEL004249	hypothetical protein
1761	AAEL004503	hypothetical protein
1762	AAEL005451	hypothetical protein
1763	AAEL001274	hypothetical protein
1764	AAEL000766	
1765	AAEL008969	
1766	AAEL008994	
1767	AAEL009151	

1768	AAEL009185	
1769	AAEL017468	
1770	AAEL009188	
1771	AAEL009201	
1772	AAEL001673	
1773	AAEL009341	
1774	AAEL009403	
1775	AAEL009496	
1776	AAEL009825	
1777	AAEL017413	
1778	AAEL010299	
1779	AAEL002047	
1780	AAEL010573	
1781	AAEL002372	
1782	AAEL017231	
1783	AAEL011447	
1784	AAEL011471	
1785	AAEL011504	
1786	AAEL011587	
1787	AAEL011656	
1788	AAEL002639	
1789	AAEL002832	
1790	AAEL002881	
1791	AAEL012944	
1792	AAEL013221	
1793	AAEL013272	
1794	AAEL003396	
1795	AAEL013533	

1796	AAEL013536	
1797	AAEL003582	
1798	AAEL005027	
1799	AAEL017536	
1800	AAEL008353	
1801	AAEL017198	
1802	AAEL016995	
1803	AAEL017590	Ref Transcript AaegL3.1_AAEL017868-RA
1804	AAEL017868	Ref Transcript AaegL3.1_AAEL017868-RA
1805	AAEL005629	ribosomal protein L35
1806	AAEL000010	ribosomal protein L36
1807	AAEL004325	ribosomal protein L5
1808	AAEL000068	ribosomal protein S25
1809	AAEL007824	ribosomal protein S29
1810	AAEL008297	Sodium channel
1811	AAEL016638	tRNA
1812	AAEL017826	U1 spliceosomal RNA
1813	AAEL017609	U1 spliceosomal RNA
1826	AAEL010379	P-glycoprotein (PgP)
1827	AAEL007823	Argonaute-3
1828	JF924909.1	Cytochrome p450 (CYP9J26)

Table 2B, cont.

Table 3 (male sterility)

Seq ID	Gene Symbol
166	AAEL000442
167	AAEL000888
168	AAEL001371
169	AAEL002079
170	AAEL003077
171	AAEL004266
172	AAEL004492
173	AAEL004517

174	AAEL004651
175	AAEL004933
176	AAEL005232
177	AAEL007609
178	AAEL008182
179	AAEL008605
180	AAEL009383
181	AAEL010737
182	AAEL011339
183	AAEL011380
184	AAEL011433
185	AAEL012330
186	AAEL012340
187	AAEL012341
188	AAEL012344
189	AAEL012345
190	AAEL012349
191	AAEL012350
192	AAEL012706
193	AAEL012710
194	AAEL012715
195	AAEL014031
196	AAEL014218
197	AAEL014238
198	AAEL014339
199	AAEL014904
200	AAEL014916
201	AAEL014920
202	AAEL014921
203	AAEL015390
204	AGAP000005
205	AGAP000005
206	AGAP000005
207	AGAP000306
208	AGAP000306
209	AGAP000306
210	AGAP000670
211	AGAP000670
212	AGAP000670
213	AGAP001652
214	AGAP001879
215	AGAP001879
216	AGAP001879

217	AGAP002353
218	AGAP002872
219	AGAP002872
220	AGAP002872
221	AGAP003500
222	AGAP003501
223	AGAP003519
224	AGAP003519
225	AGAP003519
226	AGAP003545
227	AGAP003545
228	AGAP003545
229	AGAP003796
230	AGAP004096
231	AGAP004096
232	AGAP004096
233	AGAP004840
234	AGAP004840
235	AGAP004840
236	AGAP005130
237	AGAP005130
238	AGAP005130
239	AGAP005733
240	AGAP005733
241	AGAP005733
242	AGAP006237
243	AGAP006237
244	AGAP006237
245	AGAP007242
246	AGAP007242
247	AGAP007242
248	AGAP008084
249	AGAP008084
250	AGAP008084
251	AGAP008374
252	AGAP008374
253	AGAP008374
254	AGAP008642
255	AGAP008642
256	AGAP008642
257	AGAP009091
258	AGAP009091
259	AGAP009091

260	AGAP009442
261	AGAP009442
262	AGAP009442
263	AGAP010909
264	AGAP010909
265	AGAP010909
266	AGAP010958
267	AGAP012380
268	AGAP012380
269	AGAP012380
270	CPIJ000025
271	CPIJ000367
272	CPIJ001133
273	CPIJ001692
274	CPIJ001739
275	CPIJ001883
276	CPIJ002710
277	CPIJ002715
278	CPIJ002718
279	CPIJ002719
280	CPIJ002726
281	CPIJ002789
282	CPIJ005348
283	CPIJ005588
284	CPIJ006105
285	CPIJ007600
286	CPIJ008100
287	CPIJ008391
288	CPIJ008494
289	CPIJ008983
290	CPIJ013307
291	CPIJ013432
292	CPIJ014043
293	CPIJ014354
294	CPIJ014659
295	CPIJ014870
296	CPIJ015607
297	CPIJ015663
298	CPIJ015791
299	CPIJ018368
300	CPIJ019419
301	CPIJ019949

Table 3, cont.

Table 4 (male sterility)

Seq ID	Gene Symbol
302	AAEL001340
303	AAEL001606
304	AAEL002425
305	AAEL002792
306	AAEL003660
307	AAEL004696
308	AAEL004974
309	AAEL006254
310	AAEL006488
311	AAEL006492
312	AAEL008042
313	AAEL008587
314	AAEL008844
315	AAEL008924
316	AAEL008958
317	AAEL009114
318	AAEL009174
319	AAEL009340
320	AAEL009969
321	AAEL010565
322	AAEL010789
323	AAEL010792
324	AAEL011474
325	AAEL011478
326	AAEL011663
327	AAEL011757
328	AAEL011921
329	AAEL014330
330	AGAP000460
331	AGAP000460
332	AGAP000460
333	AGAP000471
334	AGAP000471
335	AGAP000471
336	AGAP000662
337	AGAP000662
338	AGAP000662
339	AGAP001177
340	AGAP001177

341	AGAP001177
342	AGAP001179
343	AGAP001179
344	AGAP001179
345	AGAP001271
346	AGAP001271
347	AGAP001271
348	AGAP001278
349	AGAP001278
350	AGAP001278
351	AGAP001293
352	AGAP001293
353	AGAP001293
354	AGAP001335
355	AGAP001335
356	AGAP001335
357	AGAP001337
358	AGAP001337
359	AGAP001337
360	AGAP001339
361	AGAP001339
362	AGAP001339
363	AGAP001367
364	AGAP001367
365	AGAP001367
366	AGAP001388
367	AGAP001388
368	AGAP001388
369	AGAP001463
370	AGAP001463
371	AGAP001463
372	AGAP001478
373	AGAP001478
374	AGAP001478
375	AGAP001481
376	AGAP001481
377	AGAP001481
378	AGAP001498
379	AGAP001498
380	AGAP001498
381	AGAP002471
382	AGAP002471
383	AGAP002471

384	AGAP002801
385	AGAP004050
386	AGAP004416
387	AGAP004416
388	AGAP004416
389	AGAP004645
390	AGAP004930
391	AGAP006887
392	AGAP006887
393	AGAP006887
394	AGAP007963
395	AGAP008806
396	CPIJ001185
397	CPIJ001186
398	CPIJ001187
399	CPIJ001560
400	CPIJ003158
401	CPIJ003766
402	CPIJ004057
403	CPIJ004058
404	CPIJ004318
405	CPIJ005975
406	CPIJ005976
407	CPIJ007071
408	CPIJ007072
409	CPIJ007101
410	CPIJ007172
411	CPIJ007789
412	CPIJ008481
413	CPIJ008673
414	CPIJ009011
415	CPIJ009270
416	CPIJ011557
417	CPIJ011558
418	CPIJ011708
419	CPIJ012810
420	CPIJ013126
421	CPIJ015620
422	CPIJ015622
423	CPIJ017065
424	CPIJ017887
425	CPIJ019248
426	CPIJ019249

427	FBgn0127180

Table 4, cont.

Table 5 (female sterility)

SEQ ID NO.	**Name or access number**	**Description**
1829	AeSCP-2 (AF510492.1)	Aedes aegypti sterol carrier protein-2
1830	AeAct-4 (AY531222.2)	Aedes aegypti pupal-specific flight muscle actin mRNA, complete cds.
1831	AAEL002000	Aedes aegypti zinc carboxypeptidase partial mRNA
1832	AAEL005747	Aedes aegypti hypothetical protein partial mRNA (testicle target)
700	AAEL005656	Aedes aegypti AAEL005656-RA partial mRNA.
678	AAEL017015	Aedes aegypti AAEL017015-RA mRNA
652	AAEL005212	Aedes aegypti AAEL005212-RA mRNA.
733	AAEL005922	Aedes aegypti AAEL005922-RA mRNA. Aedes aegypti AAEL005922-RB partial mRNA.
722	AAEL000903	Aedes aegypti AAEL000903-RA (ENY2_AEDAE), mRNA.
638 1753 1827	AAEL005049 AAEL007698 AAEL007823	Aedes aegypti AAEL005049-RA mRNA. PIWI protein (Aub) PIWI protein (AGO3)

Table 5, cont.

As used herein, the term “downregulates an expression” or “downregulating expression" refers to causing, directly or indirectly, reduction in the transcription of a desired gene (as described herein), reduction in the amount, stability or translatability of transcription products (e.g. RNA) of the gene, and/or reduction in translation of the polypeptide(s) encoded by the desired gene.

Downregulating expression of a pathogen resistance gene product of a mosquito can be monitored, for example, by direct detection of gene transcripts (for example, by PCR), by detection of polypeptide(s) encoded by the gene (for example, by Western blot or immunoprecipitation), by detection of biological activity of polypeptides encode by the gene (for example, catalytic activity, ligand binding, and the like), or by monitoring changes in the mosquitoes (for example, reduced motility of the mosquito etc). Additionally or alternatively downregulating expression of a pathogen resistance gene product may be monitored by measuring pathogen levels (e.g. viral levels, bacterial levels etc.) in the mosquitoes as compared to wild type (i.e. control) mosquitoes not treated by the agents of the invention.

According to a specific embodiment the nucleic acid larvicide downregulates (reduces expression of) the target gene by at least 20 %, 30 %, 40 %, 50 %, or more, say 60 %, 70 %, 80 %, 90 % or more even 100 %, as compared to the expression of the same target gene in an untreated control in the same species and developmental stage.

In some embodiments of the invention, the nucleic acid agent is a double stranded RNA (dsRNA). As used herein the term "dsRNA" relates to two strands of anti-parallel polyribonucleic acids held together by base pairing. The two strands can be of identical length or of different lengths provided there is enough sequence homology between the two strands that a double stranded structure is formed with at least 80%, 90%, 95 % or 100 % complementarity over the entire length. According to an embodiment of the invention, there are no overhangs for the dsRNA molecule. According to another embodiment of the invention, the dsRNA molecule comprises overhangs. According to other embodiments, the strands are aligned such that there are at least 1, 2, or 3 bases at the end of the strands which do not align (i.e., for which no complementary bases occur in the opposing strand) such that an overhang of 1, 2 or 3 residues occurs at one or both ends of the duplex when strands are annealed.

It will be noted that the dsRNA can be defined in terms of the nucleic acid sequence of the DNA encoding the target gene transcript, and it is understood that a dsRNA sequence corresponding to the coding sequence of a gene comprises an RNA complement of the gene's coding sequence, or other sequence of the gene which is transcribed into RNA.

The inhibitory RNA sequence can be greater than 90 % identical, or even 100 % identical, to the portion of the target gene transcript. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript under stringent conditions (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 60 degrees C hybridization for 12-16 hours; followed by washing). The length of the double-stranded nucleotide sequences complementary to the target gene transcript may be at least about 18, 19, 21, 25, 50, 100, 200, 300, 400, 491, 500, 550, 600, 650, 700, 750, 800, 900, 1000 or more bases. In some embodiments of the invention, the length of the double-stranded nucleotide sequence is approximately from about 18 to about 1000, about 18 to about 750, about 18 to about 510, about 18 to about 400, about 18 to about 250 nucleotides in length.

The term "corresponds to" as used herein means a polynucleotide sequence homologous to all or a portion of a reference polynucleotide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For example, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The present teachings relate to various lengths of dsRNA, whereby the shorter version i.e., x is shorter or equals 50 bp (e.g., 17-50), is referred to as siRNA or miRNA. Longer dsRNA molecules of 51-600 are referred to herein as dsRNA, which can be further processed for siRNA molecules. According to some embodiments, the nucleic acid sequence of the dsRNA is greater than 15 base pairs in length. According to yet other embodiments, the nucleic acid sequence of the dsRNA is 19-25 base pairs in length, 30-100 base pairs in length, 100-250 base pairs in length or 100-500 base pairs in length. According to still other embodiments, the dsRNA is 500-800 base pairs in length, 700-800 base pairs in length, 300-600 base pairs in length, 350-500 base pairs in length or 400-450 base pairs in length. In some embodiments, the dsRNA is 400 base pairs in length. In some embodiments, the dsRNA is 750 base pairs in length.

The term "siRNA" refers to small inhibitory RNA duplexes (generally between 17-30 basepairs, but also longer e.g., 31-50 bp) that induce the RNA interference (RNAi) pathway. Typically, siRNAs are chemically synthesized as 21mers with a central 19 bp duplex region and symmetric 2-base 3'-overhangs on the termini, although it has been recently described that chemically synthesized RNA duplexes of 25-30 base length can have as much as a 100-fold increase in potency compared with 21mers at the same location. The observed increased potency obtained using longer RNAs in triggering RNAi is theorized to result from providing Dicer with a substrate (27mer) instead of a product (21mer) and that this improves the rate or efficiency of entry of the siRNA duplex into RISC.

It has been found that position of the 3'-overhang influences potency of an siRNA and asymmetric duplexes having a 3'-overhang on the antisense strand are generally more potent than those with the 3'-overhang on the sense strand (Rose et al., 2005). This can be attributed to asymmetrical strand loading into RISC, as the opposite efficacy patterns are observed when targeting the antisense transcript.

The strands of a double-stranded interfering RNA (e.g., an siRNA) may be connected to form a hairpin or stem-loop structure (e.g., an shRNA). Thus, as mentioned the RNA silencing agent of some embodiments of the invention may also be a short hairpin RNA (shRNA).

The term "shRNA", as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The number of nucleotides in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11. Some of the nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. Examples of oligonucleotide sequences that can be used to form the loop include 5'-UUCAAGAGA-3' (Brummelkamp, T. R. et al. (2002) Science 296: 550, SEQ ID NO: 428) and 5'-UUUGUGUAG-3' (Castanotto, D. et al. (2002) RNA 8:1454, SEQ ID NO: 429). It will be recognized by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.

As used herein, the phrase "microRNA (also referred to herein interchangeably as "miRNA" or "miR") or a precursor thereof" refers to a microRNA (miRNA) molecule acting as a post-transcriptional regulator. Typically, the miRNA molecules are RNA molecules of about 20 to 22 nucleotides in length which can be loaded into a RISC complex and which direct the cleavage of another RNA molecule, wherein the other RNA molecule comprises a nucleotide sequence essentially complementary to the nucleotide sequence of the miRNA molecule.

Typically, a miRNA molecule is processed from a “pre-miRNA” or as used herein a precursor of a pre-miRNA molecule by proteins, such as DCL proteins, present in any plant cell and loaded onto a RISC complex where it can guide the cleavage of the target RNA molecules.

Pre-microRNA molecules are typically processed from pri-microRNA molecules (primary transcripts). The single stranded RNA segments flanking the pre-microRNA are important for processing of the pri-miRNA into the pre-miRNA. The cleavage site appears to be determined by the distance from the stem-ssRNA junction (Han et al. 2006, Cell 125, 887-901, 887-901).

As used herein, a “pre-miRNA” molecule is an RNA molecule of about 100 to about 200 nucleotides, preferably about 100 to about 130 nucleotides which can adopt a secondary structure comprising an imperfect double stranded RNA stem and a single stranded RNA loop (also referred to as "hairpin") and further comprising the nucleotide sequence of the miRNA (and its complement sequence) in the double stranded RNA stem. According to a specific embodiment, the miRNA and its complement are located about 10 to about 20 nucleotides from the free ends of the miRNA double stranded RNA stem. The length and sequence of the single stranded loop region are not critical and may vary considerably, e.g. between 30 and 50 nucleotides in length. The complementarity between the miRNA and its complement need not be perfect and about 1 to 3 bulges of unpaired nucleotides can be tolerated. The secondary structure adopted by an RNA molecule can be predicted by computer algorithms conventional in the art such as mFOLD. The particular strand of the double stranded RNA stem from the pre-miRNA which is released by DCL activity and loaded onto the RISC complex is determined by the degree of complementarity at the 5′ end, whereby the strand which at its 5′ end is the least involved in hydrogen bounding between the nucleotides of the different strands of the cleaved dsRNA stem is loaded onto the RISC complex and will determine the sequence specificity of the target RNA molecule degradation. However, if empirically the miRNA molecule from a particular synthetic pre-miRNA molecule is not functional (because the "wrong" strand is loaded on the RISC complex), it will be immediately evident that this problem can be solved by exchanging the position of the miRNA molecule and its complement on the respective strands of the dsRNA stem of the pre-miRNA molecule. As is known in the art, binding between A and U involving two hydrogen bounds, or G and U involving two hydrogen bounds is less strong that between G and C involving three hydrogen bounds.

Naturally occurring miRNA molecules may be comprised within their naturally occurring pre-miRNA molecules but they can also be introduced into existing pre-miRNA molecule scaffolds by exchanging the nucleotide sequence of the miRNA molecule normally processed from such existing pre-miRNA molecule for the nucleotide sequence of another miRNA of interest. The scaffold of the pre-miRNA can also be completely synthetic. Likewise, synthetic miRNA molecules may be comprised within, and processed from, existing pre-miRNA molecule scaffolds or synthetic pre-miRNA scaffolds. Some pre-miRNA scaffolds may be preferred over others for their efficiency to be correctly processed into the designed microRNAs, particularly when expressed as a chimeric gene wherein other DNA regions, such as untranslated leader sequences or transcription termination and polyadenylation regions are incorporated in the primary transcript in addition to the pre-microRNA.

According to the present teachings, the dsRNA molecules may be naturally occurring or synthetic.

The dsRNA can be a mixture of long and short dsRNA molecules such as, dsRNA, siRNA, siRNA+dsRNA, siRNA+miRNA, or a combination of same.

The nucleic acid larvicide is designed for specifically targeting a target gene of interest. It will be appreciated that the nucleic acid larvicide can be used to down-regulate one or more target genes (e.g., belonging to groups (i) to (iv), as described above). If a number of target genes are targeted, a heterogenic composition which comprises a plurality of nucleic acid larvicides for targeting a number of target genes is used. Alternatively the plurality of nucleic acid larvicides are separately formulated. According to a specific embodiment, a number of distinct nucleic acid larvicide molecules for a single target are used, which may be separately or simultaneously (i.e., co-formulation) applied.

For example, in order to silence the expression of an mRNA of interest, synthesis of the dsRNA suitable for use with some embodiments of the invention can be selected as follows. First, the mRNA sequence is scanned including the 3' UTR and the 5' UTR.

Second, the mRNA sequence is compared to an appropriate genomic database using any sequence alignment software, such as the BLAST software available from the NCBI server (wwwdotncbidotnlmdotnihdotgov/BLAST/). Putative regions in the mRNA sequence which exhibit significant homology to other coding sequences are filtered out.

Qualifying target sequences are selected as template for dsRNA synthesis. Preferred sequences are those that have as little homology to other genes in the genome to reduce an "off-target" effect.

It will be appreciated that the RNA silencing agent of some embodiments of the invention need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides.

According to one embodiment, the dsRNA specifically targets a gene selected from the group consisting of sodium channel (AAEL008297), P-glycoprotein (AAEL010379), Argonaute-3 (AAEL007823), cytochrome p450 (CYP9J26, JF924909.1), Aub (AAEL007698), AeSCP-2 (AF510492.1), AeAct-4 (AY531222.2), AAEL002000, AAEL005747, AAEL017015, AAEL005212, AAEL005922, AAEL000903, AAEL005656 or AAEL005049.

Thus, a combination of two or more silencing agents e.g., dsRNAs, for a single target gene or distinct genes is contemplated according to the present teachings.

Thus, for example, a combination of dsRNA targeting the genes Aubergine (Aub, AAEL007698) and Argonaute-3 (AAEL007823) is contemplated herein. When referring to targeting together it is understood that the larvae may be administered two silencing agents, e.g., dsRNAs, concomitantly or subsequently to one another (e.g. hours or days apart).

According to one embodiment, the dsRNA is selected from the group consisting of SEQ ID NOs: 1822-1825 and 1857-1868.

According to a specific embodiment, the dsRNA comprises SEQ ID NOs: 1858 and 1832.

