Supplementary Material

Title: Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides

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Supplementary Table 1. Number of *Cimex lectularius* P450 families, subfamilies and individual genes in each insect P450 clan

Number	CYP2	Mitochondrial	CYP3	CYP4	Total
Family	6 (CYP15, 18, 303, 305, 306, 307)	5 (CYP301, 302, 314, 315, 394)	8 (CYP6, 395-400, 404)	1 (CYP4)	20
Subfamily	6	6	11	5	28
Individual gene	6	6	23	7	42

Supplementary Table 2. Annotation and differential expression of P450s in susceptible and resistant bed bug populations

No.	Clan	Sequence Name	Length (nt)	Gene name	Relative expression/rpl8		
					LA-1 (Mean±SE)	CIN-1 NS (Mean±SE)	NY-1 (Mean±SE)
1	2	GF6M21K05FYWJI	267	CYP15A1	0.096±0.02	0.072±0.01	0.051±0.00
2	2	contig04179	1501	CYP18A1	0.254±0.01	0.152±0.02	0.172±0.02
3	2	FTWEJGT01DYMNN	480	CYP303A1	0.053±0.01	0.016±0.00	0.023±0.00
4	2	contig08495	977	CYP305B1	0.143±0.03	0.090±0.01	0.062±0.01
5	2	contig09685	877	CYP306A1	0.058±0.01	0.054±0.00	0.082±0.01
6	2	contig01282	2371	CYP307B1	1.731±0.22	1.327±0.11	1.944±0.13
7	Mito	contig03102	1725	CYP301A1	0.017±0.00	0.017±0.00	0.024±0.00
8	Mito	contig03015	1742	CYP301B1	0.403±0.06	0.357±0.01	0.390±0.01
9	Mito	contig10417	819	CYP302A1	0.132±0.02	0.118±0.00	0.145±0.01
10	Mito	contig01141	2464	CYP314A1	0.658±0.09	0.528±0.02	0.747±0.08
11	Mito	FTWEJGT02G37T9	480	CYP315A1	0.167±0.02	0.107±0.01	0.194±0.02
12	Mito	contig02495	1880	CYP394A1	0.035±0.01	0.010±0.00	0.007±0.00
13	3	contig11249	762	CYP395A1	1.609±0.09	1.963±0.27	1.289±0.11
14	3	contig14132	554	CYP395A2	4.133±0.25	4.607±0.50	3.038±0.46
15	3	contig14522	531	CYP395A3	2.866±0.21	3.318±0.30	2.381±0.34
16	3	contig07081	1114	CYP395A4	0.170±0.01	0.100±0.02	0.058±0.00
17	3	contig08940	938	CYP395A5	1.011±0.16	0.508±0.02	0.787±0.01
18	3	contig10838	792	CYP395A6	1.019±0.12	0.935±0.06	1.502±0.13
19	3	contig02337	1925	CYP395A7	0.033±0.01	0.012±0.00	0.012±0.00
20	3	contig07631	1054	CYP395A8	1.095±0.05	0.806±0.09	1.159±0.11
21	3	contig08655	963	CYP395B1	0.541±0.06	0.518±0.05	0.769±0.15
22	3	contig03201	1704	CYP396A1	0.001±0.00	0.001±0.00	0.001±0.00
23	3	contig11345	752	CYP397A1	6.788±0.56	16.718±2.09	29.863±4.71
24	3	contig03764	1591	CYP398A1	2.364±0.13	1.556±0.07	5.550±0.63
25	3	contig02971	1757	CYP399A1	1.170±0.11	0.737±0.01	1.120±0.10
26	3	contig09058	927	CYP400A1	0.342±0.07	0.308±0.06	0.851±0.04
27	3	FTWEJGT02GUA4S	464	CYP404	0.002±0.00	0.001±0.00	0.004±0.00
28	3	contig06517	1175	CYP6DL1	0.980±0.13	0.845±0.04	0.907±0.28
29	3	contig18189	318	CYP6DL2	0.112±0.01	0.097±0.01	0.031±0.01
30	3	contig08845	941	CYP6DM1	0.238±0.03	0.233±0.03	0.263±0.01
31	3	contig02437	1896	CYP6DM2	0.888±0.14	1.169±0.04	1.359±0.30
32	3	contig04490	1460	CYP6DN1	0.653±0.19	0.788±0.06	1.551±0.11
33	3	contig04426	1469	CYP6DN2	0.716±0.05	0.700±0.12	0.172±0.00
34	3	contig07269	1092	CYP6DN3	0.991±0.11	0.784±0.11	1.446±0.10
35	3	contig03136	1718	CYP6DN4	0.343±0.05	0.255±0.02	0.111±0.01
36	4	contig04099	1524	CYP4CM1	0.301±0.01	0.635±0.10	1.278±0.12
37	4	contig02986	1755	CYP4CN1	0.524±0.05	0.514±0.02	0.382±0.03
38	4	contig03005	1748	CYP4CP1	0.251±0.04	0.289±0.02	0.318±0.02

39	4	contig01154	2455	CYP4CQ1	0.492±0.09	0.141±0.02	0.144±0.01
40	4	contig02002	2037	CYP4G52	0.601±0.09	0.528±0.01	0.665±0.01
41	4	contig03206	1705	CYP4G53	0.027±0.01	0.019±0.00	0.027±0.00
42	4	contig02404	1905	CYP4G54	0.085±0.01	0.083±0.01	0.119±0.01

Supplementary Table 3. Annotation and differential expression of esterases, GST, cuticular proteins, and Abc transporters in susceptible and resistant bed bug populations

Category	Contig Number	Sequence Length (nt)	Gene Name	Relative expression/rpl8		
				LA-1 Mean±SE	CIN-1 S Mean±SE	*P* value (t-test)
Esterase	03262	1690	ClCE21331	0.002±0.00	1.682±0.34	<0.001
	N/A	N/A	ClCE3959*	10.098±1.70	9.187±1.24	0.421
GST	N/A	N/A	GSTs1*	0.707±0.15	0.609±0.16	0.411
Cuticular protein	09967	856	C1	0.203±0.04	0.165±0.02	0.156
	15313	480	C2	0.991±0.17	1.474±0.12	<0.05
	12749	648	C3	0.084±0.00	0.041±0.00	<0.05
	12072	700	C4	0.012±0.00	0.021±0.00	<0.05
	10057	847	C5	0.003±0.00	0.020±0.00	<0.05
	14205	551	C6	0.192±0.01	0.127±0.00	<0.05
	18423	308	C7	0.039±0.00	0.076±0.01	<0.05
	09970	855	C8	0.010±0.00	0.041±0.00	<0.05
	14186	550	C9	0.124±0.00	0.081±0.00	<0.05
	02621	1840	C10	0.311±0.05	0.943±0.07	<0.05
	12101	691	C11	0.007±0.00	0.032±0.00	<0.05
	16495	409	C12	0.011±0.00	0.041±0.00	<0.05
	08158	1004	C13	0.748±0.03	2.653±0.11	<0.05
	14441	537	C14	0.067±0.00	0.086±0.01	<0.05
	06115	1223	C15	0.028±0.00	0.038±0.00	<0.05
Abc transporter	N/A	N/A	Abc1**	0.106±0.01	0.197±0.02	0.001
	04658	1420	Abc2	0.711±0.05	0.013±0.00	<0.001
	16653	400	Abc3	0.618±0.05	0.888±0.07	0.006
	09770	871	Abc4	0.954±0.02	0.883±0.07	0.167
	04780	1404	Abc5	1.017±0.04	1.061±0.04	0.192
	01139	2453	Abc6	0.273±0.00	0.226±0.00	0.001
	06994	1124	Abc7	0.025±0.02	0.083±0.00	<0.001
	08506	976	Abc8	0.138±0.01	0.438±0.01	<0.001
	02154	1984	Abc9	0.258±0.01	0.526±0.04	<0.001
	05955	1243	Abc10	0.253±0.01	1.074±0.02	<0.001
	09403	902	Abc11	0.253±0.01	0.831±0.04	<0.001
	14750	514	Abc12	0.318±0.05	0.308±0.01	0.742
	14756	514	Abc13	0.027±0.00	0.032±0.00	0.033

