농학박사학위논문

꿀벌과 제브라피쉬에서 대사체학과 단백질체학을 이용한 살충제 **abamectin**의 독성학적 비교

Comparative toxicometabolomics and proteomics of insecticide abamectin in honeybee and zebrafish

2021년 2월

서울대학교 대학원
농생명공학부 응용생명화학전공
이 정 학

A Dissertation for the Degree of Doctor of Philosophy

Comparative toxicometabolomics and proteomics of insecticide abamectin in honeybee and zebrafish

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Comparative toxicometabolomics and proteomics of insecticide abamectin in honeybee and zebrafish

Advisor: Jeong-Han Kim

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Abstract

Abamectin is an abamectin insecticide known to be isolated from fermentation of *Streptomyces avermitilis*, a naturally occurring soil Actinomycete. Due to its high toxicity in bees and fish, this study investigated the toxic mechanisms of abamectin in honeybee (*Apis mellifera*) and zebrafish (*Danio rerio*) using targeted metabolomics approach by gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-high resolution mass spectrometry (LC-orbitrap-HRMS). All homogenized samples were extracted with 80% methanol (honeybee) or 50% methanol (zebrafish) and derivatized with TMS for GC-MS/MS analysis. In the targeted metabolomics approach, multiple reaction monitoring (MRM) mode of a GC-MS/MS for 396 metabolites was used to detect 239 metabolites in honeybee and 243 metabolites in zebrafish. With the results of metabolites detected in each sample, statistical analysis such as partial least squares-discriminant analysis (PLS-DA), variable importance in the projection (VIP) and analysis of variance (ANOVA) were performed to identify important biomarkers. Metabolic pathways associated with those biomarkers were constructed using MetaboAnalyst 5.0. In the exposure experiment of honeybee to abamectin as a targeted metabolomics using GC-MS/MS and non-targeted metaboloimcs using LC-orbitrap-HRMS, metabolic pathways such as tyrosine metabolism, phenylalanine/tyrosine/tryptophan biosynthesis, citrate cycle, ascorbate/aldarate metabolism, and alanine/aspartate/glutamate metabolism were identified as the significantly perturbed pathways. While, zebrafish showed several metabolic pathways such as aminoacyl tRNA biosynthesis, glyoxylate/dicarboxylate metabolism, citrate cycle, and tryptophan metabolism were identified by exposure of abamectin. Such significant disturbance of important metabolites within key biochemical pathways by abamectin could result in biologically hazardous effects in honeybee and zebrafish. In toxicoproteomics study, the toxicological effects of abamectin in honeybee and adult zebrafisha were investigated using a label-free quantitative proteomic approach on LC-HRMS. The proteins of honeybee and

zebrafish samples were extracted with 0.1M phosphate buffer (pH 7.4) and 200 μg of proteins were digested with trypsin using the in-solution filter-aided sample preparation (FASP) method. After LC-HRMS analysis, a total of 670 proteins were identified and 32 proteins were selected as biomarkers through volcano analysis in honeybee. In zebrafish, 2189 proteins were identified and 1050 proteins were selected as biomarkers by statistically analysis.

Key words: abamectin, pathway, metabolomics, proteomics, LC-orbitrap-HRMS, GC-MS/MS, honeybee, zebrafish

