(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 February 2016 (04.02.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/018937 A1

(51) International Patent Classification:
A01N 63/02 (2006.01)

(21) International Application Number:
PCT/US2015/042522

(22) International Filing Date:
28 July 2015 (28.07.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/029,863 28 July 2014 (28.07.2014) US

(71) Applicant: **THE JOHNS HOPKINS UNIVERSITY** [US/US]; Technology Ventures, 100 N. Charles Street, 5th Floor, Baltimore, MD 21201 (US).

(72) Inventors: **ROKITA, Steven**; 4415 Bedford Pl, Baltimore, MD 21218 (US). **PHATARPHEKAR, Abhishek**; 8715 First Ave., Apt 725c, Silver Spring, MD 20910 (US). **EUN, Suk, Ho**; Technology Ventures, 100 North Charles Street, 5th Floor, Baltimore, MD 21201 (US). **CHEN, Xin**; Technology Ventures, 100 North Charles Street, 5th Floor, Baltimore, MD 21201 (US).

(74) Agents: **CORLESS, Peter, F.** et al.; Mintz Cohn Ferris Glovsky And Popeo, P.c., One Financial Center, Boston, MA 02111 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

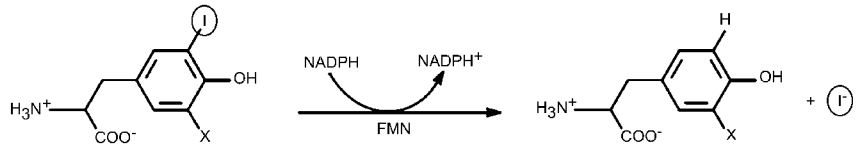
Published:

— *with international search report (Art. 21(3))*

WO 2016/018937 A1

(54) Title: COMPOSITIONS AND METHODS FOR CONTROLLING INSECT PROLIFERATION

FIG. 1



X = H (monoiodotyrosine, MIT), or I (diiodotyrisine, DIT)

(57) Abstract: The present invention relates to methods of controlling pest populations through inhibition of spermatogenesis.

COMPOSITIONS AND METHODS FOR CONTROLLING INSECT PROLIFERATION

RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/029,863, filed on July 28, 2014, which is hereby incorporated herein by reference in its entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with government support under RO1 DK084186 awarded by the National Institute of Health (NIH). The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Many strategies exist for blocking insect reproduction. However, there is a pressing need to develop an approach to selectively control insect populations without damage to the environment.

SUMMARY OF THE INVENTION

The invention provides a method of preventing, reducing, or inhibiting insect reproduction comprising administering to the insect a composition comprising an agent that inhibits spermatogenesis (e.g., late stage spermatogenesis) in the insect, thereby preventing, reducing, or inhibiting reproduction in the insect. Preferably, the agent that inhibits spermatogenesis in the insect comprises an agent that inhibits expression or activity of iodotyrosine deodinase (IYD) in the insect (i.e., in the male insect). For example, the agent inhibits expression or activity of IYD in a testis of the insect.

The methods described herein reduce a population size of the insect, i.e., the methods described herein reduce/control insect proliferation. For example, the methods described herein reduce an insect population, i.e., the methods reduce/decrease the number of insects in a defined environment by at least 1%, e.g., at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%.

The methods described herein also reduce fecundity and/or fertility of the insect. For example, the methods described herein reduce/decrease fecundity and/or fertility of the insect

by at least 1%, e.g., at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or by 100%.

In some cases, the agent for inhibiting expression or activity of IYD comprises an antibody or fragment thereof, a binding protein, a polypeptide, or any combination thereof.

In other cases, the agent for inhibiting expression or activity of IYD comprises a small molecule. A small molecule is a compound that is less than 2000 Daltons in mass. The molecular mass of the small molecule is preferably less than 1000 Daltons, more preferably less than 600 Daltons, e.g., the compound is less than 500 Daltons, less than 400 Daltons, less than 300 Daltons, less than 200 Daltons, or less than 100 Daltons.

Small molecules are organic or inorganic. Exemplary organic small molecules include, but are not limited to, aliphatic hydrocarbons, alcohols, aldehydes, ketones, organic acids, esters, mono- and disaccharides, aromatic hydrocarbons, amino acids, and lipids. Exemplary inorganic small molecules comprise trace minerals, ions, free radicals, and metabolites. Alternatively, small molecules can be synthetically engineered to consist of a fragment, or small portion, or a longer amino acid chain to fill a binding pocket of an enzyme. Typically small molecules are less than one kilodalton.

Suitable small molecule inhibitors include 4-OH-2',3,4',5,6'-PCB; 4-OH-2',3,4',6'-TCB; 4-OH-2,2',5,5'-TCB; 4,4'-diOH-3,3',5,5'-TCB; 3-OH-2,2',5,5'-TCB; 4'-OH-BDE-17; 4-OH-BDE-42; 4'-OH-BDE-49; 4-OH-BDE90; 2-OH-BDE-15; 2'-OH-BDE-28; Bromoxynil; Oxyclozanide; Tribromsalan; Closantel; Triclosan; Bithionol; Nitroxynil; Benzbromarone Rose Bengal; Erythrosine B; Phloxine B; D,L-3-(2-pyridon-5-yl)alanine; and D,L-3-(N-methyl-2-pyridon-5-yl)alanine; D,L-3-(N-ethyl-2-pyridon-5-yl)alanine; and D,L-3-(N-isopropyl-2-pyridon-5-yl)alanine. In a preferred aspect, the small molecule inhibitor is L-3-(N-methyl-2-pyridon-5-yl)alanine.

In other aspects, the agent comprises a nucleic acid molecule. For example, the nucleic acid molecule comprises double stranded ribonucleic acid (dsRNA), small hairpin RNA or short hairpin RNA (shRNA), or antisense RNA, or any portion thereof.

A variety of administration routes are available. For example, the agent is administered topically, orally, via inhalation, or via injection.

The composition is in the form of a gas, a liquid, a solid, or a semi-solid. In a preferred aspect, the composition comprises sugar water. In another example, the agent is

administered as a gaseous or liquid spray. In some cases, the agent is soluble in water. Preferably, the agent is nontoxic.

The effective amount of the agent is from 0.001 mg/kg to 250 mg/kg body weight, e.g., 0.001 mg/kg, 0.05 mg/kg 0.01 mg/kg, 0.05mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 225 mg/kg, or 250 mg/kg body weight.

In some cases, the agent is administered at least once per day, at least once per week, or at least once per month. The agent is administered for a duration of one day, one week, one month, two months, three months, six months, 9 months, or one year. For example, the compositions described herein are placed in an insect "trap" as food for ingestion by the insects. The "traps" are replenished with fresh composition as needed, e.g., at least once per day, at least once per week, at least once per month, or at least once every three moths.

The compositions and methods described herein are useful in inhibiting reproduction in any insect. For example, suitable insects include cockroaches (e.g., brown-banded cockroaches, German cockroaches, American cockroaches, Oriental cockroaches), beetles (woodworms, death watch beetles, fur beetles, varied carpet beetles, spider beetles, mealworm beetles, centipedes, red flower beetle, pine beetle), earwigs, flies (e.g., bottle flies, blue bottle flies, green bottle flies, house flies, fruit flies, horse flies, black flies, deer flies, house flies, teste flies), cicadas, hoppers, ants (e.g., Argentine ants, carpenter ants, fire ants, odorous house ants, pavement ants, pharaoh ants, thief ants, a leafcutter ant, a mountain fire ant, a Jerdon't jumping ant), aphids, bed bugs, bees (e.g., a common eastern bumble bee, a bumble bee, a honeybee, an alfalfa leafcutter bee), wasps, mantids, earthworms, terrestrial crabs, firebrats, fleas, snails, slugs, crickets (e.g., house crickets), hornets, locusts, lice, moths (e.g., almond moths, Indianmeal moths, cloths moths, gypsy moths (common clothes moths), brown house moths), red spiders, silverfish, woolice, termites (e.g., dampwood termites, subterranean termites), ticks, wasps, mosquitos, gnats, millipedes, stink buds, weevils, mites, silk worm, daphnia, fleas, Assassin bugs (kissing bugs), eye gnats, or rat fleas and woolly bears (e.g., hairy caterpillar).

The compositions and methods are also useful in inhibiting reproduction in the following insects: *Culex quinquefasciatus* (Southern house mosquito), *Drosophila willistoni* (Fruitfly), *Drosophila mojavensis* (Fruitfly), *Drosophila pseudoobscura* (Fruitfly), *Drosophila grimshawi* (Fruitfly), *Drosophila virilis* (Fruitfly), *Drosophila ananassae* (Fruitfly), *Ceratitis capitata* (Mediterranean fruitfly), *Drosophila sechellia* (Fruitfly),

Drosophila persimilis (Fruitfly), *Drosophila simulans* (Fruitfly), *Drosophila melanogaster* (Fruitfly), *Anopheles darling* (Mosquito), *Anopheles gambiae* (Mosquito), *Musca domestica* (Housfly), *Drosophila erecta* (Fruitfly), *Drosophila yakuba* (Fruitfly), *Aedes aegypti* (Mosquito, yellow fever), *Bombyx mori* (Silk worm), *Tribolium castaneum* (red flour beetle), *Nasonia vitripennis* (wasp), *Apis mellifera* (honeybee), *Apis dorsata* (honeybee), *Megachile rotundata* (alfalfa leafcutter bee), *Dendroctonus ponderosae* (mountain pine beetle), *Bombus impatiens* (common eastern bumble bee), *Bombus terrestris* (bumble bee), *Acromyrmex echinatior* (leafcutter ants), *Solenopsis invicta* (fireant), *Apis florea* (honeybee), *Harpegnathos saltator* (Jerdon's jumping ant), *Cerapachys biroi* (ant), *Daphnia pulex* (daphnia), *Camponotus floridanus* (ant), and *Pediculus humanus corporis* (lice).

In some cases, the methods described herein are useful for inhibiting the reproduction of any invertabrate, such as a crab, a lobster, a snail, a clam, an octopus, a starfish, asea-urch or a worm.

In one aspect, the compositions are administered within a device, e.g., a "trap," that attracts an insect to the device in order to administer the compositions described herein to inhibit spermatogenesis within the insect.

Definitions

By "agent" is meant any small molecule compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.

"Controlling pests" as used in the present invention means killing pests, or preventing pests from developing, or from growing or preventing pests from infecting or infesting. Controlling pests as used herein also encompasses controlling insect progeny (development of eggs). Controlling pests as used herein also encompasses inhibiting viability, growth, development or reproduction of the insect, or decreasing pathogenicity or infectivity of the insect.

"Detect" refers to identifying the presence, absence, or amount of the nucleic acid (e.g., RNA) to be detected.

By “detectable label” is meant a composition that when linked to a molecule of interest renders the latter detectable, via, for example, spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels may include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, or haptens.

By “effective amount” is meant the amount of an agent required to inhibit reproduction in an insect.

By “fragment” is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

“Hybridization” means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "inhibitory nucleic acid" is meant a double-stranded RNA, siRNA, shRNA, or antisense RNA, or a portion thereof, or a mimetic thereof, that when administered to a mammalian cell results in a decrease (e.g., by 10%, 25%, 50%, 75%, or even 90-100%) in the expression of a target gene. Chitosan compositions are useful for the delivery of polynucleotides, such as inhibitory nucleic acid molecules. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. For example, an inhibitory nucleic acid molecule comprises at least a portion of any or all of the nucleic acids delineated herein.

By “isolated polynucleotide” is meant a nucleic acid (e.g., RNA, DNA, cDNA, etc.) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other

sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By "K_d" is meant dissociation constant, a parameter of a drug that characterizes the specificity of interaction between a drug and its target.

By "k_{cat}" is meant the turnover number for an enzyme; the number of substrate molecules each enzyme site converts to product per unit time.

By "K_m" is meant Michaelis–Menten constant, which represents the substrate concentration at which an enzymatic reaction rate is half of V_{max}.

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

By "modulate" is meant alter (increase or decrease). Such alterations are detected by standard art known methods such as those described herein.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

As used herein, the terms "prevent," "preventing," and "prevention," refer to reducing or inhibiting reproduction in an insect.

"Primer set" means a set of oligonucleotides that may be used, for example, for PCR. A primer set would consist of at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 80, 100, 200, 250, 300, 400, 500, 600, or more primers.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9. With respect to sub-ranges, "nested sub-ranges" that extend from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By "reference" is meant a standard or control condition.

A "reference sequence" is a defined sequence used as a basis for sequence comparison or a gene expression comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 40 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 or about 500 nucleotides or any integer thereabout or there between.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e-3 and e-100 indicating a closely related sequence.

In some cases, by "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Alternatively, by "subject" is meant an insect.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All published foreign patents and patent applications cited herein are incorporated herein by reference. Genbank and NCBI submissions indicated by accession number cited herein are incorporated herein by reference. All other published references, documents, manuscripts and scientific literature cited herein are incorporated herein by reference. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic showing the reductive deiodination of mono- and diiodotyrosine catalyzed by IYD (iodotyrosine deiodinase) and its bound cofactor, flavin mononucleotide (FMN and in its reduced form, $FMNH_2$).

Figure 2A and Figure 2B are schematics showing the co-crystal structure of mouse IYD-MIT (monoiodotyrosine). Figure 2A is a molecular model of the α_2 dimer of mmIYD-MIT (mouse iodotyrosine deiodinase). The monomers are depicted in green and purple cartoon representation. The FMN (Flavin mononucleotide) and MIT molecules are shown in stick representation and colored according to atom. Figure 2B is a molecular model of the α_2 dimer of mmIYD-MIT showing the residues involved in substrate recognition, Y157, E153 and K178. T235 provides an important hydrogen bond to the N5 of the FMN.

Figure 3 is a multiple sequence alignment showing that IYD homologs are present in diverse organisms. Determinants of IYD are outlined in red, residues interacting with FMN are marked with (+), residues interacting with substrate are marked with (*), residues marked in red are fully conserved, lid-forming sequences are boxed, and secondary structural elements derive from the structure of mmIYD-MIT. Numbering of the amino acids for each protein is indicated on the left and right of the alignment. Secondary structure elements are derived from the crystal structure of mmIYD bound to DIT (diiodotyrosine).

Figure 4 is a diagram depicting the phylogenic analysis of catalytic domains. All proteins containing the signature residues (E153, Y157 and K178) promote deiodination of DIT. The closest structural analog (Blub) lacks these residues, and does not support deiodination of DIT (diiodotyrosine). The branch lengths are proportional to the number of amino acid substitutions per aligned residue.

Figure 5 is a graph showing the expression of IYD mRNA in Drosophilia. Tissue specific mRNA expression profiling for *Drosophila melanogaster* (fly) indicated very high levels if IYD (gene CG6279 in flybase) in the adult male testes. Adult Drosophila are shown in red bars, and larva Drosophila are shown in blue bars.

Figure 6A and Figure 6B is a diagram and a bar graph, respectively, showing the RNAi knockdown of IYD in Drosophila testis. Figure 6A is a diagram showing the promoter dependent knockdown of IYD mRNA. Figure 6B is a bar graph showing the fertility and fecundity of eya-GAL4, UAS-dmIYD-RNAi and eya-CAL1/UAS-dmYD-RNAi.

Figure 7A, Figure 7B, and Figure 7C is a diagram, a bar graph, and a photomicrograph, respectively, showing that late stage development of *Drosophila* is sensitive to IYD. Figure 7A is a diagram showing the stages of spermatogenesis in *Drosophila*. Figure 7B is bar graph showing the abnormalities detected in the testis after RNAi degradation of IYD mRNA. Figure 7C is an image showing the phase contrast of drosophila testes. Suppression of dmIYD disrupts late stage spermatogenesis.

Figure 8 is a bar graph showing the male germline cell specific expression profile of CG6279. Germline and somatic stem cells were located in niche, GB in gonioblast, S4 through S16 were spermatogonia (pre-differentiation), EC16 onwards were spermatocytes post-differentiation.

Figures 9A, Figure 9B, Figure 9C, and Figure 9D are photomicrographs showing testes of flies treated with digoxigenin-labeled riboprobes. Figure 9A is an image showing CG6279 isoform A specific antisense riboproboe. Figure 9B is an image showing CG6279 isoform A+B specific antisense riboprobe. Figure 9C is an image showing the control where isoform A specific sense riboprobes were used. Figure 9D is an image showing the control where isoform A + B specific sense riboprobes were used. (*) indicates early stages of spermatogenesis. (**) indicates later stages of spermatogenesis with strong staining for hybridization of riboprobes with CG6279 mRNA.

Figure 10 is a diagram showing the HPLC method schematic (left to right) for separation, detection and quantification of tyrosine produced from MIT. All gradients were

linear over the indicated time. A drop in the B mobile phase from 60% to30% over 2 mins after the analytical time prevented impurities from affecting the internal standard peak by delaying their elution. The column was washed with 95% B and equilibrated with A prior to next injection of analyte.

DETAILED DESCRIPTION OF THE INVENTION

As described in detail below, described herein are compositions and methods that are useful for inhibiting spermatogenesis in insects, e.g., drosophila, by decreasing the expression or activivty of iodotyrosine deiodinase (IYD). A putative IYD from *Drosophila* (dmIYD) was expressed and confirmed to catalyze deiodination. Its k_{cat}/K_m value was similar to those of all other homologues tested. Expression of dmIYD was primarily limited to the testis and its suppression by RNAi significantly disrupted spermatogenesis, particularly within the late stages of sperm development. The enzyme was critical for spermatogenesis in insects, but not mammals. Fertility and fecundity were greatly reduced when expression of this enzyme was suppressed, and hence, proliferation of insects is selectively reduced by inhibiting this enzyme. The molecular basis was consistent with iodotyrosine acting as a cellular signal and representing a progenitor of thyroxine. Prior to the invention described herein, a biological role had not been previously identified for this enzyme.

Iodotyrosine (IYD) deiodinase

IYD has been traditionally associated with iodide conservation by generating iodide (I^-) from mono- and diiodotyrosine. IYD facilitates iodide salvage in thyroid tissue in vertebrates by catalyzing deiodination of mono- (I-Tyr) and diiodotyrosine (DIT, I_2-Tyr), that are formed as halogenated byproducts of thyroid hormone biosynthesis (often in surprisingly large quantities) (Goswami, A.; Rosenberg, I. N., *Endocrinology* 1977, 101, 331-341, Gnidehou, S.et al., *FASEB J.* 2004, 18, 1574-1576 and Rokita, S. E. et al., *Biochimie* 2010, 92, 1227-1235). Prior to the invention described herein, IYD had remained poorly characterized for more than 50 years despite its significant role in intrathyroidal iodine metabolism. Patients with deficient IYD suffer from goiter, enlarged thyroid, and other symptoms associated with hypothyroidisim because iodotyrosine cannot be directly reutilized for thyroid hormone synthesis. The dehalogenation reaction catalyzed by IYD is unusual for aerobic organisms since the carbon-iodine bond is broken through a reductive process. More commonly, dehalogenation is accomplished by hydrolytic or oxidative pathways. Many of

the bacterial enzymes responsible for dehalogenation have emerged from existing enzyme superfamilies.

IYD has been associated with iodide homeostasis in humans since its mutation, first discovered over 50 years ago, was found to limit thyroid function. More recently, sequence homology and structural data identified this enzyme as the only known representative of the nitro-FMN reductase structural superfamily in humans. Members of this superfamily primarily derive from bacteria and their activities range from generating and chaperoning dihydroflavin, $FADH_2$ (Flavin Reductase Protein, FRP), to reducing nitroaromatic compounds (NOX) and degrading flavin for vitamin B_{12} biosynthesis (BluB). All IYDs are indicated by four key residues, a threonine (Thr) for hydrogen bonding to the N5 position of FMN, a glutamic acid (Glu), tyrosine (Tyr) and lysine (Lys) for coordinating to the substrate iodotyrosine.

Functional IYDs have been confirmed in Chordata, Arthropoda, Cnidaria, Eubacteria and Archaeabacteria. Chordata require the tetraiodinated hormone, thyroxine, and rely on IYD to salvage iodide from mono- and diiodotyrosine formed as byproducts of its biosynthesis. Thyroxine is not produced by invertebrates and thus IYD is unlikely to provide iodide for its synthesis. Bacteria may use IYD in a catabolic role since this enzyme is capable of dehalogenating chloro-, bromo- and iodotyrosines. As described herein, the function of IYD in Arthropoda and Cnidaria is examined in the model Arthropoda, *Drosophila melanogaster*.

Drosophila IYD

As described in detail below, this *Drosophila* gene acts as an iodotyrosine deoidinase and is critical for sperm formation in insect testes. Prior to the invention described herein, no evidence in the literature had made a connection between the catalytic activity of this enzyme and spermatogenesis. As described herein, the deiodinase gene was suppressed, and a loss in *Drosophila* fertility was observed. This organism is a well-studied model for insects.

Two isoforms have been reported for *Drosophila* IYD (dmIYD) in the NCBI databases. These two are related by alternative splicing of the CG6279 gene. Isoform B resembles IYD orthologs from other organisms and includes a membrane domain, an intermediate domain and a catalytic domain (Phatarphekar, A. et al., *Mol. Biosys.* 2014, 10, 86-92 and Friedman, J. E. et al. *J. Biol. Chem.* 2006, 281, 2812-2819). Isoform A is unique to the *Drosophila* genus by the added presence of a large N-terminal domain (470 amino

acids) that does not coincide with any known protein domains listed in the conserved domain database (CDD).

The amino acid sequence of *Drosophila melanogaster* IYD (A Isoform) is shown below:

```
  1 mepkhrhcyf krsassclvt pmhlhssris sshsqsaggi kpalasldpp ktpnsiektk
 61 ksggflsflf gkrdrrarnq setngnhisl edhdvrklir lysksvselr scleelrtlf
121 ereytiiera rrkcgeimsp siryyneflf lvphlnihfd kdlpasgtsp laagkllqnp
181 ianpemlcqk ltnftdfpla gfpgnlltkr pkkdpqdrpn kkikpipdqe nakqenpieq
241 eiqeaekvad hevsveekis gledqeqkda lspseqkniq nskdktshhh snckmsshek
301 dlvstnltcp ckpevessqs pvscpwiaen skceppcret ppqstrnsvs spqnsdises
361 skepahlmek epsgqesans qqvqmlcdmi rtelttapdf iffdakwrii eilrevhkrq
421 lvhqkaipln ntrrpippqk rkpgepnqml knpkcmsdrs ahekgqqrit mdvdelisss
481 kllkhwpslf itlaliwivk rlffkgnrvl ktynldeqve eevehfadlg delqpaledk
541 phvpfvpgqn lnpngakrly elmrgrrsir sfnshpkpdl sviedciraa gtapsgahte
601 pwtycvvqep elkrsireiv eqeelvnysq rmhpqwvtdl rplqtnhvke ylteapylil
661 ifkqtyglse ngkrmrrhyy neistsiaag illcalqaag laslvttpln cgpalrnllg
721 rpvnekllil lpvgypkdgc tvpdlarknl snimvtf
```

(SEQ ID NO: 1) GenBank Accession No: NP_648433 (Version 1), incorporated herein by reference.

The nucleic acid sequence of *Drosophila melanogaster* IYD (A Isoform) is shown below:

```
   1 aaaaaacttc acagtgacac ttacattcac cccaaaaaaa gtaaaaaaaa aatagtcagg
  61 attacaaaag ttatatttcc agataatggt acaaaatttt ttcgatttat aatgctgttg
 121 ccattaaaga acccaaagac cgttgaaagc gaggtgaacc cgaatgtgac gctactgaag
 181 cgccagatcg ccgaggatgg aaccaaaaca caggcactgt tacttcaagc gttccgcttc
 241 gagttgccta gtgacgccaa tgcatctcca ctcctccagg atcagttcca gccacagtca
 301 gtcagcagga ggcattaagc ccgctttggc atcactagat ccacctaaaa cacccaatag
 361 catcgagaaa acaaagaaat ccgggggatt tctctctttt ttgtttggca aacgagacag
 421 gcgggcgagg aatcagagtg agacaaatgg aaaccatatc tcattggagg atcatgatgt
 481 caggaagctt atacgtctct atagcaaaag cgtctcggaa ctgagatcct gtctggagga
 541 gctaaggacg ctctttgagc gggaatacac cattatcgaa cgtgctcgaa ggaagtgtgg
 601 agagataatg agtccttcaa ttcgctacta taatgaattt cttttcctgg tgccacatct
 661 taatatacac ttcgacaagg atcttcctgc cagtggaact agcccactcg ccgccggaaa
 721 acttttgcaa aatccgatag ccaacccaga gatgttgtgc caaaagttga ccaatttcac
 781 ggattttcct ctggccggtt ttcccggcaa tctactaacc aagaggccca aaaaagaccc
 841 ccaagaccgg ccaaacaaga agatcaaacc aattcctgat caagaaaatg cgaagcaaga
 901 aaatcccata gagcaggaaa ttcaggaggc agaaaaagtc gctgatcatg aagtttcggt
 961 agaagagaaa atcagcgggt tggaggatca ggaacagaaa gacgcactct ctccatcgga
1021 gcagaagaac atacaaaaca gtaaggataa aaccagtcat catcattcca attgcaagat
1081 gtccagccat gagaaggatt tagtatccac caatttgacc tgtccctgca agccggaagt
1141 ggaatcctca caaagcccag tttcctgccc ctggatagcc gaaaattcaa agtgtgaacc
1201 cccgtgtcgg gaaacaccac cacaatctac gcgaaattcc gtatccagtc cgcaaaattc
1261 agatatttct gaatccagca aagaaccggc tcacttgatg gagaaggaac catccggtca
1321 ggaatccgcg aatagccagc aagtgcagat gctttgcgat atgatacgta ccgagctaac
1381 cactgctccc gattttatat tcttcgatgc caagtggcgg atcatcgaga ttctgagaga
1441 ggtgcacaag cgccaattgg ttcaccagaa ggccattcca ctcaacaata cacgccgacc
1501 gattccgccg caaaaacgaa aaccgggtga gcccaatcag atgcttaaga atccaaaatg
1561 catgtcggat aggagtgccc acgaaaaggg acaacagagg atcaccatgg atgtggacga
1621 actgatcagc agctccaagc tactcaagca ttggccaagt ttatttatca ctctggcgct
```

```
1681 gatatggatc gtaaaaagat tgtttttaa agggaatcgc gtattaaaaa catataattt
1741 agatgaacaa gtcgaggagg aggttgaaca ctttgccgat cttggcgatg aactgcaacc
1801 ggctctggag gataagcccc atgtgccctt cgttcccggc cagaatctca accctaatgg
1861 agccaaacgc ttatatgaac tcatgcgtgg acgccgtagc attcgctcct ttaatagcca
1921 tcccaaaccg gatctcagcg tgatagagga ctgcattcgg gcggcgggaa cagctcccag
1981 tggcgcacac acagaaccct ggacgtactg cgtcgtacag gagccggaac tcaagcgatc
2041 cattcgggaa attgtggaac aggaggagct ggtcaactac agccagagga tgcatccgca
2101 gtgggtcacc gatctgcgac ccctgcaaac caatcatgtc aaggagtatc tcaccgaggc
2161 gccgtacctt atactgatct tcaagcagac ctatggattg tcggagaatg gaaagcgaat
2221 gaggaggcat tactacaacg agatatccac ttcgattgcg gccggtatcc ttttgtgtgc
2281 ccttcaagcg gcaggactcg catcgctggt caccactcct ttgaactgcg gtccagcctt
2341 gagaaacctg ctcgggcgac ccgtcaatga aaaattactc atcctactgc ccgttggtta
2401 tccgaaagat ggatgcacag ttcccgactt ggcgagaaag aatttgtcaa atattatggt
2461 tacttttaa ggtttattta cgtccgtgca gcttacgaaa tagttcaata aaaccaaact
2521 aaaatttatg gt
```

(SEQ ID NO: 2) GenBank Accession No: NM_140176 (Version 2), incorporated herein by reference.

