**Table S1** Primer sequences

|  |  |  |
| --- | --- | --- |
| **Primer name** | **Sequence 5’-3’** | **Function** |
| CYPF19AF8 | GCTCAGAGTCCAGATATTGTAC | RT-PCR long |
| CYPF19AF10 | ATGAAACAATTAGCGAAGTCGAG | RT-PCR long |
| CYP419A1seqF1 | CAATTGGTTGAGAGAATCACC | RT-PCR long |
| CYP419A1seqR2 | GCTTTTCCATTTTGTCTTTCAGA | RT-PCR long |
| CYP419A1seqR3 | CGTTCAGCAATTTCAATTTGCTT | RT-PCR long |
| BPH\_p2\_F | TTGAACGAGCGGGGAATTAT | cDNA del only qPCR |
| BPH\_p2\_R | AGATGGACAGCGTACCGATT | cDNA del only qPCR |
| BPH\_p3\_F | AGTTGCCCTATTTGAACGCC | Flies + cDNA qPCR |
| BPH\_p3\_R | ATGCCACTTCATTCGCTGTC | Flies + cDNA qPCR |
| sdha\_F | CACGACCCTCCATGATCTCG | Flies housekeeping |
| sdha\_R | CGGATGTCTCATCACCGAGG | Flies housekeeping |
| rpl\_F | GCCCAAGGGTATCGACAACA | Flies housekeeping |
| rpl\_R | GCGCTTGTTCGATCCGTAAC | Flies housekeeping |
| Act F | TAACGAGAGGTTCCGTTGCC | qPCR HK N. lugens |
| Act R | GACAGGACAGTGTTGGCGTA | qPCR HK N. lugens |
| Tub F | CCACCCTGGAACACTCTGAC | qPCR HK N. lugens |
| Tub R | CGAAGCAGTGATCGAGGACA | qPCR HK N. lugens |
| qPCR-NlDel F | ACGCTGTCCATCTCAAGTCT | gDNA qPCR |
| qPCR-NlDel R | GAGGCCTTTAACTGAGTGCA | gDNA qPCR |
| qPCR-NlCYP419A1\_F | CACGACTTGACAATGACACCA | gDNA qPCR |
| qPCR-NlCYP419A1\_R | CATCGGTGGCATCGGTTAAG | gDNA qPCR |
| VGSC F | CACCATTGTCACACAGCAGC | gDNA qPCR |
| VGSC R | CCCTGGAGTAGTGCTTGTCG | gDNA qPCR |

**Table S2** Species list

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species** | **Abbreviation** | **Subphylum** | **Class** | **Order** | **Family** |
| *Parasteatoda tepidariorum* | (Ptep) | Chelicerata | Arachnida | Araneae | Theridiidae |
| *Aculops lycopersici* | (Alyc) | Chelicerata | Arachnida | Trombidiformes | Eriophyidae |
| *Dermatophagoides pteronyssinus* | (Dpte) | Chelicerata | Arachnida | Sarcoptiformes | Pyroglyphidae |
| *Ixodes scapularis* | (Isca) | Chelicerata | Arachnida | Ixodida | Ixodidae |
| *Chamberlinius hualienensis* | (Chua) | Myriapoda | Diplopoda | Polydesmida | Paradoxosomatidae |
| *Hyalella azteca* | (Hazt) | Crustacea | Malacostraca | Amphipoda | Hyalellidae |
| *Daphnia pulex* | (Dpul) | Crustacea | Branchiopoda | Diplostraca | Daphniidae |
| *Tigriopus japonicus* | (Tjap) | Crustacea | Hexanauplia | Harpacticoida | Harpacticidae |
| *Folsomia candida* | (Fcan) | Hexapoda | Collembola | Entomobryomorpha | Istomidae |
| *Sinella curviseta* | (Scur) | Hexapoda | Collembola | Entomobryomorpha | Entomobryidae |
| *Acerentomon species* | (Acer) | Hexapoda | Protura | Suborder: Acerentomata | Acerentomidae |
| *Machilis hrabei* | (Mhra) | Hexapoda | Insecta | Archaeognatha | Machilidae |
| *Thermobia domestica* | (Tdom) | Hexapoda | Insecta | Zygentoma | Lepismatidae |
| *Calopteryx splendens* | (Cspl) | Hexapoda | Insecta | Odonata | Calopterygidae |
| *Locusta migratoria* | (Lmig) | Hexapoda | Insecta | Orthoptera | Acrididae |
| *Pediculus humanus* | (Phum) | Hexapoda | Insecta | Psocodea | Pediculidae |
| *Cimex lectularius* | (Clec) | Hexapoda | Insecta | Hemiptera | Cimicidae |
| *Myzus persicae* | (Mper) | Hexapoda | Insecta | Hemiptera | Aphididae |
| *Nilaparvata muiri* | (N.mui) | Hexapoda | Insecta | Hemiptera | Delphacidae |
| *Nilaparvata lugens* | (N.lug) | Hexapoda | Insecta | Hemiptera | Delphacidae |
| *Sogatella furcifera* | (S.furc) | Hexapoda | Insecta | Hemiptera | Delphacidae |
| *Laodelphax striatellus* | (L.str) | Hexapoda | Insecta | Hemiptera | Delphacidae |
| *Idiosystatus acutiusculus* | (I.acu) | Hexapoda | Insecta | Hemiptera | Delphacidae |
| *Scolypopa species* | (Scolypopa) | Hexapoda | Insecta | Hemiptera | Ricanilidae |
| *Catonia nava* | (C.nava) | Hexapoda | Insecta | Hemiptera | Achilidae |
| *Chondrodire chilensis* | (C.chil) | Hexapoda | Insecta | Hemiptera | Dictyopharidae |
| *Yucanda albida* | (Y.alb) | Hexapoda | Insecta | Hemiptera | Dictyopharidae |
| *Thionia simplex* | (T.sim) | Hexapoda | Insecta | Hemiptera | Issidae |
| *Acanalonia conica* | (A.con) | Hexapoda | Insecta | Hemiptera | Acanalonidae |
| *Jamella australiae* | (J.aus) | Hexapoda | Insecta | Hemiptera | Flatidae |
| *Nasonia vitripennis* | (Nvit) | Hexapoda | Insecta | Hymenoptera | Pteromalinae |
| *Apis mellifera* | (Amel) | Hexapoda | Insecta | Hymenoptera | Apidae |
| *Athalia rosae* | (A.ros) | Hexapoda | Insecta | Hymenoptera | Athaliidae |
| *Nicrophorus vespilloides* | (Nves) | Hexapoda | Insecta | Coleoptera | Silphidae |
| *Dendroctonus ponderosae* | (Dpon) | Hexapoda | Insecta | Coleoptera | Curculionidae |
| *Tribolium castaneum* | (Tcas) | Hexapoda | Insecta | Coleoptera | Tenebrionidae |
| *Anopheles gambiae* | (Agam) | Hexapoda | Insecta | Diptera | Culicidae |
| *Drosophila melanogaster* | (Dmel) | Hexapoda | Insecta | Diptera | Drosophilidae |
| *Plutella xylostella* | (Pxyl) | Hexapoda | Insecta | Lepidoptera | Plutellidae |
| *Bombyx mori* | (Bmor) | Hexapoda | Insecta | Lepidoptera | Bombycidae |
| *Helicoverpa armigera* | (Harm) | Hexapoda | Insecta | Lepidoptera | Noctuidae |
| *Manduca sexta* | (Msex) | Hexapoda | Insecta | Lepidoptera | Sphingidae |