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Preface

Some pesticides have caused serious adverse effects as a result of direct contamination of ecosystem such as water, soil and air (Yoon et al., 2016b). Abamectin is a mixture of abamectins containing about 80% abamectin B1a and 20% abamectin B1b. The abamectins are 16- membered macrocyclic lactones produced during fermentation by the soil microorganism, *Strepto- myces avermitilis* (Fisher and Mrozik, 1984). Several mode of actions for abamectin have been known to bind on GABA-gated Cl^- channel (Huang and Casida, 1997) while, additional site of action is glutamate gated Cl^- channels in invertebrates (!!! INVALID CITATION !!! (Cully et al., 1994; Cully et al., 1996; Huang and Casida, 1997)). The exposed parasites are subsequently paralysed resulting in uncoordinated movement, starvation and ultimately death due to inhibition of pharyngeal pumping (Bai and Ogbourne, 2016). The relevance between the mechanism of action and the side effects of avemectins in mammals is not clearly known. For example, in mammals, GABA is present in brain cells and hence secured by the blood-brain barrier, which makes this drug relatively safe for vertebrates (Õmura, 2008). However, GluCl channels that exist in insects, nematodes and mites, are closely related to the cys-loop channel receptor family, which include the GABA type A receptors found in mammals (Nakao et al., 2015).

Abamectin has been widely used for 35 years on various crops from introducing in 1985. It also has been used for veterinary drugs, which are known to be excreted through faeces either unchanged or as active metabolites (McKellar and Benchaoui, 1996) and can effect on reproduction, biological function and survival of non-target aquatic and terrestrial organisms (Beesley, 1991; Halley et al., 1993; Moore et al., 1993; Steel et al., 2002). They are readily adsorbed by organic

matter, soil and sediment particles and they also could pose a risk to the aquatic environment, especially when used frequently in large numbers of animals.

The purpose of this study is to evaluate the toxic effects of abamectin in honeybee and zebrafish using metabolomics and proteomics approaches. For efficient and accurate analysis of various metabolites, GC-MS/MS and LC-orbitrap-HRMS were used as analytical methodology.

This study compriese two chapters, metabolomics and proteomics studies. In Chapter I, toxicometabolomics studies of abamectin in honeybee and adult zebrafish were conducted using GC-MS/MS and LC-orbitrap-HRMS to elucidate the toxicological effects of abamectin.

In Chapter II, toxicological effects of abamectin were also demonstrated through toxicoproteomics studies of abamectin in honeybee and adult zebrafish using LC-orbitrap-HRMS.

This study emphasizes the application of high quality mass spectrometry for understanding the toxic mechanisms of pesticides at the molecular level and this metabolomics and proteomics research will contribute to the risck assessment of abamectin.

Chapter I

Toxicometabolomics of Abamectin in Honeybee (*Apis mellifera*) and Zebrafish (*Danio rerio*)