The amino acid sequence of *Drosophila melanogaster* IYD (B Isoform) is shown below:

```
   1 mdvdelisss kllkhwpslf itlaliwivk rlffkgnrvl ktynldeqve
eevehfadlg
  61 delqpaledk phvpfvpgqn lnpngakrly elmrgrrsir sfnshpkpdl sviedciraa
 121 gtapsgahte pwtycvvqep elkrsireiv eqeelvnysq rmhpqwvtdl rplqtnhvke
 181 ylteapylil ifkqtyglse ngkrmrrhyy neistsiaag illcalqaag laslvttpln
 241 cgpalrnllg rpvnekllil lpvgypkdgc tvpdlarknl snimvtf
```

(SEQ ID NO: 3) GenBank Accession No: NP_001163414 (Version 1), incorporated herein by reference.

The nucleic acid sequence of *Drosophila melanogaster* IYD (B Isoform) is shown below:

```
   1 atcgctatca gcaatagccg tgacccactt aaccaagtga taagccaatc cgagcgcaaa
  61 agcaaagcca ctttctctcc gtcgcaaaag cagttacatc ttgaagcacc atggatgtgg
 121 acgaactgat cagcagctcc aagctactca agcattggcc aagtttattt atcactctgg
 181 cgctgatatg gatcgtaaaa agattgtttt ttaaagggaa tcgcgtatta aaaacatata
 241 atttagatga acaagtcgag gaggaggttg aacactttgc cgatcttggc gatgaactgc
 301 aaccggctct ggaggataag ccccatgtgc ccttcgttcc cggccagaat ctcaacccta
 361 atggagccaa acgcttatat gaactcatgc gtggacgccg tagcattcgc tcctttaata
 421 gccatcccaa accggatctc agcgtgatag aggactgcat tcgggcggcg ggaacagctc
 481 ccagtggcgc acacacagaa ccctggacgt actgcgtcgt acaggagccg gaactcaagc
 541 gatccattcg ggaaattgtg gaacaggagg agctggtcaa ctacagccag aggatgcatc
 601 cgcagtgggt caccgatctg cgacccctgc aaaccaatca tgtcaaggag tatctcaccg
 661 aggcgccgta ccttatactg atcttcaagc agacctatgg attgtcggag aatggaaagc
 721 gaatgaggag gcattactac aacgagatat ccacttcgat tgcggccggt atccttttgt
 781 gtgcccttca agcggcagga ctcgcatcgc tggtcaccac tcctttgaac tgcggtccag
 841 ccttgagaaa cctgctcggg cgacccgtca atgaaaaatt actcatccta ctgcccgttg
 901 gttatccgaa agatggatgc acagttcccg acttggcgag aaagaatttg tcaaatatta
 961 tggttacttt ttaaggttta tttacgtccg tgcagcttac gaaatagttc aataaaacca
1021 aactaaaatt tatggt
```

(SEQ ID NO: 4) GenBank Accession No: NM_001169943 (Version 1), incorporated herein by reference.

Human IYD

Iodotyrosine deiodinase (IYD) is a membrane bound enzyme in humans that is primarily expressed in thyroid tissue to recover iodide from mono- and diiodotyrosine (MIT, DIT) generated during thyroxine biosynthesis. This enzyme is highly unusual for its (*i*) ability to promote reductive dehalogenation and (*ii*) reliance on flavin to catalyze this process (Figure 1). *In vivo*, NADPH appears to act as the electron donor for this reaction but IYD is not directly reduced by NADPH. *In vitro*, dithionite serves as an alternative electron donor. IYD also effects debromination and dechlorination of the corresponding halotyrosines. A review on the enzyme iodotyrosine deiodinase focusing on its role in mammals is found in Rokita, S.E. et al., Biochimie 2010, 92, 1227-1235, incorporated herein by reference. A review on the rare occurrence of reductive dehalogenases in aerobic organisms is found in Rokita, S.E., Handbook in Flavoproteins, vol. 1 DeGruyter Berlin, 2013. P. 337-350, incorporated herein by reference.

The amino acid sequence of Human IYD is shown below:

```
  1 myfltpilva ilcilvvwif knadrsmekk kgeprtraea rpwvdedlkd
ssdlhqaeed
 61 adewqeseen vehipfshnh ypekemvkrs qefyellnkr rsvrfisneq vpmevidnvi
121 rtagtapsga htepwtfvvv kdpdvkhkir kiieeeeein ymkrmghrwv tdlkklrtnw
181 ikeyldtapi lilifkqvhg faangkkkvh yyneisvsia cgillaalqn aglvtvtttp
241 lncgprlrvl lgrpahekll mllpvgypsk eatvpdlkrk pldqimvtv
```

(SEQ ID NO: 5) GenBank Accession No: NP_981932 (Version 1), incorporated herein by reference.

The nucleic acid sequence of Human IYD is shown below:

```
   1 gaagagttct gcactttgtt ccttccctgg cacacctgtg tctgcattcc
ttctatctcc
  61 cggcattctc cactcctgtc tctgtgtgtt taaaaaccgg tgtgggaagt gtgcacgcct
 121 gtgacgtcag actccagacc atgtatttcc tgactcccat cttggtagcc attctctgca
 181 ttttggttgt gtggatcttt aaaaatgccg acagaagcat ggagaaaaag aaggggggagc
 241 ctagaaccag ggccgaagct cgcccctggg tggatgaaga cttaaaagac agcagtgacc
 301 tgcaccaagc agaagaagat gctgatgaat ggcaagaatc agaagaaaat gttgaacaca
 361 tcccccttctc tcataaccac tatcctgaga aggaaatggt taagaggtct caggaatttt
 421 atgaacttct caataagaga cggtcagtca ggttcataag taatgagcaa gtcccaatgg
 481 aagtcattga taatgtcatc agaacggcag gaacagcccc gagtggggct cacacagagc
 541 cctggacctt cgtggttgtg aaggacccag acgtgaagca caagattcga aagatcattg
 601 aggaggaaga ggagatcaac tacatgaaaa ggatgggaca tcgctgggtc acagacctca
 661 agaaactgag aaccaactgg attaaagagt acttggatac tgcccctatt ttgattctca
 721 ttttcaaaca agtacatggt ttcgccgcaa atggcaagaa aaaagtccac tactacaatg
 781 agatcagtgt ttccatcgct tgtggcatcc tgctagctgc cctgcagaat gcaggtctgg
 841 tgactgtcac taccactcct ctcaactgtg gccctcgact gagggtgctc ctgggccgcc
 901 ccgcacatga aaagctgctg atgctgctcc ccgtggggta ccccagcaag gaggccacgg
```

961 tgcctgacct caagcgcaaa cctctggacc agatcatggt gacagtgtag gcagggcccc
1021 ccaagggagt ggcagggaga tggcgcccct gcttttccct gagcctctcg cctgctcctc
1081 ttgggtctct tggctgctct ttctccaggt gtcaggtccc ctcattgctc ttctcaggtg
1141 gccacactat gtcaagaagc ctctccacac tctgtggcac ttccagtccc ataaatcctg
1201 tttcttatcc actttggaaa tgcatgaaca ctttacaaag aacatgcccg ggtttttaca
1261 ttttaaaagt tattctagac aatcactatt ggctttttttc ttttattttt aaaaaactca
1321 catagaggag acaatcagaa atttaccata gtcccaagaa ttcagctaca tgatgactcg
1381 aatttaaatt tagattaatc aaatggtctc ctgttctctg atttctggtg gcttttagac
1441 actaattttt gagaactact ttttttttttt taccaaactt cagggacact ctgctagttt
1501 tgaataagta accatcaaag ttactaaaac atggccaggc gcagtggctc atgcctgtaa
1561 tcccagcact ttgggaggcc gaggtgggca gattgcctga gctcaagagt tcgagaccag
1621 cctggccaac atggtgaaac cccatctcta ctaaaaatac aaaaaattag ctgggcatgc
1681 acttgtaatt ccagctactc aggaggctga ggcaagagaa ctgcttgaat ccgggaggta
1741 gagattgcag tgagtcaaga tgctgagatg ccaccactgc actccagcct gggcaacaga
1801 gcgagaacct gtctcaaaaa aaacaaaaac aaagttacta aaatgtcacc ttcacagaac
1861 aggacagggt acccttgggt cgcacgggcc tggctggcat gtaaacggtc agttgcactg
1921 cctagtggtt tggtagatct gacttgtaag cagttcatag aagctgctca atactcaaga
1981 atcttgcaag acagttatta aacattcata gcttgaaatt gcccatggtg ggagagttta
2041 catcacaacc actacaaatc agcacttgct ttcttcttct taaagagagc tgcttaccat
2101 acatagcacc cacagtgtct cccttctctt ctaccccaac ccctgaaaca cactgacccc
2161 tattctccag atgacagcac agctgtttgg gactgatagg gtcacagttg ccaggttggg
2221 aatgctagat gtagtcttca cccatcacct tctgtcgtcc tcccaggggt gtcatattta
2281 cagaggcaaa ctcttacagc ttggatgatt tctcatcaag caactctact cacctctttt
2341 tccccttcc ctgtgcgttt cttcttcttg tcctcatttt ccttgagaaa actctctcta
2401 taaaaaattt ccatagagta tgtggctgat aaacaacaga aatttgtttc tcacagttct
2461 ggaggctgga agtccaaggt cggggtgccc tcatggtcag gtgctggtga ggaccctgat
2521 ctgggttgca gactgctgac ttcttattgt gtcctcgtgg tagaaagagg gttagagcct
2581 ctggcatgcc ttttataagg gtactaatcc catcatgagg ccccgtcctt atgacctcat
2641 cacctcccaa aggacccccct cctaatacca tcacattggg ggttagggtt tcaacatatg
2701 aattgcgggg gacacaaaca ttccgtccat ggcacctact tcatagggtt tggcatgctg
2761 ctcggtacat ggttaacatt cataaatggt gggggcccttt actgtggtag aaaccattgt
2821 cactgcaagg cagccctgct tgggattcag gtgaacaaat gtaacgtggg tgggtgaggg
2881 actccctcct cttcagagaa gccctcctgt ggctcagggg cagctgcagg gccagatgga
2941 gttggcctgc agggagtcag ccttacccca tcagagacca acttgaaaga ttttttttctc
3001 tgtgtagttc gtgttatcaa tctgatctgc agccaggtta tttacttctg aagcattgga
3061 gttatcgatt gccattgccc catggttctc actctttgca ggcactcttc aaatttttat
3121 tttaaaaatt ataatgcaaa catctggcca tcaccttaac caagtgacaa agtcctagca
3181 caccattaat ggtaggataa ctgacattaa gtgttcctgc tgggatgaat tatgaagtat
3241 tccttccaaa gatgtcaatg gaaactaatc gagtcttaca cctaattttc agtttacagt
3301 aaatacagag gataaagaag ttaatggcat ggtggaataa ccaaaatgtg tatcattcta
3361 caaaaaaata ggcctggact atttaaggag tgagtgtcac tgtgggaaga ataagagggg
3421 gactgtcttg tccttgctac tcaaagagtg gtcctgggcc tgtatcatca ttgggaccac
3481 ctgggcgctt gttagaatca aagactctca ggcctcacct agacatccag aatcaaaatc
3541 tgcattttaa caagatgccc aggtgatctg catgcctgtt aaagtctgag ctctgttgca
3601 gataaccaga gacccaacaa ccaaaggcaa agcgtaagcc ttgattggat tttggtttag
3661 gaaaaatctc ataggtataa aagacaactg ggaatgttga atgtggatct ggtggtaggt
3721 gatattgtta gtgatgtgag tgatgcctgc tgaagtactg agagatgaag catcatattg
3781 acaacttact ccaggaaaaa aggtgtgtgt ataggtgtgt gtttggagag tgtgagcgag
3841 aactaacatg gtaaaatgtt aggtggagca tatacagttc tcattatact cttattttaa
3901 atttatattt gaacacagtc aaatctgaat ggaaatacat ttaaataagc atttctttca
3961 gtcatataag taatttgcct aaaaatgccc tctaagtcca tcattaggtt aagcgattta
4021 ctgaaggtgc agatatcatg cttatttcat tgggttgttt tggaatgtcc cccacttgga
4081 gagtttgagg aatggaaatg agaatcggag gagaaataag gccagaggct cacattgctt
4141 gcagggaacc gactgagtaa tgaagaagag gaaggaagcc cttggaagga acgtaaaatc

4201 catttcagcc tgccgcccct tggaatggag ggtcttctag ggaccagaaa caccagacac
4261 ttcttctgcc ccaagcacac cccacactaa tttctgctgc agcatcacga tcagggcaaa
4321 tcagcacagc cccccagcca tcagtggtgt ctagaaaagt aaggataatt ttcttccttc
4381 tcactgcagc cccaagctct tattaacctt cttttatgtg ttcattaaat gcagtcatgt
4441 gacaactgtg ttcaaagtta gaaatagtgg tcggggaggc ttatagatcc tcttgctgtt
4501 ctgaggcctt aaaaccaaaa aaataatgga atgattgcat cactgcgagg aagtatactt
4561 aattaagaaa gttgaaaatg gtttgtgtct tactaatagg actcacctga agactctact
4621 ttgccaagag ggaattttta actcaaagtt gtgtcagcca gcacggggta gagccatggt
4681 cctcatactt gctggcatcc ctcatctggt ggtctgttaa agcacagatt gctgggccgc
4741 accctcaggg tgtgtgattg tgtgattcag tattgctggg tggtgcccaa atgctccagt
4801 tctaatgtat tcccaggtgt tgctgatgct ggtgattggg taccacattt tgagaaccat
4861 ggggcattgg caatcaacaa atgcaatgct tgtgacttga cgtgataaaa gttcatttat
4921 tgggcaggca aagtcctctg caggcctgga aaccctccga gacaattact tgcatggggt
4981 aacccagtaa tactgtggga tgcaacttct agggcatggc tgcctccttc ctagaggcag
5041 caggggggaga aaacactgga agaggtcacc ccaacaattc catcgtacct tggcctagga
5101 gtgacctcct cagtctcaca acccctttggc tagaactagt catgtgacct cacaggaggt
5161 ggagcagtgt cgtcaatgtt atcatccccc tgcacctgca aggaaggagg cccagatgtg
5221 tgtaggtgct ggatgtctct tccccaaaac atataactct ggatttggaa aacaaaactg
5281 gatataacag aaaagaaaag gaggggagaa ggaaagttga agaaattggt gaaatcagaa
5341 taggacagtg aaaggcataa agattttctt ctgaaacgct aagatcggtt gatatttgtc
5401 taatacagct ttaaatagag tatcactttg ggccaaagaa aattgatata aacatacata
5461 aagtggaact aagattgact tcttcctaga catctctgcc tctgtccaca aaatggaaac
5521 ttccatcaag gagtactgga ggacagttca gtatttcttc ttatttgctc ctccctatga
5581 aaggaagagt gacttctctg ctgcatgata ttgggggtaa atagttattg cacatttgct
5641 caaaccccag caaagtgcac cccatttaa gccaaattta caaacttaca aagatgatcc
5701 accaatagtt ttctctattt actttattct tgctctgtca cccaggctag aatgcagtgg
5761 tgtgattata gctcactgca gcctccaact tctggactca agtgatcctc ccaccttagc
5821 ttcccaggta gctgggatac aggtgcacgc caccacaccc aggctagttt ttctaaatat
5881 tggagttccc aaagagtatt caaactgatg tgatatttta aaagacattt tgtaagcatc
5941 ttttaggaat atcagtaagt ggaagaaaaa cacatagtta tcagtggata ttatgccaga
6001 gaggcatgag aaatataatt ttattttgct aggtatcagc agagtgccct cttataattt
6061 gtgtgaaatg gaaacaaagg taacatcgtg ttttcaattc acacatatat acaagtaatg
6121 agaggtccag aagaaatgtg gcttcagctc tgctgctact gtgcctccct tctcctgccc
6181 cactcagccc acaaaatagg ctggacactc aaaaaacgtt gcgtttatct acctttaga
6241 gagggtgaat agcagagaac tggaggtggg aatggtaagg aactcccagc agggtagtgg
6301 agggaatggg ctgacgcatc taaggctgat gccaggtctg ctccctatct gggtggcctc
6361 agcaaatgac gtccagcaca tccaggggca ggctcaaggg agaacagccc ccaaagctaa
6421 gatcctgcca agctaaatac agtagttcta atgaaatgtg agaggctata atcccatttg
6481 ggaaattcct aaaaagtcat gaggcagggg attggtttat gttattatca tgacctgaga
6541 gtcatggctc agagccaaat gttcaggatt gaattcaaca gcatttaaat gtctttagag
6601 caggatggaa atatgttagc aatgcctgca gagtgccaag taaacgcaaa agccaatgag
6661 atcataaagg aagttgttag ctaacctagg tggagtcgcc aacttccttc tactctaata
6721 attaaaaata aaaataatac ttgggaggta actggaataa aggttctaaa atcaaaaccc
6781 tctgaagggt gaaaactggg agcctcctgt tcccatagta accacagcac tcagggcact
6841 gtctcccagc gctggagtac tgtcttatga ccagagatcc taagcaacct ctgctcatct
6901 gagttgtcca ccatattgtg ggcatgagtc cttgacaata gtaaatagca cctctgttcc
6961 cttattgggt aaatgatttt ccaactctgg gaatgtgtag aattcattat ggaaataatg
7021 caataattca aatccataat attgatactt tcatgttaag tttaggacta atcttgtgta
7081 tgctccttaa gtgatttgaa tctttaaaaa gcttatgatt ccaatttgaa atgtgaaatt
7141 gattttacgt ttgtgatttg aagttgaaag gtataagaat atttaactta gctcatgaaa
7201 agtattagac tagatttact ataagtttaa tgtattagat ttacaagaga tgcttaaata
7261 tatgagaatg ttttgtctta attggttata atcttgtcat atcaatgatt tgaagtgcta
7321 aaatagaaaa ttaaatatga taaattacac aagaagttta gaatgtttaa aagattttaa
7381 taaacaaagc ctataactaa gaaaaaaaaa aaaaaaaaa

(SEQ ID NO: 6) GenBank Accession No: NM_203395 (Version 2), incorporated herein by reference.

The amino acid sequence of mouse (*mus* musculus) IYD is shown below:

```
  1 mflltpvlva vvcilvvwvf knadrnlekk keeaqvqpwv dedlkdsted lqveedaeew
 61 qeaeesvehi pfshtrypeq emrmrsqefy ellnkrrsvr fissehvpme vienvikaag
121 tapsgahtep wtfvvvkdpd mkhkireiie eeeeinymkr mgkrwvtdlk klrtnwikey
181 ldtapvlili fkqvhgfaan gkkkvhyyne isvsiacgll laalqnaglv tvtttplncg
241 prlrvllgrp shekllvllp vgypsrdatv pdlkrkaldq imvtv
```

(SEQ ID NO: 7) GenBank Accession No: NP_081667 (Version 1), incorporated herein by reference.

The nucleic acid sequence of mouse (*mus* musculus) IYD is shown below:

```
   1 aggtcggact tcagaccatg tttctcctca ccccagtctt ggtagcagtt gtctgtatct
  61 tggttgtatg ggtctttaaa aatgccgaca ggaacctaga gaaaaagaag gaggaggctc
 121 aagttcagcc ctgggtggat gaagacttga aagacagcac agaagacctt caagtggaag
 181 aagatgctga ggagtggcaa gaggcagagg agagtgtgga gcacatcccg ttctctcaca
 241 cccgataccc agaacaggaa atgaggatga ggtcccagga attctatgag ctcctcaata
 301 agagacgctc cgtcaggttt atcagcagcg agcacgtccc aatggaagtc attgaaaatg
 361 tcatcaaagc agcaggaaca gctccaagtg gggcccatac agagccctgg acctttgtgg
 421 tggtgaagga cccagacatg aagcataaga tcagagagat tatcgaagag gaggaagaaa
 481 taaattacat gaaaaggatg ggaaagcgat gggtcacaga cctgaagaaa ctgagaacca
 541 actggattaa ggagtacttg gacaccgccc cagttctgat cctcattttc aaacaagtcc
 601 atggttttgc tgcgaatgga aagaagaagg tccactacta caacgagatc agtgtgtcca
 661 tcgcctgtgg cctcctgctg gctgcactgc agaatgcagg gctagtgacg gtcactacca
 721 ctcccctcaa ctgtggtcct cgtctgaggg tgctcctggg ccgcccctca catgagaagc
 781 tgttggtgct acttcctgtg gggtacccca gcagagatgc cacagtgcct gacctcaagc
 841 ggaaagctct ggaccagatc atggtgacag tatagccagc caacctaagg agatgctgga
 901 atgctgggat gacccccact tctttcctcc cggggctgct ggcacctctt actctctgag
 961 tcaggtcttc tgaaaccagt gtacagggaa ggccactttc cttgtagtga ttttttcat
1021 actttctaga actttcagtc cagtaagtta tttttaatgg actgttttag tgaatatatg
1081 gggcaagagg ccaaaaagca ggcataactt aatttctcta caaaattata aatgattttt
1141 tatttacata taaaagtcat aaagaagtca cattagaaga tttgctatag ttcctaaagc
1201 tacctatgta atacttttta atttaaatat tgactaatca agtggtcctt ctgctctctg
1261 ctttctggtg gcattgagtc taagaattta ctactgagat ctattctgtc actaagcctc
1321 atagggattc tgccaatctt gaatgagtga cctttaacat aagcaaaata tgacactcac
1381 agaaccagag aggcatgtct aacctgccat ggatgtagct ggcaattatc tgaacatgag
1441 ttgcatggag tgtgccggag ctggcttgtg ctagcacatg gaagatggtg gttaaatatt
1501 ctgaaattat acaacaaatt attaaaccat ttgtactttg
```

(SEQ ID NO: 8) GenBank Accession No: NM_027391 (Version 3), incorporated herein by reference.

The amino acid sequence of porcine (*Sus scrofa*) IYD is shown below:

```
  1 mflltpvlva vvcilmvwif knadgatkrr eeepraraea rpwvdedlkd stdvlqveed
 61 adewqeseee vehvpfshtr ypekemvrrs qefyellnkr rsvrfisner vpmevidnvi
121 kaagtapsga htepwtfvvv kdpdvkhrir eimeeeekin ylkrmgprwv tdlkklrtnw
181 ikeywdtapv lilifkqvhg faangkkkih yyneisvsia cgiflaalqn aglvtvtttp
241 fncgprlrvf lnrpanekll mflpvgypsk eatvpdlprk pldqimvtv
```

(SEQ ID NO: 9) GenBank Accession No: NP_999581 (Version 1), incorporated herein by reference.

The nucleic acid sequence of porcine (*Sus scrofa*) IYD is shown below:

```
   1 ccaccatgtt tcttctgact ccagtcttgg tagccgttgt ctgcattttg atggtgtgga
  61 tctttaaaaa tgctgatgga gccaccaaga gaagggaaga ggagcctcga gccagggctg
 121 aggctcgacc ctgggtggat gaagacttga aagacagcac tgatgtcctc caggtggagg
 181 aagatgctga tgaatggcaa gagtcagagg aagaggtgga acacgtcccg ttctctcata
 241 cccgctatcc tgagaaggaa atggttaggc gatcccagga gttttatgag cttctcaata
 301 agagacggtc ggttagattc ataagcaatg agcgagtccc aatggaagtc atcgataatg
 361 tcatcaaggc agcaggaaca gccccaagtg gggcccacac ggagccctgg acttttgtgg
 421 ttgtgaaaga cccggacgtg aagcatagga ttcgggaaat catggaggag gaagagaaaa
 481 taaactacct gaaaaggatg ggacctcgat gggtcacaga cctgaagaaa ctcaggacca
 541 actggataaa agagtactgg gacacggccc ctgttttgat tctcatcttc aaacaagtac
 601 atggttttgc tgcaaatggc aagaagaaga tccactacta caatgagatc agcgtttcca
 661 tcgcttgcgg catcttttta gctgccctgc agaacgcagg gctggtgact gttaccacca
 721 ctcccttcaa ctgtggcccc cgactgaggg tgtttttgaa ccgccctgcc aatgaaaagc
 781 tgctgatgtt tctcccggtg gggtacccca gcaaagaggc cacggtaccc gacctccccc
 841 ggaaacccct ggaccagatc atggtgaccg tgtaggcagg atgtgggcag ggagatgctg
 901 gaaggaggtg ccccctcccc cggatcctgc tcccctgggt atacaggctg ttttttccct
 961 gggtgtttgg tctccccagt gctttactgg gcgggggggtg gggggagaac tcaagaactt
1021 tgccgctgag aagccctgat tcccacagtg ggaatagctg gacgcacagg agaggtgaac
1081 cagcaggttt ttttccattt tttatccact ttggaaatgc attaaaattt tgttaagcaa
1141 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
```

(SEQ ID NO: 10) GenBank Accession No: NM_214416 (Version 1), incorporated herein by reference.

The amino acid sequence of Honeybee IYD is shown below:

```
   1 mltelfpfwt kywyhvliti vayiflklfy kilkkdaifi eknkqnfdis niikqlketn
  61 qetedseesa lpkdlkhipy eykrpsemel fcqasefyki vtarrtirff ssdpvpkevi
 121 yeiikaagta psgahtepwt fvavsnqkik eqiryivese eeinykkrmg vkwttdllpl
 181 rtnwikeylt tapylllvfk qiygilpngk kkihyynems tciacgilit aiqyaglvtl
 241 tstplncgpa irnllgrpsn eklvvllpvg ypakdatvpd lqrkslsdil veid
```

(SEQ ID NO: 11) GenBank Accession No: XP_397179 (Version 2), incorporated herein by reference.

The nucleic acid sequence of Honeybee IYD is shown below:

```
    1 ttaataaaag tcattgtcta aagccagcgt cgatgaatta attttttta
atagcctgag
   61 tctttttac gctgaataat taaatcgttt tcatgtattg cttaaagatt tactatatgt
  121 ttcgatagat aatattctct ttttctaaga cagaaggtac aaaagacaac ttcaaatact
  181 tgctaattat atatgataaa aattataatt agtgcttttc acatttaaaa aattagaaac
  241 ttattaagca tatcatgtat atgttaaaat acaagtactt aaatacttcg ttgtactgta
  301 aatgattgat gaaaaattca tgaatatgta aatctatgta ttagtatata ataataatgg
  361 gatacatcat ctgtttaacg cgataactta gatattgtag gtcagatata tcctactctt
  421 gttgtaacac atatcgttac ttacattgtc ttgaatcgtc aaaacaattg aatataatac
  481 tcaatcttcg ttttcgaaat aaatattaaa tttttattaa gaatgcttac tgaacttttt
  541 ccattttgga cgaaatactg gtaccacgtg ttaattacta ttgtagccta tatttttta
  601 aaattatttt ataaaatatt gaaaaaggac gctatattta ttgaaaaaaa taaacaaaat
  661 tttgatattt caaatatcat aaagcaatta aaagaaacta atcaagaaac agaagatagt
  721 gaagaatctg cattaccaaa agatttaaaa catattcctt atgaatataa aagaccttct
  781 gaaatggagt tattctgtca agcttctgag ttttataaaa tagtaactgc tagaagaact
  841 ataagatttt tcagttctga ccctgtacca aaagaagtca tatatgagat aataaaagct
  901 gcaggcactg cgcctagtgg tgcacataca gaaccatgga cattcgtagc ggtgtcaaat
  961 cagaagataa aagaacaaat acgttatatt gttgaaagcg aagaagaaat aaattataaa
```

```
1021 aaaagaatgg gagtaaagtg gacaactgac ttacttccat taagaacaaa ttggattaaa
1081 gaatacctga ctaccgctcc ttatttgttg cttgtattta aacaaattta tggaatttta
1141 ccaaatggaa aaaagaaaat tcattattat aacgaaatga gcacatgtat tgcttgtggt
1201 attctcatca ccgctataca gtatgcaggt ttggtaactc tgacttctac tcctttaaat
1261 tgtggacctg ccatccgcaa ccttcttgga cgtccttcta atgaaaagct tgttgtacta
1321 ctgccagttg gttatccagc aaaggatgct acagtgcctg atcttcagcg aaaatctcta
1381 tctgatattt tagtagaaat tgattgacat atattataac aaattatggg aattaaagta
1441 cctttgttta atattttatc agatacttgc ttgtaggaca atacaaaaat gagtacacat
1501 aattatagat ttatataaat cctgtttttg taaaaaaact tatatttgtt ttgagta
```

(SEQ ID NO: 12) GenBank Accession No: XM_397179 (Version 5), incorporated herein by reference.