The dsRNA may be synthesized using any method known in the art, including either enzymatic syntheses or solid-phase syntheses. These are especially useful in the case of short polynucleotide sequences with or without modifications as explained above. Equipment and reagents for executing solid-phase synthesis are commercially available from, for example, Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the capabilities of one skilled in the art and can be accomplished via established methodologies as detailed in, for example: Sambrook, J. and Russell, D. W. (2001), "Molecular Cloning: A Laboratory Manual"; Ausubel, R. M. et al., eds. (1994, 1989), "Current Protocols in Molecular Biology," Volumes I-III, John Wiley & Sons, Baltimore, Maryland; Perbal, B. (1988), "A Practical Guide to Molecular Cloning," John Wiley & Sons, New York; and Gait, M. J., ed. (1984), "Oligonucleotide Synthesis"; utilizing solid-phase chemistry, e.g. cyanoethyl phosphoramidite followed by deprotection, desalting, and purification by, for example, an automated trityl-on method or HPLC.

According to a specific embodiment, large scale dsRNA preparation is performed by PCR using synthetic DNA templates, such as with the Ambion® MEGAscript® RNAi Kit. dsRNA integrity is verified on gel and purified by a column based method. The concentration of the dsRNA is evaluated both by Nano-drop and gel-based estimation. This dsRNA serves for the following experiments.

According to a specific embodiment, the cell is devoid of a heterologous promoter for driving recombinant expression of the dsRNA (exogenous), rendering the nucleic acid molecule of the instant invention a naked molecule. The nucleic acid agent may still comprise modifications that may affect its stability and bioavailability (e.g., PNA).

The term “recombinant expression” refers to an expression from a nucleic acid construct.

As used herein "devoid of a heterologous promoter for driving expression of the dsRNA" means that the cell doesn't include a cis-acting regulatory sequence (e.g., heterologous) transcribing the dsRNA in the cell. As used herein the term "heterologous" refers to exogenous, not-naturally occurring within the native cell (such as by position of integration, or being non-naturally found within the cell).

Although the instant teachings mainly concentrate on the use of dsRNA which is not comprised in or transcribed from an expression vector (naked), the present teachings also contemplate an embodiment wherein the nucleic acid larvicide is ligated into a nucleic acid construct comprising additional regulatory elements. Thus, according to some embodiments of the invention there is provided a nucleic acid construct comprising an isolated nucleic acid agent comprising a nucleic acid sequence larvicide.

For transcription from an expression cassette, a regulatory region (e.g., promoter, enhancer, silencer, leader, intron and polyadenylation) may be used to modulate the transcription of the RNA strand (or strands). Therefore, in one embodiment, there is provided a nucleic acid construct comprising the nucleic acid larvicide. The nucleic acid construct can have polynucleotide sequences constructed to facilitate transcription of the RNA molecules of the present invention are operably linked to one or more promoter sequences functional in a host cell. The polynucleotide sequences may be placed under the control of an endogenous promoter normally present in the host genome. The polynucleotide sequences of the present invention, under the control of an operably linked promoter sequence, may further be flanked by additional sequences that advantageously affect its transcription and/or the stability of a resulting transcript. Such sequences are generally located upstream of the promoter and/or downstream of the 3' end of the expression construct. The term "operably linked", as used in reference to a regulatory sequence and a structural nucleotide sequence, means that the regulatory sequence causes regulated expression of the linked structural nucleotide sequence. "Regulatory sequences" or "control elements" refer to nucleotide sequences located upstream, within, or downstream of a structural nucleotide sequence, and which influence the timing and level or amount of transcription, RNA processing or stability, or translation of the associated structural nucleotide sequence. Regulatory sequences may include promoters, translation leader sequences, introns, enhancers, stem-loop structures, repressor binding sequences, termination sequences, pausing sequences, polyadenylation recognition sequences, and the like. In some embodiments, the host is an algae, and promoter and other regulatory elements are active in algae.

As mentioned, the composition-of matter of some embodiments comprises cells, which comprises the nucleic acid larvicide.

As used herein the term “cell” or “cells” refers to a mosquito larvae ingestible cell.

Examples of such cells include, but are not limited to, cells of phytoplankton (e.g., algae), fungi (e.g., Legendium giganteum), bacteria, and zooplankton such as rotifers.

Specific examples include, bacteria (e.g., cocci and rods), filamentous algae and detritus.

The choice of the cell may depend on the target larvae.

Analyzing the gut content of mosquitoes and larvae may be used to elucidate their preferred diet. The skilled artisan knows how to characterize the gut content. Typically the gut content is stained such as by using a fluorochromatic stain, 4',6-diamidino-2-phenylindole or DAPI.

Cells (also referred to herein as “host cells”) of particular interest are the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae; Bacillaceae; Rhizobiceae; Spirillaceae; Lactobacillaceae; and phylloplane organisms such as members of the Pseudomonadaceae.

An exemplary list includes Bacillus spp., including B. megaterium, B. subtilis; B. cereus, Bacillus thuringiensis, Escherichia spp., including E. coli, and/or Pseudomonas spp., including P. cepacia, P. aeruginosa, and P. fluorescens.

Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such as Schizosaccharomyces; and Basidiomycetes, Rhodotorula, Aureobasidium, Sporobolomyces, Saccharomyces spp., and Sporobolomyces spp.

According to a specific embodiment, the host cell is an algal cell.

Various algal species can be used in accordance with the teachings of the invention since they are a significant part of the diet for many kinds of mosquito larvae that feed opportunistically on microorganisms as well as on small aquatic animals such as rotifers.

Examples of algae that can be used in accordance with the present teachings include, but are not limited to, blue-green algae as well as green algae.

According to a specific embodiment, the algal cell is a cyanobacterium cell which is in itself toxic to mosquitoes as taught by Marten 2007 Biorational Control of Mosquitoes. American mosquito control association Bulletin No. 7.

Specific examples of algal cells which can be used in accordance with the present teachings are provided in Marten, G.G. (1986) Mosquito control by plankton management: the potential of indigestible green algae. Journal of Tropical Medicine and Hygiene, 89: 213-222, and further listed infra.

Green algae

Actinastrum hantzschii
Ankistrodesmus falcatus
Ankistrodesmus spiralis
Aphanochaete elegans
Chlamydomonas sp.
Chlorella ellipsoidea
Chlorella pyrenoidosa
Chlorella variegata
Chlorococcum hypnosporum
Chodatella brevispina
Closterium acerosum
Closteriopsis acicularis
Coccochloris peniocystis
Crucigenia lauterbornii
Crucigenia tetrapedia
Coronastrum ellipsoideum
Cosmarium botrytis
Desmidium swartzii
Eudorina elegans
Gloeocystis gigas
Golenkinia minutissima
Gonium multicoccum

Nannochloris oculata

Oocystis marssonii

Oocystis minuta

Oocystis pusilla

Palmella texensis

Pandorina morum

Paulschulzia pseudovolvox

Pediastrum clathratum

Pediastrum duplex

Pediastrum simplex

Planktosphaeria gelatinosa

Polyedriopsis spinulosa

Pseudococcomyxa adhaerans

Quadrigula closterioides

Radiococcus nimbatus

Scenedesmus basiliensis

Spirogyra pratensis

Staurastrum gladiosum

Tetraedron bitridens

Trochiscia hystrix

Blue-green algae

Anabaena catenula

Anabaena spiroides

Chroococcus turgidus

Cylindrospermum licheniforme

Bucapsis sp. (U. Texas No.1519)

Lyngbya spiralis

Microcystis aeruginosa

Nodularia spumigena

Nostoc linckia

Oscillatoria lutea

Phormidiumfaveolarum

Spinilina platensis

Other

Compsopogon coeruleus

CTyptomonas ovata

Navicula pelliculosa

The nucleic acid larvicide is introduced into the cells. To this end cells are typically selected exhibiting natural competence or are rendered competent, also referred to as artificial competence.

Competence is the ability of a cell to take up nucleic acid molecules e.g., the nucleic acid larvicide, from its environment.

A number of methods are known in the art to induce artificial competence.

Thus, artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to the nucleic acid larvicide by exposing it to conditions that do not normally occur in nature. Typically the cells are incubated in a solution containing divalent cations (e.g., calcium chloride) under cold conditions, before being exposed to a heat pulse (heat shock).

Electroporation is another method of promoting competence. In this method the cells are briefly shocked with an electric field (e.g., 10-20 kV/cm) which is thought to create holes in the cell membrane through which the nucleic acid larvicide may enter. After the electric shock the holes are rapidly closed by the cell's membrane-repair mechanisms.

Yet alternatively or additionally, cells may be treated with enzymes to degrade their cell walls, yielding. These cells are very fragile but take up foreign nucleic acids at a high rate.

Exposing intact cells to alkali cations such as those of cesium or lithium allows the cells to take up nucleic acids. Improved protocols use this transformation method, while employing lithium acetate, polyethylene glycol, and single-stranded nucleic acids. In these protocols, the single-stranded molecule preferentially binds to the cell wall in yeast cells, preventing double stranded molecule from doing so and leaving it available for transformation.

Enzymatic digestion or agitation with glass beads may also be used to transform cells.

Particle bombardment, microprojectile bombardment, or biolistics is yet another method for artificial competence. Particles of gold or tungsten are coated with the nucleic acid agent and then shot into cells.

Astier C R Acad Sci Hebd Seances Acad Sci D. 1976 Feb 23;282(8):795-7, which is hereby incorporated by reference in its entirety, teaches transformation of a unicellular, facultative chemoheterotroph blue-green Algae, Aphanocapsa 6714. The recipient strain becomes competent when the growth reaches its second, slower, exponential phase.

Vázquez-Acevedo M[1]Mitochondrion. 2014 Feb 21. pii: S1567-7249(14)00019-1. doi: 10.1016/j.mito.2014.02.005, which is hereby incorporated by reference in its entirety, teaches transformation of algal cells e.g., Chlamydomonas reinhardtii, Polytomella sp. and Volvox carteri by generating import-competent mitochondria.

According to a specific embodiment the composition of the invention comprises an RNA binding protein.

According to a specific embodiment, the dsRNA binding protein (DRBP) comprises any of the family of eukaryotic, prokaryotic, and viral-encoded products that share a common evolutionarily conserved motif specifically facilitating interaction with dsRNA. Polypeptides which comprise dsRNA binding domains (DRBDs) may interact with at least 11 bp of dsRNA, an event that is independent of nucleotide sequence arrangement. More than 20 DRBPs have been identified and reportedly function in a diverse range of critically important roles in the cell. Examples include the dsRNA-dependent protein kinase PKR that functions in dsRNA signaling and host defense against virus infection and DICER.

Alternatively or additionally, an siRNA binding protein may be used as taught in U.S. Pat. Application No. 20140045914, which is herein incorporated by reference in its entirety.

According to a specific embodiment the RNA binding protein is the p19 RNA binding protein. The protein may increase in vivo stability of an siRNA molecule by coupling it at a binding site where the homodimer of the p19 RNA binding proteins is formed and thus protecting the siRNA from external attacks and accordingly, it can be utilized as an effective siRNA delivery vehicle.

According to a specific embodiment, the target-oriented peptide is located on the surface of the siRNA binding protein.

According to specific embodiments of the invention, whole cell preparations, cell extracts, cell suspensions, cell homogenates, cell lysates, cell supernatants, cell filtrates, or cell pellets of cell cultures of cells comprising the nucleic acid larvicide can be used.

For feeding adult mosquitoes, the cells or may be further combined with food supplements which are typically consumed by adult mosquitoes.

Adult mosquitoes typically feed on blood (female mosquitoes) and nectar of flowers (male mosquitoes), but have been known to ingest non-natural feeds as well. Mosquitoes can be fed various foodstuffs including but not limited to egg/soy protein mixture, carbohydrate foods such as sugar solutions (e.g. sugar syrup), corn syrup, honey, various fruit juices, raisins, apple slices and bananas. These can be provided as a dry mix or as a solution in open feeders. Soaked cotton balls, sponges or alike can also be used to providing a solution (e.g. sugar solution) to adult mosquitoes.

Feed suitable for adult mosquitoes may further include blood, blood components (e.g. plasma, hemoglobin, gamma globulin, red blood cells, adenosine triphosphate, glucose, and cholesterol), or an artificial medium (e.g., such a media is disclosed in U.S. Application No. 8,133,524 and in U.S. Patent Application No. 20120145081, both of which are incorporated by reference herein).The composition of some embodiments of the invention may further comprise at least one of a surface-active agent, an inert carrier vehicle, a preservative, a humectant, a feeding stimulant, an attractant, an encapsulating agent, a binder, an emulsifier, a dye, an ultra-violet protector, a buffer, a flow agent or fertilizer, micronutrient donors, or other preparations that influence the growth of the plant.

Additionally, the composition may be supplemented with larval food (food bait) or with excrements of farm animals, on which the larvae feed.

According to one embodiment, the composition is administered to the larvae by feeding.

Feeding the larva with the composition can be effected for about 2 hours to 120 hours, about 2 hours to 108 hours, about 2 hours to 96 hours, about 2 hours to 84 hours, about 2 hours to 72 hours, for about 2 hours to 60 hours, about 2 hours to 48 hours, about 2 hours to 36 hours, about 2 hours to 24 hours, about 2 hours to 12 hours, 12 hours to 24 hours, about 24 hours to 36 hours, about 24 hours to 48 hours, about 36 hours to 48 hours, for about 48 hours to 60 hours, about 60 hours to 72 hours, about 72 hours to 84 hours, about 84 hours to 96 hours, about 96 hours to 108 hours, or about 108 hours to 120 hours.

According to a specific embodiment, the composition is administered to the larvae by feeding for 48-96 hours.

According to one embodiment, feeding the larva with the composition is affected until the larva reaches pupa stage.

According to one embodiment, prior to feeding the larva with dsRNA, the larvae are first soaked with dsRNA.

Soaking the larva with the composition can be effected for about 2 hours to 96 hours, about 2 hours to 84 hours, about 2 hours to 72 hours, for about 2 hours to 60 hours, about 2 hours to 48 hours, about 2 hours to 36 hours, about 2 hours to 24 hours, about 2 hours to 12 hours, 12 hours to 96 hours, about 12 hours to 84 hours, about 12 hours to 72 hours, for about 12 hours to 60 hours, about 12 hours to 48 hours, about 12 hours to 36 hours, about 12 hours to 24 hours, or about 24 hours to 48 hours.

According to a specific embodiment, the composition is administered to the larvae by soaking for 12-24 hours.

Thus, for example, larvae (e.g. first, second, third or four instar larva, e.g. third instar larvae) are first treated (in groups of about 100 larvae) with dsRNA at a dose of about 0.001-5 μg/μL (e.g. 0.2 μg/μL), in a final volume of about 3 mL of dsRNA solution in autoclaved water. After soaking in the dsRNA solutions for about 12-48 hours (e.g. for 24 hrs) at 25-29 °C (e.g. 27 °C), the larvae are transferred into containers so as not to exceed concentration of about 200-500 larvae/1500 mL (e.g. 300 larvae/1500 mL) of chlorine-free tap water, and provided with food containing dsRNA (e.g. agarose cubes containing 300 μg of dsRNA, e.g. 1 μg of dsRNA/larvae). The larva are fed once a day until they reach pupa stage (e.g. for 2-5 days, e.g. four days). Larvae are also fed with additional food requirements, e.g. 2-10 mg/100 mL (e.g. 6 mg/100 mL) lab dog/cat diet suspended in water.

Feeding the larva can be effected using any method known in the art. Thus, for example, the larva may be fed with agrose cubes, chitosan nanoparticles, oral delivery or diet containing dsRNA.

Chitosan nanoparticles: A group of 15-20 3rd-instar mosquito larvae are transferred into a container (e.g. 500 ml glass beaker) containing 50-1000 ml, e.g. 100 ml, of deionized water. One sixth of the gel slices that are prepared from dsRNA (e.g. 32 μg of dsRNA) are added into each beaker. Approximately an equal amount of the gel slices are used to feed the larvae once a day for a total of 2-5 days, e.g. four days (see Insect Mol Biol. 2010 19(5):683-93).

Oral delivery of dsRNA: First instar larvae (less than 24 hrs old) are treated in groups of 10-100, e.g. 50, in a final volume of 25-100 μl of dsRNA, e.g. 75 μl of dsRNA, at various concentrations (ranging from 0.01 to 5 μg/μl , e.g. 0.02 to 0.5 μg/μl-dsRNAs) in tubes e.g. 2 mL microfuge tube (see J Insect Sci. 2013;13:69).

Diet containing dsRNA: larvae are fed a single concentration of 1-2000 ng dsRNA/mL, e.g. 1000 ng dsRNA/mL, diet in a diet overlay bioassay for a period of 1-10 days, e.g. 5 days (see PLoS One. 2012; 7(10): e47534.).

Diet containing dsRNA: Newly emerged larvae are starved for 1-12 hours, e.g. 2 hours, and are then fed with a single drop of 0.5-10 μl, e.g. 1 μl, containing 1-20 μg, e.g. 4 μg, dsRNA (1-20 μg of dsRNA/larva, e.g. 4 μg of dsRNA/larva) (see Appl Environ Microbiol. 2013 Aug;79(15):4543-50).

According to a further specific embodiment, the composition may further comprise a chemical larvicide, a biochemical larvicide (a biopesticide) or a combination of same.

According to the U.S. Environmental Protection Agency (EPA), Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Biopesticides fall into three major classes: (1) Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. The most widely used microbial pesticides are subspecies and strains of Bacillus thuringiensis, or Bt. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. (2) Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. (3) Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones, that interfere with mating, as well as various scented plant extracts that attract insect pests to traps.

Exemplary compounds mostly used as larvicides include, but are not limited to, organophosphates and surface oils and films.

Further examples of larvicides include, but are not limited to, waste oil or diesel oil products. Paris green dust is an arsenical insecticide, used along with undiluted diesel oil, and dichloro-diphenyl-trichloroethane (DDT), used as both an adulticide and a larvicide, malathion, an organophosphate (OP) compound, increased, but resistance was soon observed. The term organophosphate (OP) refers to all pesticides containing phosphorus, acting through inhibition of the activity of cholinesterase enzymes at the neuromuscular junction. Temephos is currently the only OP registered for use as a larvicide in the US.

Biolarvicides are comprised of two major categories: (1) Microbial agents (*e.g.*, bacteria) and (2) Biochemical agents (*e.g.*, pheromones, hormones, growth regulators, and enzymes). Regarding microbial agents, controlled-release formulations of at least one biological pesticidal ingredient are disclosed in U.S. Pat. No. 4,865,842; control of mosquito larvae with a spore-forming *Bacillus* ONR-60A is disclosed in U.S. Pat. No. 4,166,112; novel *Bacillus thuringiensis* isolates with activity against dipteran insect pests are disclosed in U.S. Pat. Nos. 5,275,815 and 5,847,079; a biologically pure culture of a *Bacillus thuringiensis* strain with activity against insect pests of the order Diptera is disclosed in U.S. Pat. No. 5,912,162; a recombinantly derived biopesticide active against Diptera including cyanobacteria transformed with a plasmid containing a *B. thuringiensis* subsp. *israelensis* dipteracidal protein translationally fused to a strong, highly active native cyanobacteria's regulatory gene sequence is disclosed in U.S. Pat. No. 5,518,897 and a formulation of Bacillus thuringiensis subspecies Israelensis and Bacillus sphaericus to manage mosquito larvicide resistance U.S. Pat. No. US 7,989,180 B2.

Biochemical agents such as Insect Growth Regulators (IGRS) mimics naturally occurring insect biochemicals and Methoprene (a juvenile hormone (JH) analog) is a commercially available insecticide of this class.

According to one embodiment, the larvicide is selected from the group consisting of Temephos, Diflubenzuron, Methoprene, or a microbial larvicide such as Bacillus sphaericus or Bacillus thuringiensis israelensis.

According to one embodiment, the larvicide comprises an adulticide.

Exemplary adulticides include, but are not limited to, deltamethrin, malathion, naled, chlorpyrifos, permethrin, resmethrin or sumithrin.

According to a specific embodiment, the cells are formulated by any means known in the art. The methods for preparing such formulations include, e.g., desiccation, lyophilization, homogenization, extraction, filtration, encapsulation centrifugation, sedimentation, or concentration of one or more cell types.

In one embodiment, the composition comprises an oil flowable suspension. For example, in some embodiments, oil flowable or aqueous solutions may be formulated to contain lysed or unlysed cells, spores, or crystals.

In a further embodiment, the composition may be formulated as a water dispersible granule or powder.

In yet a further embodiment, the compositions of the present invention may also comprise a wettable powder, spray, emulsion, colloid, aqueous or organic solution, dust, pellet, or colloidal concentrate. Dry forms of the compositions may be formulated to dissolve immediately upon wetting, or alternatively, dissolve in a controlled-release, sustained-release, or other time-dependent manner.

Alternatively or additionally, the composition may comprise an aqueous solution. Such aqueous solutions or suspensions may be provided as a concentrated stock solution which is diluted prior to application, or alternatively, as a diluted solution ready-to-apply. Such compositions may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (silicone or silicon derivatives, phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like).

The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be employed as foams, suspensions, emulsifiable concentrates, or the like. The ingredients may include Theological agents, surfactants, emulsifiers, dispersants, or polymers.

As mentioned, the dsRNA of the invention may be administered as a naked dsRNA. Alternatively, the dsRNA of the invention may be conjugated to a carrier known to one of skill in the art, such as a transfection agent e.g. PEI or chitosan or a protein/ lipid carrier.

The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, microencapsulated, desiccated, or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. Suitable agricultural carriers can be solid, semi-solid or liquid and are well known in the art. The term "agriculturally-acceptable carrier" covers all adjuvants, e.g., inert components, dispersants, surfactants, tackifiers, binders, etc. that are ordinarily used in pesticide formulation technology.