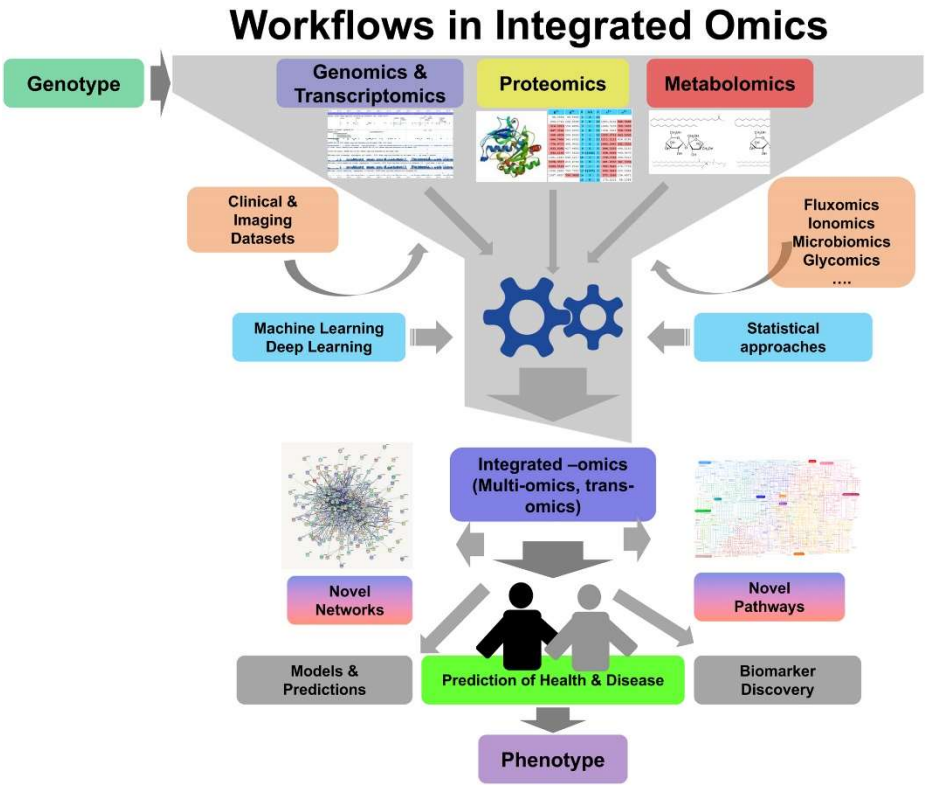
Introduction

The metabolome was known as a set of small molecules that was synthesized by a cell (Tweeddale et al., 1998). In addition, a study called metabolomics emerged in the field of analytical biochemistry, showing chemical fingerprinting and specific cellular processes (Tweeddale et al., 1998). Metabolomics consists of identifying, quantifying, and studying the characteristics of living organisms (German et al., 2005) which are chemically transformed during metabolism in response to a particular stimulus or a disease state, giving the *in vivo* information about the actual status of the body (Rochfort, 2005). In addition, metabolomics is being applied worldwide as a new "omics" through linkage with genomics, proteomics, and transcriptomics (**Fig.1.**).

In addition, metabolomics has a significant impact on various fields such as food, plant science and drug development toxicology. Metabolites are the final steps expressed in higher concepts such as genes, transcripts, and enzymes, and can be used as an important measure for understanding animals, humans and cells. Therefore, this study can also be applied to assess the disease, drug efficacy and environmental risk of organisms (Gowda and Djukovic, 2014b).

In the case of Toxicometabolomics, it is a study that explores the biochemical reactions of organisms in order to elucidate the mechanism of toxicity, so that the effect of chemical substances on organisms and the environment can be understood (Lindon and Nicholson, 2008; Keum et al., 2010). Existing toxicological endpoints are inefficient in distinguishing toxic substances that produce similar phenotypes. (Mishra et al., 2017). In practice, metabolite changes indicate toxicity, genetic alteration, or disorder due to environmental factors and disease states (Jia et al., 2018). Therefore, metabolomics has become an important study in life science to explain biological changes to specific stimuli (Ribbenstedt et al., 2018b).

Fig. 1. The diagram of the interaction between omics sciences (Misra et al., 2019)


Workflows in Integrated Omics
Genotype
Genomics & Transcriptomics
Proteomics
Metabolomics
Clinical & Imaging Datasets
Fluxomics
Ionomics
Microbiomics
Glycomics
....
Machine Learning Deep Learning
Statistical approaches
Integrated –omics (Multi-omics, trans-omics)
Novel Networks
Novel Pathways
Models & Predictions
Prediction of Health & Disease
Biomarker Discovery
Phenotype

Methodology of metabolomics

The basic research structure of metabolomics consists of pattern recognition and statistical analysis (Clayton et al., 2006). That is, it consists of sample preparation, sample analysis, data analysis and metabolic pathway analysis (Yang et al., 2019).

Sample preparation usually consists of extraction and compound separation, and the quality of the sample is critical to the success of the experiment. Often a large amount of pretreatment is required, including sample storage, management, and extraction so that metabolic information is not altered by sample processing. When analyzing a sample with, for example, nmr, a specific pH and ionic strength of the sample is required. In purification during sample preparation, it is important to remove macromolecules and recover small molecules as much as possible. (Yang et al., 2019).