**Table S3** Sensitivity of transgenic Drosophila strains expressing CYP419A1 and CYP419A1del to ethiprole, imidacloprid and fipronil

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Insecticides | Strains | LC50 (ppm) | Slope ± SE | 95% CI | RR |
| Fipronil | No transgene | 3.60 | 4.34 ± 0.48 | 3.17-4.00 |  |
| CYP419A1del | 3.00 | 3.32 ± 0.26 | 2.16-4.03 | 0.9 |
| CYP419A1 | 2.86 | 2.78 ± 0.26 | 1.83-4.05 | 0.8 |
| Ethiprole | No transgene | 137.94 | 3.56 ± 0.35 | 100.42-178.17 |  |
| CYP419A1del | 484.57 | 3.03 ± 0.30 | 359.28-614.33 | 3.5 |
| CYP419A1 | 429.73 | 3.15 ± 0.36 | 277.14-561.23 | 3.1 |
| Imidacloprid | No transgene | 754.711 | 3.60 ± 0.35 | 559.34-936.13 |  |
| CYP419A1del | 1125.56 | 2.51 ± 0.24 | 793.81-1464.54 | 1.49 |
| CYP419A1 | 1332.15 | 3.90 ± 0.49 | 790.40-1734.30 | 1.76 |

**Table S4**

1. Ligand study WT CYP419A1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Binding energies ethiprole | Ebound (Kcal/mol) | Emax (Kcal/mol) | Esurface (Kcal/mol) | Ea (Kcal/mol) | 𝜟EBS (Kcal/mol) |
| Tunnel 1 | 13.1 | 26.9 | -6.8 | 33.7 | 19.8 |
| Tunnel 2 | 16.2 | 21.8 | -1.1 | 22.9 | 17.3 |
| Tunnel 4 | 13.1 | 41.9 | 9.9 | 32.0 | 3.2 |
| Tunnel 5 | 11.6 | 42.0 | -6.1 | 48.1 | 17.7 |
| Tunnel 7 | 13.0 | 44.3 | 33.1 | 11.2 | -20.1 |
| Tunnel 8 | 13.0 | 26.9 | 6.8 | 20.1 | 6.2 |
| Tunnel 9 | 13.1 | 26.9 | -6.7 | 33.6 | 19.8 |

1. Ligand study CYP419A1del

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Binding energies ethiprole | Ebound (Kcal/mol) | Emax (Kcal/mol) | Esurface (Kcal/mol) | Ea (Kcal/mol) | 𝜟EBS (Kcal/mol) |
| Tunnel 1 | -7.1 | 16.1 | 0.7 | 15.4 | -7.8 |
| Tunnel 2 | -6.0 | 26.9 | 11.5 | 15.4 | -17.5 |
| Tunnel 4 | -6.4 | 31.1 | -4.1 | 35.2 | -2.3 |
| Tunnel 5 | -7.1 | 23.4 | 22.5 | 0.9 | -29.6 |
| Tunnel 6 | -6.9 | 20.4 | -3.0 | 23.4 | -3.9 |
| Tunnel 7 | -7.4 | 20.3 | -4.6 | 24.9 | -2.8 |
| Tunnel 8 | -5.9 | 22.0 | 1.5 | 20.5 | -7.4 |
| Tunnel 9 | -7.1 | 25.2 | -2.0 | 27.2 | -5.1 |