* Transcriptional expression of these genes was reported by Adelman et al., 2011 [18]
**Differential expression between insecticide resistant and susceptible strains was reported by Mamidala et al., 2012 [20]

Supplementary Table 4. The distribution of haplotypes representing mutations in sodium channel gene of 24 bed bug populations

No.	Name	Location	Collect Time	Haplotype
1	LA-1	Los Angeles, CA	Aug 14 2006	A
2	LEX-7	Lexington, KY	Dec 21 2011	D/C
3	LEX-3	Lexington, KY	Nov 10 2011	A
4	LEX-10	Lexington, KY	Jan 10 2012	C
5	LEX-4	Lexington, KY	Nov 30 2011	A
6	NY-1	Plainview, NY	Early 2008	C
7	LEX-1	Lexington, KY	Nov 18 2011	A
8	LEX-12	Lexington, KY	Feb 14 2012	B
9	CIN-1 S	Cincinnati, OH	Dec 2 2011	A
10	LEX-9	Lexington, KY	Jan 9 2012	B
11	LEX-13	Lexington, KY	Feb 17 2012	C
12	LEX-11	Lexington, KY	Dec 22 2011	B
13	LEX-5	Lexington, KY	Dec 2 2011	D
14	LEX-6	Lexington, KY	Dec 21 2011	B/C
15	CHI-1	Chicago, IL	Jan 3 2012	B
16	CHI-2	Chicago, IL	Jan 3 2012	C
17	CIN-5	Cincinnati, OH	Jan 3 2012	C
18	LEX-2	Lexington, KY	Nov 10 2011	D
19	LEX-8	Lexington, KY	Jan 9 2012	C
20	CHI-3	Chicago, IL	Jan 3 2012	B
21	LOU-1	Louisville, KY	Dec 21 2011	B
22	LOU-2	Louisville, KY	Dec 21 2011	D/C
23	CIN-4	Cincinnati, OH	Jan 3 2012	C
24	CIN-3	Cincinnati, OH	Dec 19 2011	C

Supplementary Table 5. Primers used for dASPCR, qRT-PCR, and RNAi

Category	Gene Name	Sequence Name	Length (nt)	Primer Name	Function	Sequence
Cytochrome P450s	GF6M21K05FYWJI	267	CYP15A1	qCle_cyp15a1F	qRT-PCR	5’ GGGAAGTACACAGAGAAACCACTCGT 3’
				qCle_cyp15a1R	qRT-PCR	5’ GCTTGCATATGGACAAAGAGCATTGGG 3’
	contig04179	1501	CYP18A1	qCle_cyp18a1F	qRT-PCR	5’ CGACAGGCAGCTGCAACAAATCAT 3’
				qCle_cyp18a1R	qRT-PCR	5’ TCGGGCTCGTGAAGCATGTAGATT 3’
	FTWEJGT01DYMNN	480	CYP303A1	qCle_cyp303a1F	qRT-PCR	5’ TGGCCTGTCTTGAAGTGTCGGTAA 3’
				qCle_cyp303a1R	qRT-PCR	5’ AGCAAATCGTTGGGTTTCGGCTTC 3’
	contig08495	977	CYP305B1	qCle_cyp305b1F	qRT-PCR	5’ TGAGCCTGTTCCTCTTTCTGTCGT 3’
				qCle_cyp305b1R	qRT-PCR	5’ GATTGTTGCACCATCGAGCCCTTT 3’
	contig09685	877	CYP306A1	qCle_cyp306a1F	qRT-PCR	5’ TGACAGGCCCTACAATGCCGATTA 3’
				qCle_cyp306a1R	qRT-PCR	5’ TCTTCACTCCATCGGCAACGTGAT 3’
	contig01282	2371	CYP307B1	qCle_cyp307b1F	qRT-PCR	5’ GCATGGGCTACAAGTTGACGCAAT 3’
				qCle_cyp307b1R	qRT-PCR	5’ TTGCTCGATATTGCAAGGTT 3’
	contig03102	1725	CYP301A1	qCle_cyp301a1F	qRT-PCR	5’ TGACTTCCTCGACGGCAACATTCT 3’
				qCle_cyp301a1R	qRT-PCR	5’ TGGTCAGTGGAACAGAACCGAAGA 3’
	contig03015	1742	CYP301B1	qCle_cyp301b1F	qRT-PCR	5’ GGCCCATTTGGCATAAACATCGGA 3’
				qCle_cyp301b1R	qRT-PCR	5’ GTGCATTGGAAGGCGTTATGCTGA 3’
	contig10417	819	CYP302A1	qCle_cyp302a1F	qRT-PCR	5’ TTACACAGAATTGGGTTGCGT 3’
				qCle_cyp302a1R	qRT-PCR	5’ TGCGAGTCTTCTAGCGATGCATGT 3’
	contig01141	2464	CYP314A1	qCle_cyp314a1F	qRT-PCR	5’ TCCAAATCGCTTCTTCCCTCACGA 3’
				qCle_cyp314a1R	qRT-PCR	5’ GCGAACAACAAAGCCCTTGACGAT 3’
	FTWEJGT02G37T9	480	CYP315A1	qCle_cyp315a1F	qRT-PCR	5’ AGTTTCTGAACAAGGTCGACGGGTGTCA 3’
				qCle_cyp315a1R	qRT-PCR	5’ TCGAGTTGTATCGGCATTCCCTGT 3’
	contig02495	1880	CYP394A1	qCle_cyp394a1F	qRT-PCR	5’ TCGCTGTGAAGTTCCTGCCAACTACAAT 3’
				qCle_cyp394a1R	qRT-PCR	5’ TGCTGACTTCGATGAGCGTGTGTA 3’
	contig11249	762	CYP395A1	qCle_cyp395a1F	qRT-PCR	5’ AGGCAAACCTCATAGCGAGGCATAT 3’
				qCle_cyp395a1R	qRT-PCR	5’ ATTCAATCCACATGGACCCGACCT 3’
	contig14132	554	CYP395A2	qCle_cyp395a2F	qRT-PCR	5’ TCAGGCACATGTCCAGGTAAGTCA 3’
				qCle_cyp395a2R	qRT-PCR	5’ ACAGGAACGAGCGAGAAATGAGGTCATA 3’
	contig14522	531	CYP395A3	qCle_cyp395a3F	qRT-PCR	5’ CAGGGTTGAAACCGAACGCGAAAT 3’
				qCle_cyp395a3R	qRT-PCR	5’ AATGACCGAGATCGGCGAGAACTT 3’
	contig07081	1114	CYP395A4	qCle_cyp395a4F	qRT-PCR	5’ GCCTTCGAATTAACCGCCTTCCAATT 3’
				qCle_cyp395a4R	qRT-PCR	5’ AGTCGTTCCTTCGGACGTTCTTCT 3’
	contig08940	938	CYP395A5	qCle_cyp395a5F	qRT-PCR	5’ ACGCTTCAGCCCTGATGTATTCCAGA 3’
				qCle_cyp395a5R	qRT-PCR	5’ CATGAGGACCTTTGCAAGGCAGAA 3’
	contig10838	792	CYP395A6	qCle_cyp395a6F	qRT-PCR	5’ TCTTTAGGGTTGAAAGGCAAGCCGTA 3’
				qCle_cyp395a6R	qRT-PCR	5’ TTCTCCATGGACGTGATCGGTTCT 3’
	contig02337	1925	CYP395A7	qcle_cyp395a7F	qRT-PCR	5’ ACAAGGCCTCCAGTTCTCTATGCT 3’
				qcle_cyp395a7R	qRT-PCR	5’ TGGGTCGAGTGTGTTTGCACAATG 3’
	contig07631	1054	CYP395A8	qcle_cyp395a8F	qRT-PCR	5’ ATTTCATCTGCTGCAAGGCGAACCTC 3’
				qcle_cyp395a8R	qRT-PCR	5’ TGACCCTGACAAATTCGACCCTGA 3’
	contig08655	963	CYP395B1	qCle_cyp395b1F	qRT-PCR	5’ TGAACAGGTTGATGCTGAGTGGGT 3’
				qCle_cyp395b1R	qRT-PCR	5’ TTGTCAAGGACTTCTCCAGCTTCG 3’
	contig03201	1704	CYP396A1	qCle_cyp396a1F	qRT-PCR	5’ TCGTCGTTAATGATCCCGAGCTGA 3’
				qCle_cyp396a1R	qRT-PCR	5’ CAAGTTGAACAGCGCTTTGGACGA 3’
	contig11345	752	CYP397A1	qCle_cyp397a1F	qRT-PCR	5’ TATTGGAGTCGACAGGGCGTGAAA 3’
				qCle_cyp397a1R	qRT-PCR	5’ TGACATCGCCCAATTGCTTGTAGC 3’