The amino acid sequence of Mosquito (*Anopheles darling*) IYD is shown below:

```
   1 masyleswvd slisssalvn ywnyifpmlv glylaislyd khryggrkkl
riispptdst
  61 tdatrkldgm eyvgeldhdg eegyelspal eekphvpyrg atvtfakdps eaadkfyaiv
 121 nnrrsvrkfs qrpvdrdive rciqaagtgp sgahtepwtf clvsdrsvke sireiiesee
 181 linytqrmak qwvtdlrpiq tnhikeylse apylilvfkq tyshkadgtk kqhyyneist
 241 siatgillca lqsaglsslv ttplncgpal rnllerppne kllvllpvgy aaedctvpdl
 301 qrkpldeliv ry
```

(SEQ ID NO: 13) GenBank Accession No: ETN68090 (Version 1), incorporated herein by reference.

The nucleic acid sequence of Mosquito (*Anopheles darling*) IYD is shown below:

```
   1 aactgtttat gctattggtg tcacagagta ccatgcgtat ggttgcagag ctccactcat
  61 acacagctgt ttccgtggtg tagtggttat cacatccgcc taacacgcgg aaggcccccg
 121 gttcgatccc gggcggaaac aggcgatatc tttttgcttc ctccaaacac ctccttgtgt
 181 gtggatcatc ttttttcttt tgcgaaccca cgttcttttc gcgttctcaa gcgatccact
 241 atgcacctca cagaaacatt ctcaatttga aaatatcata cagcagcaac acgcgatatt
 301 taaggatagt tgattgttga caaataagga ccaatagatg gttttattga tttttagtta
 361 tgctcatcac taataacgaa agacttagat gagctttact tcctcttctt accggcaaat
 421 ttcttcttcg ttatcccgcc accaccacct ccactgcgtt gtttactgat gaaacgcttc
 481 ttgctactcg cctgcatccg gtttttgcca ctaaaccgag actgctccca ggccatctgt
 541 ttggcggatg aattatcctg attgaaaact tcaccagcgg taccgtgcgc cccggcggat
 601 tgtaatttct gtttgtgctt gcctcccttt gctttcccgt cggctcccga cgatccaaca
 661 ttgcgctcaa gcagctccga gaagtcatcg gccgcagcaa acagtgagtt catgtcggac
 721 gacttgagct tccgggcaaa gtctcgttca ctcacgccgg tacgtaacat ctgtttcttc
 781 gccttcttcc tcactggctc ctcatcttct tcttctgggt catcgtcatc atcatcgtca
 841 tcatctccgg catcgaagtc atcatcttcc tcgagcgaga tgctaccacc atcactgaac
 901 tcttcatctt catcgtctcc accaaaacca cgtggttcat catcgctgtt atcgccctcc
 961 tcgtcgtctt catcatcatc ccccggtacg tcatcccaat catccaacgc accatcgtcg
1021 tcctcttcat cttcatcatc agcatcctgc tgacccttct tcttaccctt cttcttgctc
1081 gctttacgct ccatgtcctg ttccagctcg gtcatgaagt caagctccga ccgatccata
1141 ttggcaccat catcccgccc accaggaaca cccaaacgat ccaagtatgc atcgaactcg
1201 tcgtcatcga gtgagtcaac gtcggaatca acctccgcct cgtcttcttc ttcacccgac
1261 ttctttcctt cggcctttag cttcagtgcc tttttgcgtt cctccgctcg gcggaatcga
1321 tctcgtttct tctccagaaa tctgcagaaa ttagaaataa ttagttgtta ccacagtgga
1381 atcgattcca cagagtaata caacagttgg acttactgga agatgtactg ctcatcctcg
1441 gtgcactgtt ccttggtgag tgactcgacc ggtagagctc gtgagccggt aggctggtaa
1501 tctcccttgc gttgtgccac accgagcggt cttggacggt ttacctcttg gcccgtcgct
1561 tcgtcgattg ccttttgggg tttcttcgga ttcttaaacg caaatcgatc caggaaatgg
```

1621 ccgagtgaga aatcccgcaa tggatcacca tagtagctaa gtggggcatt gctaagaata
1681 cattcggcga acttttgtac cgttggatga aagtattcga gataacgggc cagctcgtac
1741 cggagcgtgt acttagcccc ggcaaactcc gatgcacgat ggaaagcatc ataaccgcga
1801 acaaccgatg aattgctaac agttcttccg gcacctttag gtggtttcac atcaagctct
1861 ttctgatcct cgtcgtcatc gtcgtccttt ggtgccaaga taggtccatc gcggtcgtcg
1921 tccttctttg aagcggacag agctgtggcc tctgctggtg ccggtagacc atcgatcagc
1981 agttgacgac gctttcggag cactttgctg atgatgatca gagtgccaca aactcgagcc
2041 ggtgggaaat aaaatgccac ctgtagcaga cgcttgatga aggcttgcgc acgatcaggg
2101 atcggatcgt tctgcatggc acggtgtata atgtagaaga acacggttga gatgcgtgga
2161 ccgacggagg aaagctgagg atcaagcagc ttgcggtata gcgtattgta gaaacggttc
2221 tgctcacgac cagggcgctc cgtcacttcc agcaacaacg agagcccctg gcaaccgatc
2281 gcaatgtctg ccagatgcac cattcgatag ataacgttca gcagctcggg ctgcacgata
2341 tcagctactg cgatgtcacg tttcacgcta ccgatcgctt tgcgtagaca ggccagtatg
2401 gcctgcatcg tccgcgagtt gatctcacca cgttcggtca gaatcttgaa gaaggcaaag
2461 cacacgttaa tcagcttctg gcacgtggca aagtcaccgt acgaggcgat cgaggccagg
2521 aacgagagcg cataatgctg cgctgtcgct gagacgttgt tgcgaaagag caatttctcc
2581 gtttccgtgg caaccactag tgccatggcg ctgtgtttcc gcacgacctg caacagatga
2641 tagagcgcct ttacggctac gcgccgtgac ggatcgccaa gtttgttgac gagcaccgtc
2701 agcaaaaagg cttctttctc cggtatgtcc gcaaacagac gtgccgtgtg aacgatcgcc
2761 ttgcacttgc tatcctcctg tcccgagtgc agtatcaccg atatgttggt cagaaagtta
2821 aagtacagct cgcgtagctg atcctcgaag tgccagtgcg ccagcagtgg ttcgcgaatg
2881 gatttcgaca gctcttgccg ctgtacgtta cgccaatcag ccccacgaag tggaatcggt
2941 attaactttc tcgaggacgg aaggagtgat ttcagcatca gctcggttaa cacttccacc
3001 acgtcaaggt ggcccttgtt cgagggtttc accatcccga tcaccgtctc cagagcggac
3061 agattgcaga acgggttcga ctgtacgagt agcgctccgg aactggctcg atcacgcgat
3121 gttcccttct cgatcgcgct ctgcaaccag cgggcatcgg atgggttgtc cttcatgcgg
3181 acacgacatt cggcgtcgaa ggccgcgcgg cacagtttcc taagttcctg gatttcgggg
3241 tctttcagct caaccacctc gccaccgacc gtattgtatc cgtcgaaatg ctcgtaccag
3301 cgtttcacct tctcgtcgtc tcccggcagg ttgaaactac tgccccgtcg accggatttc
3361 gcattcttcc ttttaccgcc agctgcctgc tccgtgcgtg tttcttcatc cgccggctcg
3421 tgttcgatgg gatcgctgat cggcacttcc tgttgttctc catcttcgct gaagacgatt
3481 ttcttcggct tccggttgtt atcgagcaca gcggtttcac ttggtttctc aaagtatcgt
3541 ttcgagtctt ttttccgtac ttttcctcct tcgatagcca tcgtgaacac agaaccgcga
3601 ttttttctgt ttatctcgaa acacgcggtg cagtgcgttt atgctgtggc ggggtcggtt
3661 tgacagcaca atcgggtgac cagataaaaa ataaacacgt agatgtcacg gacgggacaa
3721 gcgcgctacg tctagccagt caaagcgact agtcagagtg tagagaagtg tttacaaaat
3781 cccgcgaaaa gtgcaataat ttgttaaagt gagtgaaact ctcaagtaaa atgcctccga
3841 aacgaaaatc caccaccgca acggacgagg aagacgagga gtatcgcaag aagagggacc
3901 gcaataatca ggtaacgagt taggtcgtcg atttcatcat cctgcatctt aacgatacgg
3961 tttccttttg ctcaaaggcg gtgaaacgga gccgcgtaaa gtcgaagatg aaaacggaag
4021 aaacgcagca gcgtgtgaac gaactacgcg taaagaacca gctgctcgag gaaaagatcg
4081 ataaccagca caaggagcta aagtttttga aggaactttt cctaacccag gcccaggcga
4141 aggccgacaa actggtcggg gtcaacctga acgatctttt gcgctgctcg gacgaggaaa
4201 gcgacagtgc agatcaaggg accagcaagc agaagaagaa cgagtcctca aaaggtgaag
4261 gcagcagcgg gagcaaacgg tccagaaggt gagaggccac tcctggaatc cacatcctgc
4321 atacgagtac taatttaag caagcatttc cagacaattg aggaaagcat aaggcattaa
4381 gatttttggg taaagttaag taagcaactc aacacgcaag tgtataaaat acttttaatt
4441 gcatcatgtt agttttaata tatcagcgta agttttaata cacctgtact attacacatc
4501 attcaaccga tgttcaaacc ttctttgatg cttgttaaag tagaaagacg taaaggtaaa
4561 atgagaaaat actcatataa cggagtaatc catggatatt gtgtggaatg aattttgcgt
4621 gcagccttgc tgaggccgtg tggcctaatg gagaaggcgt cggacttcgg atccgaagat
4681 tgcaggttcg agtcctgtca cggtcgtgct cgtgctgaga tgattttctt tatttatgta
4741 caacagacac atagactcct tttcaacaaa cgggtaaaat aaaacataga aatataggac
4801 gtccagtagg tagtagtatc cagtgaaacc ttttactctt tacttttccc attgtattaa

4861 caatcgattt tgaaaaaaaa tccaacctca gtacgacaaa aatacaaaag atttcttcca
4921 agcgaccgtg acaggactcg aacctgcaat cttcggatcc gaagtccgac gccttctcca
4981 ttaggccaca cggccagttg gcaggtgtca acttagatta taacttggaa tcattaacat
5041 taaaaatcaa atttttggtt attttccatc gaaacaatcc tttttttttc attttactta
5101 cggcatgcta atctacaaat tttcagaatt gttttaaaga aaaaaaaaca aaatagccaa
5161 tcatgacagc cattctgggg atcaatgatt gccttaaagt tccaaagccg tttgctaaaa
5221 agcctcattg caatgtctgt ggccgcctta acattaaaac tatagtagcc atccgtttgc
5281 ttttcatcaa attttatctg gacattattt gcaaggtagc ctaggggagt ttacaacata
5341 tttaatgata tatttaagca tggcaccctt cgccagaatg gtcaccattg ctgtatttt
5401 tatatttatt ttataaaaaa ctccacaaat actcttcaaa agttgtagta tcgtttgcct
5461 tcaacaaaaa tataaaaatc tactcagttc cagtacaaaa tcacatgaat gtgccatttt
5521 ttgctaaatc acaccaaatc cctttctaag cacccagttt tcaactggcc gtgtggccta
5581 atggagaagg cgtcggactt cggatccgaa gattgcaggt tcgagtcctg tcacggtcgc
5641 tttgtgtgaa aattactttt tgttgtgtga tatatcaaac atctctttat tgctcttata
5701 ttaaatcaag tgataacatt tttatcagaa ttatcagaga atcgtgaaca caatgtatta
5761 cacccgaata ttttgtgtgc gtgtgacaaa cttatcgttg tttacatggt tccggcggca
5821 gcagcacgtg gtgttatgca gttccccatc ccatcgccta gctccatcgt cgttatcgtc
5881 gttttatcaa acttatctaa aatcagcgcc cgacttttt tgcaaccgac cgtctggaca
5941 gtcaacataa ggaaacgcta atccttatac cctcagtagc gcacgatgag ttcgtccaac
6001 ggtttccgtt gcaggtcagg tacggtgcaa tcttcggcag cgtatcctac gggtagcaac
6061 acgagaagct tctcgttcgg aggacgctcc aacagatttc gcagggctgg gccacagtta
6121 agcggggtgg tgacgagcga actcaatccc gcgctctgca gtgcgcagag tagtataccg
6181 gtcgcgatgg aggtcgatat ttcgttgtag taatgttgct tttttgtacc gtccgccttg
6241 tggctatagg tttgcttgaa caccaaaatc aaatatggcg cctcggagag gtactcctta
6301 atgtggttcg tttgaatcgg gcgcaaatcc gtgacccact gcttggccat gcgttgcgtg
6361 tagttgataa gctcttccga ttcaattatc tcccgaatac tctctttgac cgaccggtcg
6421 gacaccaaac agaaggtcca aggttctgta tgtgccccac tcggtccagt tccagctgct
6481 tggatacatc gttccacgat atcccgatca accggccgtt ggctaaattt ccgcacgctc
6541 cgtcgattgt tcacgatcgc gtagaactta tcggctgcct cagaaggatc tttggcgaac
6601 gtgacagtcg ctccacggta tggaacatgt ggtttctcct ccaaagcagg tgatagctca
6661 taccttctt caccatcgtg atccagttct gctcggaata aagaacacat taatagtcag
6721 ccatggtttg cacacacagt tcataagttc ggttacctcc aacatactcc ataccgtcca
6781 gcttcctagt tgcatccgta gttgagtcag tgggagggga gataattctc agcttctttc
6841 ggccaccata tcgatgtttg tcgtacagag aaatggccag atagaggcct accaacattg
6901 gaaaaatgta attccaatag tttactaacg ccgaactgct aatcagtgag tcgacccaac
6961 tctccagata cgaagccatt gcctatccga tggggcgatg gagttcgaat gatgcgaact
7021 gaaaagataa tcggggtggc ctctgtgcag ctcaactgca actcgcgaac tgttgcgagt
7081 tttacgtttc gcacagaatc tcccgagccg tgcatccccc ttcggtccat tatctagcgt
7141 gtttctcgct cccccaatcg gcgtccccaa gagaatcgtt tccatgtctg tgggacaagg
7201 gcgcacacac tcagccaatt tgaaccgtct cccatgtagg gtgggtgggt tgcaatgatg
7261 atgacgatgg atatggggtt agaaactgca ggccgcgtga ccttgtaact gttagcgtgc
7321 ctctaacatg ctccggcgct tgcacaaatg atagacctcg gcctcaggtg catcgtattg
7381 atcaccgagc cggactggct cgcgatcagg atcctttccc tgtggatcat atcagataca
7441 attatagacc ctgcaattta tgccgtttta ccctgggtgg catattttat ccgtaccttg
7501 aaacagctgg agaacagcta tcacaaataa aatttacaac gatcatggga ataagtgctc
7561 caaacgatct tgttatattg ctgaaagttt aatgtgatag atcatcaaat gtaagagata
7621 aatttaactg ctgatggttg gtaaaaaaac tgtttgtgcc ggttaaggaa agtgaaccgc
7681 cgcctttcga aacggagcaa atcagcttca actaggagat ataaacatag ttgtgtgctg
7741 ctcgatttta tgacatttcg agttcgagtt cgaacagttc actatggaat acgatgtggt
7801 aaaaaacaag tggaaaaatt gatggagggc tacttaaatt acttttacat ttttctatga
7861 aaattgaccc tttcaaaact attccgtgaa agtttctcat caatcgaagc agaaagaaca
7921 gagctacgat tttttaaaga tcgtcatgct acaagcgatt tttgttatgc gtgacgccat
7981 cttgaaccac acagcaacat cgcacaggtc gaacgagtcg cgctggtatc ttttcgctga
8041 tttctgctca gaactgttct tttcttttgt gaatcttgtg ctgtttacct gaaacaatcc

8101 ttttcacgag tctctcgacg tgcccgcacc gtgcgtgtga tgcgataatg tgaaaatcga
8161 cgggtgttta gtgacgaaaa tgtaaggcca agagataaac atatttgcag gtgatccgtt
8221 gagggctggc ggtctaggaa cggttcggta aagcgcggta tagatagttg tgttttggat
8281 aataattttg ttggtaaaaa cgaggccagt ctgcggtcgt ggccgtcgtg tttcctagga
8341 ggtgaaggtg aaatcatgtc cgcgatttcg cgggattgct tttcgtgttg gtaaaattgg
8401 tgcaactaat tgaactcaaa gaagagcgcc aaagtagaag caaggacatt aataaaatcg
8461 cggctaaaga aagatcaaag gcaaaaagga gtcgatggaa aagcgccttg acttggtgat
8521 ttagctgcat cgctccgtgc tgtgcgtgca gttggcgctt tctgatgtac gtgtcattat
8581 gcagcgccgt gaaaattgca ccaagagaga ctcaagcggt tctaacggtg aaaaaagtat
8641 tcctagttct tggacaacgt atcggagttg aaacgggctg cccaagttac atcgaggata
8701 ccattgcggt gataaaaatg ctgattcgct ggattatttg aaattacttt tactccgtgg
8761 aaaatttgtg aaaagacggc gcacaccttg tcacgcacac atcgccatag actcttgccg
8821 cgagacgacg gtcgtttggt caatcgctca agtcgtccta aaatttaagt ggcccttggc
8881 gttttctatg ctctgttgta cattctacgg gagtcaattt taatggtgaa taatttaaaa
8941 tcacttcaat attcgaccga gaaacgctta tcactaaaac gcaagaaatt tcgtgtttat
9001 ttactctcgc tctctctcgc catcgctctc tctctctcga gaagctcaac ggagaaaggg
9061 gtaacggtac aacaacggtc cgttacgcaa aatcagagaa caacaataga aggaatgaac
9121 tagggcggct actagaagta ggtttggaag gctgggtaga ggtcagggga gagtggaatg
9181 ttttggaaac ggcgaaaaat ccgcttagtt gtgtgtgctg tgaaattccg ctcgctttcg
9241 ctgtgcgttc ttgcggtctg cggtgaattt tgtggagccc agtgcatgcg catagcttaa
9301 cagattattt ttcttgctta acatttacag atttgatctt gaaaaattgt gttgggtgca
9361 aaaaggagag aagtttgtgg tgatcgatgg tagaagacga agtttagcgt atagcgcgat
9421 cggcgacaac gacttcgcgt ctgctttctt gtgcggtgtt tgaaggaagt tgtgtaagac
9481 gaaatgctta agacatacag ccaagcatta accagcttaa aagaaccaat agaatcttca
9541 tccagtgctg ctgccactgt tgctactgta tcaccatagc aaccgagcag cggcgcagcc
9601 tgtgctatat tttgggagat tgaaggcggc agtgccgcca gttattggtg tcggattagt
9661 gcagaattaa attttgtgtc agccacgaat acaaaatgcc ccgaaacact cgtgccaaag
9721 ggtcatccgg tcccaagaca acaggaggca agcacgggca gcccgcttct gtagggtttt
9781 gcgagacctt tcgcgctctg caggtggaac cagctcaaca actgagcagc accagcaata
9841 tcaacgtcgg tttgccacag gacatcattg ctaagcgcct tgaggaggaa ccgttgtcgt
9901 cgttctttcc gtcaatttct agccgaccat atcataaccg cgctgatcag cacaccctgc
9961 acgaaaaagg agccacctcc cgtggtcgta ccggcaagcg taaaggactc tccgagttca
10021 tcaaacctga agtgttggtg gaatacgtcg tggccgatag gatgcaagaa gagcaccagc
10081 aacaccggca acaacggacg gctttcgtta atcaccactc gcaccagtct cactatatcg
10141 atgattgcga tggtgatgat ctagactcgg gaagacgtct aaatgatcag caacaggagg
10201 aggagattat caatctcagc gatggggagg acaccatcat agacatctcg tcacccaggg
10261 ctaatggaca cgatgatatg gaggtgcact cacacgatga ggagggcgaa gatgactacg
10321 aacaggacga cggcgatgaa acggaaccgg aaatagcgga cgacgacgag gacgacgacg
10381 atgaagaaga tgagaatgct gaagaatcat acggggaagc cgaagaacaa cagctggaag
10441 aggtcgacga agaggaggac gtagaattga tggaggaaga ggaagatgaa gacgctgacc
10501 aaatagagga ggacgatgac gatgttgtga tcgatatttc cgatagcgat gataaacagg
10561 aggtccaacg taaactgaag catttgatac tgcagaagcg gcaaaaacaa caacaccaaa
10621 cttccagacg aactggaggc gttaccctag atcgggagac gatcgaaatg atcgagacat
10681 ctacaattaa caccaaaggt acgtatcata atatcaacac tatattctat gtccatccag
10741 cttagatgga cttttcgggt acattcaggg tactccnnnn nnnnttaat ggttggaaag
10801 gcatgtttta ccgataaaga gtgtacgtag gccttgacct ctgccagcat gattctgcgt
10861 gttgtcttac gaagtcatac cgacaagcca tacggtgcct ctttgcttct gttttcgttt
10921 gaacgatatc gtgtctagcg acctcgatga catttttgt tttggtccca aatcgcgtct
10981 tcgtcgatat agacaacaaa gaaagttgtc caaaaggatg agattcctga tttgctcctt
11041 acaaactgtt gtgcaattgg tcgagcaata ctgattttaa tactaacgtt aaaaacgata
11101 ctcatgctca taggctacgg taatccgtaa ccatttttgg cctccaacta agggcatgcg
11161 aggagatatc ctgagctatt ggggagacat ttgtgttaaa aatagacgtc aacgacgacc
11221 actaacttgc tgtgccggcg gtattgtagg accatgggaa catggtggtc tctttgtttc
11281 atcgtattca tcgctgtgta gtttcttcgc aacgagtgtc cactaatcct aagaagaaga

11341 atcgtaagca aagtgcgttc tgcctgatgc acagtactat gggcagcggt gcattagaaa
11401 tttgcgacat aaccgacatg gaccacttct ctggttttgg ggccaatcgg tagaagagta
11461 ttggtatacg cgtgtcaatg atttagcgcg cgaatgtgtg cacgcgcata atacgattct
11521 gcttactcga ggtggttggg tgacaaacga ttttgtttgt gattttcctg aatggaatcg
11581 agagaaacat tctctcagtg agtattgcat ttccccttag cggaacagcg ttcgaggagt
11641 tgtgtacata aaaagcgcta attctgattg cattttttat gggataacac attactccat
11701 gcaaaggtgt tctaggtgcc acgcagacaa ctccacgggt gtttgccatt gaaagtgcaa
11761 cgcacgaatc atctgcttat ggttgcggag gccgggcaga actcgaacaa aaaatcatcc
11821 ttaagaacat acgtccttct tcgtaatgtc atcggtttgt ttgaggaggg cttgcttgtt
11881 gttttcggcg tttgcacgac caagtttaaa tagaaggaaa agttccgttg ctaaatcgcg
11941 gttgtgtcgc ccttgaaaga actctctaaa tagtgtcgga ccacttgcgg tgcggttggc
12001 ttactttata agaatgatgc cacgtgtact ggggaaaagg aacatacaga aaaccatagc
12061 ataagatttc atcagtaaga tgcgattagc attaagtaat cgattgtagc tattccagcg
12121 aaacgtacct cgtagtttca tgcaacagga gcatgaacat gttttcatga acatctgtga
12181 ttacaaaact ttatgtactt tagattacag cgctgtttca aagactgtat cttctgctac
12241 gtaaatatta aattgtacaa ataacaaggc aatgtgtgct acgatctaca gccgacaacg
12301 atctcaaacg gatttttact aaatccagaa gccggttcct attggggcaa ttcaaggctt
12361 tgtttgtctc ttcaagcatc ttgtttgcca tgtcacagtc taggtcatga aaatagacgt
12421 agcaaccatg gtcagtcact tcgtctgtcg cgtcctccat tcataaccat tcctgaaatg
12481 ttttggaatt ctgtgtttgc gaggagccgg gcatacgtga ggcttctcag cgacaaaacg
12541 gccaatgctg cacttcagtt tcccgccgtg tgctacaggt gtttatttga ttctccagcg
12601 tagaataaac ttttctatgt ctatgttttc gatactcgac agacttcata gtaaccggcg
12661 acaatttcat ttccggtttt tgatgttgta ttcaatagca ggtgtcggtg tgtgtgtgtg
12721 aaaatcgaga aaaactaacc cgaagcggag gctgtgagct cagtgtgcta cgccacaaac
12781 cggatacaat attatcatcc atctgttcga tgatggaatt attacaacag gatttacgca
12841 aatttgtacc acccctatag taacaattcc gttgcattct ttttagtaaa acgacaaaac
12901 aagcagctca ctatagaaat tgagtttcat ttggttggtg gcgcgcgaag ggtgcgcatg
12961 aacctccaca aaaccgtctc acgaatacac ccgtttcagc caacaacccc cctaatgctg
13021 tgactcacac gaacacactt ctcaagctcg aagtaggtca gcaaatcatc accatgctag
13081 tggcgttctg atggtttgct cttggcgctc gcctcaacgt cggctgttgg cgtctccctt
13141 taagcttccg ttttcctacc ttgagtgtag tcaatctaaa ggttgcagga tttaattttt
13201 taatcgtttg atcaaaaaga gaggggaaaa cccgcataaa tgcttcccgg ataggttcgt
13261 tggatcgatt ctttgccgaa gtaccgttcc ggtggtaacc attgacgcgc atcatatcca
13321 tataaatctg cattacactg cgatttccaa tttctggtta aatctttgat tgtctctcct
13381 tcttgggcaa ctattcgttt aaatatcgtt acccgtagtg atttttatc gtaagcaata
13441 ctgacgccaa tttaaatatt tatatttctg atgtcctttt acttttagt ggaactatcg
13501 ccggatgata aggttggatt gaaaaacttt gagttcctaa aagttctcgg ttcaggaggt
13561 gagctctttt gaaacactaa atttatctac cacgagccgt aatatcccta accatcttat
13621 ctctgtgcac cttcagccta cggtattgta tatctcgtac gcaagattga tggcgttgac
13681 gatggcaaac tgtatgcgat gaaggttctc aagaaatcgg tcgtagcggc aaagaagaaa
13741 accgccgaac acacgcgtac tgagcggcag gtaagaaaag gaggtttttt tttgttgggg
13801 agtttactgt catgaatgta atgttgtcaa taattgtgtt tttcctggat ttttcattac
13861 cctatgaagg tgcttgaagc gatacacgat gccccgtacc tggtcacgat gcattacgca
13921 ttccagacgg attccaaact ctacttgatc atggattttc taatcggagg cgagcttttc
13981 acgcacttgt accgacgcga acactttgac gagcactacg tgagaattta catcgctgag
14041 atcatactgg caatcgagca gcttcacaag gtaagattgc ctgaaggtta ctttctttta
14101 ctctattgta taataatcta taaatccttt ttttttattt aaaccgcagc tgggtatctt
14161 gtatcgggac atcaagctgg agaatatact gctcgatggg gatggccata ttgtcctgac
14221 ggacttcggg ttgtcgcgtg agctcgcggt cgaagacgag agagccctta gcttctgcgg
14281 tacaatcgag tacatggctc cggaaatact gaagggttcc tcgtacgggc acggaatggc
14341 cgtcgattgg tggtcggttg gcgtgcttat gttcgagctc cttgtcggtc aatcgccatt
14401 ccacgtcgat tctggtccga gcgaaattca gcgacgaatt tcaaatgaag aaccgatcat
14461 accagcacac ttatcagcgg aagcagccga ttttcttcgt aagctgctca caaaggatcc
14521 aaagcgacgt ctaggttgtg gcgagcagaa ggcaggggag cttaaatcgc acccttttct