In general, metabolomics uses mass spectrometry or NMR. (Dunn et al., 2005). The NMR is also performed to determine the biological response of an organism to environmental stress. (Yuk et al., 2013; Yoon et al., 2016b; Teng et al., 2018). The NMR is relatively simple and convenient, but its sensitivity is low, so the detection range of metabolites is small. (Ong et al., 2009); Also, it is difficult to identify due to low resolution and overlapping resonance between compounds. (Barding Jr et al., 2013). On the other hand, GC-MS and LC-MS have excellent sensitivity and excellent metabolite resolution. (Ong et al., 2009). When using GC-MS/MS using MRM mode along with mass spectrometer, it is possible to reduce pollutant interference and increase selectivity and sensitivity (Wei et al., 2010; Tsugawa et al., 2014b). And LC-MS recently showed great potential in the field of metabolomics through excellent selectivity and sensitivity in aquatic organisms (Gómez-Canela et al., 2018). In particular, among mass spectrometers, HRMS is known to be excellent in mass measurement accuracy (Chen et al., 2016; Gómez-Canela et al., 2016; Knolhoff and Croley, 2016).

Using pattern recognition approaches such as 'unsupervised' principal component analysis (PCA) and 'supervised' partial least squares-discriminant analysis (PLS-DA), metabolomics could provide a comprehensive assessment of the metabolite. PCA is commonly used early and often to identify patterns and trends in metabolomics data (Wang et al., 2011). Next, as a method of distinguishing samples by maximizing the covariance between independent and dependent variables such as PLS-DA, it has better distinguishing ability between groups than PCA (Wang et al., 2012). The purpose of PLS-DA is to differentiate groups, and it is used to classify samples from different groups into different classes and extract biomarkers through loading plots of endogenous metabolites (Wang et al., 2012; Zhang et al., 2012). In general, metabolomics can statistically process a large amount of data to extract potential information from the data and make biological interpretations possible, thus contributing to understanding the expression of organisms (Yang et al., 2019).

In the final step, it is linked to the biological context under study through the pathway analysis of metabolites. (Cambiaghi et al., 2017). In other words, it aims to identify significant expressed changes between each other by examining predefined groups of functionally related metabolites. Through this, biological pathways and disease states can be proposed using the list of changed metabolites. (Xia and Wishart, 2010c). Conversely, pathway analysis involves visualization of the interactions between genes, proteins or metabolites. This is to identify pathways that have many influences on a given biological process. (Xia et al., 2011). Enrichment and pathway analyses are performed using *ad hoc* software tools (**Table 1**), which map significant metabolites to known biochemical pathways on the basis of the information contained in public databases (**Table 2**) such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Ogata et al., 1999; Spicer et al., 2017).

The metabolomics method mentioned above can be divided into two concepts: target and non-target. Non-targets identify metabolites through non-

specific screening, while targets allow identification and quantification of metabolites centered around the analysis of selected metabolites (Hirata et al., 2017b). Quantitative target metabolomics can be used to establish the level of metabolites in an organism and to define the state of comparison or change between laboratories (Simmons et al., 2015). Targeted approaches require prior knowledge of specific metabolites and risk overlooked metabolic reactions of interest due to the limited range of metabolites. In contrast, non-target metabolomics has the potential to determine new biomarkers (Vrhovsek et al., 2012; Zhang et al., 2016). However, this approach can bias the analysis conditions to the type of separate column. However, the effect of analysis conditions on the analysis range is not well reported. The non-targeted approach does not have a standardized method, so metabolite drift may occur due to matrix effect. Finally, non-target metabolomics has limitations in accurate quantification and may hinder comparisons between experimental groups. (Ribbenstedt et al., 2018a). Therefore, non-targeted metabolomics often provides more information than targeted metabolomics, but targeted metabolomics typically is more quantitative (Zhang et al., 2016)

Table 1. Representative platforms for metabolomics analysis and interpretation (Booth et al., 2013; Zhang et al., 2016; Zhang et al., 2018).