				dCl_CYP397a1F	RNAi	5’ TAATACGACTCACTATAGGGTGATCTGGACATCGACATGGACC 3’
				dCl_CYP397a1R	RNAi	5’ TAATACGACTCACTATAGGGAGTTCGAAAACCGTCATGGCTC 3’
	contig03764	1591	CYP398A1	qCle_cyp398a1F	qRT-PCR	5’ TGTCGACCCAATGATGGCTCTGAA 3’
				qCle_cyp398a1R	qRT-PCR	5’ GAAATTGGAGGCCGATTTGGCGAT 3’
				dCl_CYP398a1F	RNAi	5’ TAATACGACTCACTATAGGGGCGTTATTGTTTGCTCCATGGTGG 3’
				dCl_CYP398a1R	RNAi	5’ TAATACGACTCACTATAGGGTCGTTCTCGAAGCTACAATCAGCG 3’
	contig02971	1757	CYP399A1	qCle_cyp399a1F	qRT-PCR	5’ TGTGGATCCAAAGCTGGAACCGAT 3’
				qCle_cyp399a1R	qRT-PCR	5’ AGTGTGAGTGAAGATGTGACTGCCA 3’
	contig09058	927	CYP400A1	qCle_cyp400a1F	qRT-PCR	5’ CCGTTGTCGGCAAAGACGTTGAAATCT 3’
				qCle_cyp400a1R	qRT-PCR	5’ TATACATTCGCCAAAGCCGTCGGA 3’
	FTWEJGT02GUA4S	464	CYP404	qCle_cyp404F	qRT-PCR	5’ TGGCGCTAAACCCAGATAAACAAGAA 3’
				qCle_cyp404R	qRT-PCR	5’ CGTGAAATCTCAGTACCAACCGGGTA 3’
	contig06517	1175	CYP6DL1	qCle_cyp6dl1F	qRT-PCR	5’ TTTCAGGCATGAATCGCTCTGGGTCA 3’
				qCle_cyp6dl1R	qRT-PCR	5’ TTGAGGAAATACCCTCCTGTCCCA 3’
	contig18189	318	CYP6DL2	qCle_cyp6dl2F	qRT-PCR	5’ TTTCAAAGGGAACCGTTATGGA 3’
				qCle_cyp6dl2R	qRT-PCR	5’ CGTTCTCCATGTGGATGAGATGGT 3’
	contig08845	941	CYP6DM1	qCle_cyp6dm1F	qRT-PCR	5’ CCTAGTAACTGCCCTGTCACGTTT 3’
				qCle_cyp6dm1R	qRT-PCR	5’ TGACAGATGTCGAGGCGAACTTCA 3’
	contig02437	1896	CYP6DM2	qCle_cyp6dm2F	qRT-PCR	5’ GCTACGCACTTTACGAACTGGCAATGA 3’
				qCle_cyp6dm2R	qRT-PCR	5’ TATGGCATTTCTTGCAACGCCTCG 3’
	contig04490	1460	CYP6DN1	qCle_cyp6dn1F	qRT-PCR	5’ GCGAGTCTGGGAAATTGTGCATGAAT 3’
				qCle_cyp6dn1R	qRT-PCR	5’ AATGCCCGATTACGATGTCAGGGA 3’
				dCl_CYP6dn1F	RNAi	5’ TAATACGACTCACTATAGGGCCTCATTCCGATGCAAACTCGAGG 3’
				dCl_CYP6dn1R	RNAi	5’ TAATACGACTCACTATAGGGTTACAGGCGGTTTGGAACCG 3’
	contig04426	1469	CYP6DN2	qCle_cyp6dn2F	qRT-PCR	5’ CTGTTCTTGGCGAAACGGCTTCAA 3’
				qCle_cyp6dn2R	qRT-PCR	5’ TTTCGGGACGAAGAACTAAGGGCT 3’
	contig07269	1092	CYP6DN3	qCle_cyp6dn3F	qRT-PCR	5’ TGGGACGCAAATCTTCGTATCACTCC 3’
				qCle_cyp6dn3R	qRT-PCR	5’ TTCCTATGCACAATCTCGGACCCT 3’
	contig03136	1718	CYP6DN4	qCle_cyp6dn4F	qRT-PCR	5’ AGAAATGTGGGCAGCCTCACTGT 3’
				qCle_cyp6dn4R	qRT-PCR	5’ TGTAGAAACCTCGGTGTATGGGCT 3’
	contig04099	1524	CYP4CM1	qCle_cyp4cm1F	qRT-PCR	5’ ATTGGTAACATTGGAGGCCCTGGA 3’
				qCle_cyp4cm1R	qRT-PCR	5’ AGAGATTTGCCTTACCACCAGCGA 3’
				dCl_CYP4cm1F	RNAi	5’ TAATACGACTCACTATAGGGGTTCTGCAAAATGCACAGCTAAGG 3’
				dCl_CYP4cm1R	RNAi	5’ TAATACGACTCACTATAGGGGACTGAGTGCACATCAACTCAAG 3’
	contig02986	1755	CYP4CN1	qCle_cyp4cn1F	qRT-PCR	5’ ATCCAACGGTGCCATTTATCGGGA 3’
				qCle_cyp4cn1R	qRT-PCR	5’ AATGGATGGCGTTGTGCTACGTTC 3’
	contig03005	1748	CYP4CP1	qCle_cyp4cp1F	qRT-PCR	5’ TTCATATCGGACTGCACTCTGGAC 3’
				qCle_cyp4cp1R	qRT-PCR	5’ TCCAAATGGAGCCAAGGTTTCTGC 3’
	contig01154	2455	CYP4CQ1	qCle_cyp4cq1F	qRT-PCR	5’ TGTTGGGATCATCTTCAGGGCTCAA 3’
				qCle_cyp4cq1R	qRT-PCR	5’ AACAGTTTCTTCATCGCGCCAACG 3’
	contig02002	2037	CYP4G52	qCle_cyp4g52F	qRT-PCR	5’ AGGCCTAGTGAGCTTTCTGGTGTTT 3’
				qCle_cyp4g52R	qRT-PCR	5’ TCAGAGTCAAGGAAGGCAAACCGA 3’
	contig03206	1705	CYP4G53	qCle_cyp4g53F	qRT-PCR	5’ GAACGCAGGCGCGATTAACTTTC 3’
				qCle_cyp4g53R	qRT-PCR	5’ TTTCAACCATGGCTTGGGAAT 3’
	contig02404	1905	CYP4G54	qCle_cyp4g54F	qRT-PCR	5’ TGGCTTGGACCCAGACTAATCGT 3’
				qCle_cyp4g54R	qRT-PCR	5’ AAACCACGGCTGGAAGTAGCTGTA 3’
Esterase	N/A	N/A	ClCE3959*	ClCE3959qRTF	qRT-PCR	5’ ACGTCTGGAGAAGGGCAACTGAAA 3’
				ClCE3959qRTR	qRT-PCR	5’ GACGGCCGGGTAGATGAAAACAAC 3’
	Contig03262	1690	ClCE21331	ClCE21331qRTF	qRT-PCR	5’ TCTCACGGGGACGAACTGCCTTAT 3’
				ClCE21331qRTR	qRT-PCR	5’ CCTGGTCTTCTGGGTATTTCTTCA 3’