14581 gcggccaatc gactgggaca tgttgcaaca gaagcgaatt cctgcaccct ataagcccaa
14641 cgtagaagac gatggcgata cgcgttattt cagtgaagag tttaccagca acccgaacat
14701 ggacgaacca tgtgcctcgc cgccttttgg aaatttgctg ttcaggggta tgtgtcctcg
14761 ctgggccgat gcgaccacac cattcttact gaaccaacct ttctccatct ttttaggata
14821 ttcgtatgtc gctccagatt tgctttcgtc caaggttacc aacaaaaaca agttcattcc
14881 gatccacaat acccgaccga gcgatagact tatcaagcga ctagcatcga aagtgagttg
14941 atctgcactc gggcgaacgt gatttaatta attcattttc acatgatggt tttgtttctt
15001 ccaaacagca atcaaagttc ttccagaagt acgtgttgac acgcgaccca acgatcggta
15061 caggaacgta ttctacctgt ctaaggtgcc ggccgctgca gcgggctagt gaagacgaca
15121 ataccaatta tgcagttaag gtactgttca atcacccgca aaccgccgag tacgtagagc
15181 aggaagtacg ggccttgcaa aagtgtcaag gtcatccaaa tgtagtgcgt ttcgtggaag
15241 tgctggagga tcgctattac atctatttag tgctggagct gctggaagga ggtgagctcc
15301 tgcagcgcat taagcaaaaa cagcatacac tgaccgaggg gcaggtgcgt ggctacttcc
15361 ggcagctggt ggatgcggtt tcatttctgc atcgaaacgg aatcgctcac cgcgatctca
15421 aaccggaaaa tgtgttattc gaaagcgcgt cgtccgagcg gttgaagatt attgattttg
15481 gtttcgcaca aacgtgccgc gagaatggtg accgatcttc ggcattacca tcatcgtcct
15541 ctacatcctc aaaaccaatc atatcaccgt cctggatacc agcaggcacg ctcggctatg
15601 tcgctccgga agtgctgcat ccaagcgatg cttatgccct agagtcggct gacctctggt
15661 cgctgggcgc tatactctat acgatgctgt gcgggcaggc accgttcata ccgcgagagt
15721 tctttggtca caataatctc aattcgcagg caaagctgaa gggcatcata atggacaaga
15781 tcaagcgagg ttcgtttgat ctcgctgcga tgccgtggcg tcacgtcagt gaggatgcaa
15841 gggatctcgt caatcgattg ctcaccgtta acccggacta tcggcttacg atggaagatg
15901 tgatcgagca tcggtggctt gacgaaggaa aggaagaaga agagatacga tgccagagga
15961 agcaaccctt tatcacatca tcatctgctt tcgtcgatca ctccttggcg ctcaccaacc
16021 aatcaatcga gcagctggag acgaacgttt gccacgtcta cgatgcattc cgacggatcg
16081 agcgacaagg attccgatta cgcgagattc caaagccacg cgtttcacca ccggatcgga
16141 gttgcgttac ctcacacggc aatggctctg tcgcgtcatc atcgatgagc agcaccgcac
16201 ctaatcgaac aacgtcgcat aatggaactg aggtagggta tagaggatcg tgccaccccc
16261 acacaaacca tccggatcgg ataggtgcac aaacgacgaa atcgattgag tacagcagtt
16321 cgatggaaga aagcacctcg agcggtattg gtcgaagcaa aagcagcaaa agcagcctct
16381 cgatgtcggg cggaggtagt ccggagcgtg taatcgtgat tgaggacgat gatgaggcag
16441 aagtcacgac gacgcaacat atcgtgccag catttcaaca agatatcacc aagcaggagc
16501 tggaagaaga tgacgatatt tctccggttg ttgctgtgga cgatgcttcg tcaacgtata
16561 tcggtacagc accaatcaca acaccagaac aagcagtgtt gctgcaagat gggttaattt
16621 cggtgcaaga atcatcagag aaaatccaag aaatactaga agaaaatgcc gaaagtacgg
16681 aggcaattga tgaaattgtg tctgattttg aaggggagga ggatgaagcc gatgaagaga
16741 agaagaaaga acaagaagag aaacagaaag atgaagagga agagcctcaa caagatgacc
16801 tctatgtcgc cacgaacccg acgcatcatc aatggcggct ttcgggggaa gcacagagtc
16861 ccgctattaa tctacctcta ccaatcgcct cactaatcag tcatgagagc aataataaca
16921 atctgctggc tagaaagata gagaaccccg agatagtgga ggtagtgcaa tatgataaca
16981 ggatagaaag cgacaaaaag gaacaatcgt tcagcagcga cacttctgtg caacagtcta
17041 cagtttctcc ggggcacata caaacgctag aaggcgccga cacacggcaa agtcccgaac
17101 aacgcaagcg gaagtttgtt agtccggaat catcgatgca gcataagttg caggctaaca
17161 gcgacgaact ggaagtcaca tctgctaact cgcagcctgc cgcatcagag cagccgaagg
17221 acagtcctgg tgcgaaatat ttgccagata acatgctaga gtgcgggttt aaggggatgg
17281 atgcagatga tatctttaga ggatataacg aggacataaa caactgtctg tttcgcggtt
17341 acatgcctga taccaaggaa gtgtatcgac ttaaaatacc gtcaatctat tccgaacatc
17401 aagctccacc ggcgaaacgt ggtcgaattg cggctcggcg acaggaaatt tgttcaaggg
17461 cagacttggt gcgacccgaa attctacgga agttacaacc agcttcttac aactataacc
17521 gaccctatac tagacgccaa gtgaagactt tctaagcttg gagtatcgcc gaagaagagc
17581 aagggagtct tctgggctgg gtggagagat aaagagaaag taaattttca tatatcaatt
17641 gaaaactttc gacttatgta gcgatacaca atgtacatac ttgaatagtg ccgaagttaa
17701 tattaattta ttcctcattt tctatggcat cgccaaagtt ttcgctaatc gagggcccag
17761 gaattgcctc agtagatgct tgtctccctt tgttgttctg tttattctat ttcaatttaa

```
17821 actgtttgta caacaagtta cttttctttt ttgaactttt ttcaaacaga ctactcttac
17881 tgattaaccg ttaagagaac gttgcgttaa atgtttcatt acaccgtgca tcgctctaga
17941 acgatcggag taggacgtag agagcattat tcgtcgaaaa gtagttttag tttgatatcg
18001 cttatttgc aatattatta gtatcacgaa ccacaggaca ccaggatact agagttgcat
18061 gtactagtgg cgtacacaaa taacaaaatt cattagccac aaattgttct gtagttgatg
18121 tacattgttt gcatagttta ggccatcctg ttgtaaatag ttaagcattt tctatgccac
18181 acacattacg gtatcttggt acaggtcaat agaaggaggc tttcttgaaa caggcatgtg
18241 gtttgtacgt taagagttag gaatagttaa aaaggtacac cgaccaaaca caagaagcca
18301 gagtgcgcca ggtttttaaa tagaaaaaca atgaaaaaag tgcacttaat aaggggtacc
18361 cgagttcgag cgcgcgataa tccattaaca gcatcaattg ctgctaccct tgtgtaatac
18421 tacagccgtt gttcgggcta tgcttgcttt cgaacgacga cgattggttt tggtgtgaat
18481 ctcaccatgg ttctaactcg aagtcacatg agggag
```

(SEQ ID NO: 14) GenBank Accession No: ADMH02000012 (Version 1), incorporated herein by reference.

The amino acid sequence of Silk Worm (*Bombyx mori*) IYD is shown below:

```
  1 mniislvvwt lekncfivyt alivcfigft ifhknrldih yrnveikeek lartgpkkke
 61 refpddgddd pelllpaipe dtphipyrpp rksdeeilqk sreyyelmak rrtvrcfsae
121 pipqevldni vktagtapsg ahtepwtfvv vqdpqikeai reiveqeeel nytqrmsrqw
181 vtdlkpfatk pskpylseap alvlvfrqay swrsdgkkrm hyyseistai aagfllaaiq
241 ycglvaltst plnanarlrd llsrpanerl elllpvgrph kettvpdldr kpleeimlki
```

(SEQ ID NO: 15) GenBank Accession No: XP_004925281 (Version 1), incorporated herein by reference.

The nucleic acid sequence of Silk Worm (*Bombyx mori*) IYD is shown below:

```
   1 tacattatgt atacaacagt cggctgaaga actcagccct gagtaaacgt atcgagtaat
  61 ttttaaaaat gaatattata tcgctagttg tttggacatt ggagaagaac tgttttatcg
 121 tgtacacagc attaatagtt tgttttatag gcttcacgat attccacaag aacagattgg
 181 atattcatta caggaatgtt gaaataaagg aagaaaaatt ggcgcgaacc ggcccaaaga
 241 aaaaagaacg tgaattccct gatgatgggg acgatgaccc agagttactc ctgccagcaa
 301 tcccggaaga cactccgcac ataccttacc gacctccgag gaaatccgat gaagaaatcc
 361 ttcagaagtc cagggaatat tatgagctga tggccaaaag gcgaacggtc agatgtttta
 421 gcgctgagcc tatacctcag gaagtcttgg ataatattgt gaagactgct ggcactgcac
 481 catcaggcgc tcacactgaa ccctggacct ttgtagtggt ccaagatcca caaattaagg
 541 aagcaatccg cgagattgta gaacaagaag aggaattaaa ctacacccag aggatgtcca
 601 gacaatgggt gactgatctc aaacctttg ccacaaagcc aagcaaacct tacctatctg
 661 aagcaccagc tttagttctg gtgttcagac aggcgtactc ctggagatca gatggaaaga
 721 aacggatgca ttactatagt gagatcagca cggccatcgc agcaggattt ctgctagcag
 781 ctattcagta ctgcggccta gtggcgctta catcgacacc tctaaatgcc aacgctcgtc
 841 tccgggatct cctctcgaga ccagccaatg agagactgga actccttctg ccggtaggaa
 901 ggccgcacaa ggaaacaact gtacctgatt tagatagaaa gcctttagaa gagatcatgc
 961 ttaagatctg aactgctgct tagttttaat agctcatcca aatgactgaa taaattaact
1021 atacaaaagt g
```

(SEQ ID NO: 16) GenBank Accession No: XM_004925224 (Version 2), incorporated herein by reference.

The amino acid sequence of a wasp (*Nasonia vitripennis*) IYD is shown below:

```
  1 mdtgqrypnn fsstmerads kiatgldafp ffakyghyvl atvvlvsivn lilgryskst
 61 sivskepvas kelspdeefn eyetadgped epylpkdlqh vkfeykrpse aeivkrslef
121 yeiadarrtl rffspdpvpr evirnivraa gtspsgahte pwtfvvvsnp svkenireiv
181 ereeeinylk rmgkkwttdl qplktdwrke ylatapylil vfkqdygflp ngkrkihyyk
241 emsvaiacgi litaiqyagl vtltstplnc gpalrvllnr pvneklvlll pvgypaedat
```

```
301 vpglkrkdld evlvefe
```

(SEQ ID NO: 17) GenBank Accession No: XP_008213524 (Version 1), incorporated herein by reference.

The nucleic acid sequence of a wasp (*Nasonia vitripennis*) IYD is shown below:

```
   1 agtctatgga cacgggacag agatatccga ataatttttc atcgacgatg gagcgggccg
  61 attcgaaaat cgcgaccggc ctcgacgcct tccccttttt cgccaagtac ggccactacg
 121 tcctggcgac agtcgtcctg gtgtcgatcg tcaatctcat cctcggacgt tacagcaaga
 181 gcacctcgat cgtctcgaaa gagcccgtcg ccagcaagga attgagtccg gacgaagagt
 241 tcaacgagta cgagacagcc gacggccccg aggacgagcc gtatttaccg aaggacctgc
 301 agcacgtcaa gttcgagtac aagaggccgt ccgaggcgga gatcgttaag agatcgctgg
 361 aattttacga gatagccgac gccagacgta cgctgcgctt cttcagtcct gacccagttc
 421 cccgcgaagt aattcgcaac atcgtcagag ccgcgggaac ctcccccagc ggagcccaca
 481 ccgagccctg gactttcgtc gtggtgtcga atccctcggt gaaggagaac atccgcgaga
 541 tcgtcgagcg cgaggaggag ataaactacc tcaagcgtat gggcaagaag tggacgaccg
 601 atttgcagcc gctgaagacc gactggcgca aggagtacct cgcgacggcg ccttacctca
 661 ttctcgtctt caagcaggat tacggctttc ttcctaatgg aaagaggaag attcattact
 721 acaaggagat gagcgtcgct attgcctgcg gcatactcat cactgccatt cagtacgccg
 781 gtttggtgac gctgacgtcg acgccgctga actgcggacc ggcactacgc gtcctgctca
 841 acaggccggt caacgagaag ctcgttctgc tgctgcccgt tggttacccg gccgaagatg
 901 ccacggtacc tggtctgaaa agaaaggacc ttgacgaggt gctcgtcgag ttcgagtgac
 961 gctgaagcga cgtgagattc acaatcgaca aaagacatgt ttgactttta taaaaaatgt
1021 agatttttaa caaatacatt gttcgtaagt gagaaaacgc atctttatac tttctgggga
1081 gacagcttga tcggagaaat gcattgcgta tgcaggagga aaaacgcgct gaaatacagt
1141 ttgtataaac taatgtttat tcaaatttgc gtacattatt gttaaaaata agcatgtata
1201 aaaattatat ttaaggacgc atgttagaat caacggagac gcaaattcat tcgtgtgtat
1261 acaattcaat tcacaatctt tatcatcgtt tcttttataa ttcacctcta cgcactgata
1321 aatgatccgt atagctctct cggataccgt atgtatatat gaaaataaag gaaacgtgca
1381 tttcactttt tttacgcgag taccaaatat atatagtcgc caacgatagt gtgacgaagg
1441 agaaataaaa aaaataatca ttaagtacac gacgttccgt tcgttatacg cgtattatat
1501 tatatgtaaa atacaaaaat actgagctaa aacaaccaag gggcgttaga attgttagca
1561 cgctttttt tcatcgttgc agaacttggc tttatcataa atggctttcg ttcttccttc
1621 gtttcgttat aatcttttat tactagactt acacgtatgc tgctaccgag agggactttt
1681 gttttctatg tcattaagtg tagaaatcag aaagtttcac tgtacttaca gattaaaaaa
1741 cgttgttgct ctttttcttt tcattattta ctacattaga gcaattattt tatacgacac
1801 actaaacatt atgcacttgc aatcgaaaat ggccaacaga atcaaggcaa aaatacagtt
1861 tagaaattca ttcggtgatt ttcgtctgta ataaatacta taaagaggca taaaataaat
1921 aatttaccag ctttttatcg cgattcgaca aggaatcgcg aattcatgag ggaacaaact
1981 ctttgcgatg attctggtga cgagttctct ccgttattct ataaaattaa atcttcacgc
2041 ttttacacga ttccattgac gcctaacgca catttacact aatttttcac acgttcttca
2101 agaaaaaaat aaaaaaaaaa taaaaaaaaa cggactacat agaaaataaa actttattcc
2161 aaagttgctt gcttaaacgg gtgtcgaaat tttgaaattt caacaatgat gtacaaagtt
2221 acgcgaacta ggagagtatt tttctacaat aaaaaaagga aatgctctgt ttcatcgata
2281 ttaatctttt tatagttcag gctactttca gtcgtttggt taaaaactac cgcataatgc
2341 tattgtcaac ataatacact tagaattaca agaatttaca agaggtatga gcagatcaaa
2401 atctcgccca acgataggca agaagttaat tattgttata tatatatata tatactatat
2461 atagttacaa ttcttatttt accaaggtgc agcccaatca gcttcgaatc tttcgtcgtt
2521 tcgcgaccga ggttctgaca atgtggaaca aactgtatac gcgagttact aatttaacaa
2581 cgcatcacat ggaaatttca gtcctacagt ctaggggtta aaaacgactt gttagtaaaa
2641 atcattaaaa aaaacctggt tgttttagta gtaaatcaaa aaaatcatcg tggtttagaa
2701 atatcacagc tgtttagatc attcatatat cacttctcca accgtataca aatttcccga
2761 atattcaata agatcgagtg caatgatcga ggtatattgt cagaaagaat agcgctaaaa
2821 cgtaagtcta cacaataaaa gaaacaaaaa aatctaaaaa gttcattcca catttcctta
```

```
2881 tagcaacgtt aggcgcgaca acgatttcca acgactcaca agagaagggc tccaccttga
2941 aataaaaata aaaaaaaaat aaaaaatacg tgcacgcaca cgccgagctt acttacagtc
3001 gcgagaataa acatcagaga tataccgatc ttatgcaacg cgccaaagtt acacatgtgc
3061 atccgatcgg ccgatcgatc gattgttcgc taataattga ggccagaata tatacctacg
3121 tcgacatcag acaaaaggag agaaacgcgc gcggcagact cattaaaaaa tacgcacaat
3181 tattagtcgt ctgagatcat aggatgcctg agaggataga tggatatata gacgtctgac
3241 aaacgttgca ccaatggttt ttcaactatt tcactgagta cgtatttacg taatgttcaa
3301 caaactgtag ctttctgaga ttgaatgcga gagtacaaga atatatatat atatatatat
3361 ataatgtata tgcatcgaag aaggtatgta tatatgtata cgacagattg ttctttgtga
3421 atattataag cgtcattgtt acgacccgtc ctcggtgatg tgtgagcgta agtgtgtatc
3481 tgtgtgtgtg tgtgtgcacc aagtgttgcc ctgcatatat cgcgcgctgt ttggattatt
3541 ccagatcgaa gattatcgat tatattatat gtacaattat caaccctata gtctctataa
3601 gtaactagct gccacgactg aatttcgaag gatagatcga tgagattgat acagaagcac
3661 ccatcagcaa cactcgcgga ctacaagcag cagcagtccc cctaaaagta cacaactgct
3721 tctgtaaacc ctacgctatt atttgttttc tgtttcttct ctctatattt ata
```

(SEQ ID NO: 18) GenBank Accession No: XM_008215302 (Version 1), incorporated herein by reference.

Another exemplary amino acid sequence of mosquito (*Anopheles gambie*) IYD is shown below:

```
  1 masyledlvd yflsssalvt ywnyifpvlv glylaislyd khrygrnkkl ritepaatek
 61 ngskldgiey vgdlehadyd plpaleekph vpyagatvtl aqdpleaaer fyaiannrrs
121 vrkfssrpvd vaivercira agtapsgaht epwtfclvsd psikasvrei ieaeefvnyt
181 qrmakqwvtd lrpirtnhvk eylteaphlv lvfkqtygyk vdgdatakkq hyyneistsi
241 atgmllcafq caglsslvtt plncgpalrt llerppnekl lvllpvgypa ddctvpdlqr
301 kpledilvry
```

(SEQ ID NO: 19) GenBank Accession No: XP_3115442 (Version 4), incorporated herein by reference.

Another exemplary nucleic acid sequence of mosquito (*Anopheles gambie*) IYD is shown below:

```
  1 tgttcttggc gcgcgtcgtt gttgctcggt aacaccccat ccccatccgt
ctgcagagcc
 61 atggcctcct atctggagga tttagtcgac tatttcctca gcagctcggc gctcgttacc
121 tactggaact acatcttccc cgtgctggtg ggactgtacc tggcgatctc cctgtacgac
181 aagcaccggt acggacggaa caagaagctg agaattacgg aaccggcggc aacagagaag
241 aatggcagca agctggacgg tatcgagtat gttggagacc tggaacatgc tgactacgac
301 ccactgcctg cactggagga gaaaccacac gtcccatacg ccggtgcaac ggtcacacta
361 gcccaggacc cactagaggc agccgaacgg ttctacgcca tcgccaacaa tcggcgcagt
421 gtgcgcaagt ttagctcccg cccggtggat gtggccatcg tggagcggtg catccgggcg
481 gccggtacgg cgccaagcgg tgcccacacc gaaccgtgga ccttctgtct cgtgtccgac
541 ccttcgatca aggcgagcgt gcgcgagatc atcgaggccg aggagttcgt caactacacg
601 cagcgaatgg ccaaacagtg ggtgaccgat ctgcggccca ttcgtacgaa ccacgtgaag
661 gagtacctga ccgaggcgcc ccatctggtg ctggtgttca agcagacgta cggctacaag
721 gtggatgggg acgccacggc caagaagcag cactactaca acgaaatatc gacctcgatc
781 gcgaccggca tgctgctgtg cgcgttccag tgtgccgggt tgagctcgct cgttaccacg
841 ccgctcaact gcggccctgc cctgcgcacc ctgctcgagc ggccaccgaa cgagaagctg
901 ctggtgctgc tgcccgtcgg ctacccggcg gacgactgta ccgtgccgga tctgcagcgg
961 aaaccgctgg aagacatcct ggtgcgctat tga
```

(SEQ ID NO: 20) GenBank Accession No: XM_315442(Version 4), incorporated herein by reference.

The amino acid sequence of a house fly (*Musca domestica*) IYD is shown below:

```
      1 meldeilets vflkywnvvl tilligilsk wlwnrkghkq ierednei
ladssskaee
     61 idyapaleek ehiaykgave tlpdgakefy kmmndrrsvr syrrepippi dvienciraa
    121 gtspsgahte pwtfcvitdm eikqsirdii eseeelnyaq rmhkqwttdl rplktnhvkp
    181 yltdapylil vfkqtygvtk qgkrkqhyyn eisvsiavgi llcalqaagl sslvttplnc
    241 gpalrallnr pvnekllvll pvgypaenck vpdlerkqld diivry
```

(SEQ ID NO: 21) GenBank Accession No: XP_005190343 (Version 1), incorporated herein by reference.

The nucleic acid sequence of a house fly (*Musca domestica*) IYD is shown below:

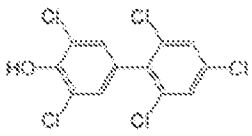
```
   1 catttttgta ttttcattca acgaatatgg aacttgacga aattttggaa acttcggtat
  61 tcctaaaata ctggaatgtt gtactgacaa tattactcat tgggattttg agtaaatggc
 121 tgtggaaccg aaagggtcat aaacaaatcg aaagggaaga tgaagataat gaaattcttg
 181 ccgattccag ttccaaagct gaagaaatag actatgcccc tgctctggaa gaaaaagaac
 241 atattgcata taaaggggca gtggaaactt tacctgatgg tgctaaagaa ttctataaaa
 301 tgatgaacga caggcgtagt gttcgctcat accgccgtga gccaattcca cccatcgatg
 361 taattgaaaa ttgtatacga gctgctggta cctcaccgag tggtgcccat acagaaccct
 421 ggacttttg tgttattacc gacatggaaa taaaacaaag tattcgagat attatcgaat
 481 cggaagagga attaaattat gctcaacgta tgcacaaaca atggacaacg gatctgagac
 541 cgttaaaaac aaatcacgta aaaccatatc taacagatgc tccctattta atattggtat
 601 ttaaacaaac gtatggagtt accaaacaag gcaaacgtaa acaacattat tacaatgaaa
 661 tatccgtatc catagcggtg ggtattctac tgtgtgcctt gcaggcagct ggtttgagtt
 721 ccttggtcac cacaccatta aattgtggcc cagctttacg tgctttgcta aatagaccgg
 781 ttaatgagaa attattggtt ctcttgccag tcggttatcc tgcggaaaat tgtaaagttc
 841 ctgatttgga acgtaagcaa ttagatgata ttattgtcag atattag
```

(SEQ ID NO: 22) GenBank Accession No: XM_005190286 (Version 2), incorporated herein by reference.

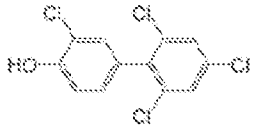
IYD Small molecule inhibitors

Several small molecule inhibitors have shown IYD-inhibitory activity. As such, these small molecules are useful as inhibitors of various IYDs. In humans, these inhibitors can disrupt thyroid hormone homeostasis by blocking iodide recycling through inhibition of IYD activity in the thyroid. Various classes of phenolic compounds comprise: PCBs (polychlorinated biphenyls), PBDEs (polybrominated diphenyl ethers), agrochemicals, antiparasitics, pharmaceuticals and food colorants.

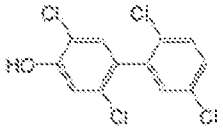
PCB examples include:



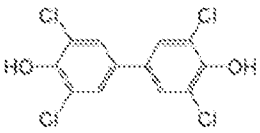
4-OH-2',3,4',5,6'-PCB



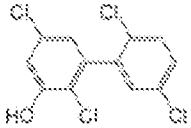
4-OH-2',3,4',6'-TCB



4-OH-2,2',5,5'-TCB

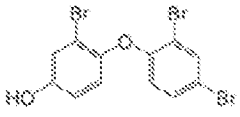


4,4'-diOH-3,3',5,5'-TCB

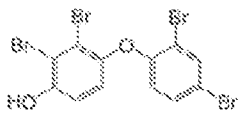


3-OH-2,2',5,5'-TCB

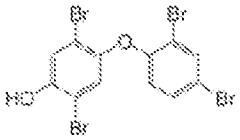
PBDE examples include:



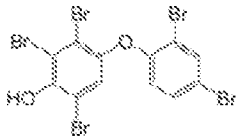
4'-OH-BDE-17



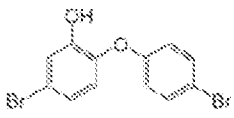
4-OH-BDE-42



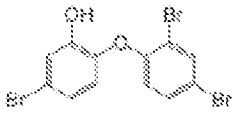
4'-OH-BDE-49



4-OH-BDE90

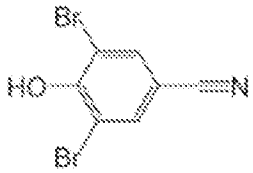


2-OH-BDE-15



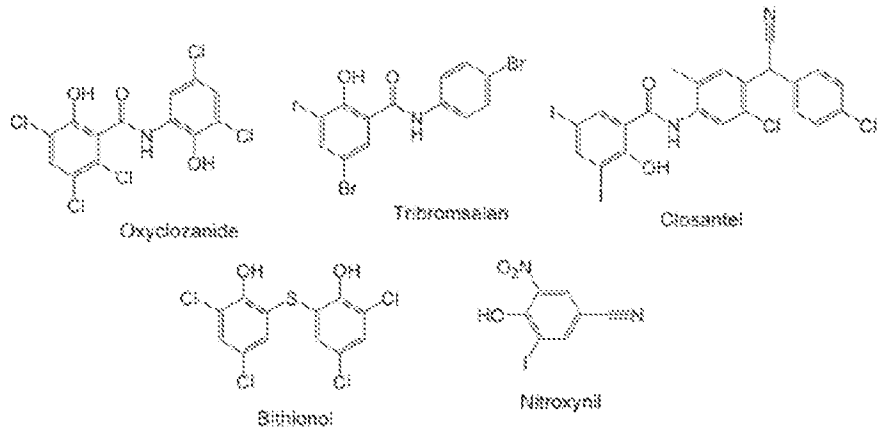
2'-OH-BDE-28

Agrochemical examples include:

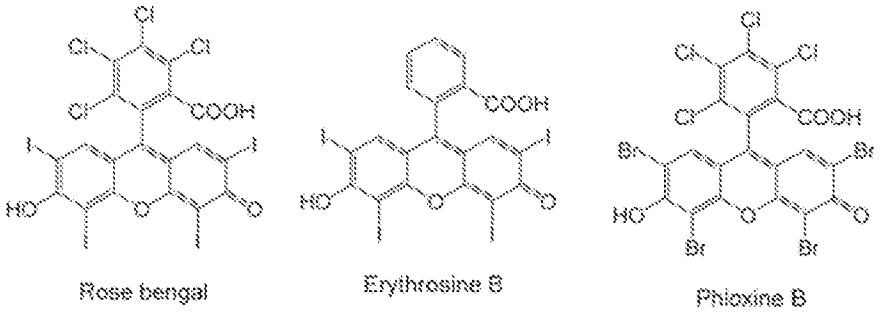


Bromoxynil

Antiparasitic examples include:



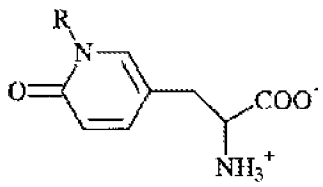
Food colorant examples include:



Competitive IYD small molecules

The pyridonyl amino acids (Table 6) were all observed to be reversible and competitive inhibitors of diiodotyrosine turnover under standard assay conditions (Kunishima, M., et al., *J. Am. Chem. Soc.* 1999, 121, 4722-4723, incorporated herein by reference).

