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Proprietor: Intrexon Corporation
Our Ref.: INX14805OP
Title: NOVEL ECDYSONE RECEPTOR/INVERTEBRATE RETINOID X RECEPTOR-BASED INDUCIBLE GENE EXPRESSION SYSTEM
Date: April 8, 2020

New Main Request (marked)

1. A gene expression modulation system comprising:

a) a first gene expression cassette that is capable of being expressed in a host cell, comprising a polynucleotide that encodes a first hybrid polypeptide comprising:

i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and

ii) an ecdysone receptor ligand binding domain; and

b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide that encodes a second hybrid polypeptide comprising:

i) a transactivation domain; and

ii) a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain.

2. The gene expression modulation system according to claim 1, further comprising a third gene expression cassette comprising:

i) a response element recognized by the DNA-binding domain of the first hybrid polypeptide;

ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and

iii) a gene whose expression is to be modulated.

3. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain (LBD) of the first hybrid polypeptide is selected from the group consisting of a spruce budworm *Choristoneura fumiferana* EcR (“CfEcR”) LBD, a beetle *Tenebrio molitor* EcR ("TmEcR") LBD, a *Manduca sexta* EcR ("MsEcR") LBD, a *Heliothies virescens* EcR ("HvEcR") LBD, a midge *Chironomus tentans* EcR (“CtEcR”) LBD, a silk moth *Bombyx mori* EcR (“BmEcR”) LBD, a fruit fly *Drosophila melanogaster* EcR (“DmEcR”) LBD, a mosquito *Aedes aegypti* EcR (“AaEcR”) LBD, a blowfly *Lucilia capitata* (“LcEcR”) LBD, a blowfly *Lucilia cuprina* EcR ("LucEcR") LBD, a Mediterranean fruit fly *Ceratisis capitata* EcR (“CcEcR”) LBD, a locust *Locusta migratoria* EcR (“LmEcR”) LBD, an aphid *Mycus persicae* EcR

("MpEcR") LBD, a fiddler crab *Celuca pugilator* EcR ("CpEcR") LBD, an ixodid tick *Amblyomma americanum* EcR ("AmaEcR") LBD, a whitefly *Bamecia argentifoli* EcR ("BaEcR") LBD, and a leafhopper *Nephotetix cincticeps* EcR ("NcEcR") LBD.

4. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain of the first hybrid polypeptide is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

5. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43 and SEQ ID NO: 59.

6. The gene expression modulation system according to claim 1, wherein the invertebrate retinoid X receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

7. The gene expression modulation system according to claim 1, wherein the invertebrate retinoid X receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

8. The gene expression modulation system according to claim 1, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and an ecdysone receptor ligand binding domain.

9. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and an invertebrate retinoid X receptor ligand binding domain.

10. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 37) and a B42 AD (SEQ ID NO: 39), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

11. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 38) and a B42 AD (SEQ ID NO: 40), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

12. A gene expression modulation system comprising:

a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising:

i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and

ii) a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain; and

b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide that encodes a second hybrid polypeptide comprising:

i) a transactivation domain; and

ii) an ecdysone receptor ligand binding domain.

13. The gene expression modulation system according to claim 12, further comprising a third gene expression cassette comprising:

i) a response element recognized by the DNA-binding domain of the first hybrid polypeptide;

ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and

iii) a gene whose expression is to be modulated.

14. The gene expression modulation system according to claim 12, wherein the invertebrate retinoid X receptor ligand binding domain of the first hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

15. The gene expression modulation system according to claim 12, wherein the invertebrate retinoid X receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

16. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide is selected from the group consisting of a spruce budworm *Choristoneura fumiferana* EcR (“CfEcR”) LBD, a beetle *Tenebrio molitor* EcR ("TmEcR") LBD, a *Manduca sexta* EcR ("MsEcR") LBD, a *Heliothies virescens* EcR ("HvEcR") LBD, a midge *Chironomus tentans* EcR (“CtEcR”) LBD, a silk moth *Bombyx mori* EcR (“BmEcR”) LBD, a fruit fly *Drosophila melanogaster* EcR (“DmEcR”) LBD, a

mosquito *Aedes aegypti* EcR ("AaEcR") LBD, a blowfly *Lucilia capitata* ("LcEcR") LBD, a blowfly *Lucilia cuprina* EcR ("LucEcR") LBD, a Mediterranean fruit fly *Ceratisis capitata* EcR ("CcEcR") LBD, a locust *Locusta migratoria* EcR ("LmEcR") LBD, an aphid *Mycus persicae* EcR ("MpEcR") LBD, a fiddler crab *Celuca pugilator* EcR ("CpEcR") LBD, an ixodid tick *Amblyomma americanum* EcR ("AmaEcR") LBD, a whitefly *Bamecia argentifoli* EcR ("BaEcR") LBD, and a leafhopper *Nephotetix cincticeps* EcR ("NcEcR") LBD.

17. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

18. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

19. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and an invertebrate retinoid X receptor ligand binding domain.

20. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) or a LexA DBD (SEQ ID NO: 35) and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

21. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

22. The gene expression modulation system according to claim 12, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and an ecdysone receptor ligand binding domain.

23. A gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain and a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain, wherein the DNA binding domain is from a nuclear receptor other than an invertebrate retinoid X receptor.

24. The gene expression cassette according to claim 23, wherein the DNA-binding domain is a GAL4 DNA-binding domain or a LexA DNA-binding domain.

25. The gene expression cassette according to claim 23, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) and a LexA DBD (SEQ ID NO: 35), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

26. The gene expression cassette according to claim 23, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32

27. A gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain and a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain, wherein the transactivation domain is from a nuclear receptor other than an invertebrate retinoid X receptor.

28. The gene expression cassette according to claim 27, wherein the transactivation domain is a VP16 transactivation domain or a B42 acidic activator transactivation domain.

29. The gene expression cassette according to claim 27, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 37) and a B42 AD (SEQ ID NO: 39), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

30. The gene expression cassette according to claim 29, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 38) and a B42 AD (SEQ ID NO: 40), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group

consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32

31. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces ligand binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;

(b) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;

(c) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces non-steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;

(d) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances ligand binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;

(e) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;

(f) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances non-steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;

(g) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein

the truncation mutation increases ligand sensitivity of the truncated invertebrate retinoid X receptor ligand binding domain; and

(h) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of a heterodimer, wherein the heterodimer comprises said truncated invertebrate retinoid X receptor ligand binding domain and a dimerization partner.

32. The isolated polynucleotide according to claim 31, wherein the dimerization partner is an ecdysone receptor polypeptide.

33. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

34. An isolated polypeptide encoded by the isolated polynucleotide according to claim 33.

35. An isolated truncated invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

36. A method of modulating the expression of a gene in a host cell comprising the steps of:

a) introducing into the host cell the gene expression modulation system according to claim 1; and

b) introducing into the host cell a ligand;

wherein the gene to be modulated is a component of a gene expression cassette comprising:

i) a response element recognized by the DNA binding domain from the first hybrid polypeptide;

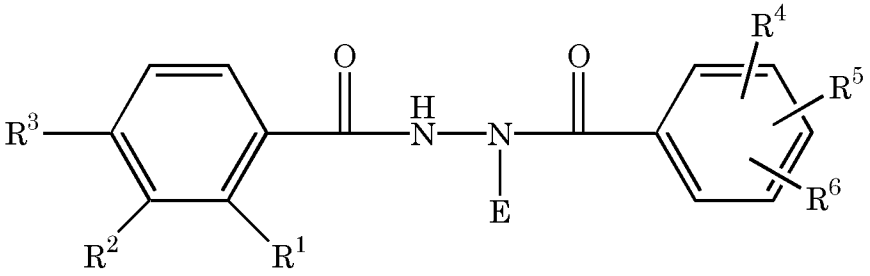
ii) a promoter that is activated by the transactivation domain of the

second hybrid polypeptide; and

iii) a gene whose expression is to be modulated;

whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

37. The method according to claim 36, wherein the ligand is a compound of the formula:



wherein:

E is a (C_4-C_6)alkyl containing a tertiary carbon or a cyano(C_3-C_5)alkyl containing a tertiary carbon;

R^1 is H, Me, Et, i-Pr, F, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, SCN, or $SCHF_2$;

R^2 is H, Me, Et, n-Pr, i-Pr, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF_2H, COEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, OCF_3, $OCHF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN, or joined with R^3 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R^3 is H, Et, or joined with R^2 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R^4, R^5, and R^6 are independently H, Me, Et, F, Cl, Br, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

38. The method according to claim 36, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

39. A method of modulating the expression of a gene in a host cell comprising the steps of:

a) introducing into the host cell the gene expression modulation system of claim 12; and

b) introducing into the host cell a ligand;

wherein the gene to be modulated is a component of a gene expression cassette comprising:

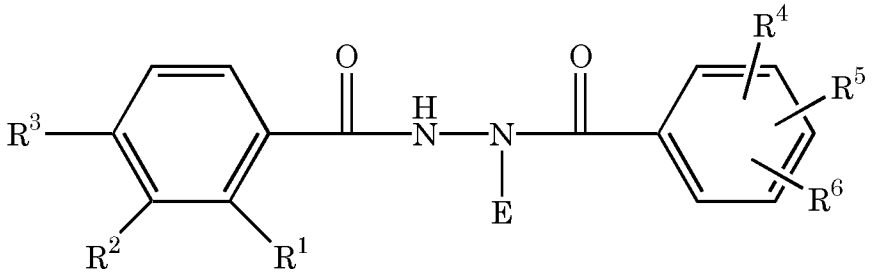
i) a response element recognized by the DNA binding domain from the first hybrid polypeptide;

ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and

iii) a gene whose expression is to be modulated;

whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

40. The method according to claim 39, wherein the ligand is a compound of the formula:



wherein:

E is a (C_4-C_6)alkyl containing a tertiary carbon or a cyano(C_3-C_5) alkyl containing a tertiary carbon;

R^1 is H, Me, Et, i-Pr, F, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, SCN, or $SCHF_2$;

R^2 is H, Me, Et, n-Pr, i-Pr, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF_2H, COEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, OCF_3, $OCHF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN, or joined with R^3 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R^3 is H, Et, or joined with R^2 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R^4, R^5, and R^6 are independently H, Me, Et, F, Cl, Br, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

41. The method according to claim 39, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

42. An isolated host cell comprising the gene expression modulation system according to claim 1.

43. The isolated host cell according to claim 42, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

44. The isolated host cell according to claim 43, wherein the mammalian cell is a murine cell or a human cell.

45. An isolated host cell comprising the gene expression modulation system according to claim 12.

46. The isolated host cell according to claim 45, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

47. The isolated host cell according to claim 46, wherein the mammalian cell is a murine cell or a human cell.

~~48. A non-human organism comprising the host cell of claim 45.~~

~~49. The non-human organism according to claim 48, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.~~

~~50. The non-human organism according to claim 49, wherein the mammal is selected from the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, and a chimpanzee.~~

~~51. A non-human organism comprising the host cell of claim 45.~~

~~52. The non-human organism according to claim 51, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.~~

~~53. The non-human organism according to claim 52, wherein the mammal is selected from the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, and a chimpanzee.~~

~~54~~48. The gene expression modulation system of claim 1, wherein said system exhibits increased ligand sensitivity compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.

~~55~~49. The gene expression modulation system of claim 1, wherein said system exhibits increased sensitivity to non-steroidal ligands compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.

~~56~~50. The gene expression modulation system of claim 12, wherein said system exhibits increased ligand sensitivity compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.

~~57~~51. The gene expression modulation system of claim 12, wherein said system exhibits increased sensitivity to non-steroidal ligands compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.