According to one embodiment, the composition is formulated as a semi-solid such as in agarose (e.g. agarose cubes).

The mosquito larva food containing dsRNA may be prepared by any method known to one of skill in the art. Thus, for example, cubes of dsRNA-containing mosquito food may be prepared by first mixing 10-500 μg, e.g. 300 μg of dsRNA with 3 to 300 μg, e.g. 10 μg of a transfection agent e.g. Polyethylenimine 25 kDa linear (Polysciences) in 10-500 μL, e.g. 200 μL of sterile water. Alternatively, 2 different dsRNA (10-500 μg, e.g. 150 μg of each) plus 3 to 300 μg, e.g. 30 μg of Polyethylenimine may be mixed in 10-500 μL, e.g. 200 μL of sterile water. Alternatively, cubes of dsRNA-containing mosquito food may be prepared without the addition of transfection reagents. Then, a suspension of ground mosquito larval food (1-20 grams/100 mL e.g. 6 grams/100 mL) may be prepared with 2 % agarose (Fisher Scientific). The food/agarose mixture can then be heated to 53-57 °C, e.g. 55 °C, and 10-500 μL, e.g. 200 μL of the mixture can then be transferred to the tubes containing 10-500 μL, e.g. 200 μL of dsRNA+PEI or dsRNA only. The mixture is then allowed to solidify into a gel. The solidified gel containing both the food and dsRNA can be cut into small pieces (approximately 1-10 mm, e.g. 1 mm, thick) using a razor blade, and can be used to feed mosquito larvae in water.

Compositions of the invention can be used to control or exterminate mosquitoes. Such an application comprises feeding larvae of the mosquitoes with an effective amount of the composition to thereby control or exterminate the mosquitoes.

According to a specific embodiment, the composition may be applied to standing water.

The pesticidal compositions of the invention may be employed in the method of the invention singly or in combination with other compounds, including, but not limited to, other pesticides (not included in the formulation as described above).

Regardless of the method of application, the amount of the active component(s) are applied at a larvicidally-effective amount, which will vary depending on factors such as, for example, the specific mosquito to be controlled, the water source to be treated, the environmental conditions, and the method, rate, and quantity of application of the larvicidally-active composition.

The concentration of larvicidal composition that is used for environmental, systemic, or foliar application will vary widely depending upon the nature of the particular formulation, means of application, environmental conditions, and degree of biocidal activity.

The larvae may be pathogenically infected as described above or uninfected larvae.

The concentration of the composition that is used for environmental, systemic, or foliar application will vary widely depending upon the nature of the particular formulation, means of application, environmental conditions, and degree of activity.

Exemplary concentrations of dsRNA in the composition include, but are not limited to, about 1 pg - 10 μg of dsRNA/μl, about 1 pg - 1 μg of dsRNA/μl, about 1 pg – 0.1 μg of dsRNA/μl, about 1 pg – 0.01 μg of dsRNA/μl, about 1 pg – 0.001 μg of dsRNA/μl, about 0.001 μg - 10 μg of dsRNA/μl, about 0.001 μg - 5 μg of dsRNA/μl, about 0.001 μg - 1 μg of dsRNA/μl, about 0.001 μg – 0.1 μg of dsRNA/μl, about 0.001 μg – 0.01 μg of dsRNA/μl, about 0.01 μg - 10 μg of dsRNA/μl, about 0.01 μg - 5 μg of dsRNA/μl, about 0.01 μg - 1 μg of dsRNA/μl, about 0.01 μg – 0.1 μg of dsRNA/μl, about 0.1 μg - 10 μg of dsRNA/μl, about 0.1 μg - 5 μg of dsRNA/μl, about 0.5 μg - 5 μg of dsRNA/μl, about 0.5 μg - 10 μg of dsRNA/μl, about 1 μg - 5 μg of dsRNA/μl, or about 1 μg - 10 μg of dsRNA/μl.

When formulated as a feed, the dsRNA may be effected at a dose of 1 pg/larvae - 1000 µg/larvae, 1 pg/larvae - 500 µg/larvae, 1 pg/larvae - 100 µg/larvae, 1 pg/larvae - 10 µg/larvae, 1 pg/larvae - 1 µg/larvae, 1 pg/larvae - 0.1 µg/larvae, 1 pg/larvae - 0.01 µg/larvae, 1 pg/larvae - 0.001 µg/larvae, 0.001-1000 µg/larvae, 0.001-500 µg/larvae, 0.001-100 µg/larvae, 0.001-50 µg/larvae, 0.001-10 µg/larvae, 0.001-1 µg/larvae, 0.001-0.1 µg/larvae, 0.001-0.01 µg/larvae, 0.01-1000 µg/larvae, 0.01-500 µg/larvae, 0.01-100 µg/larvae, 0.01-50 µg/larvae, 0.01-10 µg/larvae, 0.01-1 µg/larvae, 0.01-0.1 µg/larvae, 0.1-1000 µg/larvae, 0.1-500 µg/larvae, 0.1-100 µg/larvae, 0.1-50 µg/larvae, 0.1-10 µg/larvae, 0.1-1 µg/larvae, 1-1000 µg/larvae, 1-500 µg/larvae, 1-100 µg/larvae, 1-50 µg/larvae, 1-10 µg/larvae, 10-1000 µg/larvae, 10-500 µg/larvae, 10-100 µg/larvae, 10-50 µg/larvae, 50-1000 µg/larvae, 50-500 µg/larvae, 50-400 µg/larvae, 50-300 µg/larvae, 100-500 µg/larvae, 100-300 µg/larvae, 200-500 µg/larvae, 200-300 µg/larvae, or 300-500 µg/larvae.

According to some embodiments, the nucleic acid agent is provided in amounts effective to reduce or suppress expression of at least one mosquito gene product. As used herein "a suppressive amount" or "an effective amount" refers to an amount of dsRNA which is sufficient to downregulate (reduce expression of) the target gene by at least 20 %, 30 %, 40 %, 50 %, or more, say 60 %, 70 %, 80 %, 90 % or more even 100 %.

Testing the efficacy of gene silencing can be effected using any method known in the art. For example, using quantitative RT-PCR measuring gene knockdown. Thus, for example, ten to twenty larvae or mosquitoes from each treatment group can be collected and pooled together. RNA can be extracted therefrom and cDNA syntheses can be performed. The cDNA can then be used to assess the extent of RNAi by measuring levels of gene expression using qRT-PCR.

Compositions of the present invention can be packed in a kit including the cells which comprise the nucleic acid larvicides, instructions for administration of the composition to mosquito larvae.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, which may contain one or more dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration to the mosquito larvae.

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of” means “including and limited to”.

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

When reference is made to particular sequence listings, such reference is to be understood to also encompass sequences that substantially correspond to its complementary sequence as including minor sequence variations, resulting from, e.g., sequencing errors, cloning errors, or other alterations resulting in base substitution, base deletion or base addition, provided that the frequency of such variations is less than 1 in 50 nucleotides, alternatively, less than 1 in 100 nucleotides, alternatively, less than 1 in 200 nucleotides, alternatively, less than 1 in 500 nucleotides, alternatively, less than 1 in 1000 nucleotides, alternatively, less than 1 in 5,000 nucleotides, alternatively, less than 1 in 10,000 nucleotides.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); “Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

MATERIALS AND EXPERIMENTAL PROCEDURES

Mosquito maintenance

Mosquitoes were taken from an *Ae. aegypti* colony of the Rockefeller strain or from a mosquito field population of *Ae. aegypti* isolated from urban area of Rio de Janeiro, Brazil. Both lineages were reared continuously in the laboratory at 28 °C and 70–80 % relative humidity. Adult mosquitoes were maintained in a 10 % sucrose solution, and the adult females were fed with sheep blood for egg laying. The larvae were reared on dog/cat food unless stated otherwise.

Introducing dsRNA into a mosquito larvae

Three different approaches were evaluated for treatment with dsRNA:

A) Soaking with "naked" dsRNA

Third instar larvae were treated (in groups of 100 larvae) in a final volume of 3 mL of dsRNA solution in autoclaved water (0.5 µg/µL for sodium channel (AAEL008297), PgP (AAEL010379) and Ago3 (AAEL007823) dsRNA, or 0.1 µg/µL for CYP9J26 (JF924909.1). The control group was kept in 3 ml sterile water only. Larvae were soaked in the dsRNA solutions for 24 hr at 27 °C, and then transferred into new containers (300 larvae/1500 mL of chlorine-free tap water), which were also maintained at 27 °C, and were provided 6 mg/100 mL lab dog/cat diet (Purina Mills) suspended in water as a source of food on a daily basis. As pupae developed, they were transferred to individual vials to await eclosion and sex sorting. For bioassays purpose only females up to five days old were used. See Flowchart 1, Figure 1 for detailed explanation of this experiment.

B) Soaking with "naked" dsRNA plus additional larvae feeding with food-containing dsRNA

After soaking in the dsRNA solutions for 24 hr at 27 °C, the larvae were transferred into new containers (300 larvae/1500 mL of chlorine-free tap water), and were provided agarose cubes containing 300 µg of dsRNA once a day for a total of four days. The larvae were reared until adult stage. For bioassays purpose only females up to five days old are used. See Flowchart 2, Figure 2 for detailed explanation of this experiment.

C) Larvae feeding with food-containing dsRNA only

Third instar larvae were fed (in groups of 300 larvae) in a final volume of 1500 mL of chlorine-free tap water with agarose cubes containing 300 μg of dsRNA once a day for a total of four days. The larvae were reared until adult stage. For bioassays purpose only females up to five days old are used. See Flowchart 3, Figure 3 for detailed explanation of this experiment.

Bioassay with pyrethroid

CDC bottle bioassays - Bottles were prepared following the Brogdon and McAllister (1998) protocol [Brogdon and McAllister (1998) *Emerg Infect Dis* 4:605-613]. Fifteen-twenty non-blood-fed females from each site were introduced in 250 mL glass bottles impregnated with different concentrations of deltamethrin (Sigma-Aldrich) in 1 ml acetone. Each test consisted of four impregnated bottles and one control bottle. The control bottle contained acetone with no insecticide. At least three tests were conducted for each insecticide and population. Immediately prior to use, all insecticide solutions were prepared fresh from stock solutions. At 15, 30 and 45 min intervals, the number of live and dead mosquitoes in each bottle was recorded. The mortality criteria included mosquitoes with difficulties flying or standing on the bottle's surface. Mosquitoes that survived the appropriate dose for insecticide were considered to be resistant [Brogdon and McAllister (1998), supra].

Preparation of Mosquito Larval Food Containing dsRNA

Cubes of dsRNA-containing mosquito food were prepared as follows: First, 300 μg of dsRNA were mixed with 30 μg of Polyethylenimine 25 kD linear (Polysciences) in 200 μL of sterile water. Then, a suspension of ground mosquito larval food (6 grams/100 mL) was prepared with 2% agarose (Fisher Scientific). The food/agarose mixture was heated to 55°C and 200 μL of the mixture was then transferred to the tubes containing 200 μL of dsRNA+PEI or water only (control). The mixture was then allowed to solidify into a gel. The solidified gel containing both the food and dsRNA was cut into small pieces (approximately 1 mm thick) using a razor blade, which were then used to feed mosquito larvae in water.

RNA isolation and dsRNA production

Total RNA was extracted from groups of five *Ae. aegypti* fourth instar larvae and early adult male/female *Ae. aegypti*, using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was treated with amplification grade

DNase I (Invitrogen) and 1 μg was used to synthesize cDNA using a First Strand cDNA Synthesis kit (Invitrogen). The cDNA served as template DNA for PCR amplification of gene fragments using the primers listed in Table 6, below. PCR products were purified using a QIAquick PCR purification kit (Qiagen). The MEGAscript RNAi kit (Ambion) was then used for in vitro transcription and purification of dsRNAs. See Flowchart 4, Figure 4 for detailed explanation production off dsRNA.

Table 6: qPCR primers and dsRNA sequences for adulticide targets

Target gene	Accession number	dsRNA sequence	qPCR primers (5'-3')
P-glycoprotein (AAEL010379)	XM_001654442.1	SEQ ID NO: 1822	F: GCGCGCTCGTTCAGTATTTA (SEQ ID NO: 1814) R: ACACCCGTTACGGCACAATA (SEQ ID NO: 1815)
Argonaute-3 (AAEL007823)	XM_001652895.1	SEQ ID NO: 1823	F: TCGGCATTCGTAGCTTCGTT (SEQ ID NO: 1816) R: GCAGCTGACAGTTTGCCTTC (SEQ ID NO: 1817)
Cytochrome p450 (CYP9J26) JF924909.1	JF924909.1 XM_001649047.2	SEQ ID NO: 1824	F:CCGTTTGGTATCGGCCCAAG (SEQ ID NO: 1818) R:GTCTTTGCGCCTCGGACG (SEQ ID NO: 1819)
Sodium channel (AAEL008297)	KC107440.1 XM_001653136.1	SEQ ID NO: 1825	F:CTGGAGTCGGTGAGCGAAA (SEQ ID NO: 1820) R:TACGTATCGTAAACGCGCTC (SEQ ID NO: 1821)

qPCR analysis

Approximately 1000 ng first-strand cDNA obtained as described previously was used as template. The qPCR reactions were performed using SYBR® Green PCR Master Mix (Applied Biosystems) following the manufacturer's instructions. Briefly, approximately 50 ng/μl cDNA and gene-specific primers (600 nM) were used for each reaction mixture. qPCR conditions used were 10 min at 95 °C followed by 35 cycles of 15 s at 94 °C, 15 s at 54 °C and 60 s at 72 °C. The ribosomal protein S7 and tubulin were used as the reference gene to normalize expression levels amongst the samples. Raw quantification cycle (Cq) values normalized against those of the tubulin and S7 standards were then used to calculate the relative expression levels in samples using the $2^{-\Delta\Delta Ct}$ method [Livak & Schmittgen, (2001) Methods. 25(4):402-8.). Results (mean ± SD) are representative of at least two independent experiments performed in triplicate.

RESULTS

Characterization of insecticide resistance using two different strains of Aedes aegypti mosquitoes

Vector control strategies employed for Aedes control are mainly anti-larval measures, source reduction and use of adulticides (pyrethroids). Pyrethroids are a major class of insecticides, which show low mammalian toxicity and fast knockdown activity. Unfortunately, the intensive use of pyrethroids, including their indirect use in agriculture, has led to reports of reduced efficacy. One of the mechanisms of resistance in insects against pyrethroids is knockdown resistance (kdr) which is conferred by mutation(s) in the target site, the voltage gated sodium channel (VGSC). Several kdr mutations have been reported in many insects of agricultural and medical importance including *Ae. aegypti*. In *Ae. Aegypti*, eleven non-synonymous mutations at nine different loci have been reported [Med Vet Ent 17: 87–94.; Insect Mol Biol 16: 785–798.; Insect Biochem Mol Biol 39: 272–278.], amongst which mutations at three loci, i.e., Iso1011 (IRM/V) and Val1016 (VRG/I) in domain II and F1534 (FRC) in domain III are most commonly reported as contributing to pyrethroid resistance.

Using a population of mosquitoes that shows increased pyrethroid resistance, the present inventors target (during larval stage) several genes associated with resistance to pyrethroid in order to break resistance to insecticide at the adult stage.

A diagnostic dosage (DD) was established for the insecticide using the Rockefeller reference susceptible *Ae. aegypti* strain and a resistance threshold (RT), time in which 98-100 % mortality was observed in the Rockefeller strain, was then calculated. Using the DD (2 μg/mL of deltamethrin) (Figures 5A-C) is was possible to demonstrate that this dose killed only 63.95 % of the Rio de Janeiro strain whereas 100 % of the mosquitoes from the Rockefeller strain were dead. Therefore, it was concluded that 36.05 % of the mosquitoes in this population (RJ) are resistant to deltamethrin.

To further confirm the resistance status of the Rio de Janeiro strain, the kdr mutations reported as contributing to pyrethroid resistance were assessed. In Figures 6A-B, the present inventors show that V1016G and F1534C were both detected in the RJ strain. Indeed, the V1016G and F1534C mutation were detected in 49 % and 60 % of the mosquitoes from Rio de Janeiro strain, respectively.

Silencing of sodium channel during larval development increases the susceptibility of adult mosquitoes to pyrethroid

Using the first approach (soaking with "naked" dsRNA), mosquito larvae (RJ strain) were treated with three different dsRNA: Ago3, P-glycoprotein and Sodium channel. Treatment with dsRNA against sodium channel increased substantially the susceptibility of mosquitoes to the insecticide (Figure 7A). Interestingly, female mosquitoes showed a decreased expression in the mRNA level for sodium channel before deltamethrin treatment (Figure 7B). When compared to water-treated mosquitoes only, dead female mosquitoes previously treated with dsRNA showed a striking decrease in mRNA expression level for sodium channel (Figure 7C).

In order to test the second approach (soaking with "naked" dsRNA plus additional larvae feeding with food-containing dsRNA), mosquito larvae (L3) were first soaked with dsRNA (sodium channel, 0.5 μg/μL) for 24 hours. Then, larvae were treated 4 times with food-containing dsRNA and reared until adult stage. Although there was no obvious advantage in using this approach when compared to soaking with naked dsRNA alone, treatment with dsRNA against sodium channel increased the susceptibility of mosquitoes to deltamethrin (Figure 8).

This approach was also tested using dsRNA to target Cytochrome p450 (CYP9J26). As can be seen in the Figure 9, dsRNA-treated mosquitoes were more sensitive to deltamethrin during the first 15 minutes of contact with deltamethrin.

It is important to note that that 24 and 48 hours after the end of dsRNA treatment, decreased mRNA levels were detected in mosquito adults that were treated with PgP, Ago3 or sodium channel dsRNA as larvae (Figures 10A-C). However, PgP and Ago3 mRNA expression reached normal levels when mosquitoes became adults (Figure 11A-B, respectively).

<u>EXAMPLE 2</u>

MATERIALS AND EXPERIMENTAL PROCEDURES

Mosquito maintenance

Mosquitoes were taken from an *Ae. aegypti* colony of the Rockefeller strain, which were reared continuously in the laboratory at 28 °C and 70–80 % relative humidity. Adult mosquitoes were maintained in a 10 % sucrose solution, and the adult females were fed with sheep blood for egg laying. The larvae were reared on dog/cat food unless stated otherwise.

Introducing dsRNA into a mosquito larvae

Soaking with "naked" dsRNA plus additional larvae feeding with food-containing dsRNA

Third instar larvae were treated (in groups of 100 larvae) in a final volume of 3 mL of dsRNA solution in autoclaved water (dsRNA concentrations are shown in Table 7, below). The control group was kept in 3 ml sterile water only. After soaking in the dsRNA solutions for 24 hr at 27 °C, the larvae were transferred into new containers (300 larvae/1500 mL of chlorine-free tap water) and provided 6 mg/100 mL lab dog/cat diet (Purina Mills) suspended in water and agarose cubes containing 300 μg of dsRNA once a day for a total of two days. As pupae developed, they were transferred to individual vials to await eclosion and sex sorting. For bioassays purpose only females up to five days old were used.

The pupae mortality was calculated based on the initial number of treated larvae (300) (Mortality of pupae = Total number of pupae/300). Once the adults emerged they start to copulate.

Table 7: dsRNA concentrations

dsRNA	**Concentration (μg/μL each 100 larvae)**
Ago3 (AAEL007823)	0.5
Aub (AAEL007698)	0.5
AF510492.1	0.1
AY531222.2	0.02
AAEL017015	0.06
AAEL005212	0.06
AAEL005922	0.05
AAEL000903	0.06

Preparation of Mosquito Larval Food Containing dsRNA

Cubes of dsRNA-containing mosquito food were prepared as follows: First, 300 μg of dsRNA were mixed with 30 μg of Polyethylenimine 25 kD linear (Polysciences) in 200 μL of sterile water. Then, a suspension of ground mosquito larval food (6 grams/100 mL) was prepared with 2 % agarose (Fisher Scientific). The food/agarose mixture was heated to 55 °C and 200 μL of the mixture was then transferred to the tubes containing 200 μL of dsRNA+PEI or water only (control). The mixture was then allowed to solidify into a gel. The solidified gel containing both the food and dsRNA was cut into small pieces (approximately 1 mm thick) using a razor blade, which were then used to feed mosquito larvae in water.

Blood feeding

Five to seven days following adult emergence, dsRNA-treated or untreated control mosquitoes received defibrinated sheep blood through a membrane feeder. Thirty minutes after receiving a blood meal, three groups of 15 engorged females were separated inside a new cartoon cage to perform the oviposition assay.

Oviposition assay and hatching rate

Five days after the blood meal, an ovipositon cup was place inside each cage containing 15 females to allow the females to lay their eggs. The oviposition cup was changed every 24 hours for 3 consecutive days. The number of eggs laid was counted and used to check the viability and egg hatching rate.

To check the viability of the eggs the oviposition paper were kept to dry and embrionate for a period minimum of 5 days. After this time the ovipositions papers containing the eggs were placed inside a tray with aged water and food and wait for the eggs to hatch for a period of 24 hours. The hatching rate (HR) for each treatment were calculated as follow : HR = total number of hatched larvae/ total number of eggs oviposited). Figure 12 describes the experiment.