Table 6: Kinetic and binding constants for iodotyrosine deiodinase.



inhibitor	R	K_I (nM)
1	H	1,100 (±100)
2	Me	24 (±8)
3	Et	87 (±13)
4	*i*Pr	400 (±33)

Spermatogenesis

The method described herein comprise administering to the insect a composition comprising an agent that inhibits spermatogenesis (e.g., late stage spermatogenesis) in the insect, thereby preventing, reducing, or inhibiting reproduction in the insect.

Spermatogenesis is the process in which spermatozoa are produced from male primordial germ cells by way of mitosis and meiosis. The initial cells in this pathway are called spermatogonia, which yield primary spermatocytes by mitosis. The primary spermatocyte divides meiotically, Meiosis I, into two secondary spermatocytes; each secondary spermatocyte divides into two spermatids by Meiosis II. These develop into mature spermatozoa, also known as sperm cells. Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes, and the two secondary spermatocytes by their subdivision produce four spermatozoa.

Spermatogenesis begins immediately after meiosis, when spermatids begin to undergo the morphological changes required for sperm development. Elongation of the spermatids occurs within a syncytial cyst that becomes polarized such that all the nuclei localize to one end and the growing ends of the sperm tails are found at the other end. Spermatid elongation is accompanied by dramatic changes in nuclear shape. Following meiosis, round spermatids (alternatively, onion stage spermatids), contain spherical nuclei that are approximately 5 μm in diameter. During sperm development, the nuclei become thinner and the chromatin condenses. Following elongation and nuclear shaping, the mature sperm are invested with their own membranes in a process called individualization. Individualization requires formation of an individualization complex (IC), composed of actin cones that form around the nuclei in a mature spermatid cyst. Following individualization, each group of mature sperm becomes coiled in a process that brings the entire length of the nearly 2mm long sperm bundle into the base of the testis. After coiling, the sperm are released into the testis lumen and are transferred to the seminal vesicle, where they are stored until needed for fertilization.

Common insects

Insects are a class of invertebrates within the arthropod phylum that have an achitinous exoskeleton, a three-part body (head, thorax and abdomen), three pairs of jointed legs, compound eyes and one pair of antennae. They are among the most diverse groups of animals on the planet, including more than a million described species and representing more than half of all known living organisms. The exact number of species is estimated between six and ten million, and potentially represents over 90% of the differing

animal life forms on Earth. Insects may be found in nearly all environments, although only a small number of species reside in the oceans, a habitat dominated by another arthropod group, crustaceans.

An arthropod is an invertebrate animal having an exoskeleton (external skeleton), a segmented body, and jointed appendages. Arthropods form the phylum Arthropoda, and include the insects, arachnids, myriapods, and crustaceans. Arthropods are characterized by their jointed limbs and cuticle made of chitin, often mineralised with calcium carbonate. The arthropod body consists of segments, each with a pair of appendages. The rigid cuticle inhibits growth, so arthropods replace it periodically by moulting. Their versatility has enabled them to become the most species-rich members of all ecological guilds in most environments. They have over a million described species, making up more than 80% of all described living animal species, some of which, unlike most animals, are very successful in dry environments. Like their exteriors, the internal organs of arthropods are generally built of repeated segments. Their nervous system is "ladder-like", with paired ventral nerve cords running through all segments and forming paired ganglia in each segment. Their heads are formed by fusion of varying numbers of segments, and their brains are formed by fusion of the ganglia of these segments and encircle the esophagus. The respiratory and excretory systems of arthropods vary, depending as much on their environment as on the subphylum to which they belong.

Extrapolation of the data herein indicates that inhibiting IYD enzyme in insects (e.g., mosquitos), may provide a strategy for population control. The compositions and methods described herein are useful for inhibiting spermatogenesis in any insect, e.g., cockroaches (e.g., brown-banded cockroaches, German cockroaches, American cockroaches, Oriental cockroaches), beetles (woodworms, death watch beetles, fur beetles, varied carpet beetles, spider beetles, mealworm beetles, centipedes, red flower beetle, pine beetle), earwigs, flies (e.g., bottle flies, blue bottle flies, green bottle flies, house flies, fruit flies, horse flies, black flies, deer flies, house flies, teste flies), cicadas, hoppers, ants (e.g., Argentine ants, carpenter ants, fire ants, odorous house ants, pavement ants, pharaoh ants, thief ants, a leafcutter ant, a mountain a fireant, a Jerdon't jumping ant), aphids, bed bugs, bees (e.g., a common eastern bumble bee, a bumble bee, a honeybee, an alfalfa leafcutter bee), wasps, mantids, earthworms, terrestrial crabs, firebrats, fleas, snails, slugs, crickets (e.g., house crickets), hornets, locusts, lice, moths (e.g., almond moths, Indianmeal moths, cloths moths, gypsy moths (common clothes moths), brown house moths), red spiders, silverfish, woolice,

termites (e.g., dampwood termites, subterranean termites), ticks, wasps, mosquitos, gnats, millipedes, stink buds, weevils, mites, silk worm, daphnia, fleas, Assassin bugs (kissing bugs), eye gnats, or rat fleas and woolly bears (e.g., hairy caterpillar).

The compositions and methods are also useful in inhibiting reproduction in the following insects: *Culex quinquefasciatus* (Southern house mosquito), *Drosophila willistoni*(Fruitfly), *Drosophila mojavensis* (Fruitfly), *Drosophila pseudoobscura* (Fruitfly), *Drosophila grimshawi* (Fruitfly), *Drosophila virilis* (Fruitfly), *Drosophila ananassae* (Fruitfly), *Ceratitis capitata* (Mediterranean fruitfly), *Drosophila sechellia* (Fruitfly), *Drosophila persimilis* (Fruitfly), *Drosophila simulans* (Fruitfly), *Drosophila melanogaster* (Fruitfly), *Anopheles darling* (Mosquito), *Anopheles gambiae* (Mosquito), *Musca domestica* (Housfly), *Drosophila erecta* (Fruitfly), *Drosophila yakuba* (Fruitfly), *Aedes aegypti* (Mosquito, yellow fever), *Bombyx mori* (Silk worm), *Tribolium castaneum* (red flour beetle), *Nasonia vitripennis* (wasp), *Apis mellifera* (honeybee), *Apis dorsata* (honeybee), *Megachile rotundata* (alfalfa leafcutter bee), *Dendroctonus ponderosae* (mountain pine beetle), *Bombus impatiens* (common eastern bumble bee), *Bombus terrestris* (bumble bee), *Acromyrmex echinatior* (leafcutter ants), *Solenopsis invicta* (fireant), *Apis florea* (honeybee), *Harpegnathos saltator* (Jerdon's jumping ant), *Cerapachys biroi* (ant), *Daphnia pulex* (daphnia), *Camponotus floridanus* (ant), and *Pediculus humanus corporis* (lice).

Blocking insect reproduction

The methods described herein offer a unique and selective approach for controlling insect population without damage to the environment. Inhibiting this enzyme in insects, (e.g., mosquitos) provides a new strategy for insect population control, i.e., reducing the number of insects.

Also provided is the identification of chemical, e.g., small molecule, inhibitors of IYD. The methods described herein involve selective application of an agent within the environment to selectively target insects (e.g., mosquitos) for controlling insect population, i.e., reducing the number of insects. Alternatively, the agent may be selectively placed such that the insect of interest is the only organism susceptible to population control.

One means of population control in insects is an insect growth regulator (IGR), which is a chemical substance that inhibits the life cycle of an insect, and is commonly used to control populations of harmful pests. IGRs prevent an insect from reaching maturity by interfering with its molting process (for example, azadirachtin, hydroprene, methoprene, pyriproxyfen and tritlumuron). This results in immature insects that cannot reproduce.

Hormonal IGRs mimic or inhibit one of the two major hormones involved in insect molting. Chitin synthesis inhibitors prevent the formation of chitin, a carbohydrate used to form the insect's exoskeleton. As described herein, in some cases, the compositions of the invention are administered in conjunction with IGRs.

Additionally, insecticides are substances that are used to kill insects, and are used in agriculture, medicine, industry, and by consumers. Two major groups include systemic and contact insecticides. Systemic insecticides become incorporated and distributed systemically throughout the whole plant. When insects feed on the plant, they ingest the insecticide. Systemic insecticides have activity pertaining to their residue which is called "residual activity" or long-term activity. Contact insecticides are toxic to insects upon direct contact. These can be inorganic insecticides, which are metals and
include arsenates, copper and fluorine compounds, which are less commonly used, and the commonly used sulfur. Contact insecticides can be organic insecticides, i.e. organic chemical compounds, synthetically produced, and comprising the largest numbers of pesticides used today, or, they can be natural compounds like pyrethrum, neem oil etc. Contact insecticides usually have no residual activity. Some insecticides kill or harm other organisms in addition to those they are intended to kill. For example, birds may be poisoned when they eat food that was recently sprayed with insecticides or when they mistake an insecticide granule on the ground for food and eat it.

Dosages and frequency of administration of pest-controlling agent

The effective amount of the agent is from 0.001 mg/kg to 250 mg/kg body weight, e.g., 0.001 mg/kg, 0.005 mg/kg, 0.01 mg/kg, 0.05 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 225 mg/kg, or 250 mg/kg body weight. The agent is more effective with some insects than others. Amounts of some of the agents may be as low as 2.5 milligrams per kilogram of body weight, and possibly less. Even smaller amounts, for instance as little as 0.05 milligrams per kilogram of body weight are effective when the dosage is continued from day to day as may be desirable in some circumstances.

One treatment (dosage) may also be sufficient to alter such transient insects as mosquitoes and flies. Within a few weeks or months after treatment, it may become apparent that repeated dosages may be required to control insects, e.g., mosquitoes over a long period of time. As such, this type of insect control may be desired in particular circumstances over long periods of time. IYD inhibitors are not contact toxins, i.e., adult insects exposed to IYD

inhibitors will likely not die; however, their reproduction will be blocked. Male mosquitos live ~ 5 days and females can live for weeks, thus, the results of treatment may take days to weeks to detect in mosquitos. The effect depends on the lifetime of each target insect.

Insect traps

The In2Care® Mosquito Trap uses a unique mixture of bio-actives that target not only the mosquito and its larvae, but can also have an impact on the Dengue virus inside the mosquito. Trap activity is not limited to the trap itself, but extends to the surrounding area due to its mosquito-driven larvicide spreading features. The trap actives provide an environmentally-friendly alternative to synthetic pesticides and combines active ingredients that individually are used in other mosquito products which are WHO recommended and/or US-EPA approved.

Similar traps are utilized in the compositions and methods described herein. For example, the compositions are administered within a device, e.g., a "trap," that attracts an insect to the device in order to administer the compositions described herein to the insect.

In one case, an egg-laying female is attracted to the trap, where it picks up biological agents within the trap to prevent, inhibit, or reduce insect reproduction in a male insect. In other cases, a male insect is attracted to the trap, where it picks up biological agents within the trap to prevent, inhibit, or reduce insect reproduction. Preferably, the agent inhibits spermatogenesis (e.g., late stage spermatogenesis). For example, the agent that inhibits spermatogenesis in the insect comprises an agent that inhibits expression or activity of IYD in the insect, e.g., the agent inhibits expression or activity of IYD in a testis of the insect.

EXAMPLES

Example 1: Protein co-crystal structure of mouse IYD in complex with monoiodotyrosine (IYD-MIT) identified key amino acids

The structure of IYD from mouse and human identified key amino acids that were used to query databases of genomic information to identify the presence of the IYD gene in other organisms (Phatarphekar, A. et al., *Mol. Biosys.* 2014, 10, 86-92). The co-crystal structure of mouse IYD-MIT showed that E153, Y157 and K178 were key residues for substrate recognition (Figure 2A and Figure 2B). T235 provided an important hydrogen bond to the N5 of FMN (Flavin mononucleotide) (Figure 2B). These residues provided markers to identify IYD homologs through the tree of life. The key interacting residues, E153, Y157 and K178 were located on one monomer of the dimer, whereas the FMN molecule was

positioned between the monomers, and the adenine tail further extended into the adjacent monomer. Hydrophobic interactions between the phenyl of the MIT (monoiodotyrosine) and the flavin rings of FMN may contribute to the overall protein-ligand interaction. The structural results indicated that a small set of residues was sufficient to specify IYD activity.

Example 2: IYD Homologs are present in diverse organisms

A sequence alignment between several different organisms (human, mouse, zebrafish, lancelet, drosophilia, daphnia, sea anemone, hydra, bacteria, archaea and bacterial BluB) showed the presence of IYD homologs (Figure 3). All vertebrates and invertebrates sequenced contain IYD. Several key residues were conserved among the organisms. For example, a tryptophan residue in the active site lid, which was responsible for substrate binding. Additionally, a glycine residue within the active site lid is also conserved among the organisms. Determinant IYD residues (glutamic acid, tyrosine, and lysine) were conserved among the organisms and are outlined in red in Figure 3.

Example 3: IYD Exhibits deiodinase activity

Selected orthologs were expressed in *E. coli* and were tested for deiodination activity. All of the orthologs displayed similar catalytic efficiency for deiodination of I_2-Tyr with k_{cat}/K_m values within an order of magnitude of each other (Table 1). However, prior to the invention described herein, the function of halogenated tyrosines and the biological role of IYD was unknown in organisms such as invertebrates that are not know to produce TH. *See*, Phatarphekar, A., et al., Mol. BioSyst. 2014, 10, 86-92, incorporated herein by reference, for a discussion linking iodotyrosine deiodinase genes with deiodinase activity in organisms not known to require iodide or respond to the iodide-containing hormone thyroxine (including the first insect, honey bee).

All the proteins containing the signature residues (E153, Y157 and K178) promoted deiodination of DIT (diiodotyrosine), with similar K_m, k_{cat} and k_{cat}/K_m values) (Figure 4). For example, the k_{cat}/K_m in humans was 0.40 ± 0.08 μM/min, and 0.56 ±0.09 μM/min in drosophilia. The closest structural homolog, BluB (vitamin B_{12} biosynthesis), did not exhibit deiodinase activity (i.e., the K_m for diiodotyrosine was ≥ 133 μM). BluB, however, does not contain the signature, key residues (E153, Y157 and K178). Steady-state kinetic characterization (K_m, k_{cat} and k_{cat}/K_m) of dehalogenation catalyzed by IYD homologs (intermediate and soluble domains) from representative organisms from diverse phyla is shown in Table 1. Kinetic constants were determined by quantification of ^{125}I- released from [^{125}I]-I_2-Tyr (Goswami, A.; Rosenberg, I. N., *Endocrinology* 1977, 101, 331-341, Gnidehou,

S.et al., *FASEB J.* 2004, 18, 1574-1576 and Rokita, S. E. et al., *Biochimie* 2010, 92, 1227-1235). Reductive deoidination of iodotyrosine was common to a diverse set of organisms.

Table 1: Kinetic characterization of IYD homologs (intermediate and soluble domains)

Organism	**K_m (μM)**	**k_{cat} (min^{-1})**	**k_{cat}/K_m ($min^{-1}μM^{-1}$)**
Human[a]	31 ± 6	12.5 ± 1.0	0.4 ± 0.10
Mouse[b]	9 ± 1	4.5 ± 0.7	0.49 ± 0.09
Zebrafish[c]	8 ± 1	4.1 ± 0.2	0.51 ± 0.07
Lancet[c]	6 ± 3	7.0 ± 1.0	0.14 ± 0.04
Drosophila[d]	13 ± 2	7.1 ± 0.2	0.55 ± 0.09
Honeybee[c]	29 ± 7	8.0 ± 1.0	0.28 ± 0.07
Daphnia[c]	7 ± 1	17.6 ± 0.8	2.50 ± 0.40
Sea Anemone[c]	105 ± 26	32.0 ± 4.0	0.30 ± 0.08
Hydra[c]	4 ± 1	13.0 ± 1.0	3.50 ± 0.90
Haliscomenobacter hydrossis[c]	6.6 ± 0.9	5.4 ± 0.3	0.8 ± 0.10
Archaea (60°C)[d]	1.8 ± 0.3	5.5 ± 0.2	3.10 ± 0.5
Archaea (25°C)	1 ± 0.1	0.44 ± 0.08	0.4 ± 0.1

[a]See, Hu, J.; et al., *J. Biol. Chem.* 2015, *290*, 590-600. [b]See, Thomas, S. R.et al., *J. Biol. Chem.* 2009, *284*, 19659-19667. [c]See, Phatarphekar, A. et al., Mol Biosys. 2014, 10, 86-92.

IYD orthologs were present in all 13 species of fruit fly including *Drosophila melanogaster* that have been sequenced to date. Orthologs of IYD were also found in most all genomes of other insects including the disease carrying vectors such as *Anopheles gambiae* (malaria) and *Aedes aegypti* (yellow fever). Using Drosophila as a genetic model, the biological role of IYD in invertebrates was investigated.

Example 4: Tissue distriubtion of IYD in drosophila

The tissue expression of IYD mRNA in *Drosophila melanogaster* (fly) was disproportinally exressed in the testes as compared to other tissues (e.g., the male accessory gland, the ovaries, the trachea, the heart, and salivary glands) (Figure 5). Tissue specific mRNA expression profiling indicated very high levels of IYD (gene CG6279 in flybase) in adult male testes (Figure 5) (Chintapalli, V. R.; Wang, J.; *Nat. Genet.* 2007, 39, 715-720). The high IYD expression in the testes was observed in adult drosophilia, as compared to larva which are nearly devoid of IYD. High levels of expression in the testes was surprising, and

was further investigated (Figure 8). CG6279 was strongly expressed in testis during later stages of germline development, especially post differentiation (after meiosis).

IYD is a membrane bound enzyme in humans, and is primarily expressed in thyroid tissue. In humans, IYD assists in the recovery of iodide from mono- and diiodotyrosine (MIT, DIT) generated during thyroxine biosynthesis. IYD is crticial for spermatogenesis in insects, but not mammals. Thus, the lack of IYD in mammalian testes makes IYD an attractive target for controlling insect populations without harming mammals, including humans.

Example 5: RNAi knockdown of IYD in Drosophila testis

Selective suppression of a chosen gene *in vivo* can be performed through applications of RNAi. Application of RNAi in *Drosophila* provided the first confirmation that IYD was directly involved in spermatogenesis. As described herein, suppression of this gene produced male sterility and developmental anomalies as evident when imaging the testes. Similar effects are expected in other Diptera (e.g., flies, mosquitos, etc.) since all insects sequenced to date contain a gene for IYD. In particular, IYD from *Drosophila* and *Anopheles* share 67% identity and 82% similarity.

RNAi-based suppression using double-stranded RNA (dsRNA)

A standard method for inducing RNAi-based suppression in *Anopheles* is to inject double-stranded RNA (dsRNA) directly into the body cavity. Thus, a series of dsRNAs complementary to the IYD gene are prepared and tested for their ability to suppress IYD *in vivo*. Not all dsRNA have the same efficiency to suppress the gene and hence multiple dsRNA are tested. If fertility does not diminish with this treatment, the expression of IYD in testes is measured with qPCR to confirm its presence. Additionally, direct injection may not necessarily suppress genes in the testes.

If necessary, dsRNA is also injected along with a fluorescent marker gene into embryos to generate transgenic species with the innate ability to suppress IYD expression. As described herein, a similar strategy was used in *Drosophila* and placed RNAi suppression of IYD under control of a series of promoters induced at different stages of spermatogenesis. Loss of IYD activity is observed *in vivo* as a loss of fertility and fecundity. Again, qPCR is used to correlate gene expression with the observed phenotype.

Promoter dependent knockdown of IYD

Expression of IYD mRNA was decreased using promotor dependent knockdown (Figure 6A, Figure 6B, and Figure 6C). Promoters used for IYD knockdown are shown in Table 2.

Table 2: Promoters used for IYD knockdown

Promoter	Cell Type	Expression Stage	Fertility, Fecundity affected?
nos	germ	early	no
bam	germ	late	no
c587	somatic	early	no
tj	somatic	early	no
eya	somatic	late	yes

As described herein, *Drosophila* IYD plays a key role in spermatogenesis, and disruption of IYD dramatically lowered *Drosophila* fertility and fecundity (Figure 6B). IYD was found to be crucial to sperm development, and suppression by RNAi disrupted the elongation of spermatocytes and blocked sperm formation. The average number of progeny in eya-GAL4/UAS-dmIYD-RNAi resulted in 7 sterile *Drosophila* samples, whereas the control *Drosophila* testes, eya-GAL4 and UAS-dmIYD-RNAI resulted in no sterile *Drosophila* (Figure 6B). The additional promoters evaluated, nos, bam, c587, and tj did not affect fertility and fecundity in drosophilia.

Iodotyrosine may generate as a signal during maturation, and thus IYD may be responsible for turning the signal off. These RNAi data provide an indication that insects may use iodination/deiodination of tyrosine for regulating certain developmental processes.

Example 6: Late stage development is sensitive to IYD

Spermatogenesis in drosophila begins with mitotic division (alternatively, spermatogonia), of germ cells to somatic cells (Figure 7A). Before the transition to meiotic division, the expression of dmIYD (drosophilaIYD) is at its peak. Within meiotic division, a spermatocyte evolves into a round spermatide, at which point developmental abnormalities ensue. Elongated spermatids are generated following the formation of the round spermatids.

After RNAi degradation of IYD mRNA, abnormalities were detected in dosophilia testis (Figure 7B). In control testis, nearly 100% showed no phenotype, whereas in IYD RNAi samples, over 80% of the testis showed an abnormal phenotype.

Using phase contrast images of drosophila testis, a considerable difference in control samples versus RNAi knockdown of IYD samples was highlighted (Figure 7C). In control

samples, a sperm reservoir was developed, whereas in the RNAi sample, no sperm reservoir was developed. The reservoir serves to ensure successful fertilization by providing the appropriate number of sperm in in the appropriate physiological state for fertilization. Development of the sperm reservoir is distinctive of late stage development. No sperm reservoir was developed in IYD RNAi drosophilatestis, indicating that suppression of dmIYD disrupted late stage spermatogenesis.

Example 7: Strong expression of isoforms A and B of CG6279 in late stages of spermatogenesis.

Two isoforms have been reported for *Drosophila* IYD (dmIYD) in the NCBI databases. These two are related by alternative splicing of the CG6279 gene. Isoform B resembles IYD orthologs from other organisms and includes a membrane domain, an intermediate domain and a catalytic domain (Phatarphekar, A. et al., *Mol. Biosys.* 2014, 10, 86-92 and Friedman, J. E. et al. *J. Biol. Chem.* 2006, 281, 2812-2819). Isoform A is unique to the *Drosophila* genus by the added presence of a large N-terminal domain (470 amino acids) that does not coincide with any known protein domains listed in the conserved domain database (CDD).

In situ hybridization experiments were conducted with male *Drosophila* testes, using sequence specific probes (riboprobes) targeting distinct regions of isoform A and B of CG6279 to identify the mRNA expression pattern as well as the stage of mRNA expression during spermatogenesis. *In situ* hybridization revealed strong expression during the later stages of spermatogenesis, and both riboprobes revealed similar expression patterns (Figures 9A, Figure 9B, Figure 9C, and Figure 9D).

Example 8: Binding affinities of dmIYD Isoform B using various dehalogenation ligands.

Isoform B of the drosophila enzyme (dmIYD) has been cloned, expressed and purified to homogeneity following standard protocols (Phatarphekar, A. et al., *Mol. Biosys.* 2014, 10, 86-92). This enzyme displayed similar binding affinities for iodo-, diiodo-, bromo- and chlorotyrosine (I-Tyr, I_2-Tyr, Br-Tyr and Cl-Tyr, respectively), but a 30-fold lower affinity for fluorotyrosine (F-Tyr), as shown in Table 3. Error represents the standard deviation from an average of 3 independent experiments.

Table 3: Dissociation constants (K_D) for halotyrosines with dmIYD

Ligand (dmIYD)	**K_D (μM)**
I_2-Tyr	0.55 ± 0.08
I-Tyr	0.62 ± 0.09

Br-Tyr	0.47 ± 0.06
Cl-Tyr	0.46 ± 0.08
F-Tyr	17.60 ± 0.80

The enzyme also catalytically dehalogenated all the halotyrosine ligands but F-Tyr. The catalytic efficiency (k_{cat}/K_m) for deiodination and debromination was similar and both were 3-4 fold more efficient than dechlorination, as shown in Table 4. (*) indicated I_2-Tyr dehalogenation measured by [^{125}I]-iodide released from [^{125}I]-I_2-Tyr.

Table 4: Steady-state kinetics of dehalogenation catalyzed by dmIYD

Ligand (dmIYD)	**K_m (μM)**	**k_{cat} (min^{-1})**	**k_{cat}/K_m (min^{-1} μM^{-1})**
I_2-Tyr*	13.2 ± 2.0	7.1 ± 0.2	0.55 ± 0.09
I-Tyr	14.4 ± 4	7.3 ± 0.6	0.50 ± 0.20
Br-Tyr	8 ± 2	4.2 ±0.3	0.50 ± 0.10
Cl-Tyr	21. ± 6	3.0 ± 0.3	0.14 ± 0.14
F-Tyr	N/A	<0.002	N/A

Substrate turnover measured by production of tyrosine using HPLC separation and detection

Catalytic deiodination of halotyrosines were measured by detecting and quantifying the tyrosine produced in presence of enzyme using reverse phase HPLC. The buffer used for reaction is the same as that used for the [^{125}I]-release assay as well as for equilibrium binding measurements (100 mM potassium phosphate, 200 mM KCl at pH 7.4). Each reaction (final volume of 1 ml) was initiated be addition of 0.5% dithionite (100 μl of 5% dithionite in 5% aqueous sodium bicarbonate) rather than the 1% dithionite used for the [^{125}I]-release assay. This change minimized interference between tyrosine and dithionite during HPLC separation. The total enzyme used per reaction was 0.08 μM and the duration of the reaction was 15-20 mins. M-cresol (30 μM) was added to each reaction tube as the internal standard (IS). The reaction was quenched with formic acid (5% final concentration) and the entire reaction mixture was injected manually onto a Microsorb MV 300-5 C18 column analytical HPLC column (Agilent) protected with a manually packed C18 silica guard column. The HPLC method used for analysis is provided in Figure 10.

The concentration of tyrosine produced by IYD was be determined from a standard curve in which known concentrations of tyrosine were spiked into reaction mixtures not

containing the halotyrosine but otherwise identical. The ratio of the areas of tyrosine peak to the internal standard peak was plotted against the concentration of tyrosine spiked into the reaction and the best fit line was determined by linear regression. This equation of the line was used to determine the concentration of tyrosine produced in μM from a ratio of peak areas of tyrosine to the internal standard.