Name	Access	Database	Functions	Wepsite	References
MetaboAnalyst	Web-based	KEGG, HMDB, SMPDB	Full processing, Statistical analysis, Enrichment analysis, Pathway analysis	https://www.metaboanalyst.ca	(Xia et al., 2009; Xia and Wishart, 2010a; Xia and Wishart, 2011; Xia et al., 2015)
XCMS	Web-based, R-package	METLIN, KEGG, HMDB, Lipid Maps, NIST, MassBank	Full processing, Statistical analysis, Enrichment analysis, Pathway analysis	https://xcmsonline.scripps.edu/	(Tautenhahn et al., 2012; Gowda and Djukovic, 2014a)
IMPaLA	Web-based	KEGG, HMDB, CAS, ChEBI, PubChem, Reactome, Wikipathways	Enrichment Analysis	http://impala.molgen.mpg.de/	(Kamburov et al., 2011)
Metabox		KEGG, PubChem, UniProt, ENSEMBL, miRTarBase, BioGRID, Pathway Commons	Enrichment analysis	https://kwanjeeraw.github.io/metabox	(Rodchenkov et al., 2020)
Pathway Commons	Web-based, R-package	Reactome, NCI PID, PhosphoSitePlus, HumanCyc, HPRD, PANTHER, DIP, BioGRID, intAct, BIND, CORUM, MSigDB,	Enrichment analysis	http://www.pathwaycommons.org	(Cerami et al., 2010)

Name	Access	Database	Functions	Wepsite	References
		miRTarBase, DrugBank, Recon X, CTG, KEGG, SMPD, INOH, NetPath, WikiPathways, ChEBI, SwissProt, UniChem			
RaMP	R package, MySQL Dump, Python code to build MySQL Dump	KEGG, Reactome, WikiPathways, HMDB/SMPDB	Enrichment analysis, Pathway analysis	https://github.com/Mathelab/RaMP-DB/	(Zhang et al., 2018)
MetExplore	Web-based	Generally BioCyc related	Compound mapping, graph analysis of metabolism maps.	http://metexplore.toulouse.inra.fr	(Cottret et al., 2010)
PAPi	R Package	KEGG	Compares activity of metabolic pathways between sample types.	http://www.4shared.com/file/0v5zSobM/PAPi_10.html	(Aggio et al., 2010)
MBRole	Web-based	KEGG, HMDB, PubChem, ChEBI, SMILES	Enrichment analysis of metabolites' annotations.	http://csbg.cnb.csic.es/mbrole/	(Chagoyen and Pazos, 2011)
MPEA	Web-based	KEGG, GMD, SMPDB	Pathway enrichment analysis.	http://ekhidna.biocenter.helsinki.fi/poxo/mpea/	(Kankainen et al., 2011)

Name	Access	Database	Functions	Wepsite	References
MeltDB (MSEA)	Web-based	GMD, KEGG, ChEBI, CAS	Comprehensive preprocessing, statistical analysis and metabolite mapping, enrichment analysis.	http://www.cebitec.uni-bielefeld.de/groups/brf/software/meltdb_info/	(Neuweger et al., 2008; Xia and Wishart, 2010c)
Meta P-server	Web-based	KEGG, HMDB, LipidMaps, PubChem	Data quality control, statistical analysis, hypothesis testing.	http://metabolomics.helmholtz-muenchen.de/metap2/	(Suhre et al., 2011)
MassTrix	Web-based	KEGG, HMDB, LipidMaps	Compound mapping	http://metabolomics.helmholtz-muenchen.de/masstrix2/	(Suhre and Schmitt-Kopplin, 2008; Wägele et al., 2012)
BioCyc (Pathway Tools)	Installation required	MetaCyc	Network exploration, genome annotation, 'omics data painting.	http://biocyc.org/	(Karp et al., 2009)
Pathos	Web-based	KEGG	Compound mapping	http://motif.gla.ac.uk/Pathos/index.html	(Leader et al., 2011)
PaintOmics	Web-based	KEGG	Compound mapping	http://www.paintomics.org	(García-Alcalde et al., 2011)
MetaMapp	Web-based	KEGG	Metabolite networking	http://uranus.fiehnlab.ucdavis.edu:8080/MetaMapp/homePage	{Barupal, 2012 #239
VANTED	Installation required	KEGG	Metabolite networking, compound mapping, statistical analysis	http://vanted.ipk-gatersleben.de/	(Junker et al., 2006)