RNA isolation and dsRNA production

Total RNA was extracted from groups of five *Ae. aegypti* fourth instar larvae and early adult male/female *Ae. aegypti*, using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was treated with amplification grade DNase I (Invitrogen) and 1 μg was used to synthesize cDNA using a First Strand cDNA Synthesis kit (Invitrogen). The cDNA served as template DNA for PCR amplification of gene fragments using the primers listed in Table 8, below. PCR products were purified using a QIAquick PCR purification kit (Qiagen). The MEGAscript RNAi kit (Ambion) was then used for in vitro transcription and purification of dsRNAs sequences (Table 9, below).

Table 8: qPCR primers for fertility targets

Target gene	Accession number	qPCR primers (5'-3')
Argonaute-3 AAEL007823	XM_001652895.1	F: TCGGCATTCGTAGCTTCGTT (SEQ ID NO: 1833) R: GCAGCTGACAGTTTGCCTTC (SEQ ID NO: 1834)
AuB AAEL007698		F: CAGAATCCCAGACCCGGAAC (SEQ ID NO: 1835) R: TTGGCGAAACCGTACCTTGA (SEQ ID NO: 1836)
AeSCP-2 (AF510492.1)	AF510492	F:TAAGCGTCTGGAGAGCATCG (SEQ ID NO: 1837) R:CTCGACCAGCTGACGTTCTT (SEQ ID NO: 1838)
AeAct-4 (AY531222.2)	AY531222	F:GTTTCGCTGGTGATGATGCC (SEQ ID NO: 1839) R:GGTGAGGATACCTCGCTTCG (SEQ ID NO: 1840)
AAEL002000	XM_001660689.1	F:CGTCAAGGTGGAAGATTTCGG (SEQ ID NO: 1841) R:CGGCATCCGGATTATTGTCG (SEQ ID NO: 1842)
AAEL005747	XM_001651331.1	F:TGCTGTCCACCAGTATGAGC (SEQ ID NO: 1843) R:TCCTCCGATGGCATTGCTTT (SEQ ID NO: 1844)
AAEL005656	XM_001651169.1	F:GCGCATGAAGAAGAAGCTGG (SEQ ID NO: 1845) R:TTTGTGCCTCTGCATTTGCC (SEQ ID NO: 1846)
AAEL017015	XM_011494635.1	F:GCCTACCAAGCTCCGCAAAT (SEQ ID NO: 1847) R:GACGATGTCCTGCTGTTCGT (SEQ ID NO: 1848)
AAEL005212	XM_001650421.1	F:TGTGGACGCTAAGGAACAGCC (SEQ ID NO: 1849) R:CATCGAGCCCCAAGCATCC (SEQ ID NO: 1850)
AAEL005922	XM_001651632.1	F:GAAGATCAATGCACCACCGC (SEQ ID NO: 1851) R:GGACGCGATCTACGAGGTTT (SEQ ID NO: 1852)

AAEL000903	XM_001651555.1	F:TACCGGACACCGTCAAGAAG (SEQ ID NO: 1853) R:CTAAATATCGATACCCTCCTGCTG (SEQ ID NO: 1854)
AAEL005049	XM_001650243.1	F:ACTCGGAAGCAGTGGTAACG (SEQ ID NO: 1855) R:ATCTGCATTCCTTCCGGCTT (SEQ ID NO: 1856)

Table 9: dsRNA sequences for fertility targets

Target gene	Accession number	dsRNA sequence
Argonaute-3 AAEL007823	XM_001652895.1	SEQ ID NO: 1857
AuB AAEL007698		SEQ ID NO: 1858
AeSCP-2 (AF510492.1)	AF510492	SEQ ID NO: 1859
AeAct-4 (AY531222.2)	AY531222	SEQ ID NO: 1860
AAEL002000	XM_001660689.1	SEQ ID NO: 1861
AAEL005747	XM_001651331.1	SEQ ID NO: 1862
AAEL005656	XM_001651169.1	SEQ ID NO: 1863
AAEL017015	XM_011494635.1	SEQ ID NO: 1864
AAEL005212	XM_001650421.1	SEQ ID NO: 1865
AAEL005922	XM_001651632.1	SEQ ID NO: 1866
AAEL000903	XM_001651555.1	SEQ ID NO: 1867
AAEL005049	XM_001650243.1	SEQ ID NO: 1868

qPCR analysis

Approximately 1000 ng first-strand cDNA obtained as described previously was used as template. The qPCR reactions were performed using SYBR® Green PCR Master Mix (Applied Biosystems) following the manufacturer's instructions. Briefly, approximately 50 ng/μl cDNA and gene-specific primers (600 nM) were used for each reaction mixture. qPCR conditions used were 10 min at 95 °C followed by 35 cycles of

15 s at 94 °C, 15 s at 54 °C and 60 s at 72 °C. The ribosomal protein S7 and tubulin were used as the reference gene to normalize expression levels amongst the samples. Raw quantification cycle (Cq) values normalized against those of the tubulin and S7 standards were then used to calculate the relative expression levels in samples using the $2^{-\Delta\Delta Ct}$ method [Livak & Schmittgen, (2001) Methods. 25(4):402-8]. Results (mean ± SD) are representative of at least two independent experiments performed in triplicate.

RESULTS

Gene silencing with dsRNA during larval development decreases the number of hatchings

The sterile insect technique (SIT) is a non-insecticidal control method that relies on the release of sterile male mosquitoes that search for and mate with wild females, preventing offspring. This approach has been used successfully to control various insect pest species. Recently, a dsRNA-based method to produce sterile male mosquitoes was described [Whyard et al., Parasit Vectors. (2015) 8: 96].

The present inventors hypothesized that dsRNA could be used to produce effective sterile male/female *Ae. aegypti* mosquitoes by targeting genes expressed mainly (but not exclusively) in male testes and/or female ovary. Since sterile female insects can still damage crops and transmit disease, ideally the product will include dsRNA sequences to induce mortality in infected-mosquitoes or reduce resistance to pyrethroids.

As illustrated in Figure 10B, the present inventors were able to induce gene silencing in mosquito larvae after treatment with dsRNA against Ago3, one of the targets to induce male/female sterility. Next, larvae were treated with dsRNA against Aub and Ago3 and were reared until the adult stage. Female mosquitoes were allowed to blood fed on sheep blood and engorged females were separated in 3 cages containing 15 females each and the oviposition rate was calculated. As illustrated in Figures 13A-B, there was no difference in the oviposition rate among dsRNA-treated groups and water control. However, the number of hatched eggs decrease to 50 % in the dsRNA treated groups. Similar results were observed for treatment with dsRNA targeting AY531222.2, AAEL005922, AAEL000903, AAEL017015 or AAEL005212 (see Figures 14A-B, 15A-B and 16A-B). When larvae were treated with dsRNA targeting the combination of Ago + Aub a much stronger reduction in the hatchability was observed (Figure 16B).

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A composition-of-matter for mosquito control, comprising a cell comprising an exogenous naked dsRNA which specifically down-regulates expression of a gene being endogenous to a mosquito or which specifically down-regulated expression of a gene being endogenous to a mosquito pathogen.

2. A composition-of-matter for mosquito control, comprising a cell comprising a nucleic acid larvicide.

3. A composition-of-matter for mosquito control, comprising a cell comprising a nucleic acid larvicide affecting fertility or fecundity of a female mosquito.

4. A composition-of-matter for mosquito control comprising a nucleic acid larvicide that targets a piRNA pathway gene and/or a sterility gene.

5. A composition-of-matter for mosquito control comprising a nucleic acid larvicide that targets a gene comprising Aub (AAEL007698) and Argonaute-3 (AAEL007823).

6. The composition-of-matter of claim 4 or 5, wherein said nucleic acid larvicide comprises at least one dsRNA.

7. The composition-of-matter of claim 6, comprising a dsRNA which comprises SEQ ID NO: 1858 and a dsRNA which comprises SEQ ID NO: 1823.

8. A method of producing a larvicidal composition, the method comprising introducing into a cell a nucleic acid larvicide, thereby producing the larvicide.

9. A method of producing a larvicidal composition, the method comprising introducing into a cell a nucleic acid larvicide affecting fertility or fecundity of a female mosquito, thereby producing the larvicide.

10. The method of claim 8 or 9, wherein said introducing is effected by electroporation.

11. The method of claim 8 or 9, wherein said introducing is effected by particle bombardment.

12. The method of claim 8 or 9, wherein said introducing is effected by chemical-based transfection.

13. The composition-of-matter of claim 2 or method of claim 8, wherein said nucleic acid larvicide down-regulates a target gene selected from the group consisting of:

(i) affecting larval survival;

(ii) interfering with metamorphosis of larval stage to adulthood;

(iii) affecting susceptibility of mosquito larvae to a larvicide;

(iv) affecting susceptibility of an adult mosquito to an adulticide/insecticide; and

(v) affecting fertility or fecundity of a male or female mosquito.

14. The composition-of-matter of claim 13, wherein said target gene is selected from the group consisting of 1-427, 430-1813, 1826-1832.

15. The composition-of-matter of claim 13, wherein said target gene is selected from the group consisting of P-glycoprotein (AAEL010379), Argonaute-3 (AAEL007823), Cytochrome p450 (CYP9J26), Sodium channel (AAEL008297), Aub (AAEL007698), AeSCP-2 (AF510492.1), AeAct-4 (AY531222.2), AAEL002000, AAEL005747, AAEL005656, AAEL017015, AAEL005212, AAEL005922, AAEL000903 and AAEL005049.

16. The composition-of-matter of claim 13, wherein said target gene comprises Aub (AAEL007698) and Argonaute-3 (AAEL007823).

17. The composition-of-matter of claim 16, wherein said nucleic acid larvicide which down-regulates said target gene is a dsRNA.

18. The composition-of-matter of claim 17, wherein said dsRNA comprises SEQ ID NOs: 1858 and 1823.

19. The composition-of-matter of any one of claims 1-3, or method of claim 8-9, wherein said cell is an algal cell.

20. The composition-of-matter of any one of claims 1-3, or method of claim 8-9, wherein said cell is a microbial cell.

21. The composition-of-matter or method of claim 20, wherein said cell is a bacterial cell.

22. The composition-of-matter or method of any one of claims 1-21, wherein the composition further comprises a food-bait.

23. The composition-of-matter or method of any one of claims 1-22, wherein the composition is formulated in a formulation selected from the group consisting of technical powder, wettable powder, dust, pellet, briquette, tablet and granule.

24. The composition-of-matter or method of claim 23, wherein said granule is selected from the group consisting of an impregnated granule, dry flowable, wettable granule and water dispersible granule.

25. The composition-of-matter or method of any one of claims 1-22, wherein the composition is formulated as a non-aqueous or aqueous suspension concentrate.

26. The composition-of-matter or method of any one of claims 1-22, wherein the composition is formulated as a semi-solid form.

27. The composition-of-matter or method of claim 26, wherein said semi-solid form comprises an agarose.

28. The composition-of-matter or method of any one of claims 1-26, wherein the cell is lyophilized.

29. The composition-of-matter or method of any one of claims 1-28, wherein the cell is non-transgenic.

30. The composition-of-matter or method of any one of claims 1-28 further comprising an RNA-binding protein.

31. The composition-of-matter or method of any one of claims 2-29, wherein said nucleic acid larvicide comprises a dsRNA.

32. The composition-of-matter or method of claim 31, wherein said dsRNA is a naked dsRNA.

33. The composition-of-matter or method of claim 31, wherein said dsRNA comprises a carrier.

34. The composition-of-matter or method of claim 33, wherein said carrier comprises a polyethyleneimine (PEI).

35. The composition-of-matter or method of claim 31, wherein said dsRNA is effected at a dose of 0.001-1 μg/μL for soaking or at a dose of 1 pg to 10 μg/larvae for feeding.

36. The composition-of-matter or method of any one of claims 31-35, wherein said dsRNA is selected from the group consisting of SEQ ID NOs: 1822-1825 and 1857-1868.

37. The composition-of-matter or method of claim 1 or 31, wherein said dsRNA is selected from the group consisting of siRNA, shRNA and miRNA.

38. The composition-of-matter or method of any one of claims 1-37, wherein said cell is devoid of a heterologous promoter for driving expression of said dsRNA in the plant.

39. The composition-of-matter or method of any one of claims 1-29, wherein said nucleic acid larvicide is greater than 15 base pairs in length.

40. The composition-of-matter or method of any one of claims 1-29, wherein said nucleic acid larvicide is 19 to 25 base pairs in length.

41. The composition-of-matter or method of any one of claims 1-29, wherein said nucleic acid larvicide is 30-100 base pairs in length.

42. The composition-of-matter or method of any one of claims 1-29, wherein said nucleic acid larvicide is 100-800 base pairs in length.

43. The composition-of-matter or method of any one of claims 1-29, wherein the composition further comprises at least one of a surface-active agent, an inert carrier vehicle, a preservative, a humectant, a feeding stimulant, an attractant, an encapsulating agent, a binder, an emulsifier, a dye, an ultra-violet protector, a buffer, a flow agent or fertilizer, micronutrient donors, or other preparations that influence the growth of the plant.

44. The composition-of-matter of any one of claims 1-43 having an inferior impact on an adult mosquito as compared to said larvae.

45. The composition-of-matter or method of any one of claims 1-28, wherein the composition further comprises a chemical larvicide or a biochemical larvicide or a combination of same.

46. The composition-of-matter or method of claim 45, wherein said larvicide is selected from the group consisting of Temephos, Diflubenzuron, methoprene, Bacillus sphaericus, and Bacillus thuringiensis israelensis.

47. The composition-of-matter or method of claim 45, wherein said larvicide comprises an adulticide.

48. The composition-of-matter or method of claim 47, wherein said adulticide is selected from the group consisting of deltamethrin, malathion, naled, chlorpyrifos, permethrin, resmethrin and sumithrin.

49. A method of controlling or exterminating mosquitoes, the method comprising feeding larvae of the mosquitoes with an effective amount of the composition-of-matter of any one of claims 1-48, thereby controlling or exterminating the mosquitoes.

50. The method of claim 49, wherein said mosquitoes comprise female mosquitoes capable of transmitting a disease to a mammalian organism.

51. The method of any one of claims 45-48 or 49, wherein said mosquitoes are of a species selected from the group consisting of *Aedes aegypti* and *Anopheles gambiae*.

FIG. 1

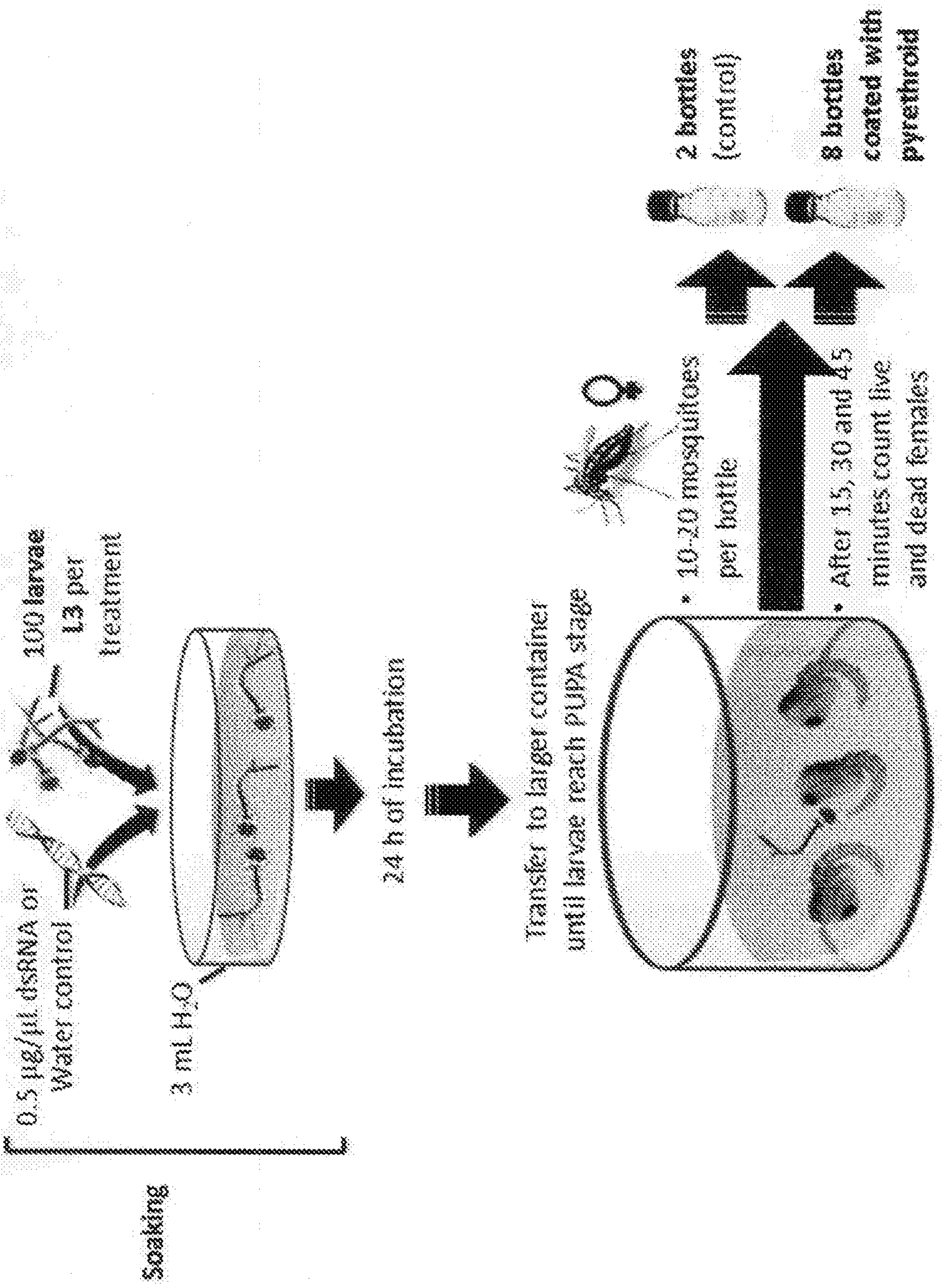
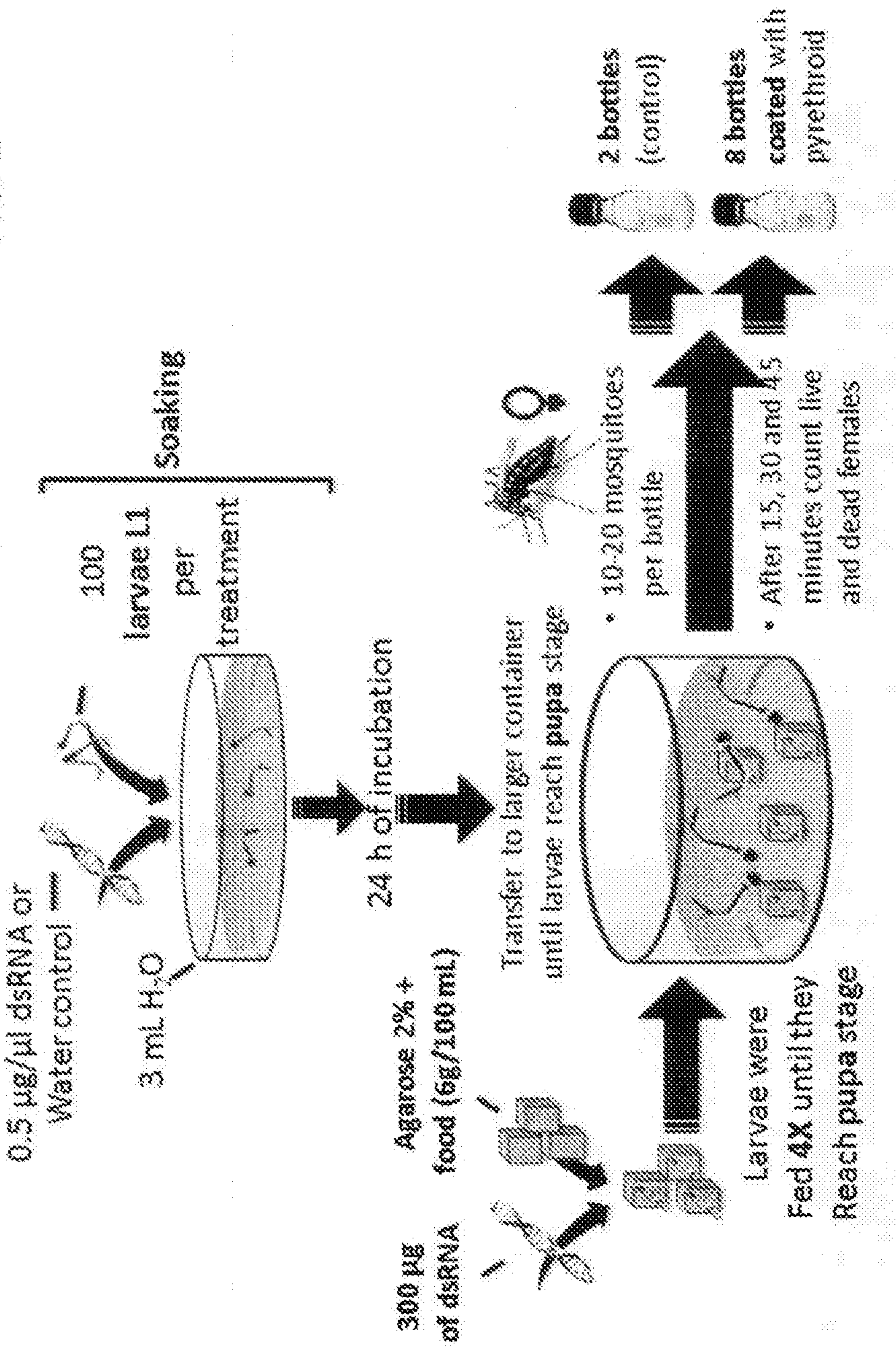