Example 9: Site-directed mutagenesis on dmIYD identified active site amino acid residues critical for catalysis

Site-directed mutagenesis studies on dmIYD were conducted to identify active site amino acid residues that are critical for catalysis. The E154Q mutation was most detrimental to catalytic efficiency of dmIYD, as shown in Table 5 and may provide a functional knockout mutation in continuing efforts to define the full role of dmIYD *in vivo*. Kinetic constants were determined by [^{125}I]-iodide released from [^{125}I]-I_2-Tyr.

Table 5: Steady-state kinetics for dehalogenation catalyzed by active site mutants of dmIYD.

Mutant	**K_m (μM)**	**k_{cat} (min^{-1})**	**k_{cat}/K_m (min^{-1} μM^{-1})**
E154Q	3400 ± 500	2.4 ± 0.2	0.0007 ± 0.0001
K179Q	700 ± 100	7.7 ± 0.5	0.0110 ± 0.0020

Example 10: The significance of the CG6279 for spermatogenesis is confirmed using CRISPER-CAS9 and site-directed mutation studies

Genetic experiments are conducted using CRISPR-CAS9 technology to remove the entire CG6279 gene from the *Drosophila* genome. Additionally, a loss of function mutation with an E154Q mutation at the genomic level is generated. These experiments confirm the significance of the CG6279 gene for spermatogenesis.

Example 11: Feeding studies using halotyrosines are performed to determine their effects on fertility and spermatogenesis

Feeding studies are performed using halotyrosines added to the standard food for drosophila to determine their effects on fertility and spermatogenesis. This determines whether or not dietary supplements mimic the effect of inhibiting IYD since both allow an unnatural high concentration of halotyrosine to accumulate *in vivo*.

Example 12: Use of small molecule inhibitors in feeding traps to inhibit reproduction of insects

The feasibility of controlling insect (e.g., mosquito) population by an inhibitor of IYD is assessed through a combination of chemistry, biochemistry and biology. The most

efficient inhibitor of IYD requires a 3-step synthesis that is available from the literature. This compound is a pyridone analog of iodotyrosine and previously designed as a mimic of an intermediate formed during enzyme catalysis. Its high affinity was originally measured with porcine IYD. However, the active site structure and catalytic mechanism of all IYDs are similar enough that this inhibitor is effective against IYD from insects. This compound along with others available commercially is used for inhibition studies *in vitro* and *in vivo*.

The sequence of IYD from *Anopheles gambiae* is available, but has not yet been studied. Data assembled from other organisms strongly predict its ability to catalyze deiodination of I_n-Tyr (n= 1,2). The gene is synthesized commercially, expressed heterologously and characterized for its catalytic specificity. Additionally, the enzyme's sensitivity to the known inhibitors, nitrotyrosines and the pyridone derivative, is measured *in vitro* to confirm their potency before use as feeding supplements.

Feeding studies to measure the effectiveness of simple inhibitors to disrupt fertility

The ability to control mosquito fertility in the field is expedited by easy access to inhibitors of IYD already described in the literature. Nitro- and dinitrotyrosine were identified decades ago as potent inhibitors of IYD *in vitro*, and more recently a pyridone analog of iodotyrosine was found to maximize affinity to IYD based on the mechanism of catalysis common to all orthologs. These compounds are purchased and synthesized respectively for supplementing mosquito food and are even be tested by direct injection into the body cavity if necessary. Measuring a dose-dependent response establishes the minimum concentration necessary to observe the diminished fertility and fecundity. Since suppression of IYD and the presumed buildup of I- and I_2-Tyr are detrimental to *Drosophila*, mosquitos are also fed with media containing increasing amounts of I- and I_2-Tyr in an attempt to overwhelm the catalytic capacity of the native IYD. These treatments recapitulate the expected results of the RNAi investigation.

The most cost effective supplement that suppresses sperm development is then tested in the field. If disruption of I- and I_2-Tyr levels is a basis for the sterility, then inhibiting the initial formation of I and I_2-Tyr also block spermatogenesis. Biosynthesis of such halogenated amino acids is typically promoted by a peroxidase. Consequently, peroxidase inhibitors such as thiourea, methimazole and propylthiouracil are tested as dietary supplements to inhibit fertility as well. Already, methimazole and propylthiouracil are used as safe and effective drugs for controlling hyperthyroidism. They act by inhibiting the iodination step of thyroxine biosynthesis.

Utilizing commercially available female mosquito traps (e.g., In2Care®), an agent that reduces activity or expression of IYD is added to the media to inhibit reproduction of subsequent generations of mosquitos. Larvae eat much more than adults, so spiking a trap like those of "In2Care®" will reach the larvae. Male mosquitoes live on sugar water (e.g., rotting fruit), so in some cases, an agent that reduces activity or expression of IYD is added to a liquid composition to inhibit the reproduction of male mosquitoes.

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

What is claimed is:

1. A method of preventing, reducing, or inhibiting insect reproduction comprising:

administering to the insect a composition comprising an agent that inhibits spermatogenesis in the insect,

thereby preventing, reducing, or inhibiting reproduction in the insect.

2. The method of claim 1, wherein the agent that inhibits spermatogenesis in the insect comprises an agent that inhibits expression or activity of iodotyrosine deodinase (IYD) in the insect.

3. The method of claim 2, wherein the agent inhibits expression or activity of IYD in a testis of the insect.

4. The method of claim 3, wherein the IYD inhibits late stage spermatogenesis.

5. The method of claim 1, wherein the method reduces a population size of the insect.

6. The method of claim 1, wherein fecundity of the insect is decreased.

7. The method of claim 1, wherein fertility of the insect is decreased.

8. The method of claim 1, wherein the agent for inhibiting expression or activity of IYD comprises an antibody or fragment thereof, a binding protein, a polypeptide, or any combination thereof.

9. The method of claim 1, wherein the agent for inhibiting expression or activity of IYD comprises a small molecule.

10. The method of claim 9, wherein the small molecule for inhibiting expression or activity of IYD comprises 4-OH-2',3,4',5,6'-PCB; 4-OH-2',3,4',6'-TCB; 4-OH-2,2',5,5'-TCB; 4,4'-diOH-3,3',5,5'-TCB; 3-OH-2,2',5,5'-TCB; 4'-OH-BDE-17; 4-OH-BDE-42; 4'-OH-BDE-49; 4-OH-BDE90; 2-OH-BDE-15; 2'-OH-BDE-28; Bromoxynil; Oxyclozanide; Tribromsalan; Closantel; Triclosan; Bithionol; Nitroxynil; Benzbromarone

Rose Bengal; Erythrosine B; Phloxine B; D,L-3-(2-pyridon-5-yl)alanine; and D,L-3-(N-methyl-2-pyridon-5-yl)alanine; D,L-3-(N-ethyl-2-pyridon-5-yl)alanine; and D,L-3-(N-isopropyl-2-pyridon-5-yl)alanine.

11. The method of claim 1, wherein the agent comprises a nucleic acid molecule.

12. The method of claim 11, wherein the nucleic acid molecule comprises dsRNA, shRNA, or antisense RNA, or any portion thereof.

13. The method of claim 1, wherein the agent is administered as a spray.

14. The method of claim 1, wherein the agent is administered topically, orally, via inhalation, or via injection.

15. The method of claim 12, wherein the composition is in the form of a gas, a liquid, a solid, or a semi-solid.

16. The method of claim 1, wherein the agent is soluble in water.

17. The method of claim 1, wherein the agent is nontoxic.

18. The method of claim 14, wherein the composition comprises sugar water.

19. The method of claim 1, wherein the agent is administered at a concentration of 0.001 mg/kg to 250 mg/kg of body weight

20. The method of claim 1, wherein the agent is administered once per day.

21. The method of claim 1, wherein the insect comprises a cockroach, a beetle, an earwig, a fly, a cicada, a hopper, an ant, anaphid, a bed bug, a bee, a wasp, a mantid, an earthworm, a terrestrial crab, a firebrat, a flea, a snail, a slug, a cricket, a hornet, a locust, lice, a moth, a red spider, a silverfish, a woolice, a termite, a tick, a wasp, a mosquito, a gnat, a millipede, a

stink bug, a weevil, a mite, a silk worm, a daphnia, a flea, an assassin bug, an eye gnat, a rat fleaor a woolly bear (hairy caterpillar).

22. The method of claim 1, wherein the insect comprises *Culex quinquefasciatus*, *Drosophila willistoni*, *Drosophila mojavensis*, *Drosophila pseudoobscura*, *Drosophila grimshawi*, *Drosophila virilis*, *Drosophila ananassae*, *Ceratitis capitata*, *Drosophila sechellia*, *Drosophila persimilis*, *Drosophila simulans*, *Drosophila melanogaster*, *Anopheles darling*, *Anopheles gambiae*, *Musca domestica*, *Drosophila erecta*, *Drosophila yakuba*, *Aedes aegypti*, *Bombyx mori, Tribolium castaneum*, *Nasonia vitripennis*, *Apis mellifera*, *Apis dorsata*, *Megachile rotundata*, *Dendroctonus ponderosae*, *Bombus impatiens*, *Bombus terrestris*, *Acromyrmex echinatior*, *Solenopsis invicta*, *Apis florea*, *Harpegnathos saltator*, *Cerapachys biroi*, *Daphnia pulex*, *Camponotus floridanus*, or *Pediculus humanus corporis*.

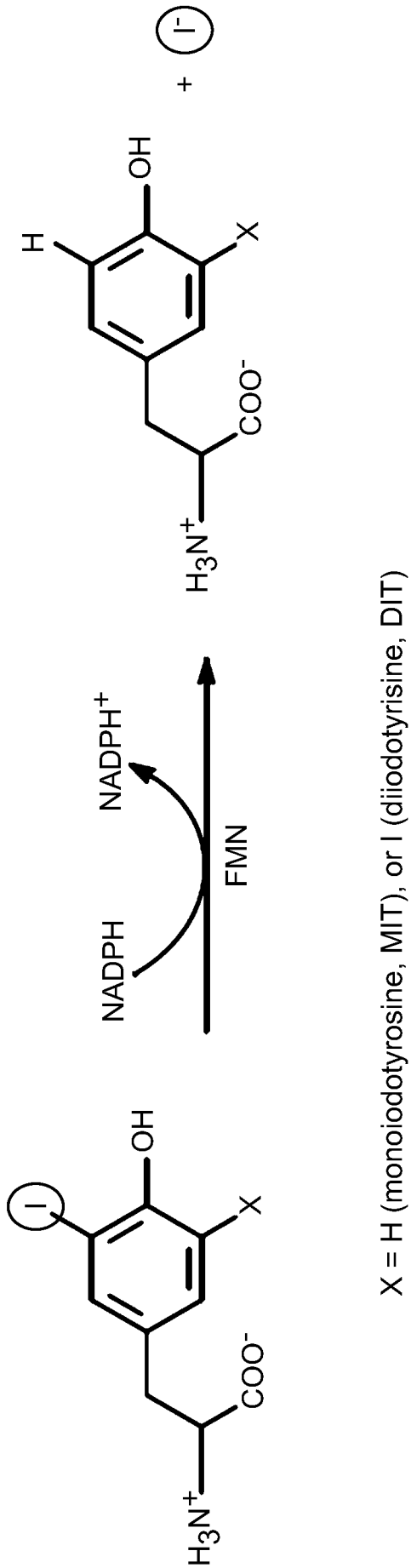

I⁻
+
OH
H
X
COO⁻
H3N+
NADPH+
NADPH
FMN
OH
I
X
COO⁻
H3N+
X = H (monoiodotyrosine, MIT), or I (diiodotyrisine, DIT)

FIG. 1

FIG. 2A

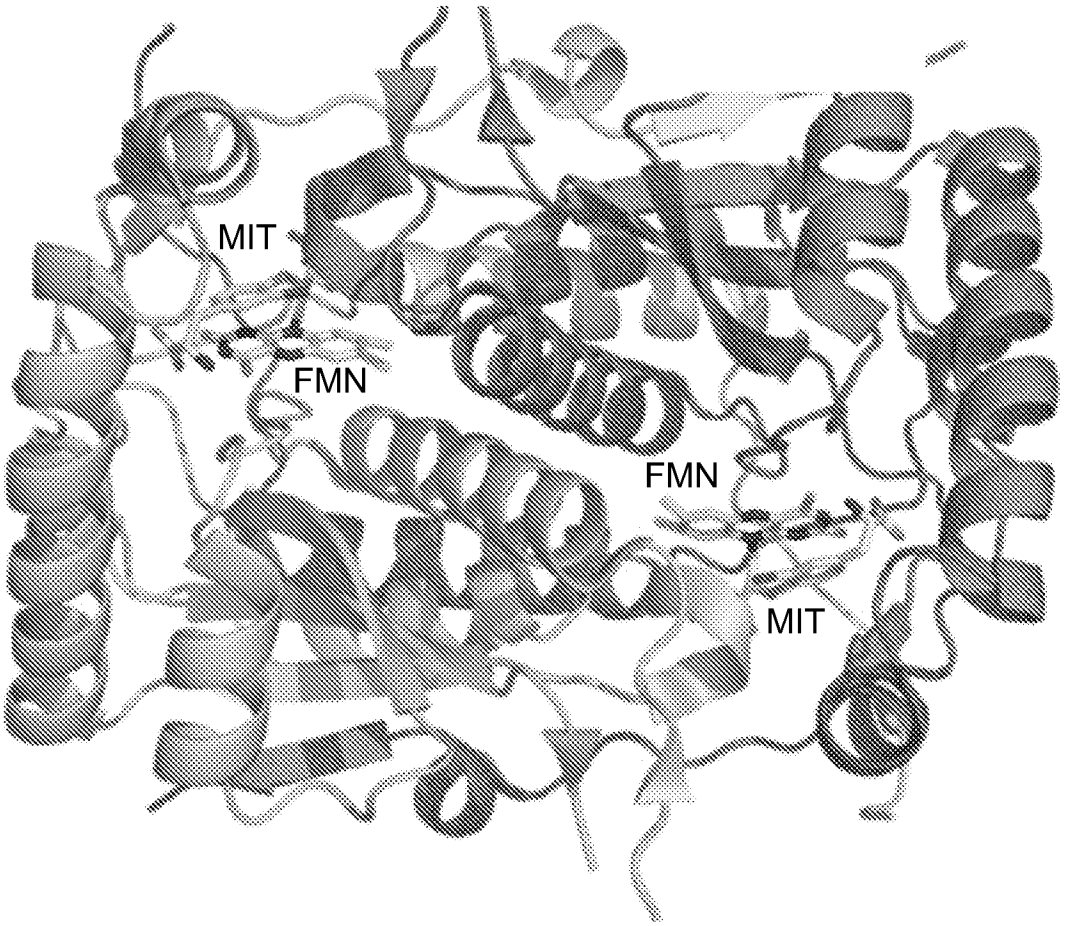
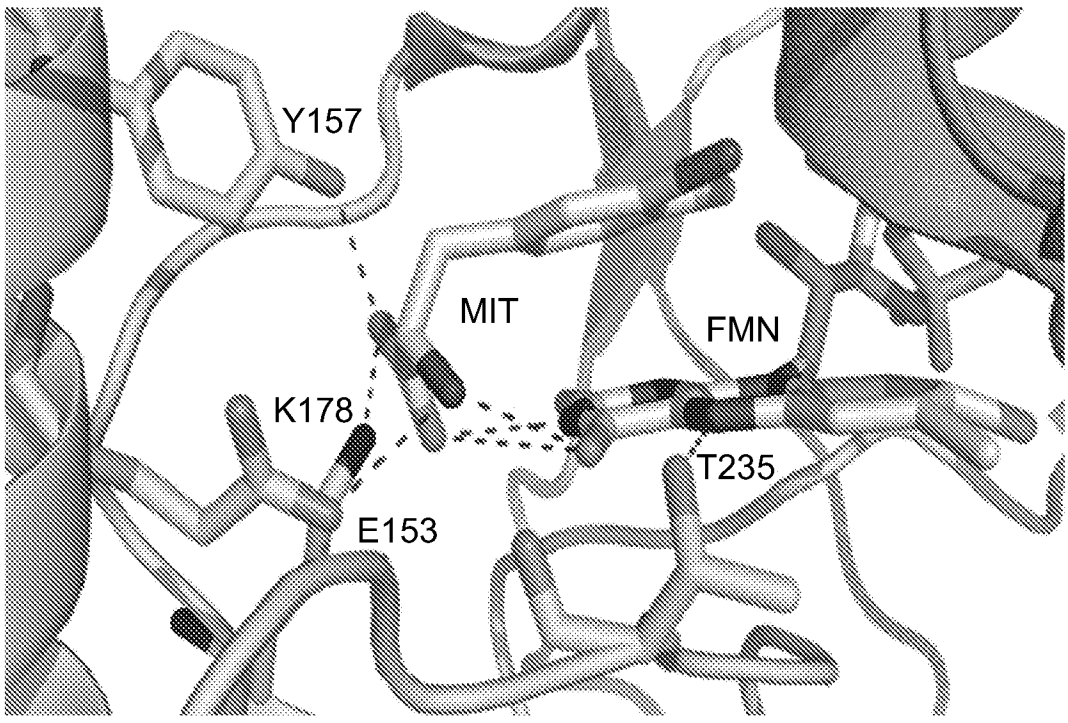


FIG. 2B



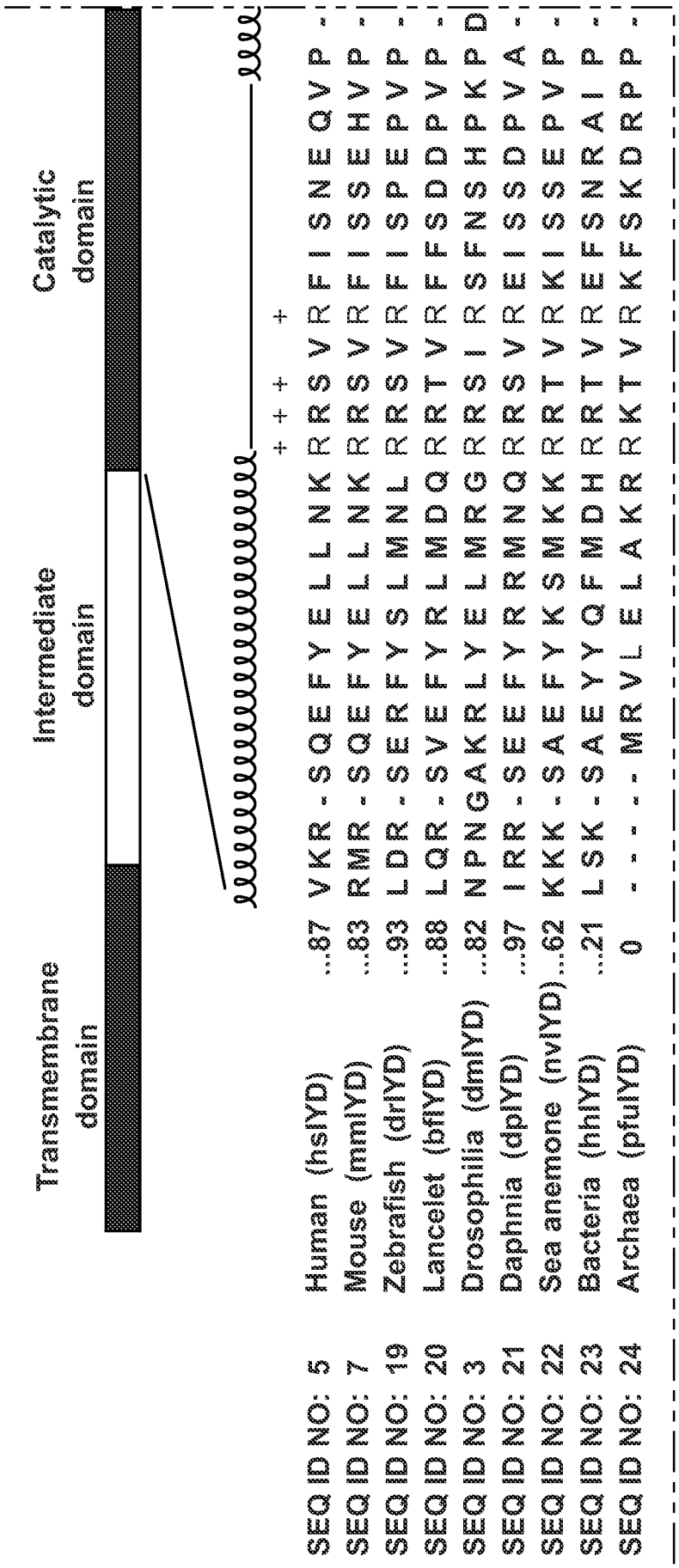

Transmembrane domain
Intermediate domain
Catalytic domain
+ + + +
SEQ ID NO: 5 Human (hsIYD) ...87 V K R - S Q E F Y E L L N K R R S V R F I S N E Q V P -
SEQ ID NO: 7 Mouse (mmIYD) ...83 R M R - S Q E F Y E L L N K R R S V R F I S S E H V P -
SEQ ID NO: 19 Zebrafish (drIYD) ...93 L D R - S E R F Y S L M N L R R S V R F I S P E P V P -
SEQ ID NO: 20 Lancelet (bfIYD) ...88 L Q R - S V E F Y R L M D Q R R T V R F F S D D P V P -
SEQ ID NO: 3 Drosophilia (dmIYD) ...82 N P N G A K R L Y E L M R G R R S I R S F N S H P K P D
SEQ ID NO: 21 Daphnia (dpIYD) ...97 I R R - S E E F Y R R M N Q R R S V R E I S S D P V A -
SEQ ID NO: 22 Sea anemone (nvIYD) ...62 K K K - S A E F Y K S M K K R R T V R K I S S E P V P -
SEQ ID NO: 23 Bacteria (hhIYD) ...21 L S K - S A E Y Y Q F M D H R R T V R E F S N R A I P -
SEQ ID NO: 24 Archaea (pfuIYD) 0 - - - - - M R V L E L A K R R K T V R K F S K D R P P -

FIG. 3A

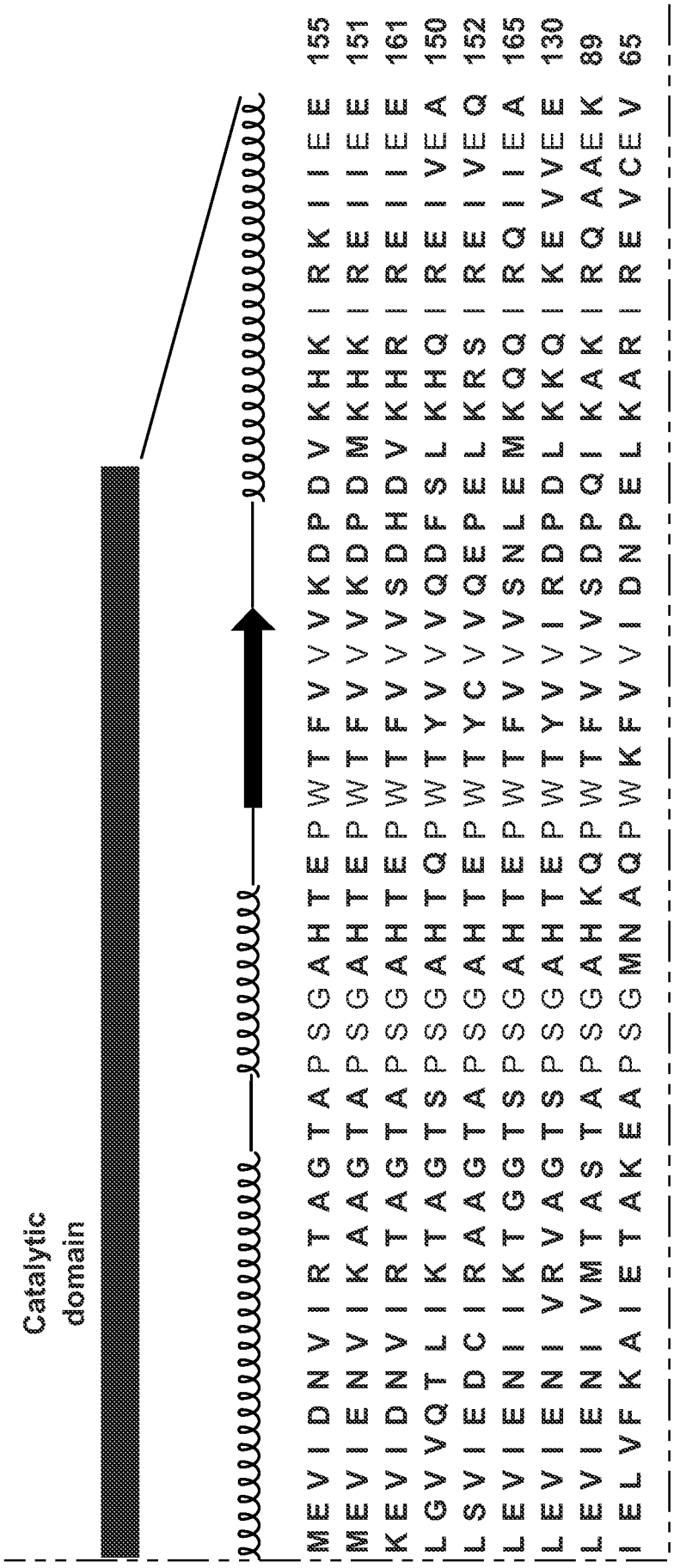

Catalytic domain
MEVIDNVIRTAGTAPSGAHTEPWTFVVVKDPDVKHKIRKIIEE 155
MEVIENVIKAAGTAPSGAHTEPWTFVVVKDPDMKHKIREIIEE 151
KEVIDNVIRTAGTAPSGAHTEPWTFVVVSDHDVKHRIREIIEE 161
LGVVQTLIKTAGTSPSGAHTQPWTYVVVQDFSLKHQIREIVEA 150
LSVIEDCIRAAGTAPSGAHTEPWTYCVVQEPELKRSIREIVEQ 152
LEVIENIIKTGGTSPSGAHTEPWTFVVVSNLEMKQQIRQIIEA 165
LEVIENIVRVAGTSPSGAHTEPWTYVVVIRDPDLKKQIKEVVEE 130
LEVIENIVMTASTAPSGAHKQPWTFVVVSDPQIKAKIRQAAEK 89
IELVFKAIETAKEAPSGMNAQPWKFVVIDNPELKARIREVCEV 65

FIG. 3B

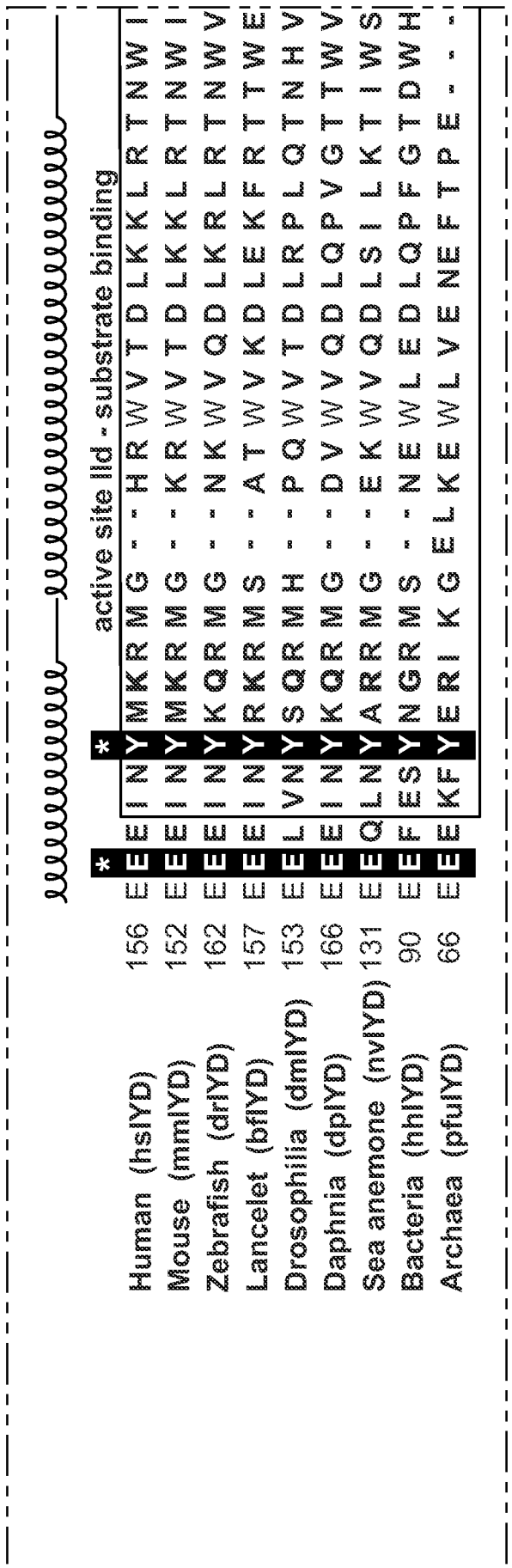

active site lid - substrate binding
Human (hsIYD) 156 E E E I N Y M K R M G - - H R W V T D L K K L R T N W I
Mouse (mmIYD) 152 E E E I N Y M K R M G - - K R W V T D L K K L R T N W I
Zebrafish (drIYD) 162 E E E I N Y K Q R M G - - N K W V Q D L K R L R T N W V
Lancelet (bfIYD) 157 E E E I N Y R K R M S - - A T W V K D L E K F R T T W E
Drosophilia (dmIYD) 153 E E L V N Y S Q R M H - - P Q W V T D L R P L Q T N H V
Daphnia (dpIYD) 166 E E E I N Y K Q R M G - - D V W V Q D L Q P V G T T W V
Sea anemone (nvIYD) 131 E E Q L N Y A R R M G - - E K W V Q D L S I L K T I W S
Bacteria (hhIYD) 90 E E F E S Y N G R M S - - N E W L E D L Q P F G T D W H
Archaea (pfuIYD) 66 E E K F Y E R I K G E L K E W L V E N E F T P E - - -