Name	Access	Database	Functions	Wepsite	References
TICL	Web-based	KEGG	Enrichment analysis.	http://mips.helmholtz-muenchen.de/proj/cmp/home.html	(Antonov et al., 2009)

Table 2. Representative biochemical databases (Go, 2010; MetabolomicsSociety, 2014)

Classification	Database	Content	Website
Comprehensive metabolomics databases	HMDB	Metabolites found in the human body, NMR, GC-MS and MS/MS data, physical, clinical and physicochemical properties, chemical structure, concentration data, pathway information, experimental methods	https://hmdb.ca/
	BiGG	Metabolic reconstruction of human metabolism designed for systems biology simulation and metabolic flux balance modeling	http://bigg.ucsd.edu/
	MetaboLights database	Metabolomics experiments and derived information with cross-species, cross-technique, metabolite structures and their reference spectra as well as their biological roles, locations, concentrations and experimental data	https://www.ebi.ac.uk/metabolights/index
Metabolic pathway databases	KEGG	Metabolic and regulatory pathways, protein–protein interactions, genes of completely and partially sequenced genome, drugs, glycans, small molecules, reactions, functional hierarchies	https://www.genome.jp/kegg/
	MetaCyc	Metabolic pathways of organisms determined from experiments, enzymes, genes, chemical	https://metacyc.org/

Classification	Database	Content	Website
		compounds, biochemical reactions, links to other databases	
	HumanCyc	Human metabolic pathways, genes, metabolic enzymes, links to other databases	https://humancyc.org/
	BioCyc	Pathway and genome database of organisms with completely or partially sequenced genome, database of chemical compounds, links to other databases	https://biocyc.org/
	Reactome	Human metabolic pathways, reactions, proteins, links to other databases	https://reactome.org/
	WikiPathways	Open, collaborative platform for capturing and disseminating models of biological pathways for data visualization and analysis	https://www.wikipathways.org/index.php/WikiPathways
Compound or compound-specific database	PubChem	Chemical compounds and structures, links to *Entrez* databases, bioactivity data from high throughput screening programs	https://pubchem.ncbi.nlm.nih.gov/
	ChEBI	Molecular entities focused on 'small' chemical compounds, structure and nomenclature information along with hyperlinks	https://www.ebi.ac.uk/chebi/
	ChemSpider	Aggregated database of organic molecules containing more than 20 million compounds	http://www.chemspider.com/

Classification	Database	Content	Website
		from many different providers, calculated physico-chemical property values	
Spectral database	HMDB	NMR, GC-MS and MS/MS data, physical, clinical and physicochemical properties, chemical structure, concentration data, experimental methods	https://hmdb.ca/
	BMRB	NMR spectral data, primarily for macromolecules, structures, structure viewing applets, nomenclature data, extensive 1D and 2D spectral peak lists, raw spectra and FIDs	http://www.bmrb.wisc.edu/metabolomics/
	MMCD	NMR and LC MS spectra, physical properties, links to chemical and metabolomic databases, chemical structure	http://mmcd.nmrfam.wisc.edu/
	MassBank	GC, ESI, and FAB-MS, MS/MS spectra, chemical structure and name, separation method, experimental conditions	http://www.massbank.jp/
	Golm Metabolome Database	GC-MS spectra, retention time, experimental methods and protocols	http://gmd.mpimp-golm.mpg.de/
	Metlin	LC/MS, LC/MS/MS, FT-MS spectra, chemical structures, retention time, link to KEGG, chemical formula	https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage

Classification	Database	Content	Website
	BML-NMR	NMR data recorded at 500 MHz using various water suppression methods and acquisition parameters, for solutions at pH values of 6.6, 7.0 and 7.4	http://www.bml-nmr.org/
	mzCloud	Searchable collection of high resolution/accurate mass spectral trees using a new third generation spectra correlation algorithm, identification of unknowns even if they are not present in library	https://www.mzcloud.org/

Application of metabolomics

Microbial metabolomics. Microorganisms are important samples in the field of metabolomics because they have been used to develop experimental procedures and establish research tactics. In metabolomics, controllable experimental conditions such as growth conditions and surrounding environment of microorganisms are required for method verification. Therefore, assay verification is one of the important applications of sample preparation and metabolite measurement (Putri et al., 2013).

One of the breakthroughs in microbial metabolomics was the phenotypic analysis of silent gene mutants (Raamsdonk et al., 2001). In these studies, silent gene knockout mutations were distinguished by metabolic snapshots and multivariate analysis, suggesting possible benefits of metabolomics phenotypes for gene function. Recent developments in metabolic phenotypes have succeeded in semi-rational screening of aging-related genes (Yoshida et al., 2010). In addition, not only research on riboneogenesis in yeast using stable isotopes such as ^{13}C (Clasquin et al., 2011), but also the latest advances in applying footprinting techniques to the analysis of co-culture systems of different microorganisms (Nakanishi et al., 2011) are note worthy.

Several methodbook studies have already been conducted on eukaryotes (Allen et al., 2003; Yoshida et al., 2010; Clasquin et al., 2011; Li et al., 2015). In *S. cerevisiae*, the relationship between metabolic flow and metabolism was found by studying the relationship between metabolite concentration and enzyme (Clasquin et al., 2011). In metabolic engineering, several studies have been conducted to improve the yield of important metabolites such as high SO_2 production in *Saccharomyces pastorianus* (Yoshida et al., 2008), through a combination of metabolomics and transcriptomics. In addition, for the purpose of optimized fermentation, the development of a metabolic engineering strategy that confers resistance to stress using *S. cerevisiae* was studied to find target genes (Hasunuma et al., 2011). The

discovery of a new synthetic pathway for glyoxylate from glycine was achieved using metabolomics and isotope labeling analysis (Villas-Bôas et al., 2005).

Metabolomics is ahead of a long development period in the study of biological systems, and the analysis of metabolites of microorganisms in the future will become an important methodology for the development of methods for higher organism research (Putri et al., 2013).

Animal metabolomics. Metabolomics has been applied to study biological phenomena in models of several organisms such as zebrafish and fruit flies. Analysis of metabolites of these organisms provided physiological, developmental and pathological information. Therefore, metabolomics has the expandability to study other species (Putri et al., 2013). Representative applications of metabolomics in animal science are prediction of vertebrate development (Hayashi et al., 2009; Hayashi et al., 2011), metabolic profiling of male and female (Ong et al., 2009), metabolic profiling of heat or cold stress (Malmendal et al., 2006; Overgaard et al., 2007) and metabolomic of age-related decline of hypoxia tolerance (Coquin et al., 2008).

In particular, fish is also a measure of water pollution among various ecotoxicity models (Park et al., 2015). The tropical fish zebrafish has become an excellent animal model for molecular biology (Gómez-Canela et al., 2017). The zebrafish has many advantages for research in laboratory conditions due to its small size, wide distribution, easy availability, and low maintenance cost. Therefore, it is also a representative model for omics research (Olivares et al., 2013; Xu et al., 2013). Additionally, zebrafish have a wide range of genome and transcriptome properties (Ortiz-Villanueva et al., 2017), and are known to be homologous to mammals physiologically and genetically (Mishra et al., 2017). Approximately 86% of human drug targets have been identified in zebrafish (Gunnarsson et al., 2008). Therefore, zebrafish are well suited to environmental risk assessment of chemical compounds

(McGrath and Li, 2008) and risk assessment for human health (Scholz et al., 2008), and also have been studied in depth with four –omics levels: genomics, transcripomics, proteomics and metabolomics (Ortiz-Villanueva et al., 2017).