Soaking
0.5 µg/µl dsRNA or
Water control
100 larvae
L3 per
treatment
3 mL H2O
24 h of incubation
Transfer to larger container
until larvae reach PUPA stage
10-20 mosquitoes
per bottle
After 15, 30 and 45
minutes count live
and dead females
2 bottles
(control)
8 bottles
coated with
pyrethroid

FIG. 2



Flowchart 2

FIG. 3

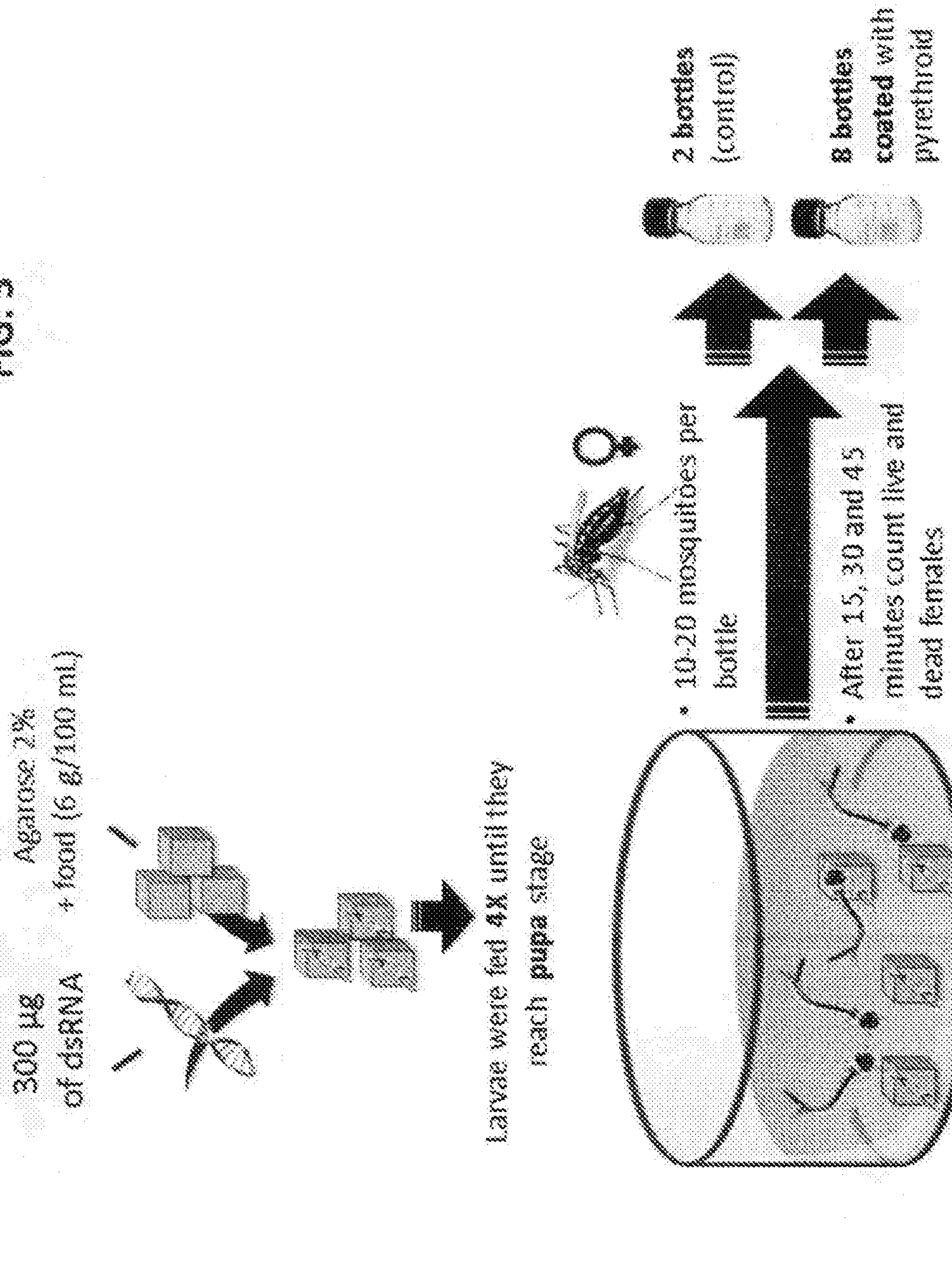
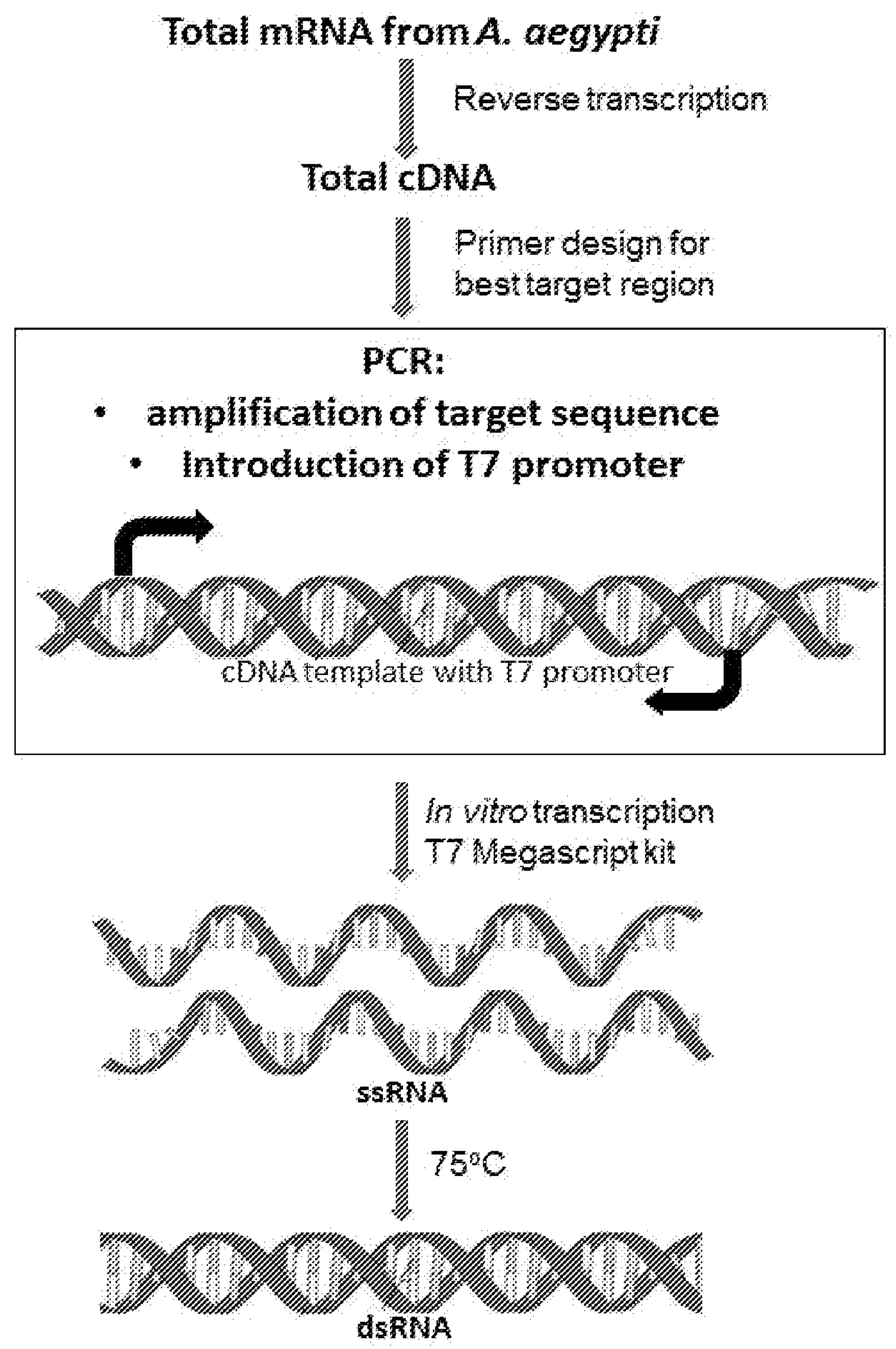

300 µg of dsRNA
Agarose 2% + food (6 g/100 ml)
Larvae were fed 4X until they reach pupa stage
• 10-20 mosquitoes per bottle
• After 15, 30 and 45 minutes count live and dead females
♀
2 bottles (control)
8 bottles coated with pyrethroid

FIG. 4



Flowchart 4

FIG. 5A

Rockfeller strain

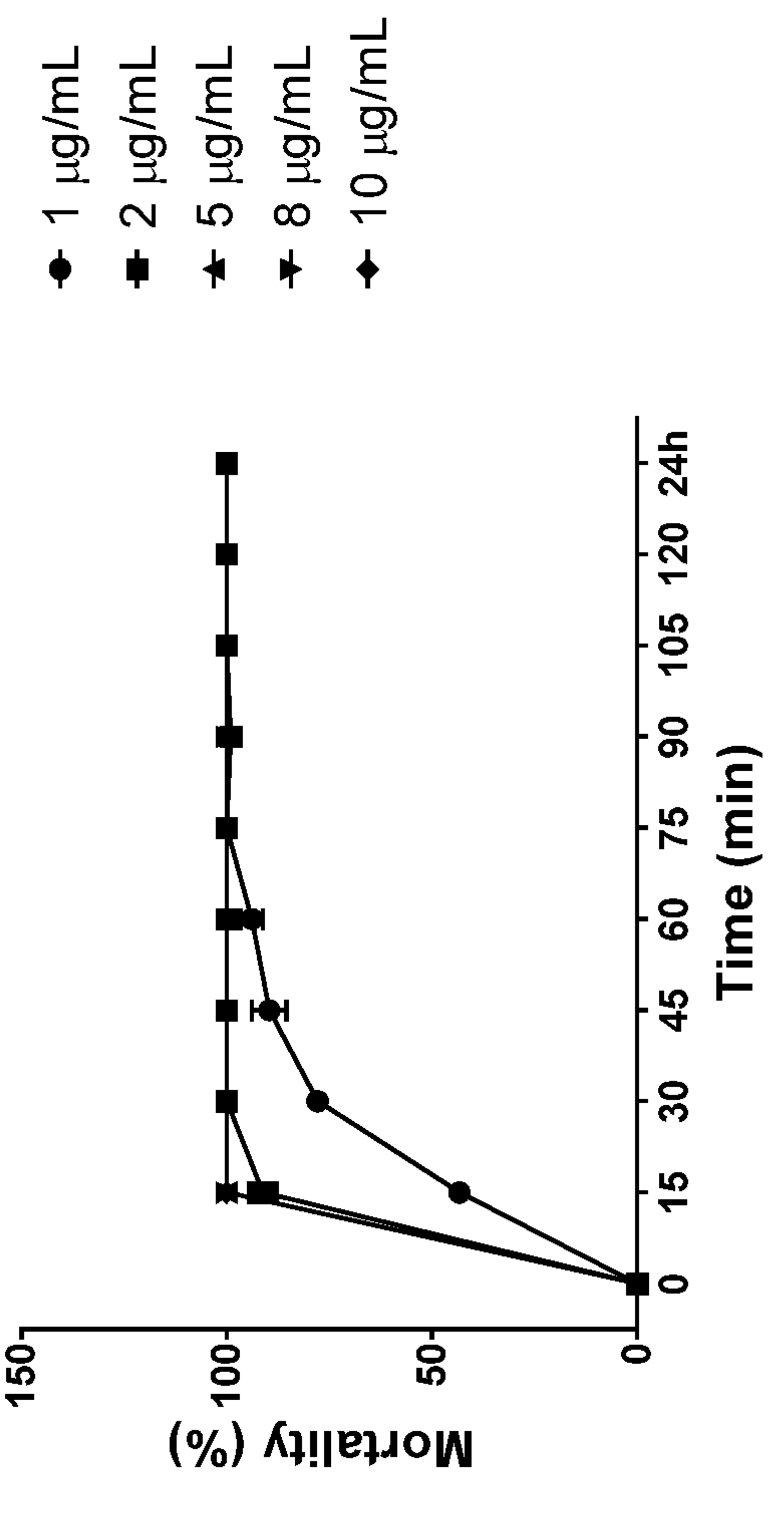


FIG. 5B

Rio de Janeiro strain

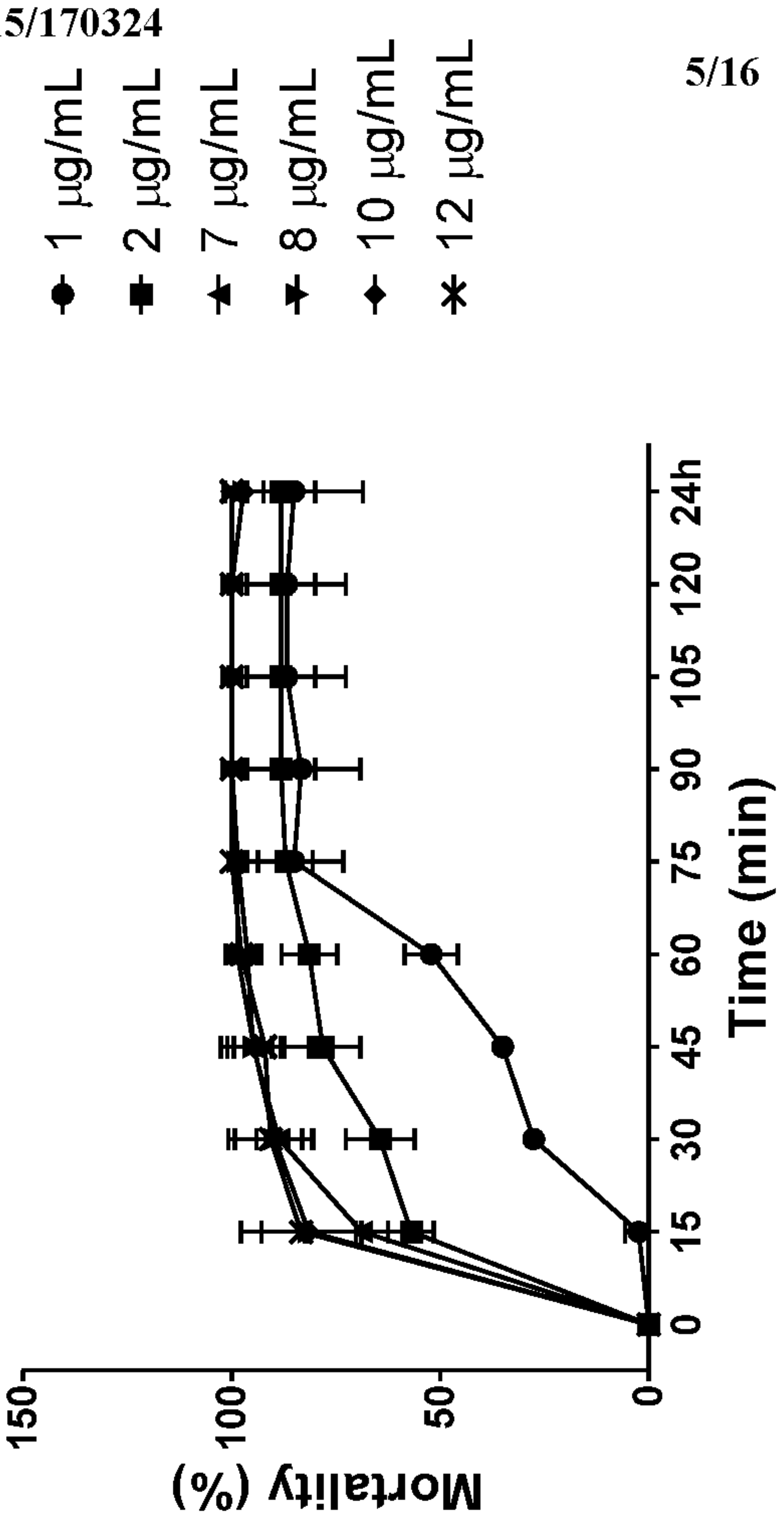
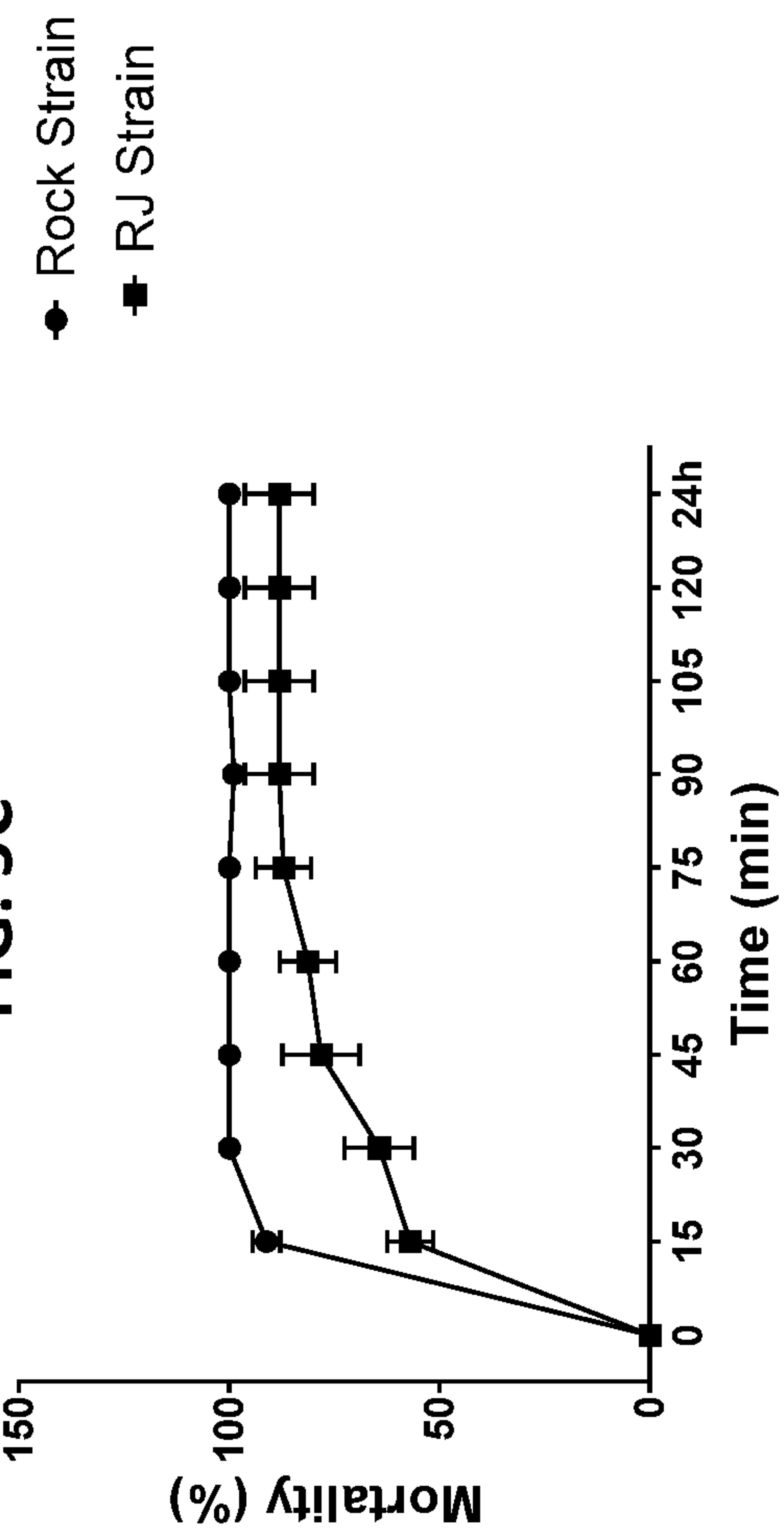


FIG. 5C



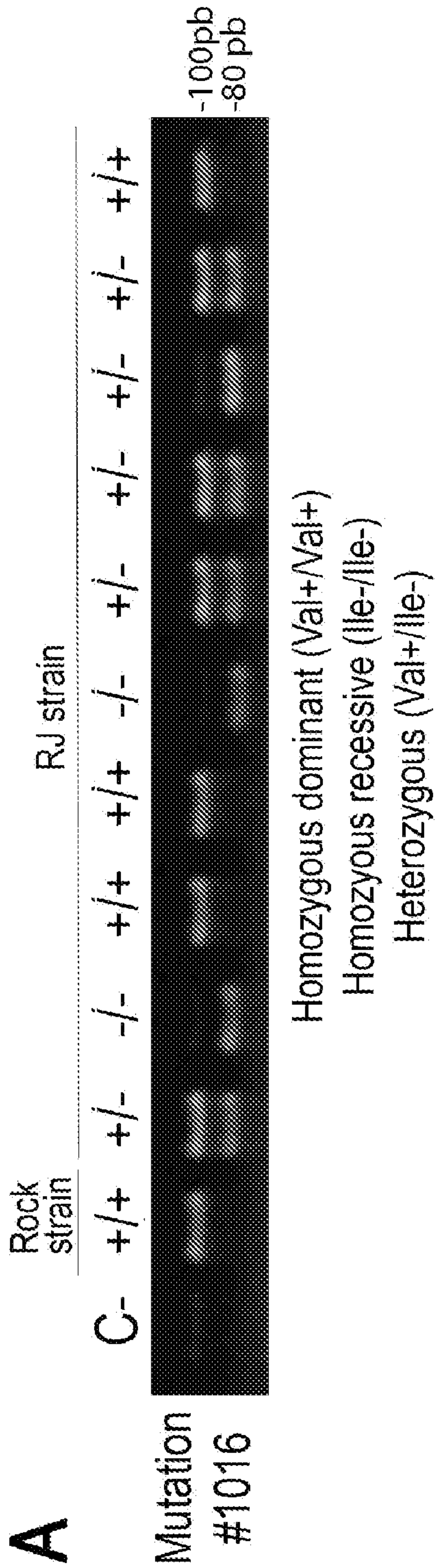

A
Mutation #1016
Rock strain
RJ strain
C- +/+ +/- -/- +/+ +/+ -/- +/- +/- +/- +/- +/+
-100pb
-80 pb
Homozygous dominant (Val+/Val+)
Homozyous recessive (Ile-/Ile-)
Heterozygous (Val+/Ile-)