FIG. 3C

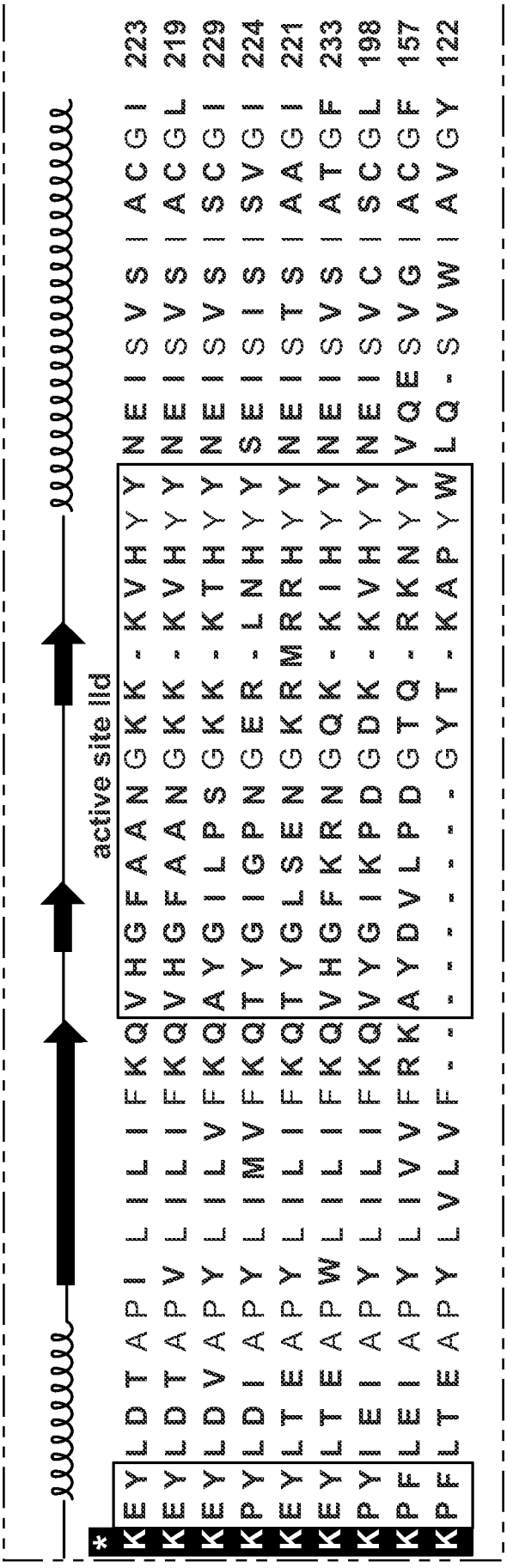

active site lid
* K E Y L D T A P I L I L I F K Q V H G F A A N G K K - K V H Y Y N E I S V S I A C G I 223
K E Y L D T A P V L I L I F K Q V H G F A A N G K K - K V H Y Y N E I S V S I A C G L 219
K E Y L D V A P Y L I L V F K Q A Y G I L P S G K K - K T H Y Y N E I S V S I S C G I 229
K P Y L D I A P Y L I M V F K Q T Y G I G P N G E R - L N H Y Y S E I S I S I S V G I 224
K E Y L T E A P Y L I L I F K Q T Y G L S E N G K R M R R H Y Y N E I S T S I A A G I 221
K E Y L T E A P W L I L I F K Q V H G F K R N G Q K - K I H Y Y N E I S V S I A T G F 233
K P Y I E I A P Y L I L I F K Q V Y G I K P D G D K - K V H Y Y N E I S V C I S C G L 198
K P F L E I A P Y L I V V F R K A Y D V L P D G T Q - R K N Y Y V Q E S V G I A C G F 157
K P F L T E A P Y L V L V F - - - - - - - - - G Y T - K A P Y W L Q - S V W I A V G Y 122

FIG. 3D

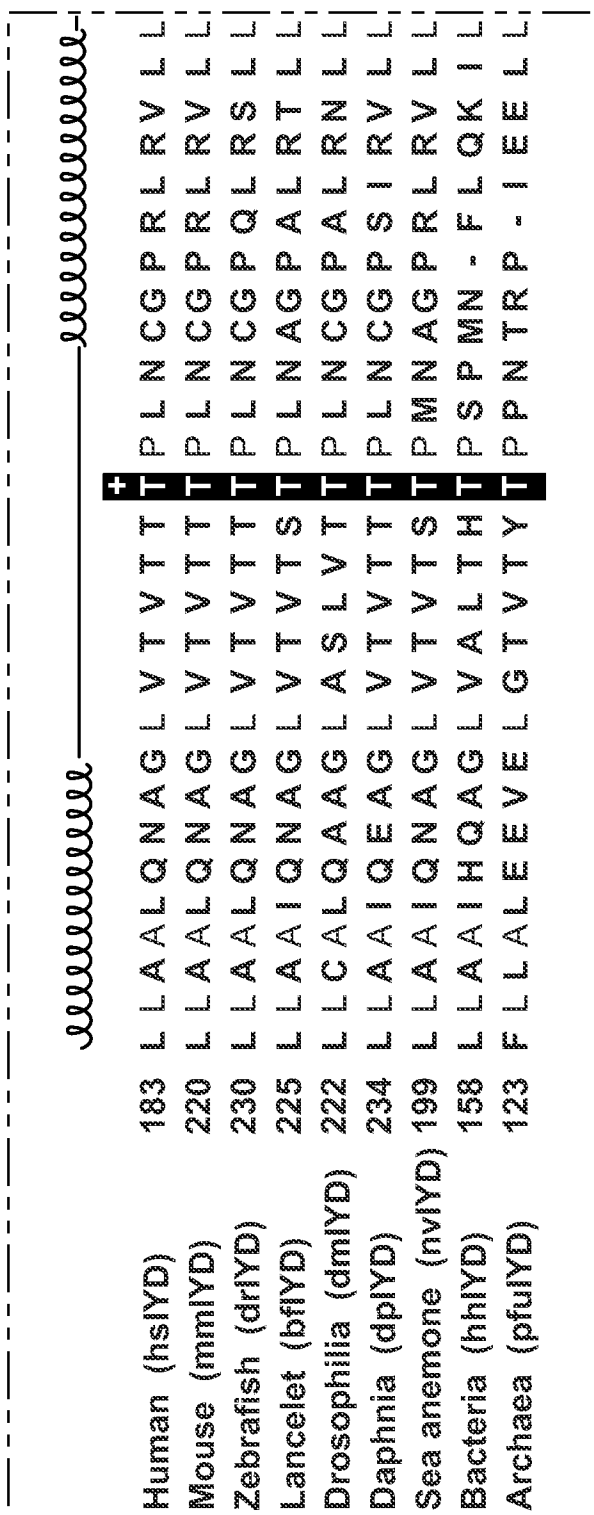

Human (hsIYD) 183 L L A A L Q N A G L V T V T T P L N C G P R L R V L L
Mouse (mmIYD) 220 L L A A L Q N A G L V T V T T P L N C G P R L R V L L
Zebrafish (drIYD) 230 L L A A L Q N A G L V T V T T P L N C G P Q L R S L L
Lancelet (bfIYD) 225 L L A A I Q N A G L V T V T S P L N A G P A L R T L L
Drosophilia (dmIYD) 222 L L C A L Q A A G L A S L V T P L N C G P A L R N L L
Daphnia (dpIYD) 234 L L A A I Q E A G L V T V T T P L N C G P S I R V L L
Sea anemone (nvIYD) 199 L L A A I Q N A G L V T V T S P M N A G P R L R V L L
Bacteria (hhIYD) 158 L L A A I H Q A G L V A L T H P S P M N - F L Q K I L
Archaea (pfuIYD) 123 F L L A L E E V E L G T V T Y P P N T R P - I E E L L

FIG. 3E

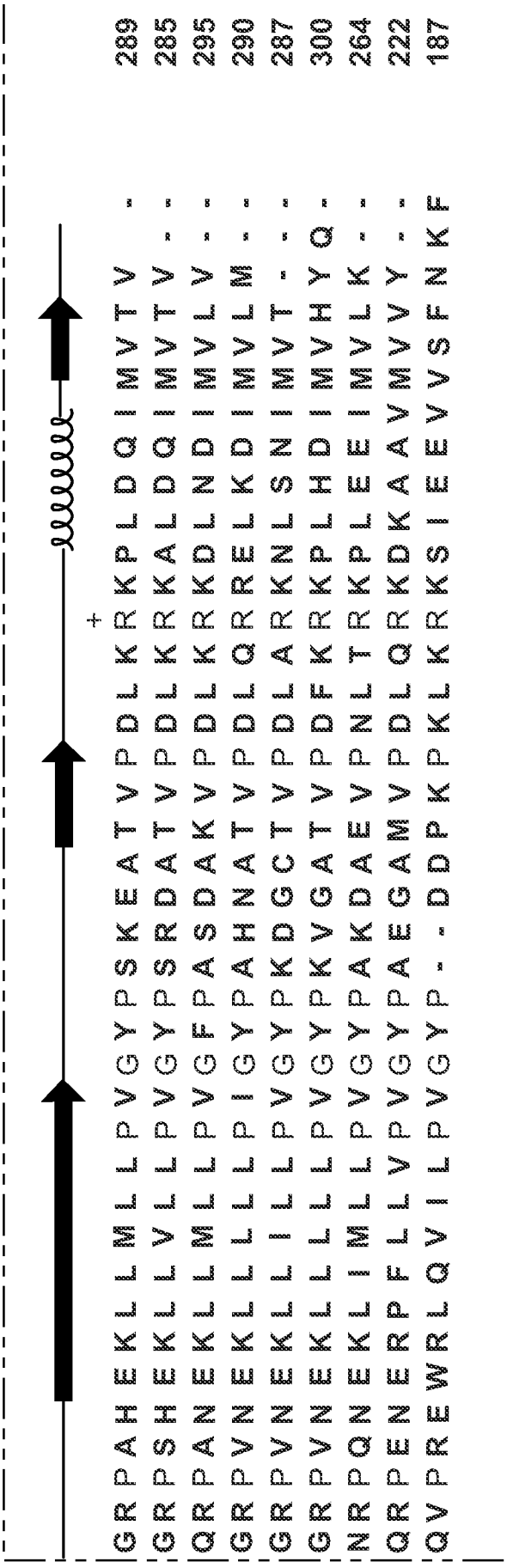


FIG. 3F

FIG. 4

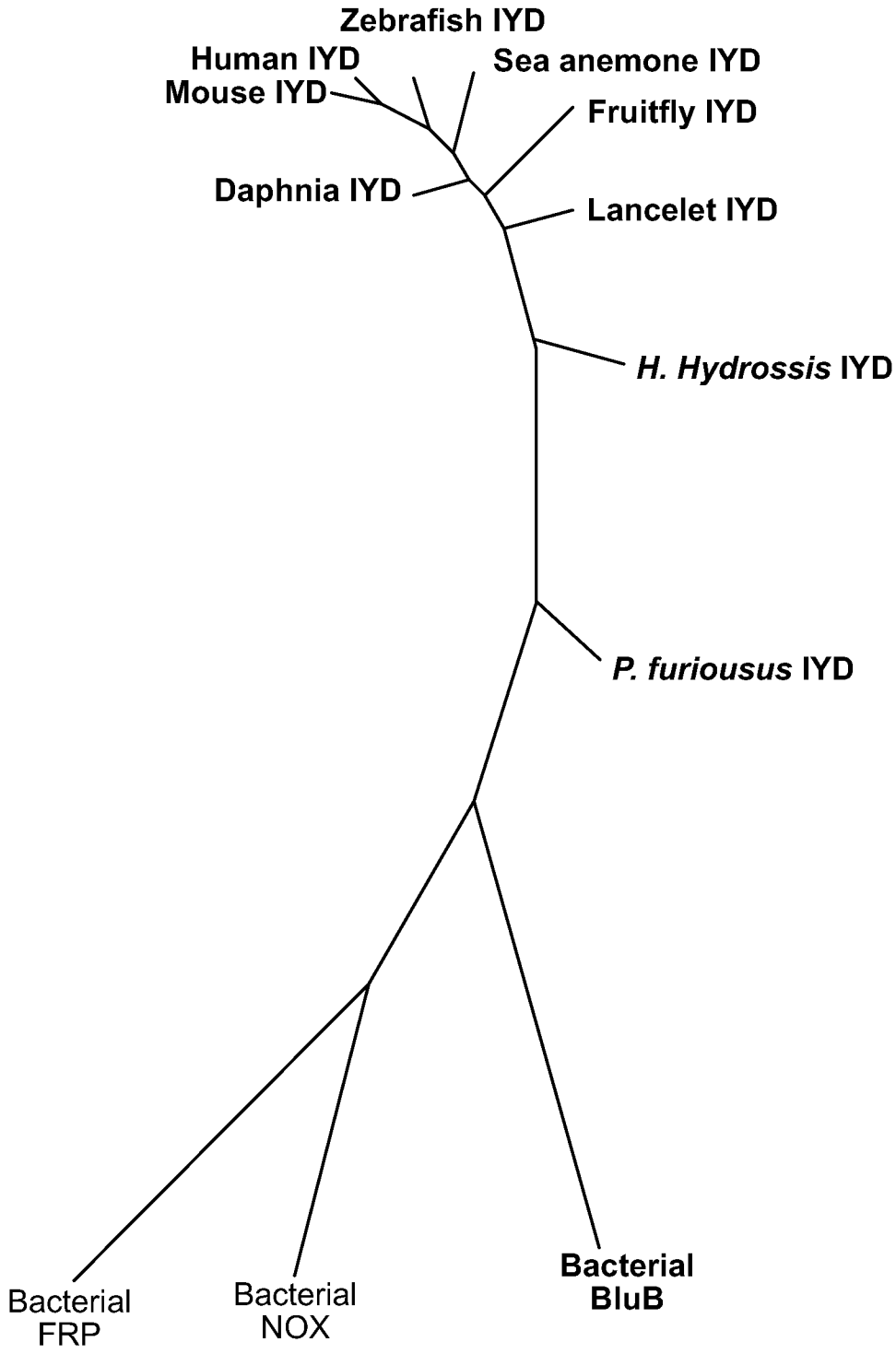

Zebrafish IYD
Human IYD
Mouse IYD
Sea anemone IYD
Fruitfly IYD
Daphnia IYD
Lancelet IYD
H. Hydrossis IYD
P. furiousus IYD
Bacterial FRP
Bacterial NOX
Bacterial BluB

FIG. 5

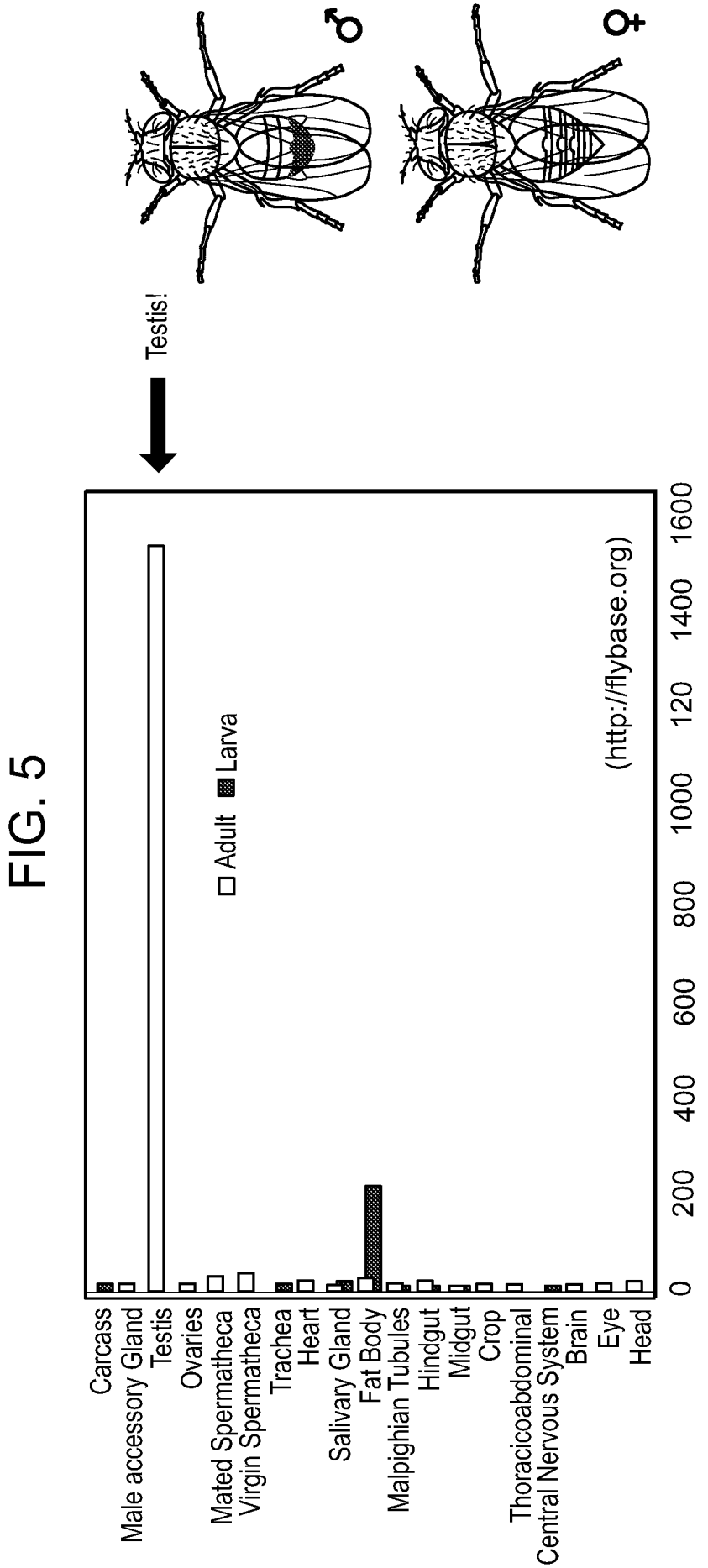

♂
♀
Testis!
Adult
Larva
(http://flybase.org)
0
200
400
600
800
1000
1200
1400
1600
Carcass
Male accessory Gland
Testis
Ovaries
Mated Spermatheca
Virgin Spermatheca
Trachea
Heart
Salivary Gland
Fat Body
Malpighian Tubules
Hindgut
Midgut
Crop
Thoracicoabdominal
Central Nervous System
Brain
Eye
Head

FIG. 6A

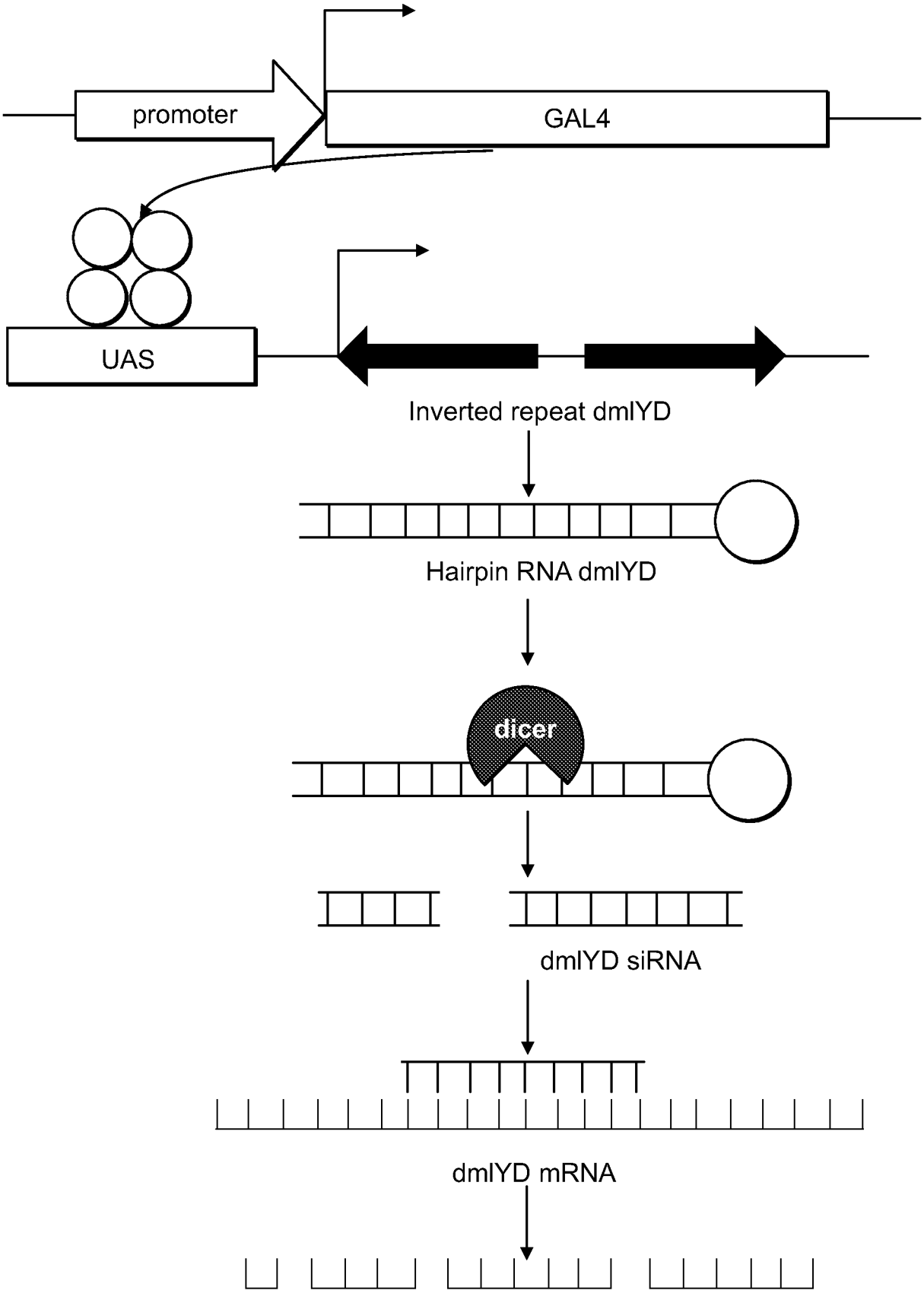

promoter
GAL4
UAS
Inverted repeat dmlYD
Hairpin RNA dmlYD
dicer
dmlYD siRNA
dmlYD mRNA

FIG. 6B

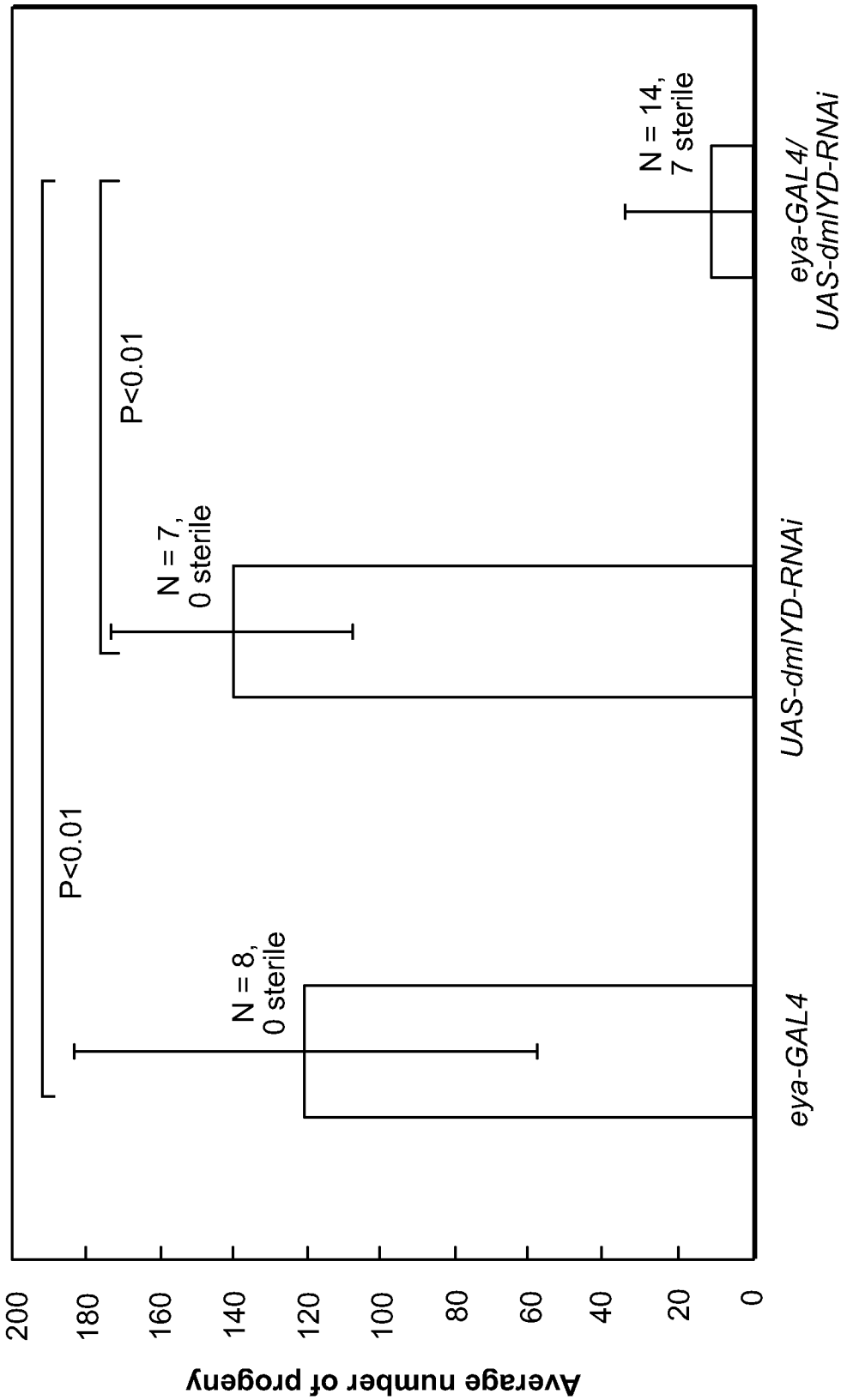

Average number of progeny
200
180
160
140
120
100
80
60
40
20
0
P<0.01
N = 8,
0 sterile
P<0.01
N = 7,
0 sterile
N = 14,
7 sterile
eya-GAL4
UAS-dmlYD-RNAi
eya-GAL4/
UAS-dmlYD-RNAi