				ClCE21331qRTF	RNAi	5’ TAATACGACTCACTATAGGGACAGGCATGCTTACATTCCTCCGA 3’
				ClCE21331qRTR	RNAi	5’ TAATACGACTCACTATAGGGTAATCTTCGTGGAGGCCGGTGAAA 3’
GST	N/A	N/A	GSTs1*	ClGSTS1qRTF	qRT-PCR	5’ AGGAGAGCCAGTTAGATTTATGTT 3’
				ClGSTS1qRTR	qRT-PCR	5’ AAGCGATTCCCACCGATTTT 3’
Cuticular proteins	09967	856	C1	ClCut09967qRTF	qRT-PCR	5’ TACACATTGTTCGCAGCTTGCCTG 3’
				ClCut09967qRTR	qRT-PCR	5’ GTCGTTGTGCCTGTTGATCTGCTT 3’
	15313	480	C2	ClCut15313qRTF	qRT-PCR	5’ ACGGAACCCATCCCGATCCTTAAA 3’
				ClCut15313qRTR	qRT-PCR	5’ ATGCCGTTACCGGTTTCGTATTCC 3’
				ClCut15313DsF	RNAi	5’ TAATACGACTCACTATAGGGCCAAGGAAAGCGGCTTCGTCAAA 3’
				ClCut15313DsR	RNAi	5’ TAATACGACTCACTATAGGGAGATAGTCGAGTGCCCTCTGGAT 3’
	12749	648	C3	ClCut12749qRTF	qRT-PCR	5’ ACTTTGCTGCGCTAGTTGCTGTTG 3’
				ClCut12749qRTR	qRT-PCR	5’ ATTTCGTTGCCGGTTTCATACGCC 3’
	12072	700	C4	ClCut12072qRTF	qRT-PCR	5’ TCTACAAACCTGCGACTGTTGCCT 3’
				ClCut12072qRTR	qRT-PCR	5’ AGCATAGTTGGCGTATGGAGCTGA 3’
	10057	847	C5	ClCut10057qRTF	qRT-PCR	5’ TTACGGAGGATACGGCTTGGGAAA 3’
				ClCut10057qRTR	qRT-PCR	5’ ACTGCGTTCCTCACTGGAAATCCT 3’
	14205	551	C6	ClCut14205qRTF	qRT-PCR	5’ TCCAACCTCAACCAGGTCCCAATA 3’
				ClCut14205qRTR	qRT-PCR	5’ TACCGTTTCCGGTTTCGTAGCTGT 3’
	18423	308	C7	ClCut18423qRTF	qRT-PCR	5’ AACAGTCCTTGCAACCAGCATTGG 3’
				ClCut18423qRTR	qRT-PCR	5’ GCGGTTCTAGTTTCGTGTTTGGCA 3’
	09970	855	C8	ClCut09970qRTF	qRT-PCR	5’ AAATCTGTCGCTGTCCCAGCTGTA 3’
				ClCut09970qRTR	qRT-PCR	5’ TTTGTTTCCATGGGCGTCTTGGTG 3’
	14186	550	C9	ClCut14186qRTF	qRT-PCR	5’ GTCTCGGCAAGCAAACCAACATGA 3’
				ClCut14186qRTR	qRT-PCR	5’ TGGTCTGGACAAGGTTTGCGGTTA 3’
	02621	1840	C10	ClCut02621qRTF	qRT-PCR	5’ ACTCCTGAAGTCCAAGCAGCAAGA 3’
				ClCut02621qRTR	qRT-PCR	5’ TCGGAAGGGTTGTCCAAATCGTGA 3’
				ClCut02621dsRF	RNAi	5’ TAATACGACTCACTATAGGGAGTACCACAATGGGTCATCAGGCA 3’
				ClCut02621dsRR	RNAi	5’ TAATACGACTCACTATAGGGACAGCGGCAACTGGTATATCTCCT 3’
	12101	691	C11	ClCut12101qRTF	qRT-PCR	5’ AGTACGCAACAATTCTGGGAGGCA 3’
				ClCut12101qRTR	qRT-PCR	5’ TGGCAGCATGGTAGTTGATCGGTA 3’
	16495	409	C12	ClCut16495qRTF	qRT-PCR	5’ ACTCAGGAAGAGTCTCGTATCGGT 3’
				ClCut16495qRTR	qRT-PCR	5’ CTGAACATGGGCATTGAAGCCTGT 3’
	8158	1004	C13	ClCut08158qRTF	qRT-PCR	5’ CCAAGCGGTCAAAGCAGCACATTT 3’
				ClCut08158qRTR	qRT-PCR	5’ AGCCTAGCGAGAGCTTGGTTGTAA 3’
				ClCut08158dsRF	RNAi	5’ TAATACGACTCACTATAGGGACAGCCGATCGGGTTACTATTGGT 3’
				ClCut08158dsRR	RNAi	5’ TAATACGACTCACTATAGGGAAATTGTACGCTCTGGCTTTGGCG 3’
	14441	537	C14	ClCut14441qRTF	qRT-PCR	5’ ATCCCAGACAGTCCTGAAGTGGAA 3’
				ClCut14441qRTR	qRT-PCR	5’ TTATTGAGACAGCGCGAGTTTGGC 3’
	06115	1223	C15	ClCut06115qRTF	qRT-PCR	5’ ACGGTCAAGCACCTGTCTCAGATT 3’
				ClCut06115qRTR	qRT-PCR	5’ AGACTGAAGCCGGAGCTTTGTGTA 3’
Abc transporters	N/A	N/A	Abc1**	BBABCtransporterF	qRT-PCR	5’ TGCTCTACATAATTCTGACAT 3’
				BBABCtransporterR	qRT-PCR	5’ GTAGGACGGTATGAGGTA 3’
	contig04658	1420	Abc2	ClABCT04658F	qRT-PCR	5’ TCGTTGCCACAGGGTTATGACACA 3’
				ClABCT04658R	qRT-PCR	5’ TGGAGTCAAGAGCAGAAGTTGCCT 3’
	contig16653	400	Abc3	ClABCT16653F	qRT-PCR	5’ AACCAGGAAAGTCTGTCGCTTTGG 3’
				ClABCT16653R	qRT-PCR	5’ CGTAGAAACGCAGTAGCAAACCTACG 3’
	contig09770	871	Abc4	ClABCT09770F	qRT-PCR	5’ AAGACAGGAAGGTGTGCGAGAAGT 3’
				ClABCT09770R	qRT-PCR	5’ CAAGCATGGCGTTAATTCCGGTGT 3’
	contig04780	1404	Abc5	ClABCT04780F	qRT-PCR	5’ CAACCTCGCTTGCCATGTCTTCAA 3’
				ClABCT04780R	qRT-PCR	5’ TTGTCGATGCCGAGCCAAAGTACA 3’