FIG. 6A

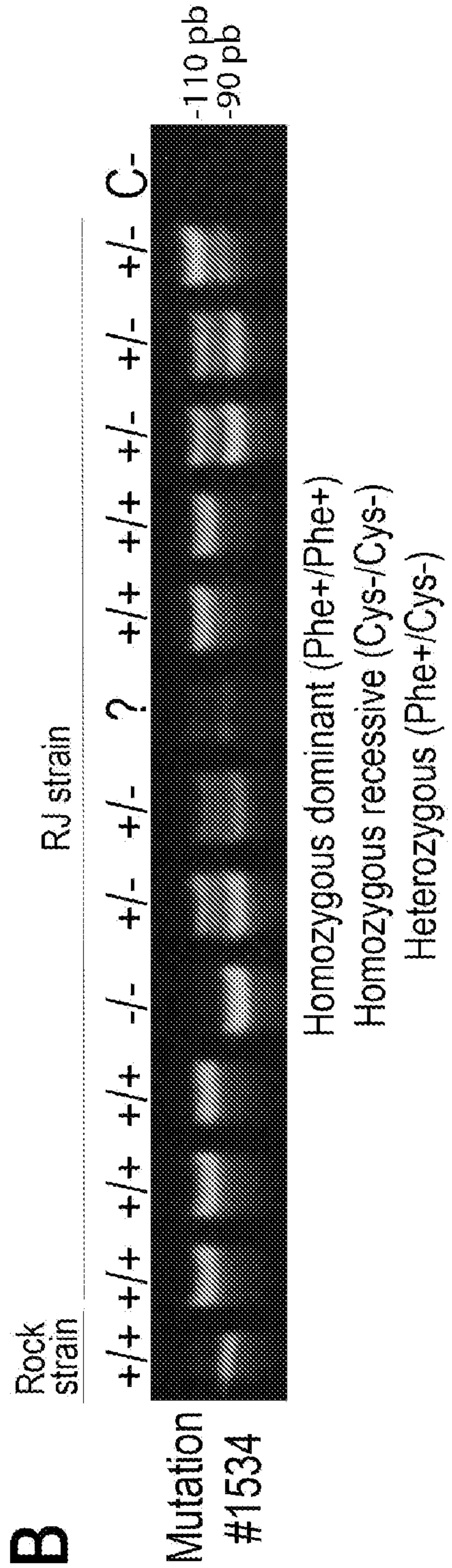

B
Mutation #1534
Rock strain
RJ strain
+/+ +/+ +/+ +/+ -/- +/- +/- ? +/+ +/+ +/- +/- +/- C-
-110 pb
-90 pb
Homozygous dominant (Phe+/Phe+)
Homozygous recessive (Cys-/Cys-)
Heterozygous (Phe+/Cys-)

FIG. 6B

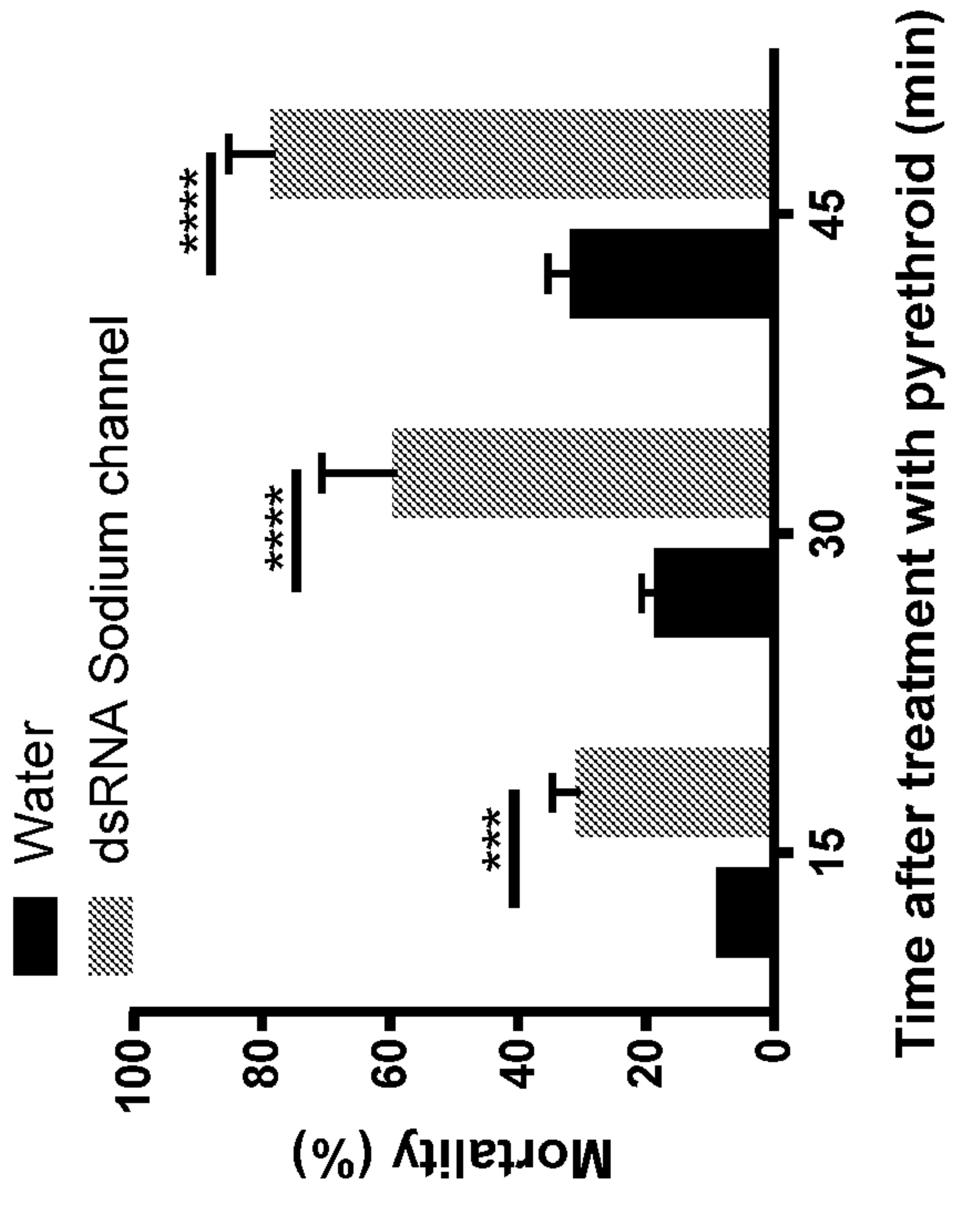


FIG. 7A

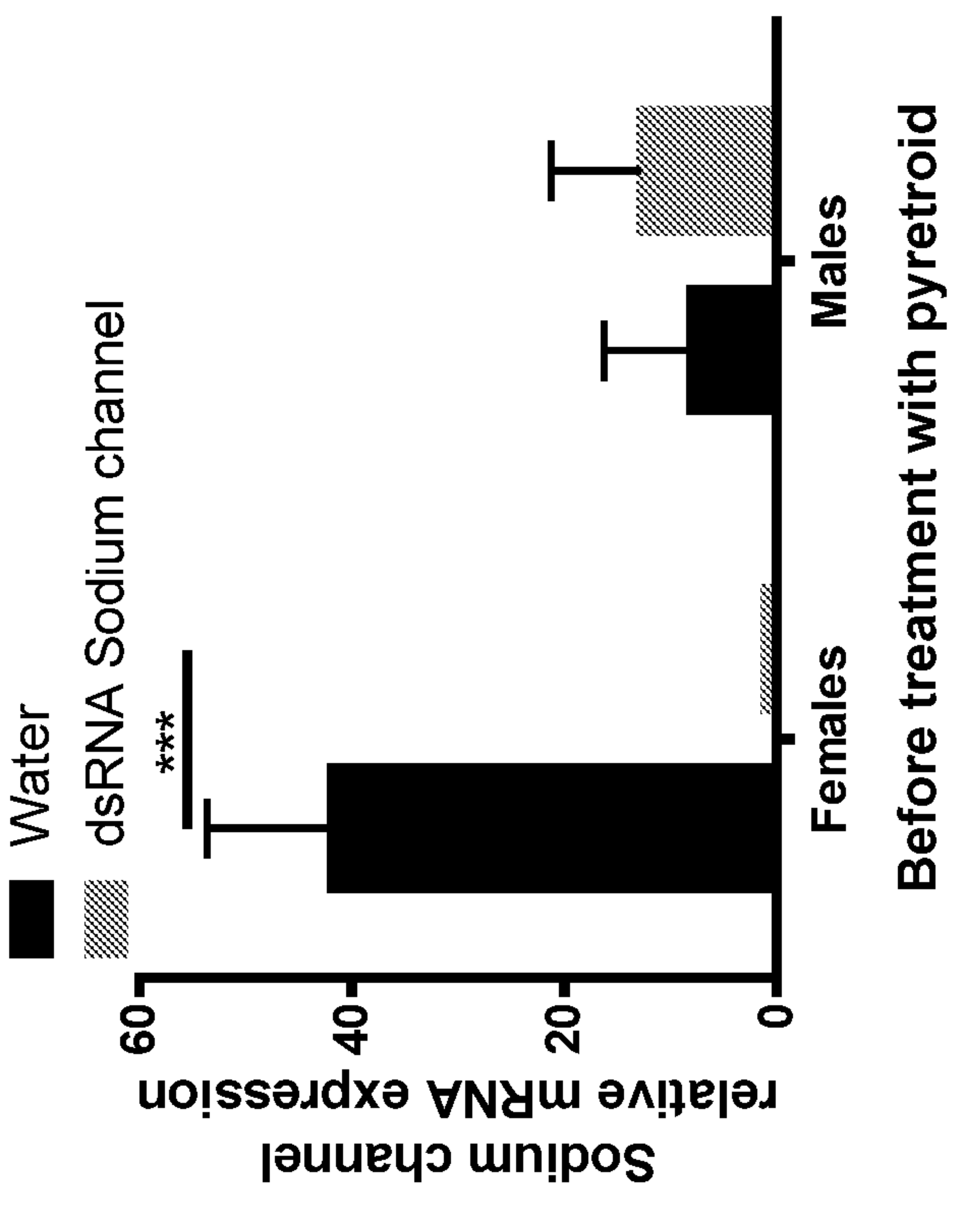


FIG. 7B

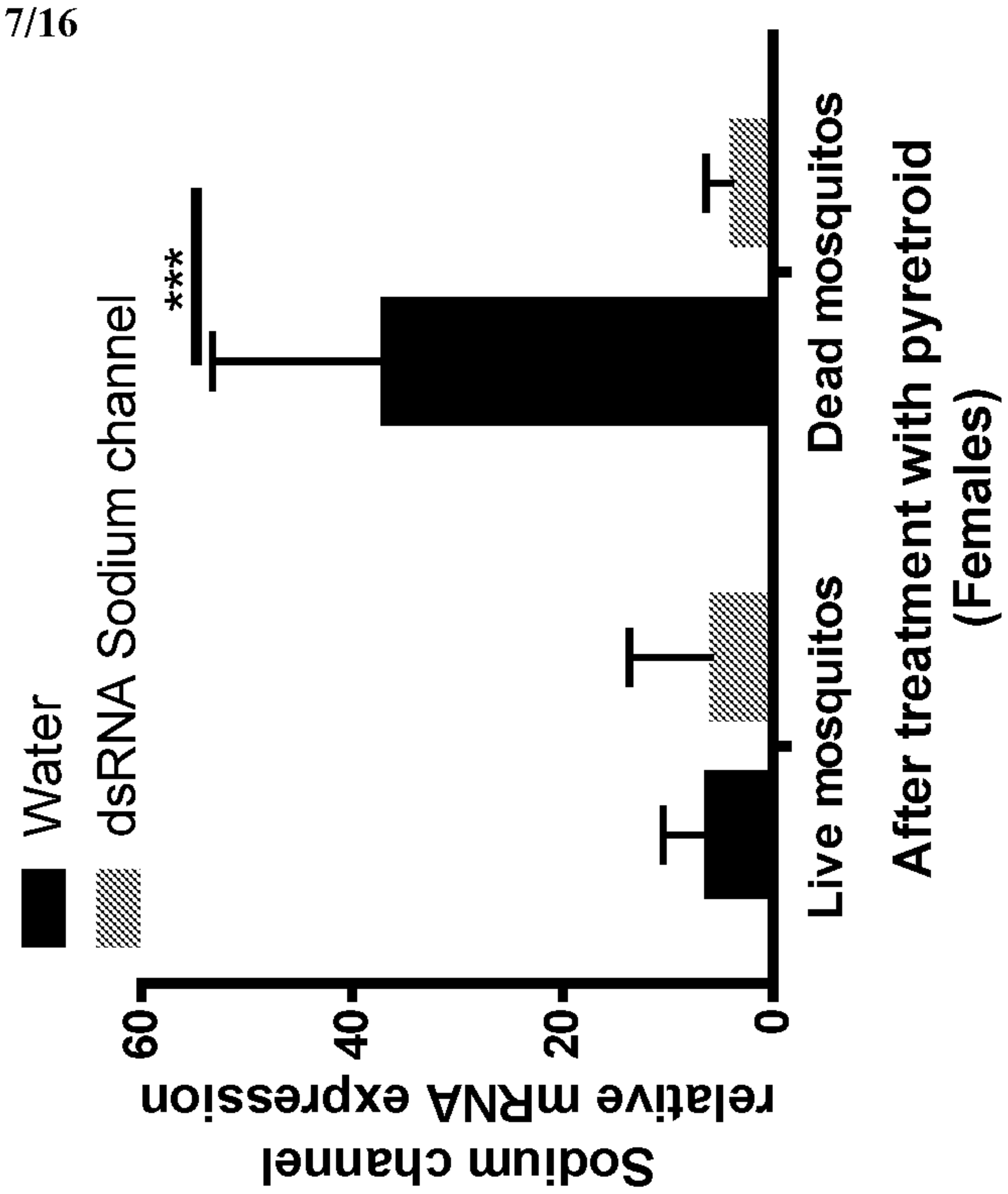


FIG. 7C

FIG. 8

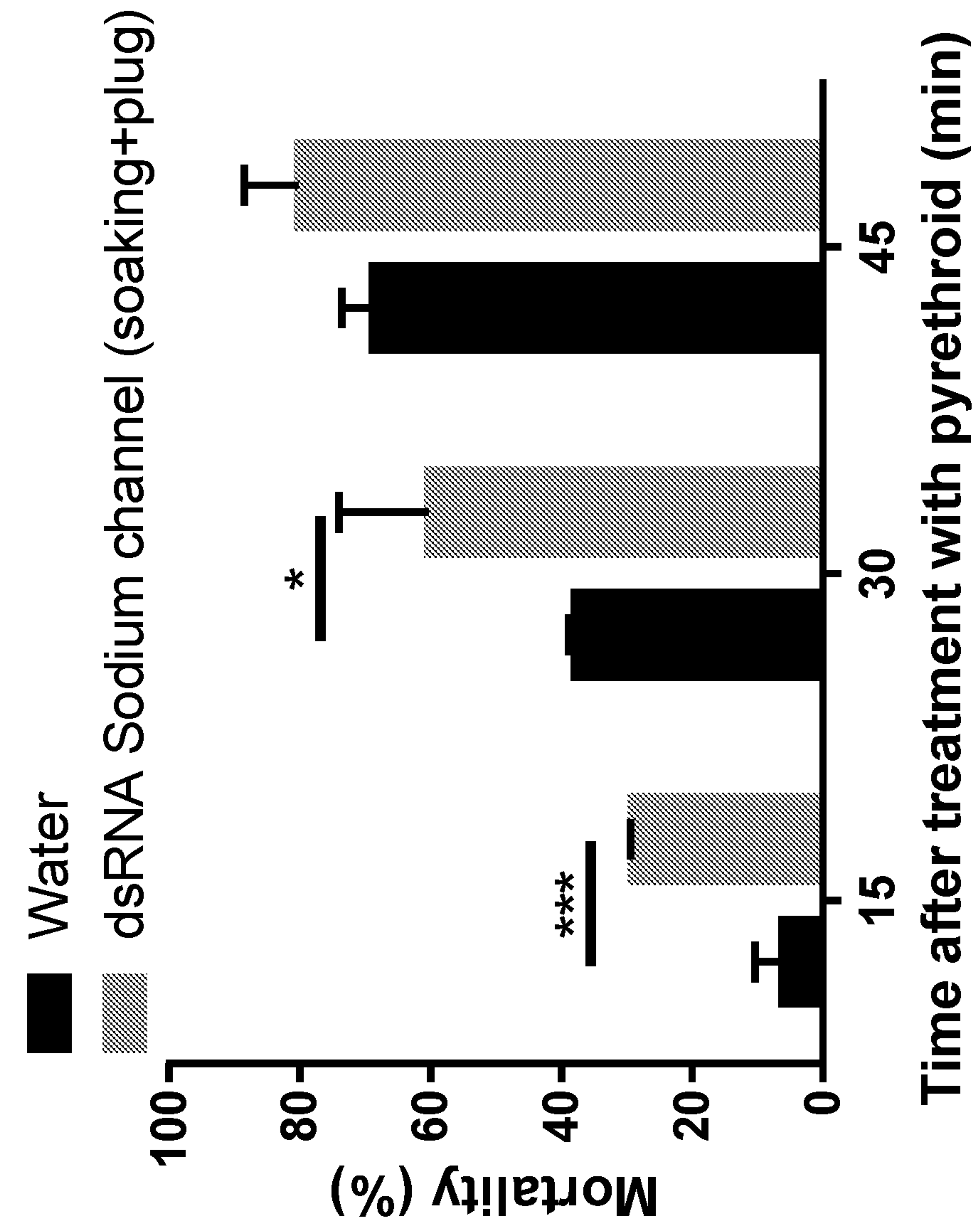
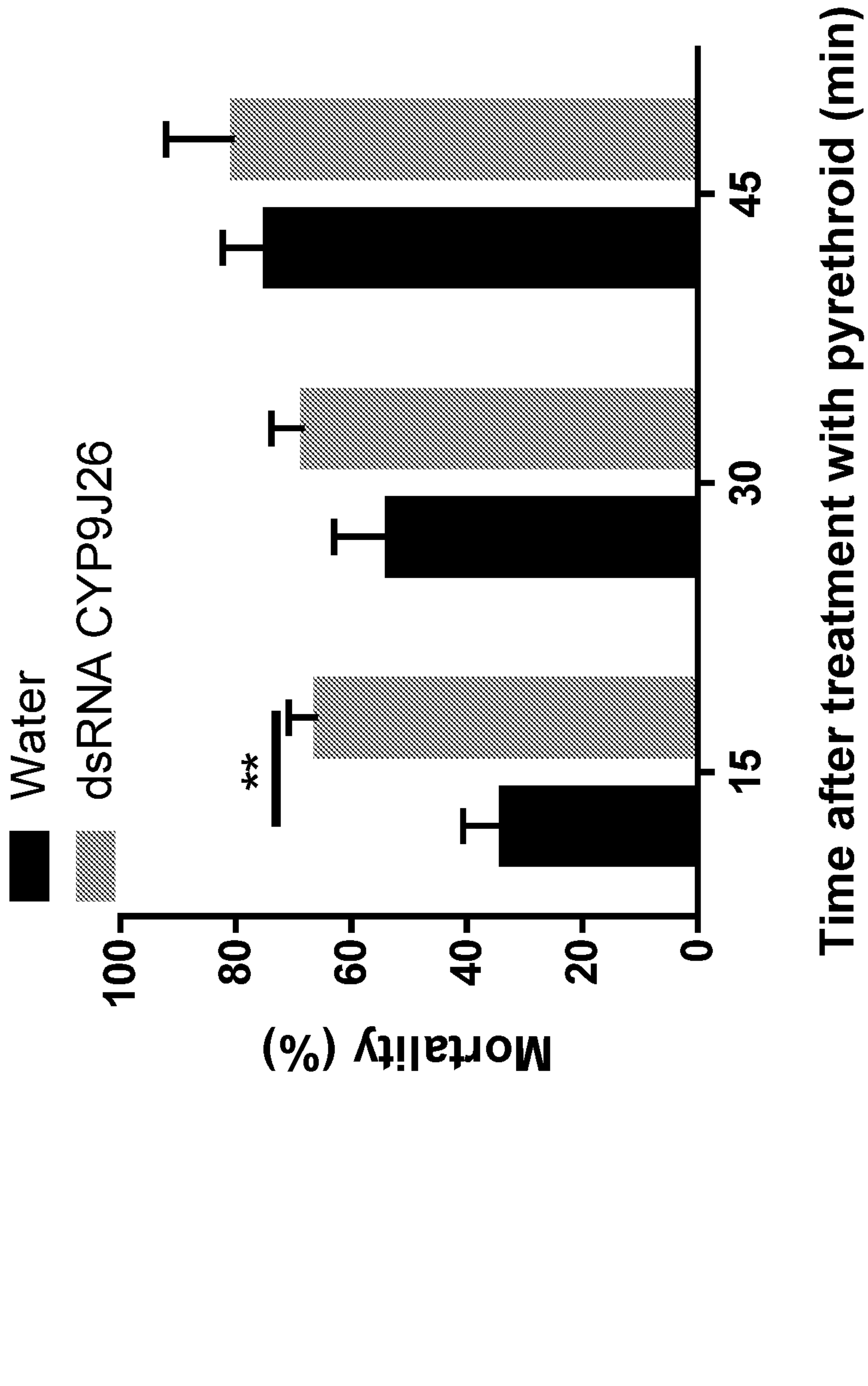