FIG. 7A

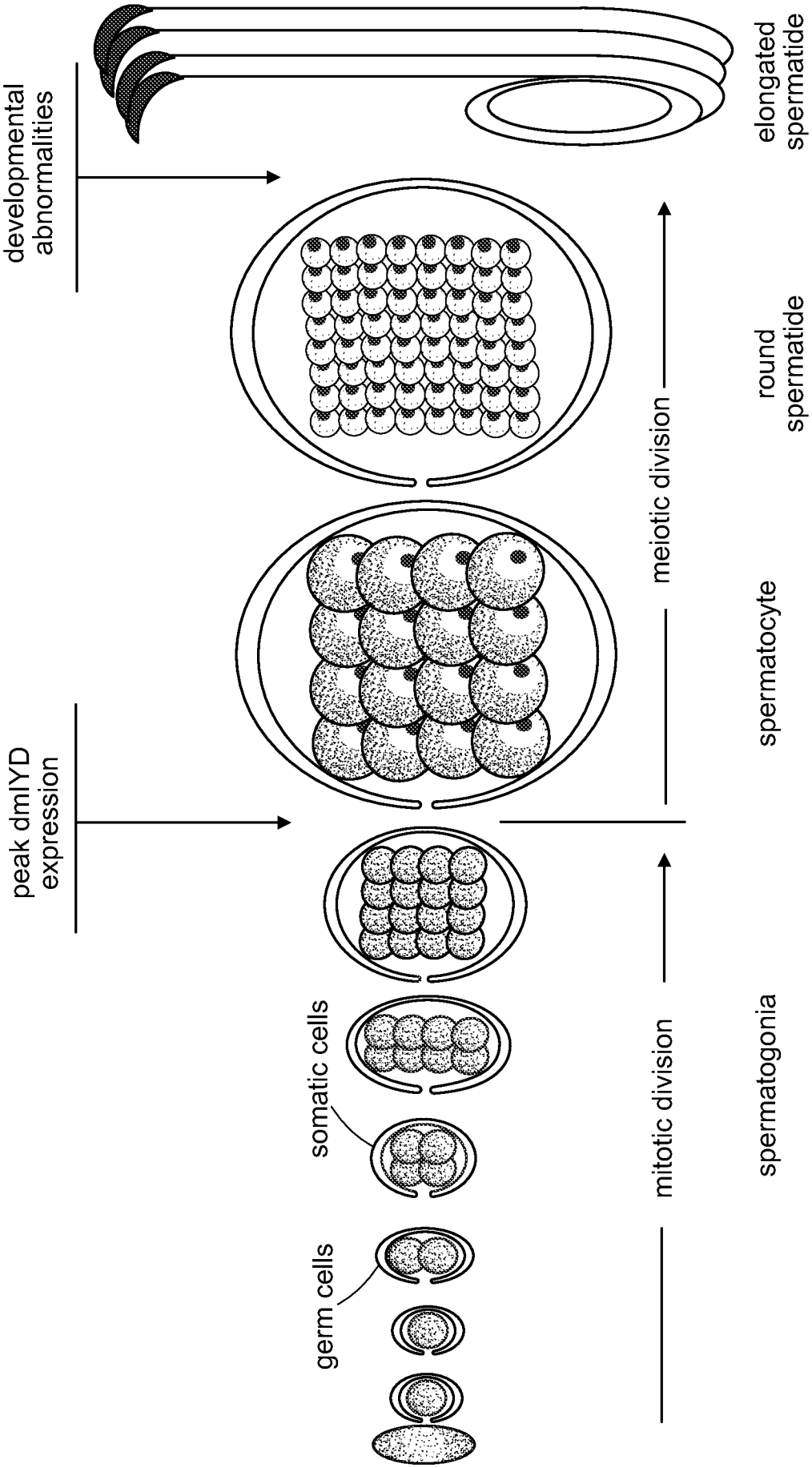

developmental abnormalities
peak dmIYD expression
germ cells
somatic cells
mitotic division
meiotic division
spermatogonia
spermatocyte
round spermatide
elongated spermatide

FIG. 7B

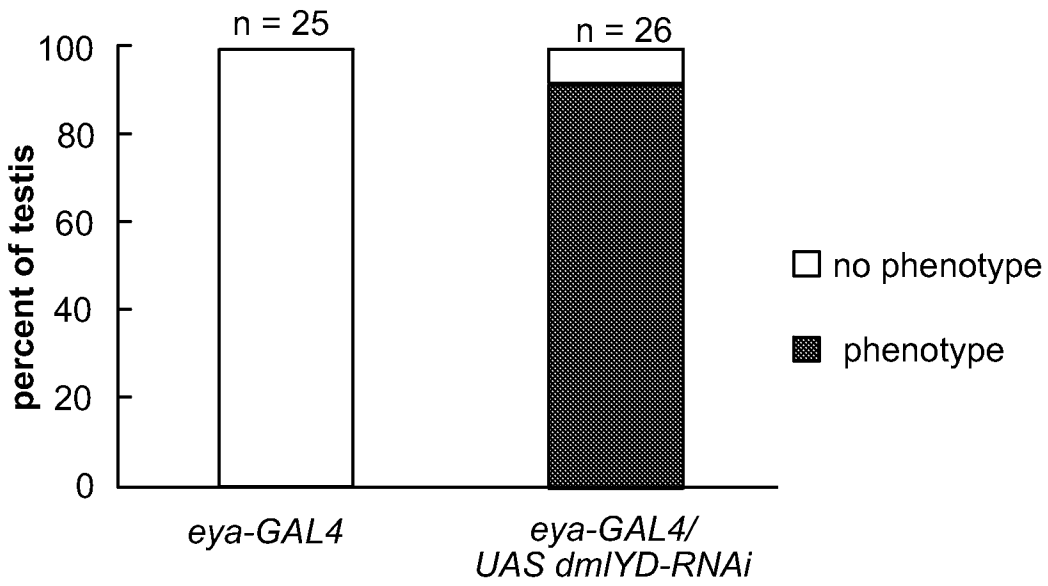

n = 25
n = 26
100
80
60
40
20
0
percent of testis
no phenotype
phenotype
eya-GAL4
eya-GAL4/
UAS dmlYD-RNAi

FIG. 7C

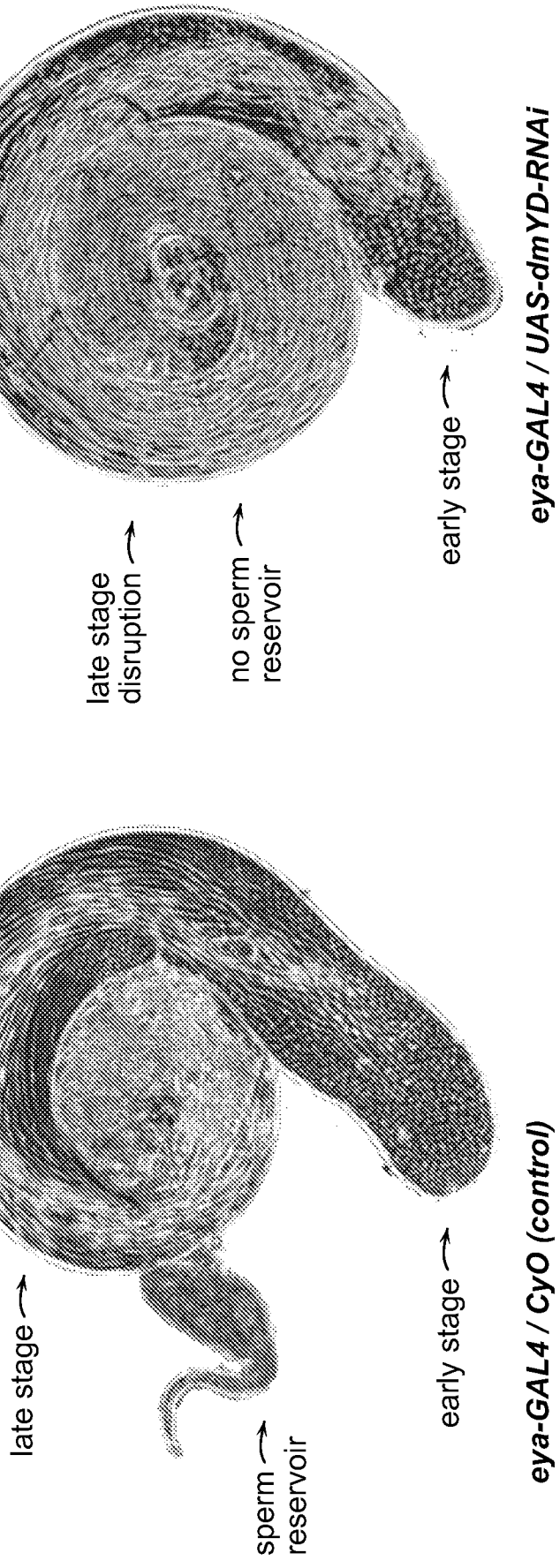

late stage
disruption
no sperm
reservoir
early stage
eya-GAL4 / UAS-dmYD-RNAi
late stage
sperm
reservoir
early stage
eya-GAL4 / CyO (control)

FIG. 8

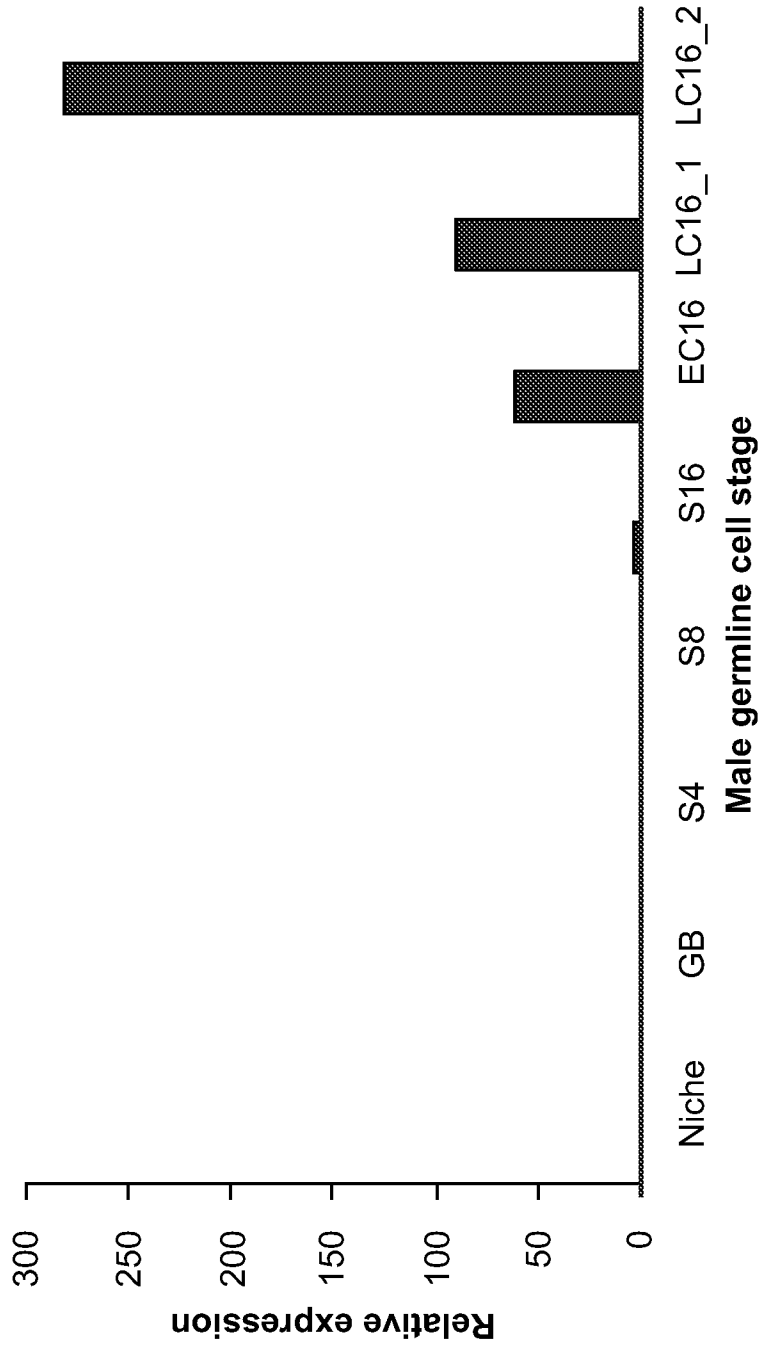

Cell Stage Specific CG6279 expression in male testis
Relative expression
300
250
200
150
100
50
0
Niche
GB
S4
S8
S16
EC16
LC16_1
LC16_2
Male germline cell stage

FIG. 9A

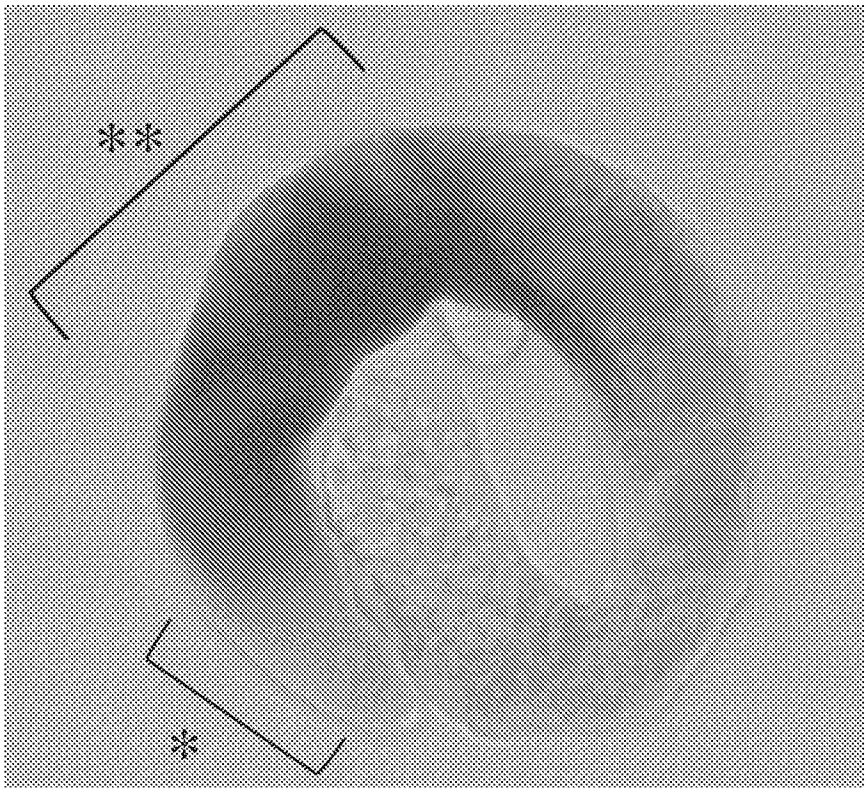


FIG. 9B

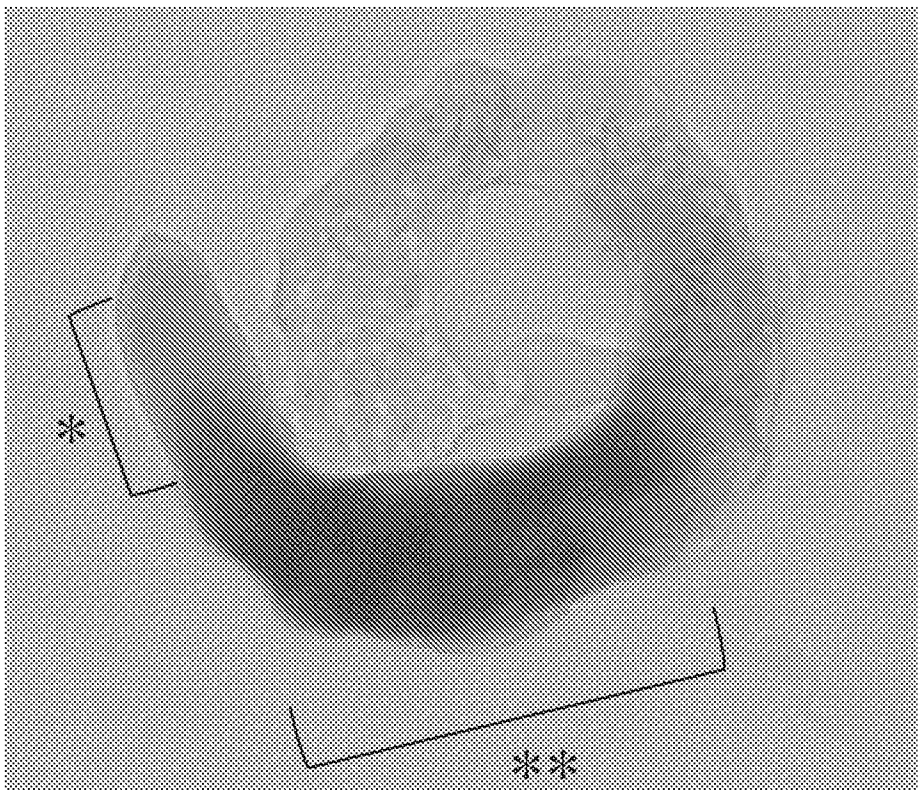


FIG. 9C

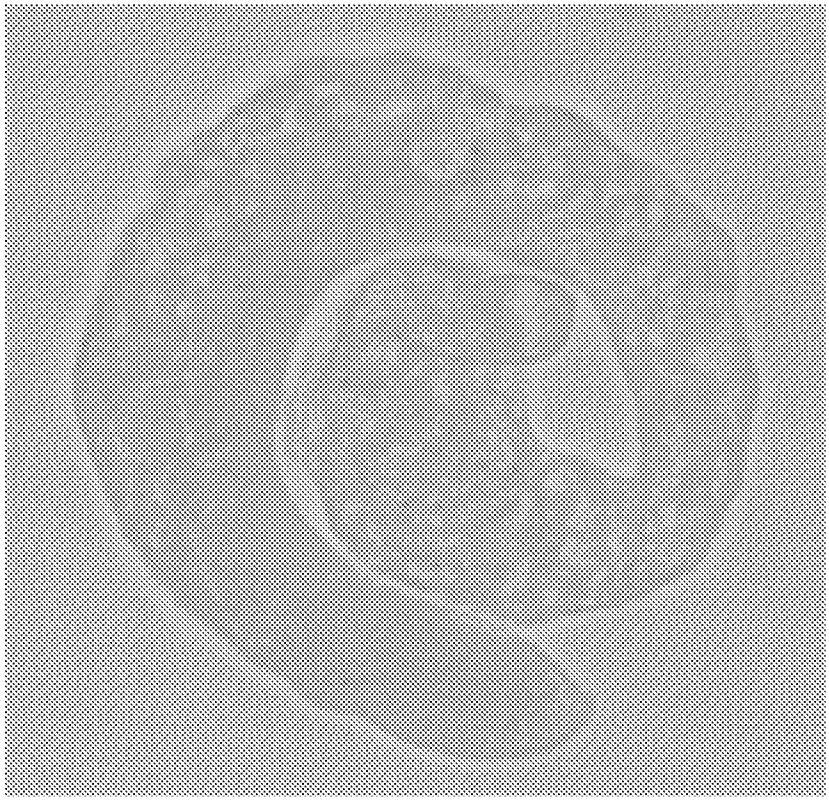


FIG. 9D

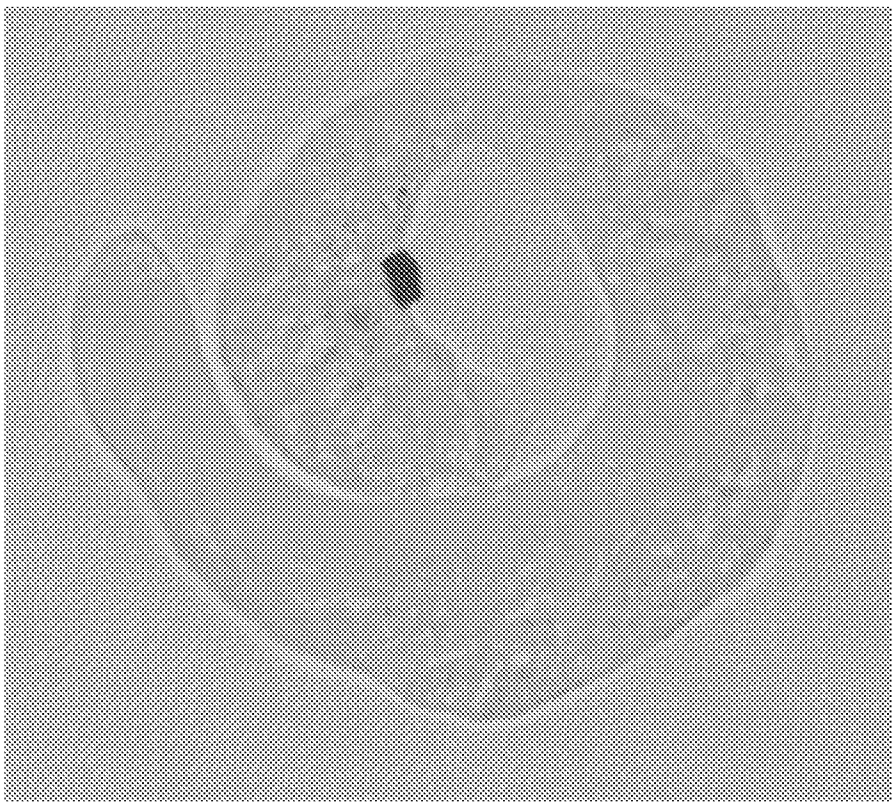
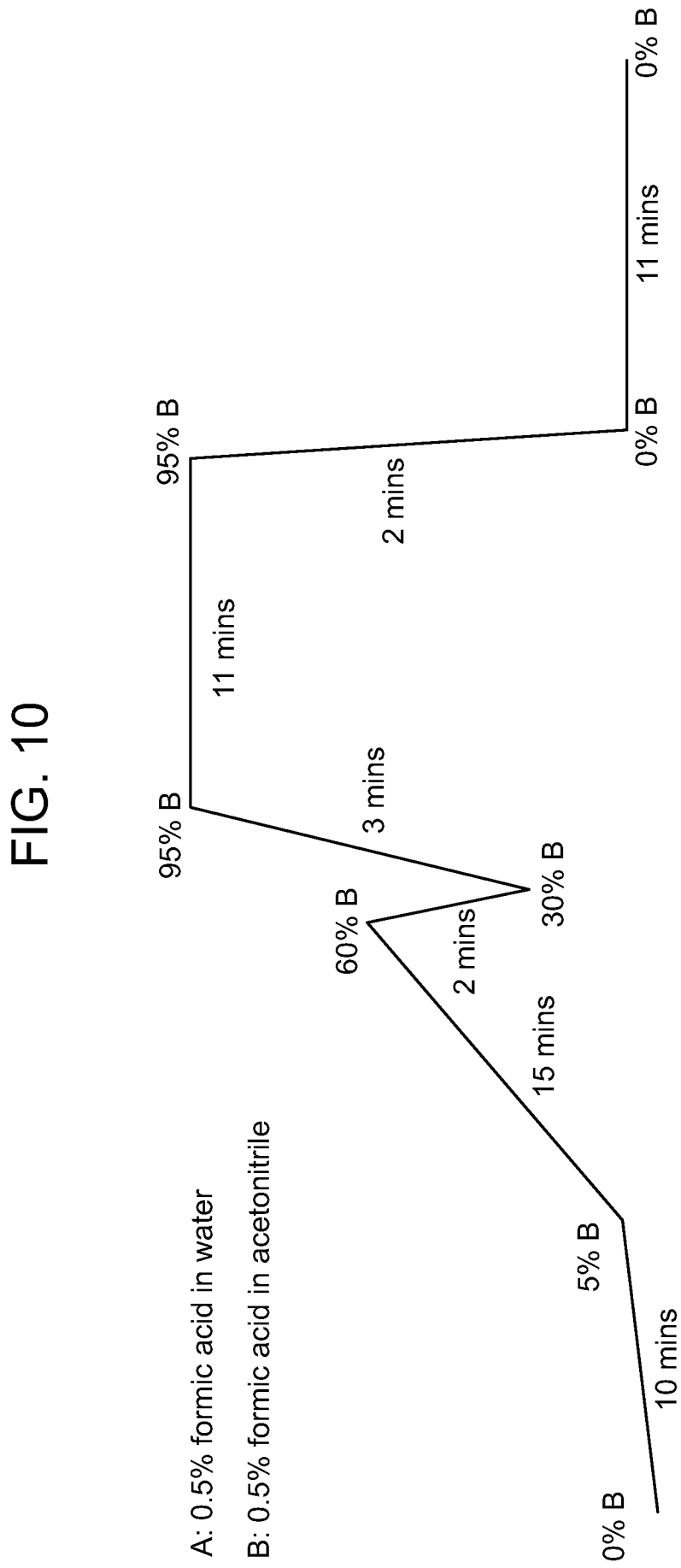


FIG. 10


A: 0.5% formic acid in water
B: 0.5% formic acid in acetonitrile
0% B
10 mins
5% B
15 mins
60% B
2 mins
30% B
3 mins
95% B
11 mins
95% B
2 mins
0% B
11 mins
0% B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/042522

A. CLASSIFICATION OF SUBJECT MATTER

A01N 63/02(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N 63/02; A01N 37/00; A01N 25/08; A01N 35/00; A01N 38/04; A01N 25/00; A01K 51/00; A01N 9/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & keywords: insect, reproduction, spermatogenesis, iodotyrosine deiodinase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4434180 A (VISSCHER) 28 February 1984 See claims 1-2 and column 1, lines 64-66.	1,5-7,11-22
A		2-4,8-10
X	US 3900567 A (LANCINI et al.) 19 August 1975 See claim 1.	1,5-7,11-22
X	US 2003-0044443 A1 (ERICKSON, JR. et al.) 06 March 2003 See claims 1, 15.	1,5-7,11-22
X	WO 2007-120461 A2 (HASS) 25 October 2007 See claims 1, 15.	1,5-7,11-22
X	US 5753615 A (THORPE et al.) 19 May 1998 See claim 14.	1,5-7,11-22

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 September 2015 (09.09.2015)	**09 September 2015 (09.09.2015)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 35208,
Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
KIM, Seung Beom
Telephone No. +82-42-481-3371

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2015/042522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4434180 A	28/02/1984	None	
US 3900567 A	19/08/1975	None	
US 2003-0044443 A1	06/03/2003	CA 2439112 A1	06/09/2002
		CN 1505504 A	16/06/2004
		EP 1372623 A1	02/01/2004
		EP 1372623 A4	06/05/2004
		US 2005-0090560 A1	28/04/2005
		US 6843985 B2	18/01/2005
		US 7423068 B2	09/09/2008
		WO 02-067914 A1	06/09/2002
WO 2007-120461 A2	25/10/2007	AU 2007-239040 A1	25/10/2007
		CA 2649351 A1	25/10/2007
		EP 2001285 A2	17/12/2008
		EP 2001285 A4	27/10/2010
		US 2007-0232188 A1	04/10/2007
		US 2008-0026673 A1	31/01/2008
		US 2010-0087121 A1	08/04/2010
		US 2010-0317742 A1	16/12/2010
		US 7597912 B2	06/10/2009
		US 7767234 B2	03/08/2010
		WO 2007-120461 A3	24/12/2008
US 5753615 A	19/05/1998	AU 1994-14194 A	26/09/1994
		AU 681630 B2	04/09/1997
		CA 2156863 A1	15/09/1994
		CA 2156863 C	05/10/1999
		EP 0751953 A1	08/01/1997
		JP 09-505552 A	03/06/1997
		WO 94-20530 A2	15/09/1994
		WO 94-20530 A3	13/10/1994