contig01139	2453	Abc6	ClABCT01139F	qRT-PCR	5’ ACTAAGCGCCAGCAAGGAAATGTG 3’
			ClABCT01139R	qRT-PCR	5’ TTGGAGCTATCGAATTCCCAGGCA 3’
contig06994	1124	Abc7	ClABCT06994F	qRT-PCR	5’ TTCAACATCCACCGAGTATGCCCT 3’
			ClABCT06994R	qRT-PCR	5’ TTGTACCTCTTGGCAGGGTCATGT 3’
contig08506	976	Abc8	ClABCT08506F	qRT-PCR	5’ ATCCTGATGGGCCGAGTAAACCAT 3’
			ClABCT08506R	qRT-PCR	5’ TTCTGGAGGTGACCGTCAAGTTGT 3’
			dClABCT08506F	RNAi	5’ TAATACGACTCACTATAGGGCCTATTTTGTTAGCCTGTTTGG 3’
			dClABCT08506R	RNAi	5’ TAATACGACTCACTATAGGGGATGGGTGAATGGAATGACTG 3’
contig02154	1984	Abc9	ClABCT02154F	qRT-PCR	5’ TTTAGCAACCGATGTGACGCAAGC 3’
			ClABCT02154R	qRT-PCR	5’ TGACCCAGACGTTGTCAACACAGA 3’
			dClABCT02154F	RNAi	5’ TAATACGACTCACTATAGGGATGGGTTTGTCATATCCTGCG 3’
			dClABCT02154R	RNAi	5’ TAATACGACTCACTATAGGGCGTCGAGAGGAAAATCAAAGTCC 3’
contig05955	1243	Abc10	ClABCT05955F	qRT-PCR	5’ TCACAGCGGTCTTCCTGGATTCTT 3’
			ClABCT05955R	qRT-PCR	5’ AACTTCTGCGCGCACATTAGAACG 3’
contig09403	902	Abc11	ClABCT09403F	qRT-PCR	5’ ATGCAGCTCAGTAGGGTCGTCTTT 3’
			ClABCT09403R	qRT-PCR	5’ CGGGCCAAAGTCAAATCAGCACAT 3’
contig14750	514	Abc12	ClABCT14750F	qRT-PCR	5’ ATTTCGCGCTTATAGATCGCACGC 3’
			ClABCT14750R	qRT-PCR	5’ TTTGCCGAGTGGTATCGTGTAGGT 3’
contig14756	514	Abc13	ClABCT14756F	qRT-PCR	5’ TGTCGAGACATTGAGTGGAGGACA 3’
			ClABCT14756R	qRT-PCR	5’ CGACTGTCGGCTCATCCAAGATTA 3’
Sodium channel			BBParaF1	1^{st} PCR	5’ AACCTGGATATACATGCCTTCAAGG 3’
			BBParaR1	1^{st} PCR, dASPCR	5’ TGATGGAGATTTTGCCACTGATG 3’
			BBParaF3	1^{st} PCR	5’ GGAATTGAAGCTGCCATGAAGTTG 3’
			BBParaR3	1^{st} PCR, dASPCR	5’ TGCCTATTCTGTCGAAAGCCTCAG 3’
			BBParaF1-AS	dASPCR	5’ ATTCCTGGGATCATTCTACCTCG 3’
			BBParaF1-AS1	dASPCR	5’ ATTCCTGGGATCATTCTACCTCC 3’
			BBParaF3-AS	dASPCR	5’ ATTATGGGCAGAACAGTGGGTGCCC 3’
			BBParaF3-AS1	dASPCR	5’ ATTATGGGCAGAACAGTGGGTGCCA 3’

* Transcriptional expression of these genes was reported by Adelman et al., 2011 [18]

**Differential expression between insecticide resistant and susceptible strains was reported by Mamidala et al., 2012 [20]

Supplementary Figure 1. Flowchart for transcriptomic analysis of *C. lectularius* sequences. Ten μg of CIN-1 NS strain cDNA was used for 454 pyrosequencing using Genome Sequencer FLX system (Roche-applied-science) available at Advanced Genomics Technology Center of University of Kentucky (http://www.uky.edu/Centers/AGTC/). The high quality reads from Roche 454 pyreosequencing were combined with four groups of expressed sequence tag (EST) resources in NCBI database including SRX028107 (Accession No.), SRX013985, SRX013984 and 7131 ESTs. Then these data was assembled through Roche *de novo* Assembler program (Newbler).

Supplementary Figure 2. Summary of *C. lectularius* transcriptomic sequences. The length distribution of *C. lectularius* transcript sequences: (A) contigs; (B) singletons.

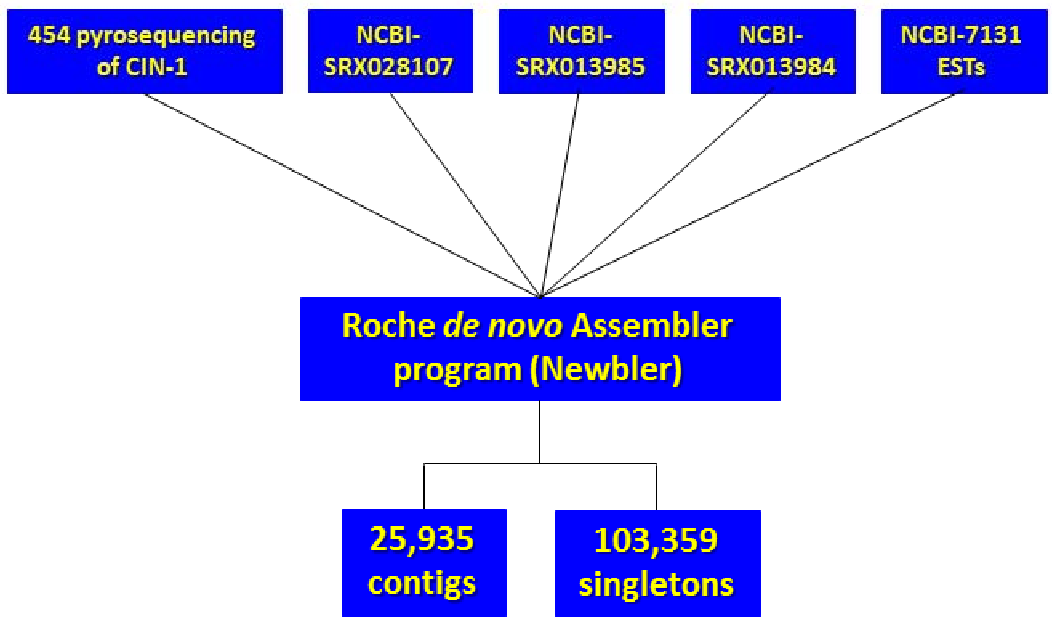
Supplementary Figure 3. Gene ontology (GO) terms for the transcriptomic sequences of *C. lectularius*. (A) molecular function; (B) biological process; (C) cellular component.

Supplementary Figure 4. Spatial expression of resistance associated genes in CIN1 NS strain. The head (H), leg (L), gut (foregut and midgut) (G), fat body (F), mesospermalege (M), ovary (O), and integument (I) were dissected from 1 week-old female adult CIN-1 NS bed bugs. The mRNA levels of 12 target genes were quantified by qRT-PCR. Relative mRNA levels were normalized using the mRNA levels of *rpl8*. The data shown are mean + SEM (n = 3). There was no significant difference among relative expression within samples with the same alphabetic letter (i.e. a, b and c) (One-way ANOVA followed by Duncan multiple mean separation, SAS v9.4).

Supplementary Figure 5. The geographic distribution of 21 bed bug field populations collected from the United States. The red point represents the cities where these bed bug populations collected. These four cities belong to three states, Kentucky, Illinois, and Ohio.

Supplementary Figure 6. Effect of RNAi on cytochrome P450 gene expression 6 days after injection of dsRNA of control *malE* or target P450s mix into CIN-1 S strain. Knockdown efficiency of four target P450s (*CYP397A1*, *CYP398A1*, *CYP4CM1*, and *CYP6DN1)* and three non-target P450s (*CYP399A1*, *CYP4CN1*, *CYP6DM1) are shown*. The results are expressed as mean+SEM (n = 6) as a ratio in comparison with the mRNA levels of *rpl8*. Means labeled with double asterisks (**) represent the significant difference in relative mRNA expression level between control and target P450s RNAi treatment (P450s-KD) (Student t-test, $P < 0.01$).