Water
dsRNA Sodium channel (soaking+plug)
Mortality (%)
100
80
60
40
20
0
***
*
15
30
45
Time after treatment with pyrethroid (min)

FIG. 9


Water
dsRNA CYP9J26
**
Mortality (%)
100
80
60
40
20
0
15
30
45
Time after treatment with pyrethroid (min)

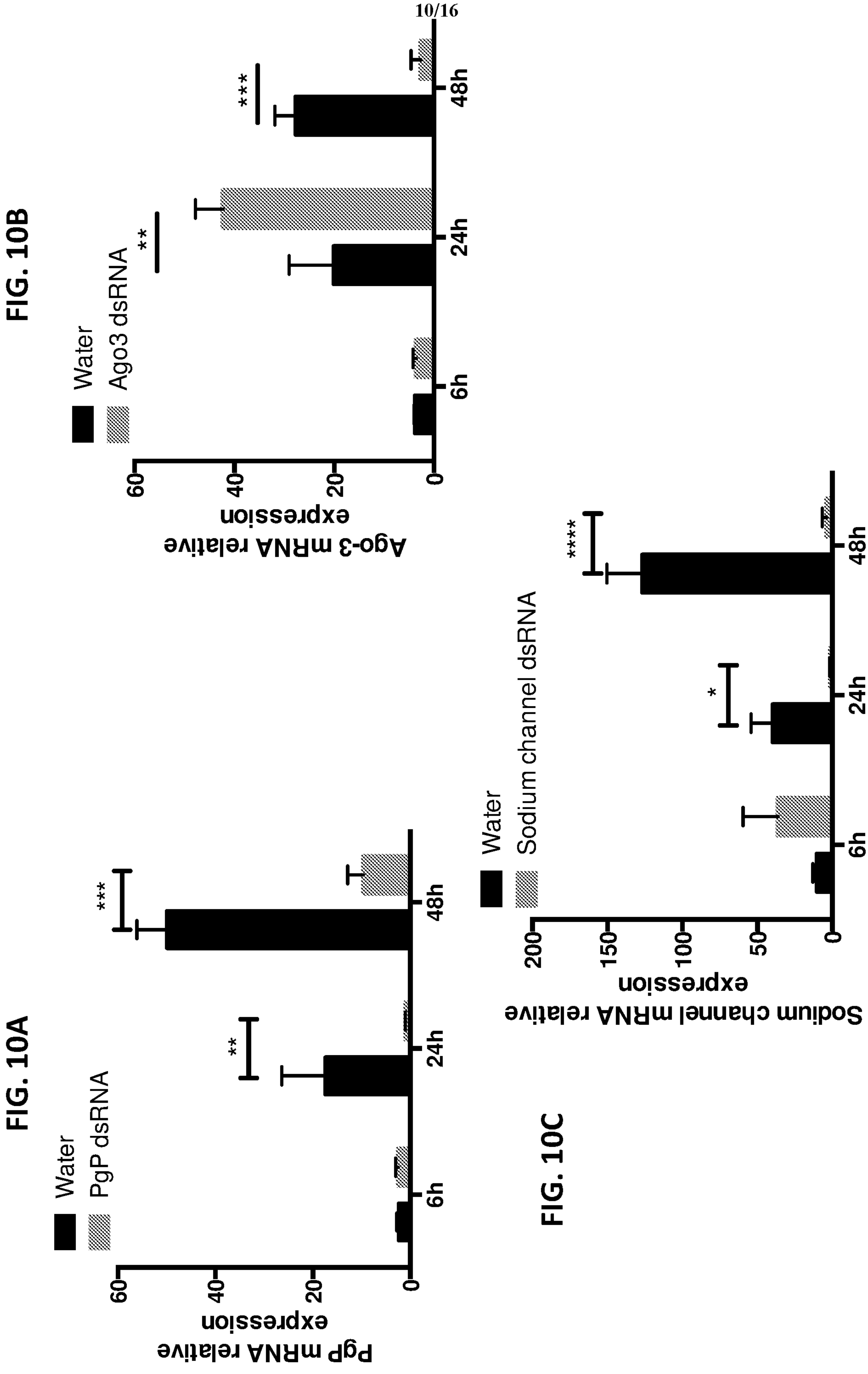


FIG. 10A

FIG. 10B

FIG. 10C

FIG. 11A

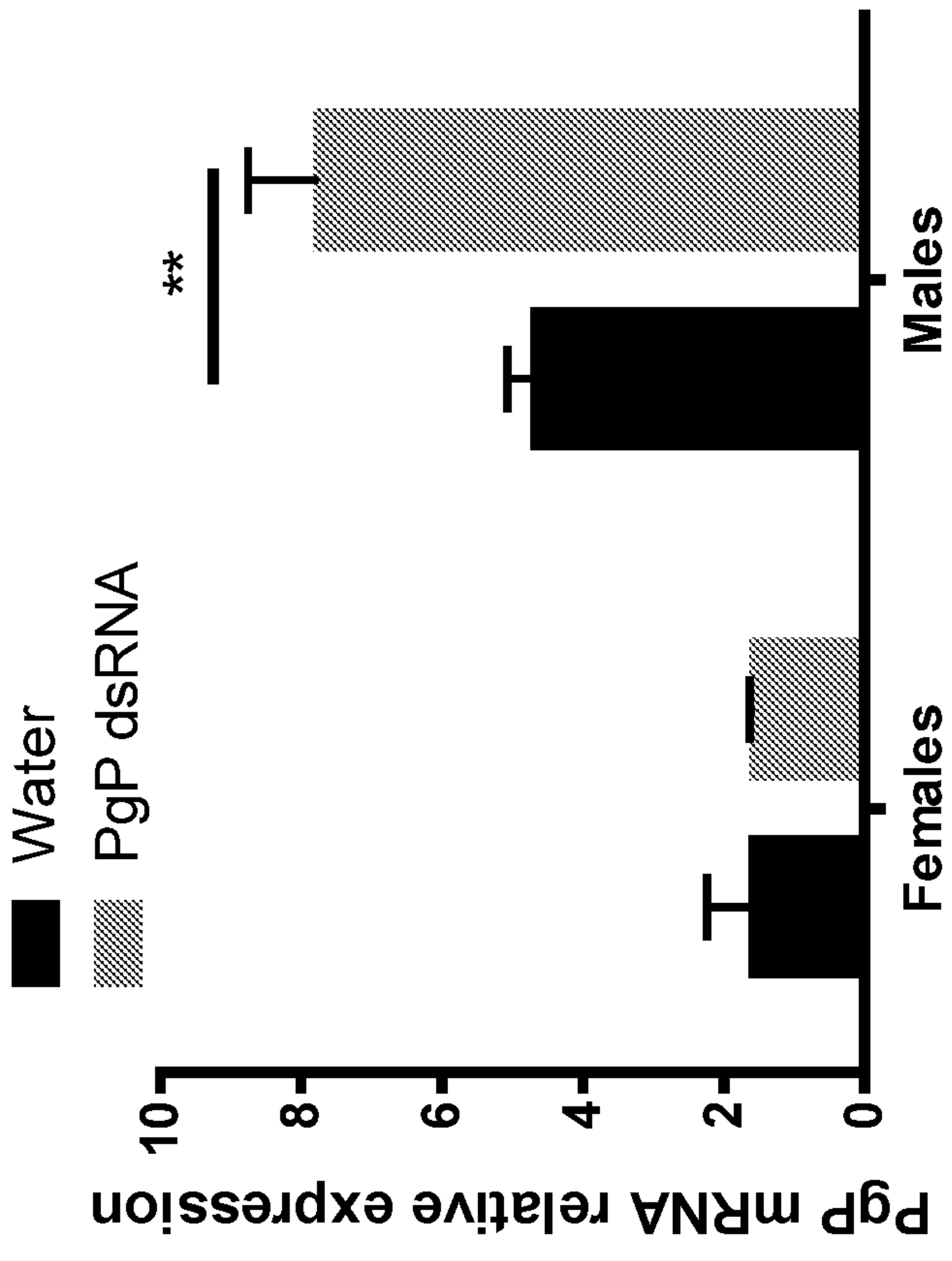


FIG. 11B

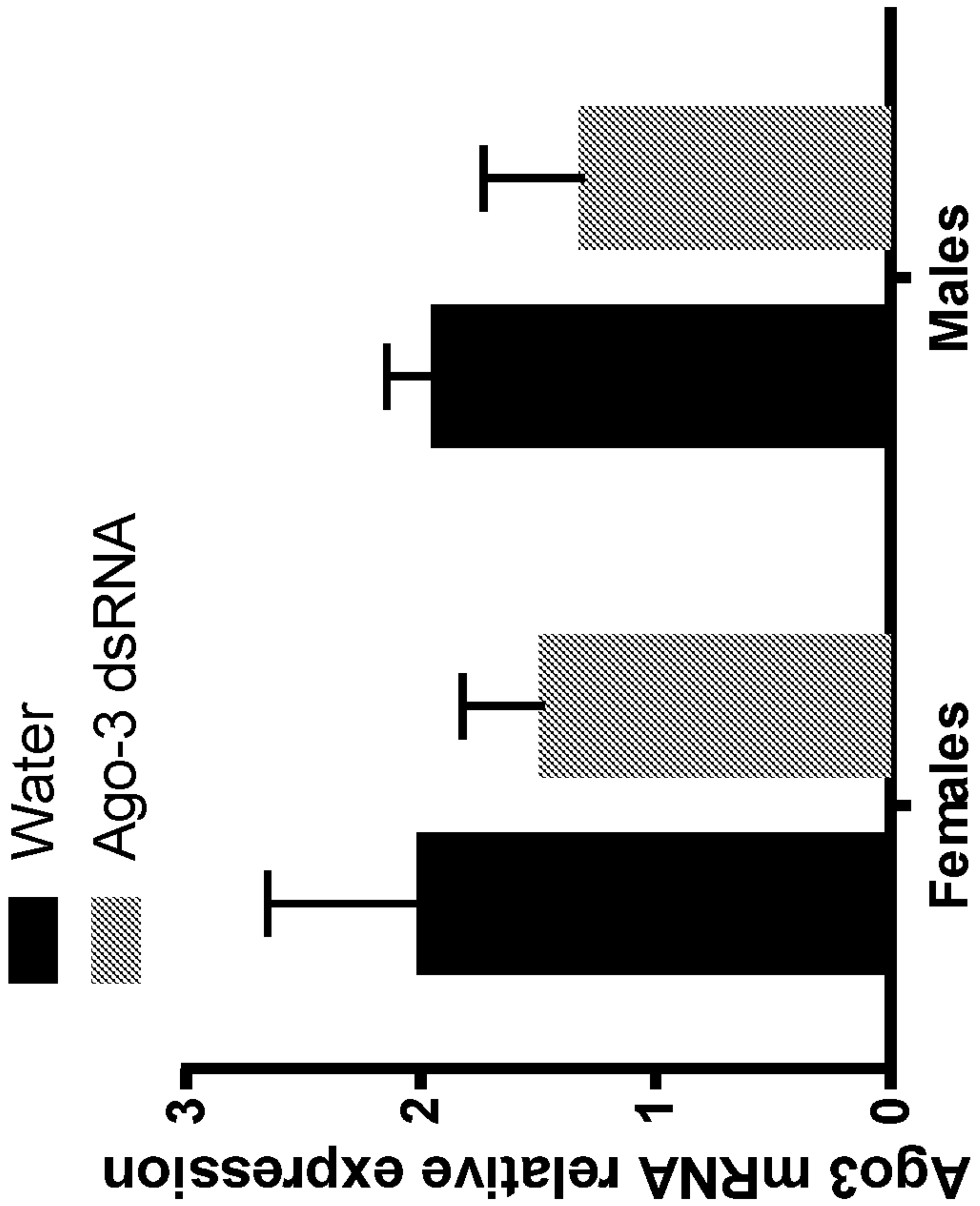


FIG. 12

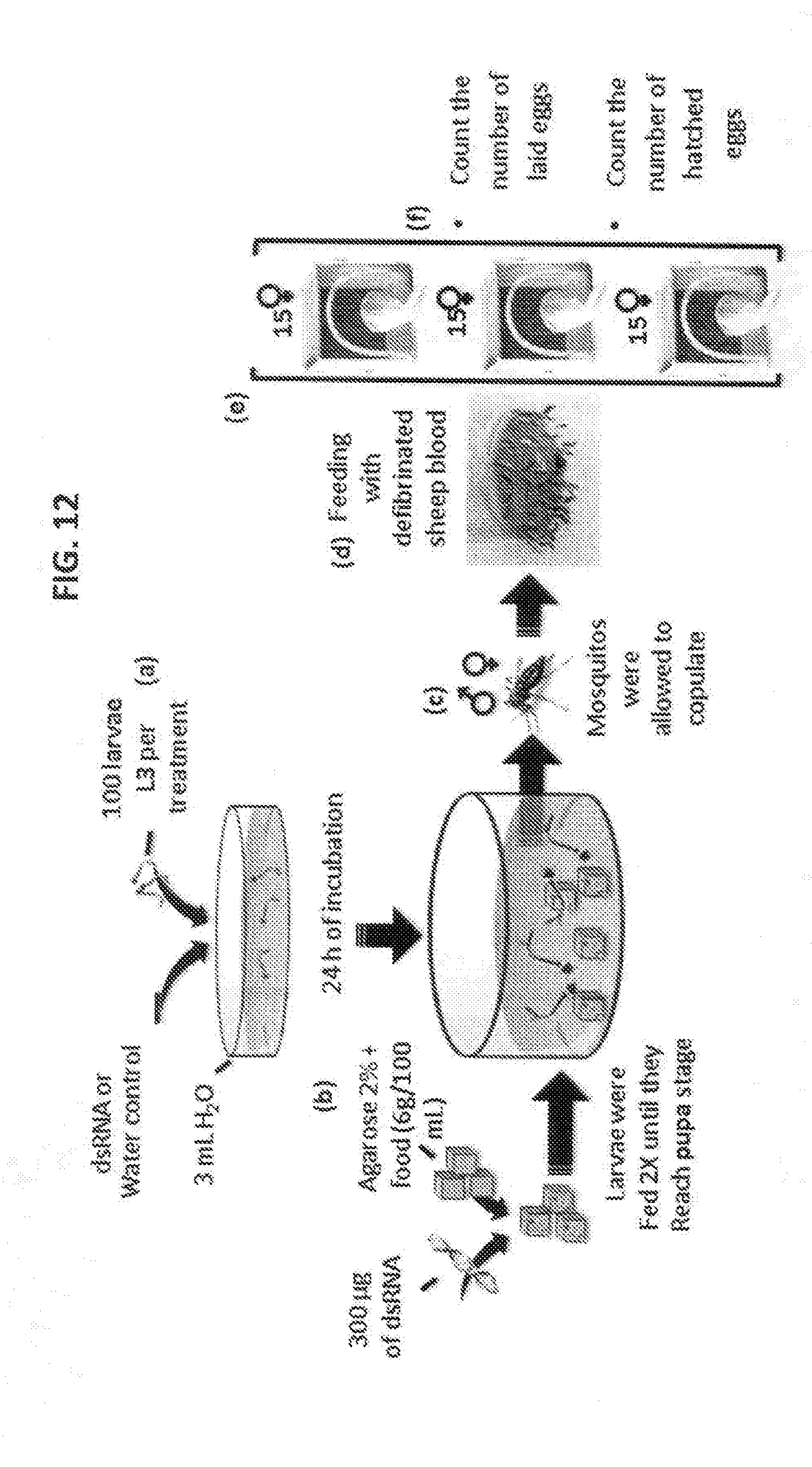

dsRNA or Water control
3 mL H_2O
100 larvae L3 per treatment (a)
24 h of incubation
(b) Agarose 2% + food (6g/100 mL)
300 μg of dsRNA
Larvae were Fed 2X until they Reach pupa stage
(c) Mosquitos were allowed to copulate
(d) Feeding with defibrinated sheep blood
(e)
15♀
15♀
15♀
(f)
Count the number of laid eggs
Count the number of hatched eggs