Figure 1S


454 pyrosequencing of CIN-1
NCBI-SRX028107
NCBI-SRX013985
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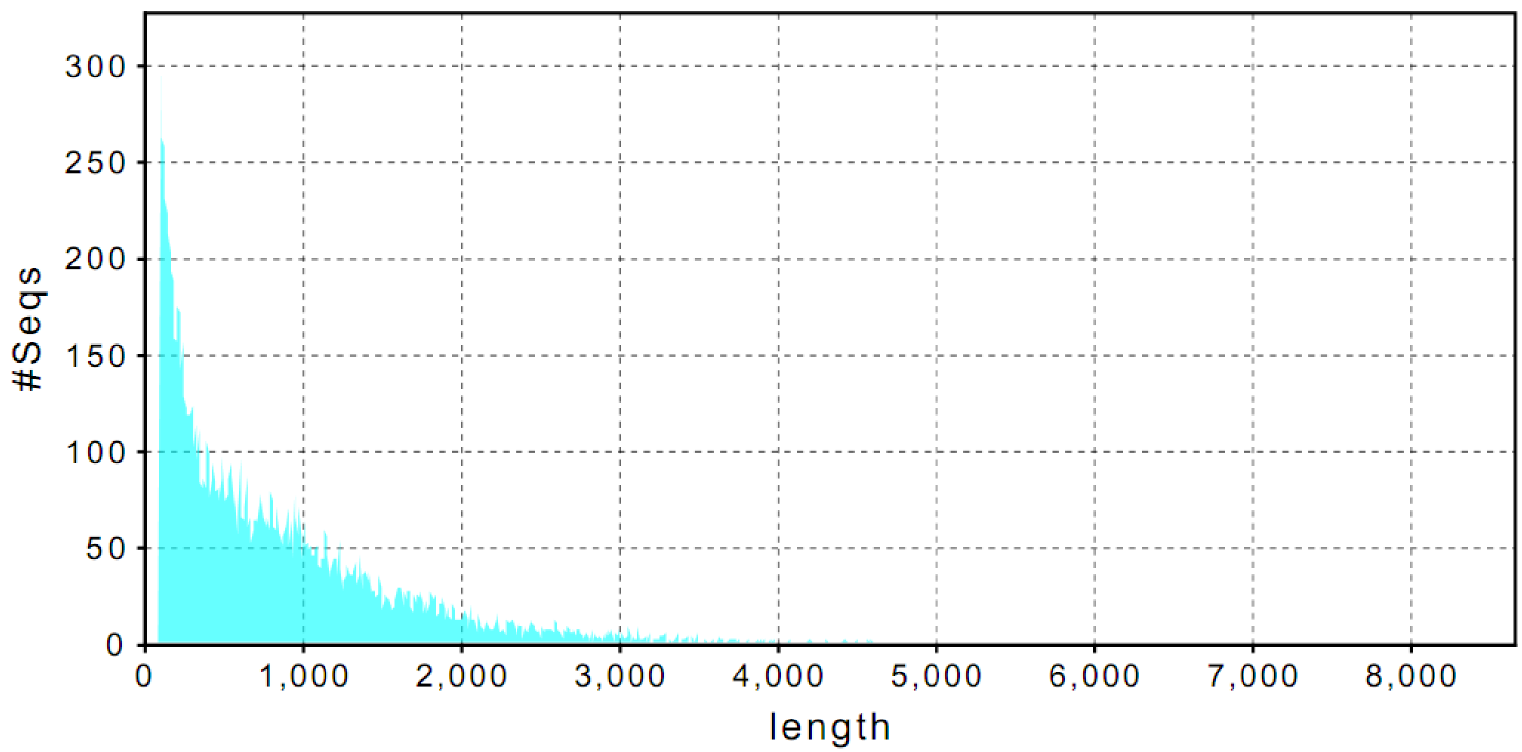
NCBI-7131 ESTs
Roche *de novo* Assembler program (Newbler)
25,935 contigs
103,359 singletons

Figure 2S A



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200
150
100
50
0
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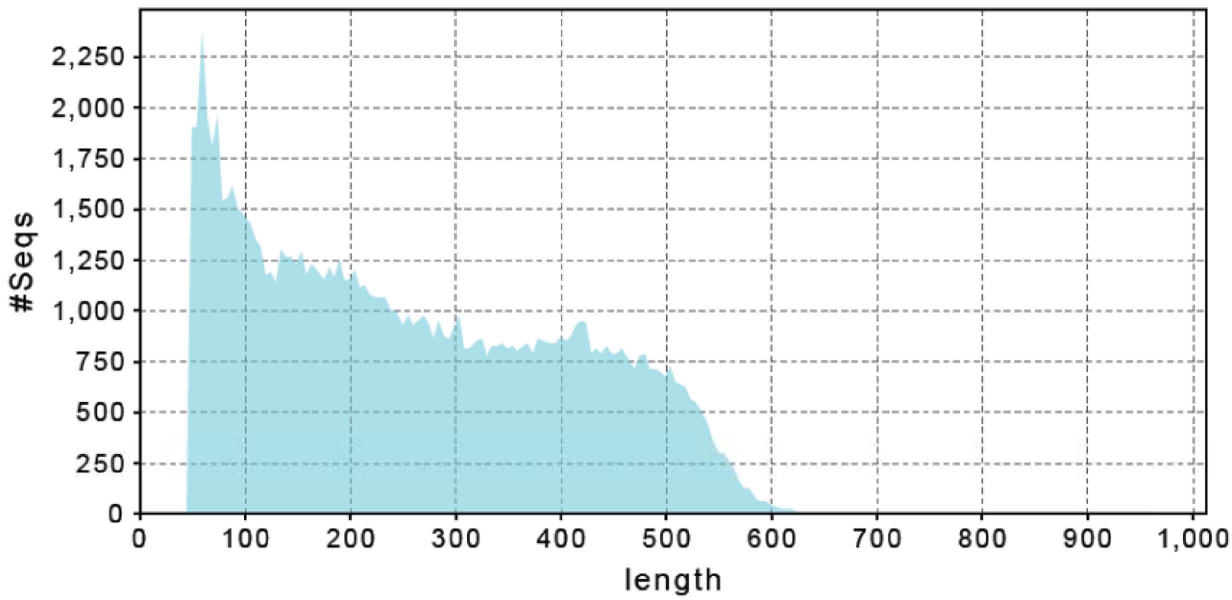
2,000
3,000
4,000
5,000
6,000
7,000
8,000
length

Figure 2S B



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2,000
1,750
1,500
1,250
1,000
750
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100
200
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400
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600
700
800
900
1,000
length

Figure 3S A

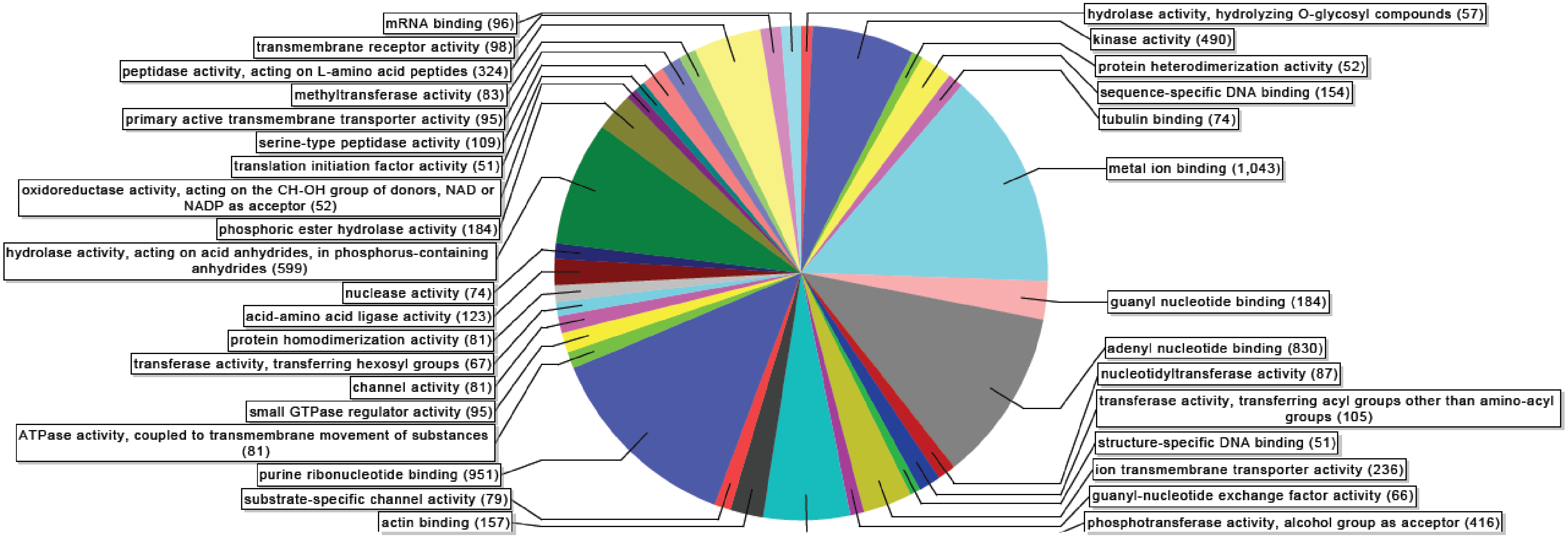

mRNA binding (96)
transmembrane receptor activity (98)
peptidase activity, acting on L-amino acid peptides (324)
methyltransferase activity (83)
primary active transmembrane transporter activity (95)
serine-type peptidase activity (109)
translation initiation factor activity (51)
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor (52)
phosphoric ester hydrolase activity (184)
hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides (599)
nuclease activity (74)
acid-amino acid ligase activity (123)
protein homodimerization activity (81)
transferase activity, transferring hexosyl groups (67)
channel activity (81)
small GTPase regulator activity (95)
ATPase activity, coupled to transmembrane movement of substances (81)
purine ribonucleotide binding (951)
substrate-specific channel activity (79)
actin binding (157)
hydrolase activity, hydrolyzing O-glycosyl compounds (57)
kinase activity (490)
protein heterodimerization activity (52)
sequence-specific DNA binding (154)
tubulin binding (74)
metal ion binding (1,043)
guanyl nucleotide binding (184)
adenyl nucleotide binding (830)
nucleotidyltransferase activity (87)
transferase activity, transferring acyl groups other than amino-acyl groups (105)
structure-specific DNA binding (51)
ion transmembrane transporter activity (236)
guanyl-nucleotide exchange factor activity (66)
phosphotransferase activity, alcohol group as acceptor (416)

Figure 3S B

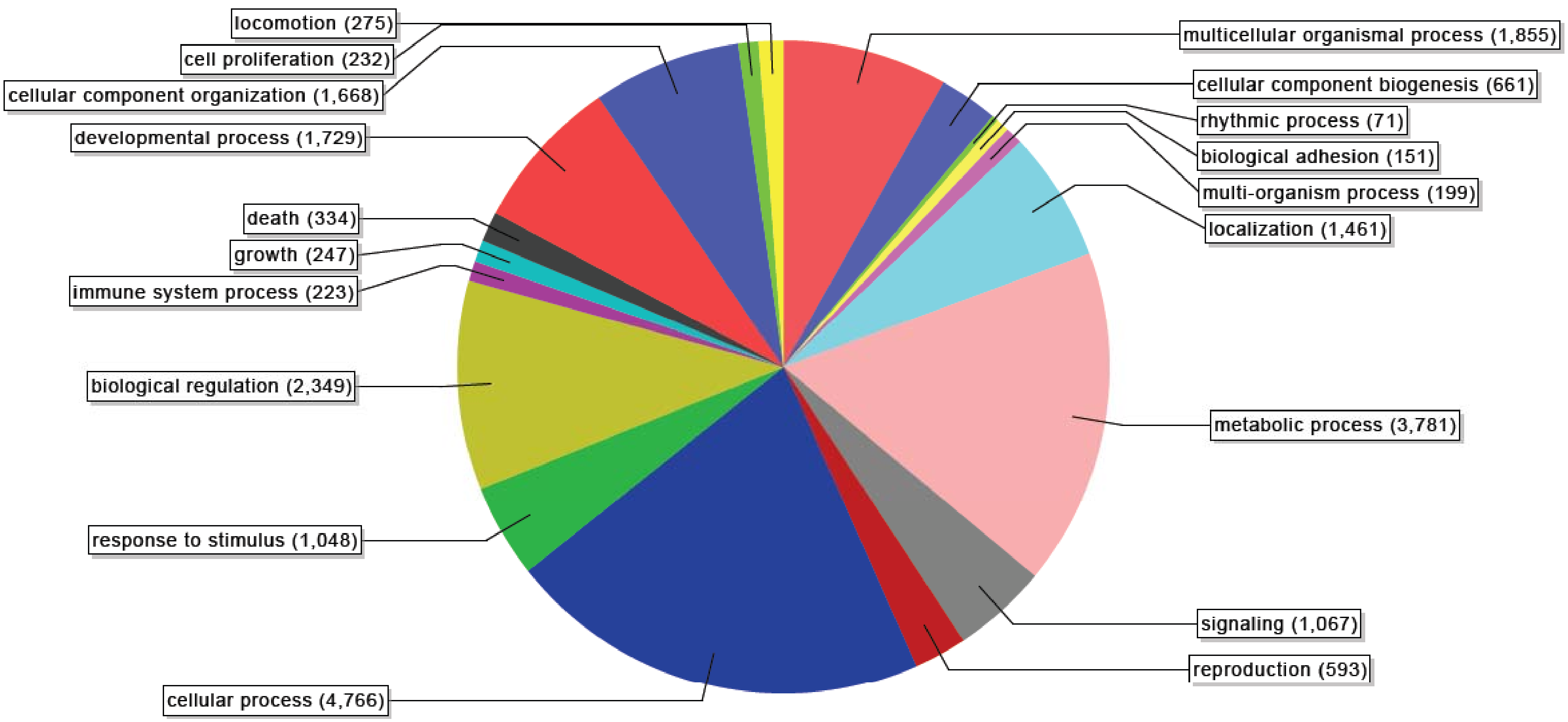

locomotion (275)
cell proliferation (232)
cellular component organization (1,668)
developmental process (1,729)
death (334)
growth (247)
immune system process (223)
biological regulation (2,349)
response to stimulus (1,048)
cellular process (4,766)
multicellular organismal process (1,855)
cellular component biogenesis (661)
rhythmic process (71)
biological adhesion (151)
multi-organism process (199)
localization (1,461)
metabolic process (3,781)
signaling (1,067)
reproduction (593)