FIG. 13A

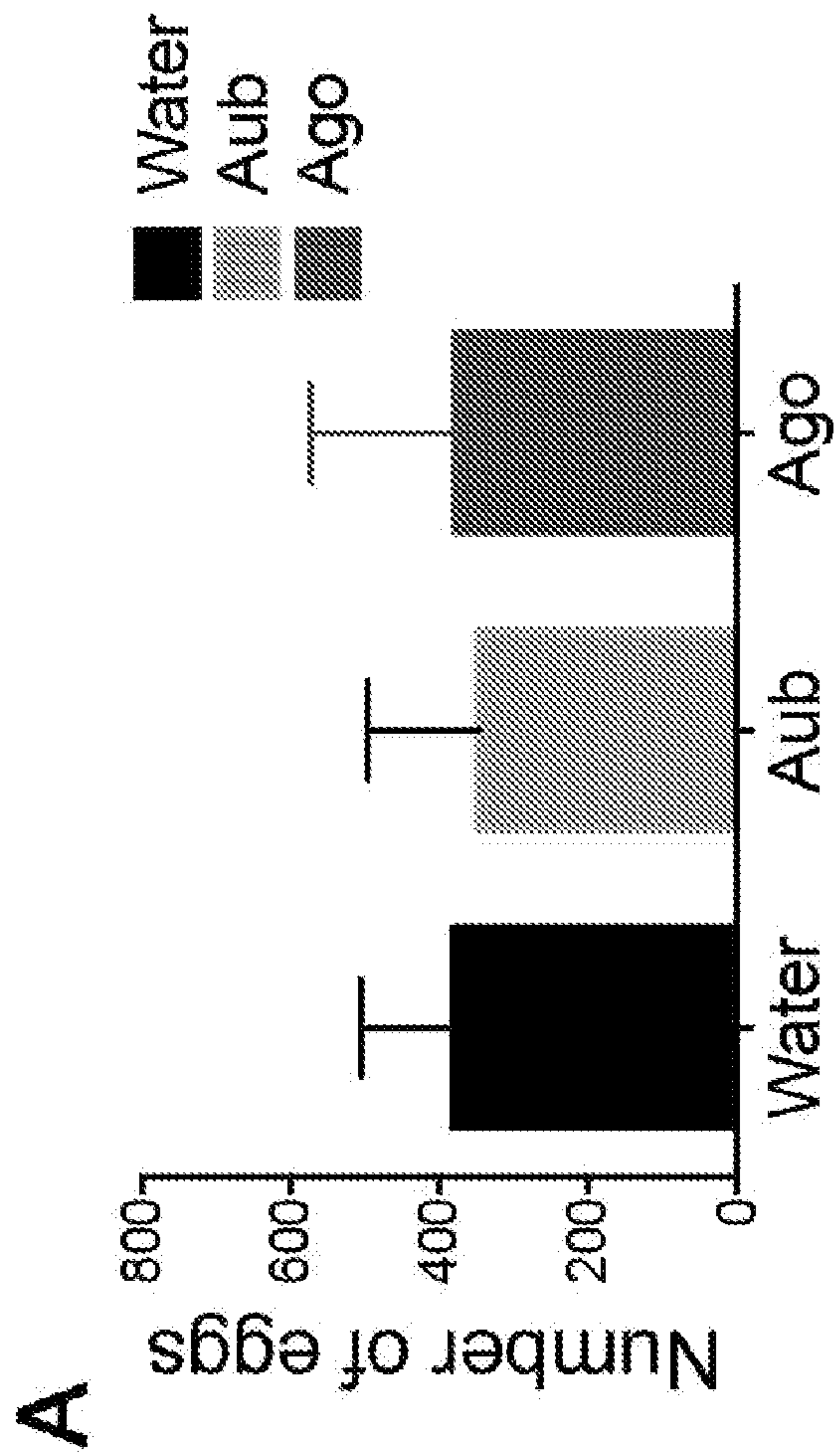


FIG. 13B

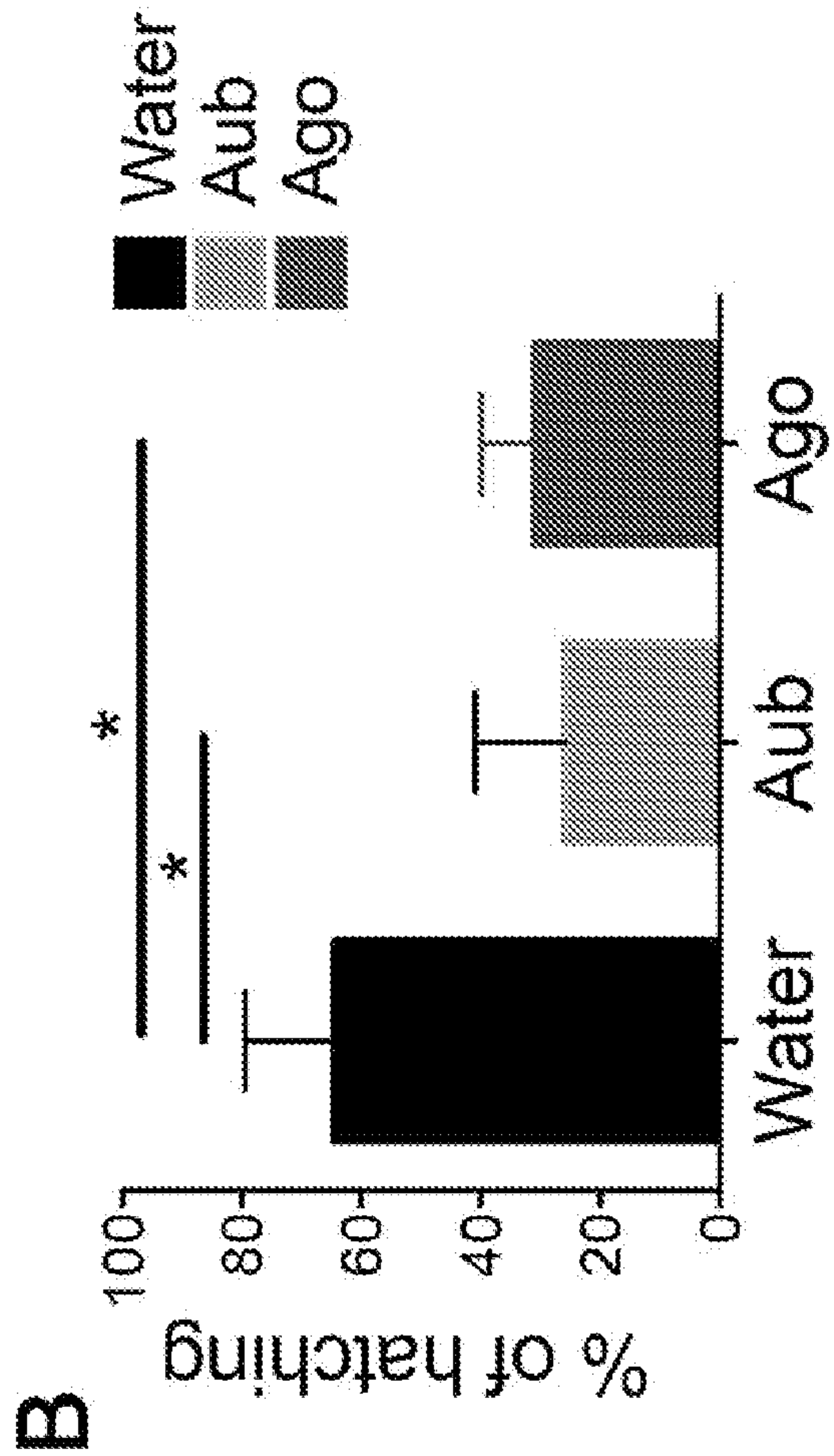


FIG. 14B

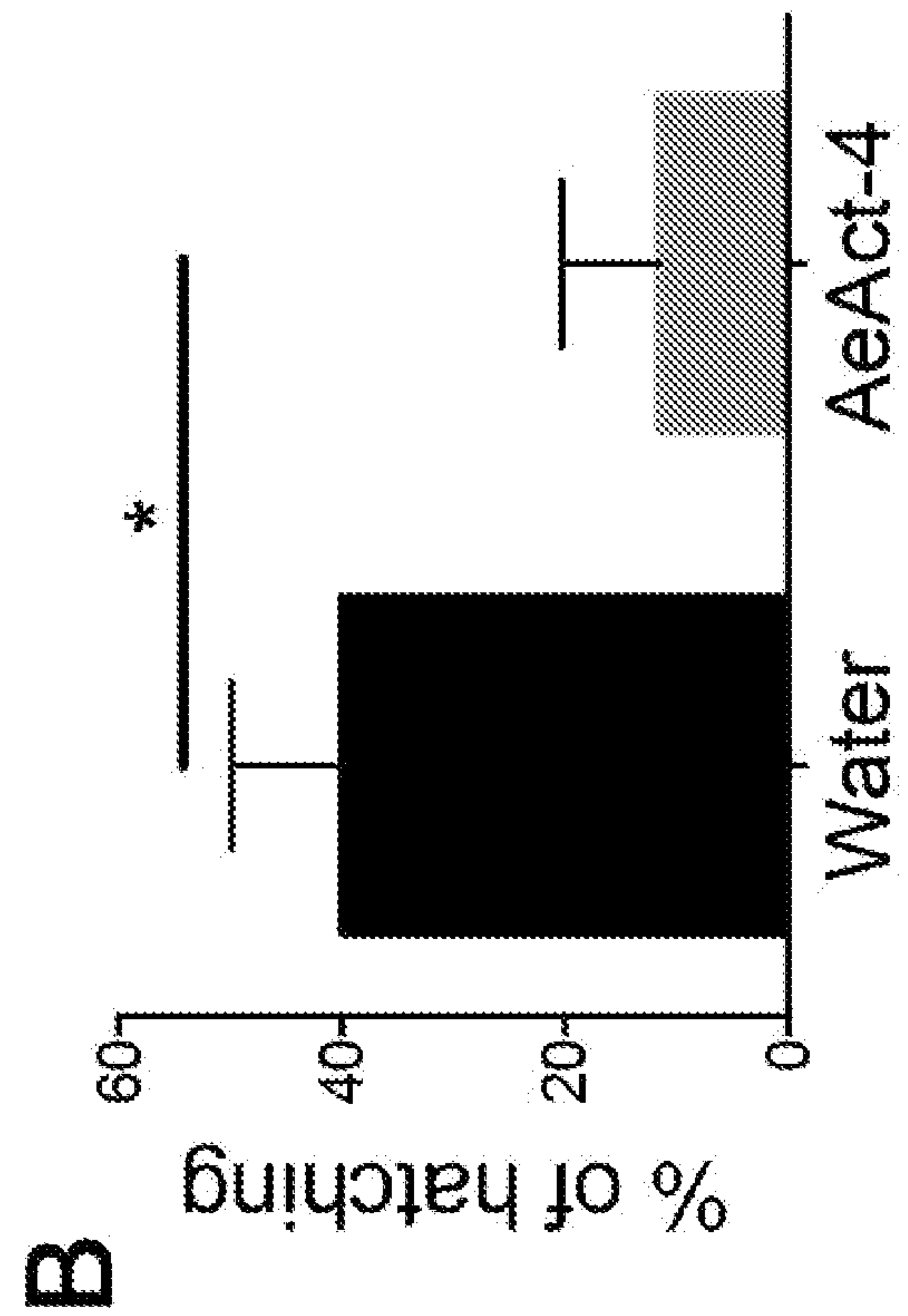


FIG. 14A

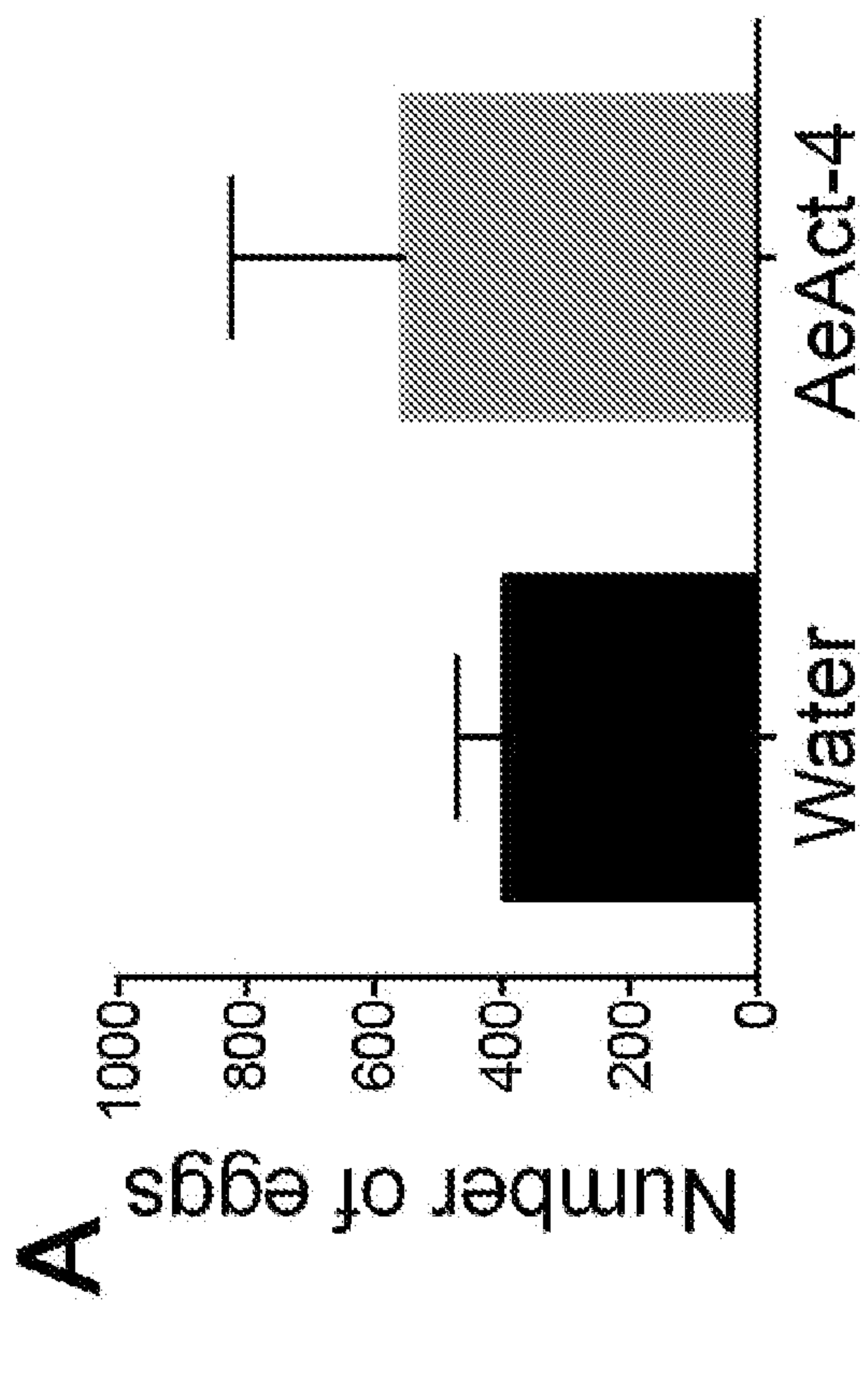


FIG. 15B

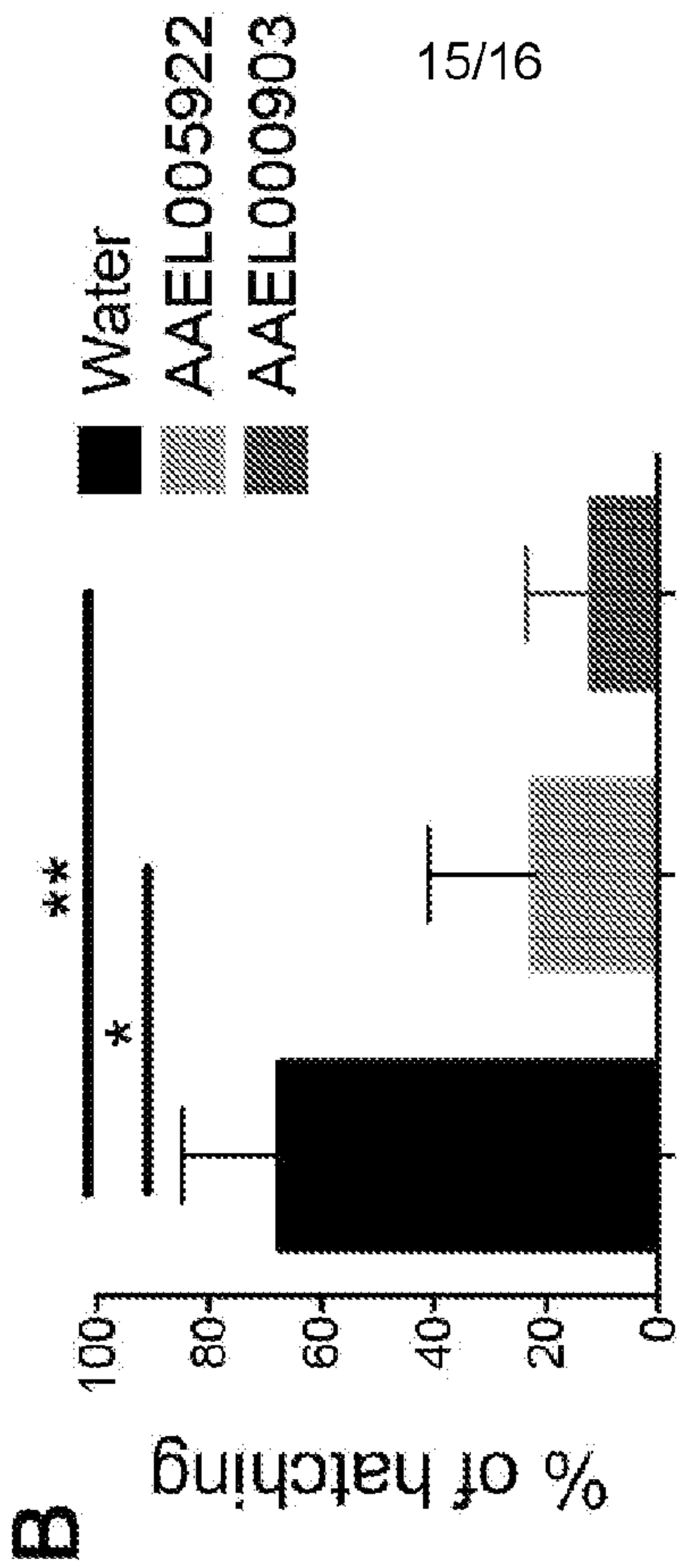


FIG. 15A

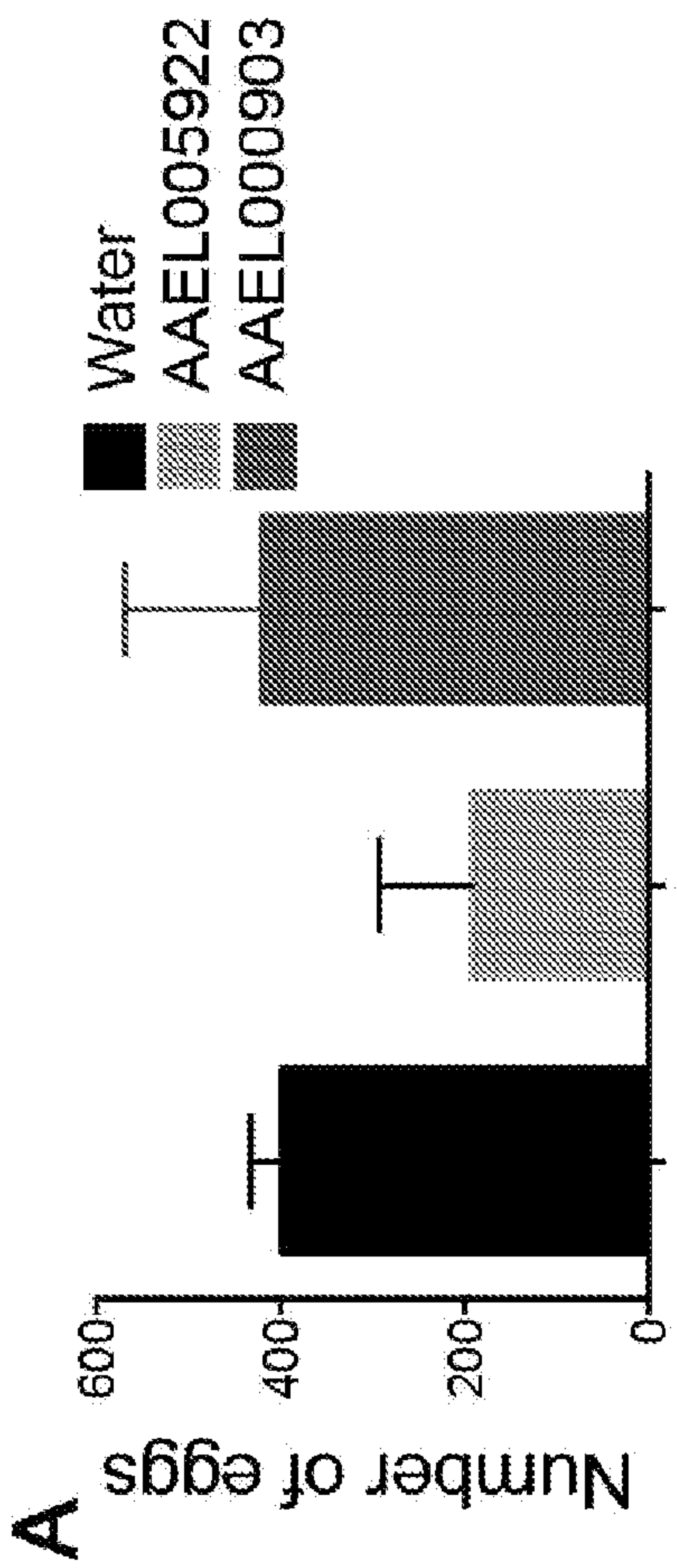


FIG. 16B

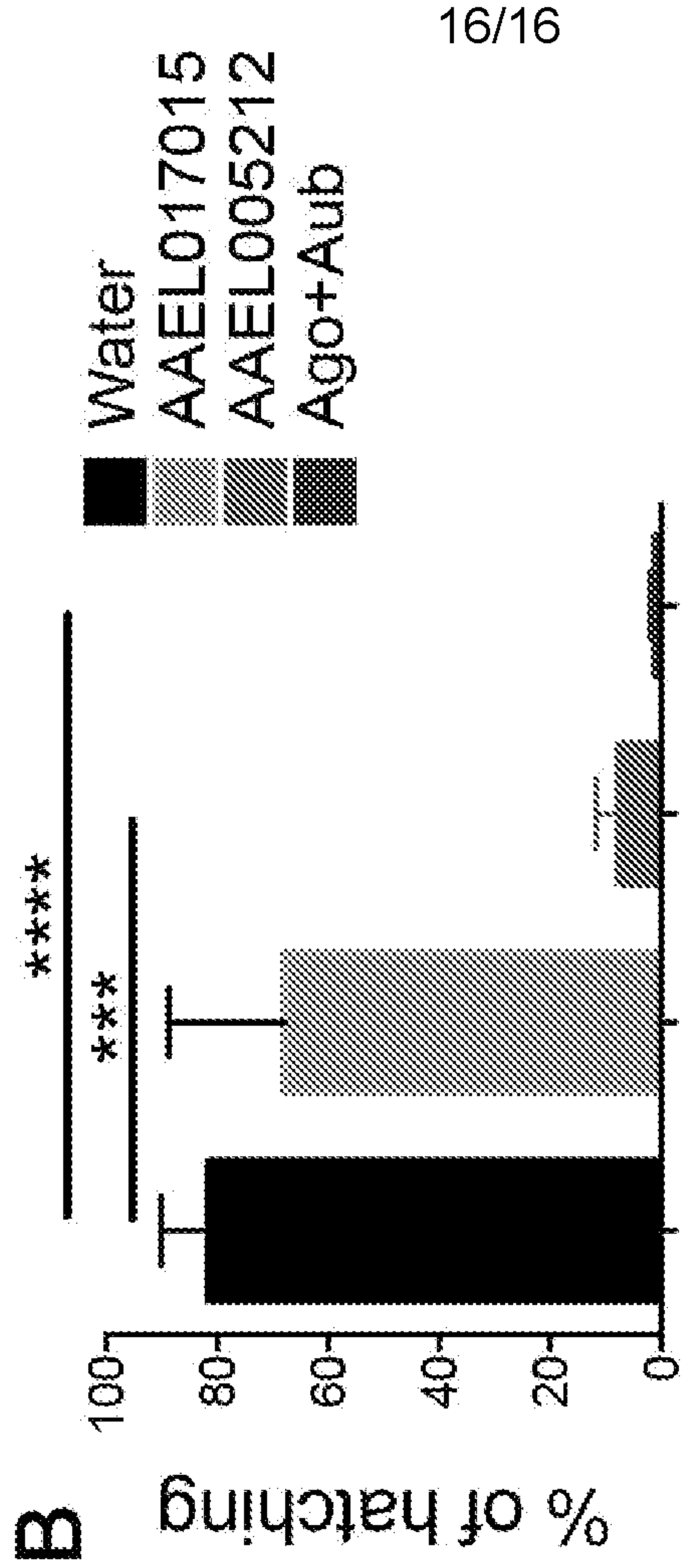


FIG. 16A