Figure 3S C

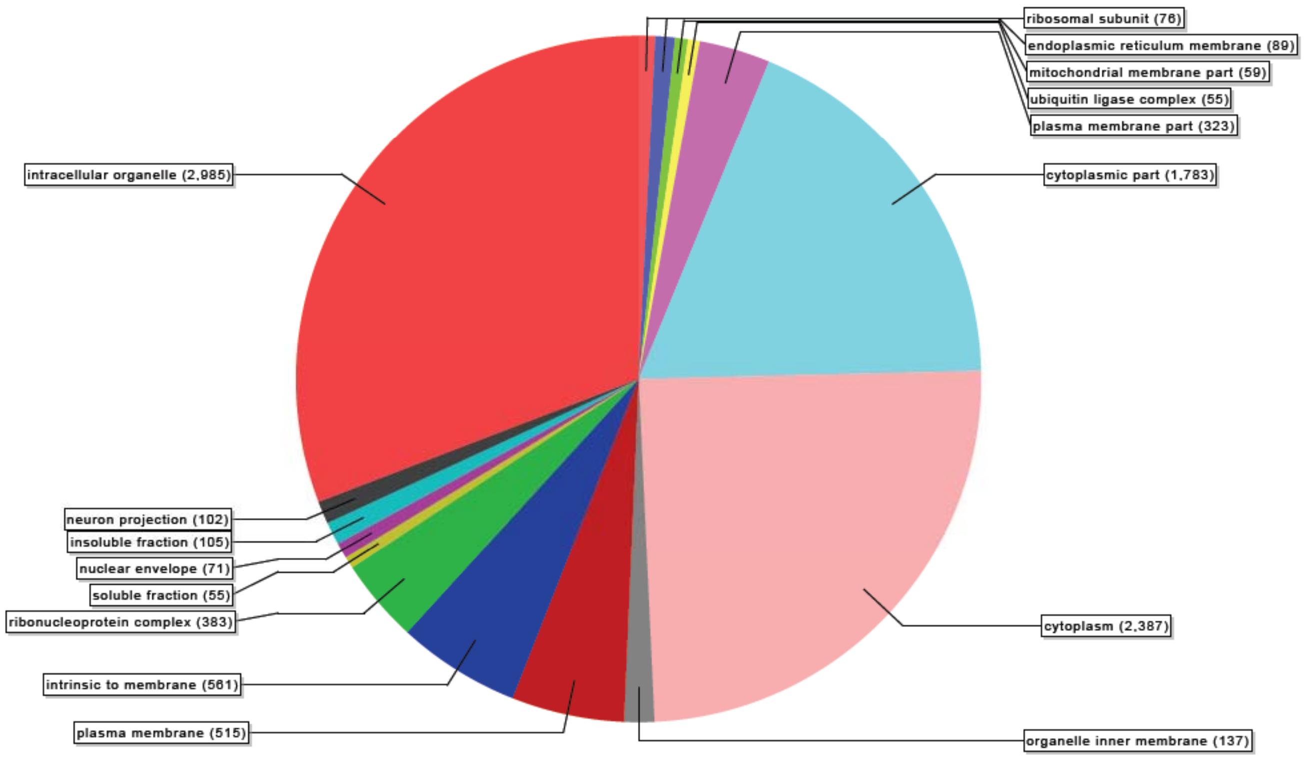

ribosomal subunit (76)
endoplasmic reticulum membrane (89)
mitochondrial membrane part (59)
ubiquitin ligase complex (55)
plasma membrane part (323)
intracellular organelle (2,985)
cytoplasmic part (1,783)
neuron projection (102)
insoluble fraction (105)
nuclear envelope (71)
soluble fraction (55)
ribonucleoprotein complex (383)
cytoplasm (2,387)
intrinsic to membrane (561)
plasma membrane (515)
organelle inner membrane (137)

Figure 4S

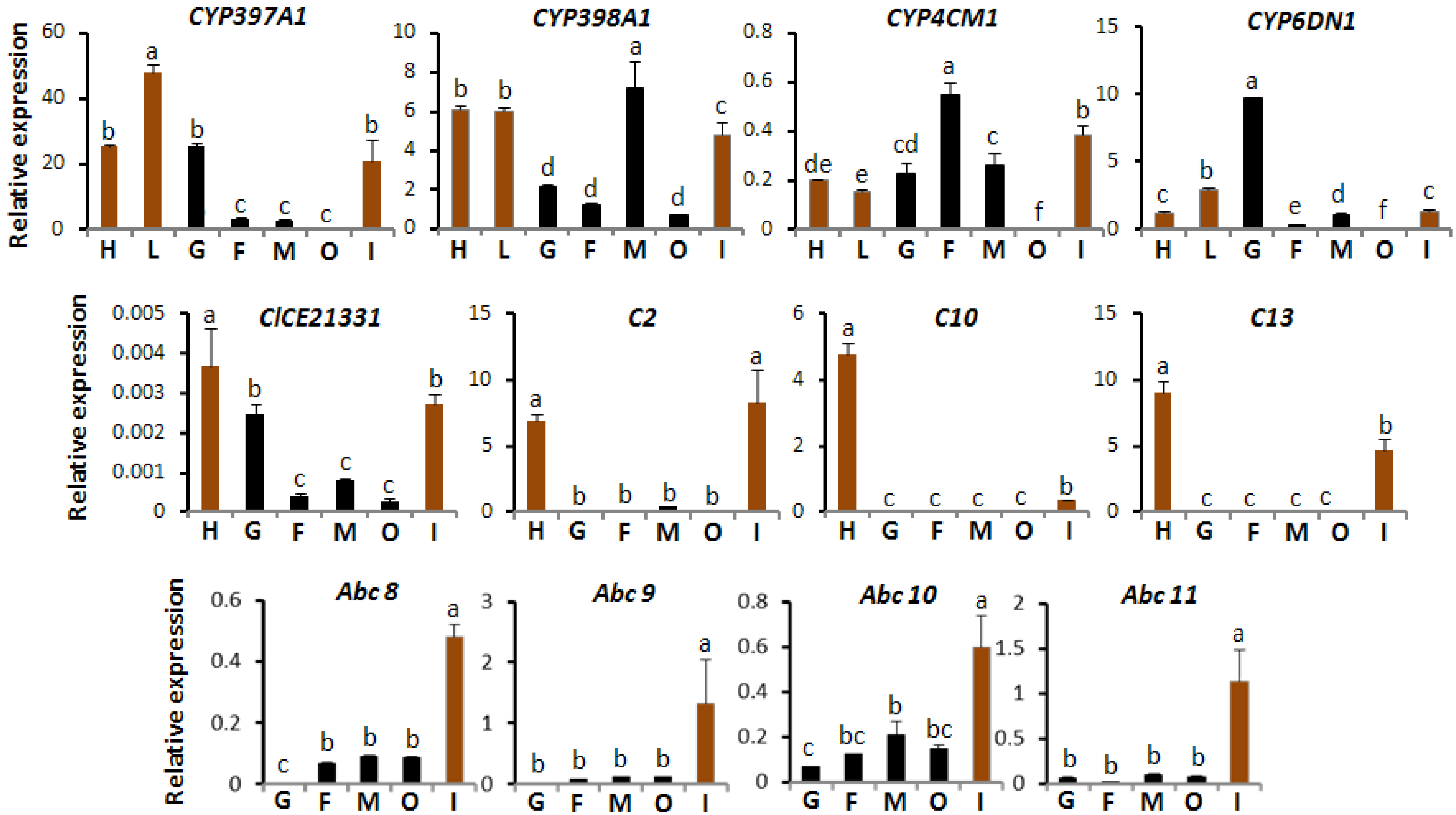
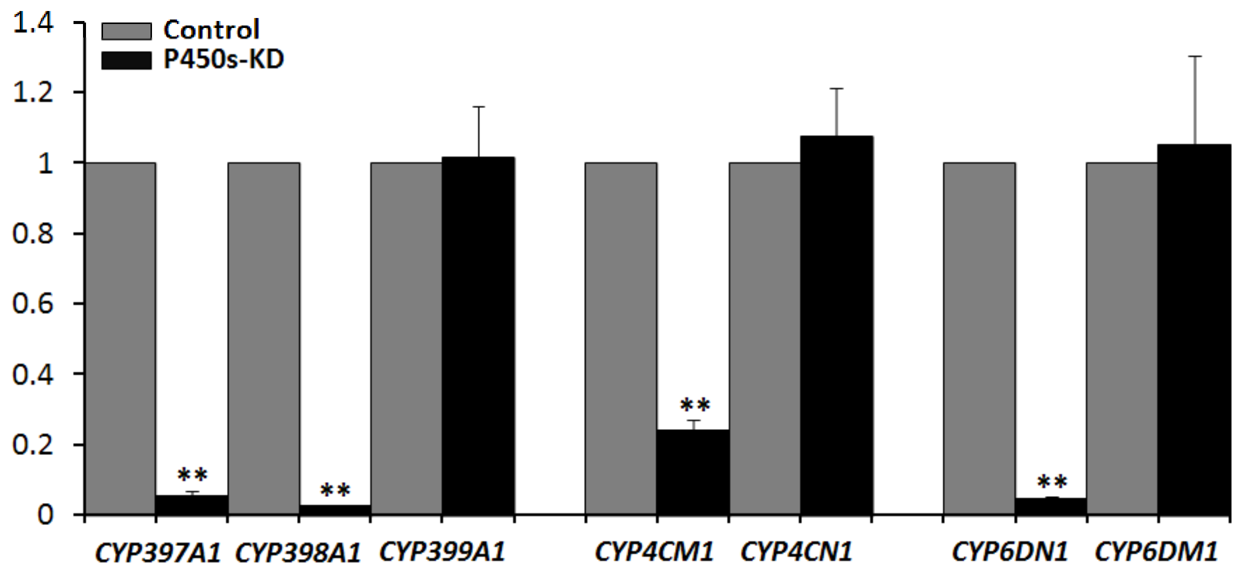

CYP397A1
CYP398A1
CYP4CM1
CYP6DN1
ClCE21331
C2
C10
C13
Abc 8
Abc 9
Abc 10
Abc 11
Relative expression
H L G F M O I
H G F M O I
G F M O I

Figure 5S

Figure 6S



Control
P450s-KD
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
**
**
**
CYP397A1
CYP398A1
CYP399A1
CYP4CM1
CYP4CN1
CYP6DN1
CYP6DM1