The application of metabolomics for biochemical research is limited in animal physiology compared to plant science and microbiology, but recent research has been steadily increasing. The metabolomics approach is a field of research that can be used to identify mutants and to broaden the limited field of research (Putri et al., 2013).

Purpose of study

In the present study, a GC-MS/MS-based targeted metabolomics and a LC-orbitrap-HRMS-based non-targeted metabolomics were used for a more comprehensive assessment of metabolic changes at the metabolite level to elucidate the toxicological effects of abamectin in honeybee and zebrafish.

Materials and Methods

Chemicals and reagents

Abamectin was purchased from Dr. Ehrenstorfer Gmb. (96.4%; Augsburg, Germany). Ribitol was purchased from Wako (Tokyo, Japan) for use as an internal standard, and MSTFA reagent (MSTFA + 1% TMCS) was from Thermo Fisher Scientific Co. (Pittsburgh, PA). Methoxyamine hydrochloride, sucrose and pyridine were purchased from Sigma-Aldrich (St. Louis, MO). Methanol and acetone purchased from Merck (Darmstadt, Germany) was of HPLC grade. All of the reagents used were the highest grade products.

Experimental animals

The honeybees (*Apis mellifera*) were collected from an apiary and the bees were kept in the stainless cage (85 (W) × 45 (D)× 65 (H) mm)) with glass slide cover. The cage was placed in incubator, breding by 30 mL syringe including 50% sucrose solution. The honeybees were stored at 25 ± 2 ℃ and kept in darkness during the whole trial period. While adult zebrafish were acclimated for 2 weeks in a glass water tank with dechlorinated tap water at 25 ± 1°C. The photoperiod was kept a constant 16:8-h light:dark cycle. Zebrafish were fed with a commercial fish food once a day during the acclimation period, and they were not fed for 1 day prior to the chemical exposure.

Chemical exposure

The test chemical was dissolved in acetone and stored at a concentration of 1,000 μg/mL as a stock solution. The exposure concentrations were set at 0.115 ng/μL for the low-exposure group and 1.15 ng/μL for the high-exposure group based on the 48-h LD_{50} value (0.0023 μg/bee), which was applied to thorax of honeybee. Before application of standard solution (1 μL), each honeybee was anaesthetized by CO_2

gas. Fifteen of honeybee in each group were exposed to each concentration of the test compound and six of honeybee in each group were collected after 48 hours of exposure. In zebrafish, The exposure concentrations were set at 6 μg/L for the low-exposure group and 60 μg/L for the high-exposure group based on the 48-h LC_{50} value (59 μg/L). Abamectin was dissolved in acetone and diluted to concentrations of 1000 mg/L and 100 mg/L for stock solution. Each 0.3 mL of stock solution was added to 10 L beakers and 0.3 mL of acetone was added as a control. After 30 adult zebrafish per exposure group were distributed into each beaker, six zebrafish in each group were sampled after 48 hours. The exposure solution was changed every day to maintain the exposure concentration of compound during the experiment.

Sample preparation

Each honeybee and zebrafish was frozen in liquid N_2 and pulverized by a ball-mill instrument (MM 400, Retsch, GmbH, Haan, Germany) in 2 mL of round bottom centrifuge tube. The sample was vortexed for 10 min at 4°C with 1 mL of 80% methanol (honeybee) and 50% methanol (zebrafish) solution containing 0.2 μg/mL of ribitol as an internal standard. After the vortexed extracts were centrifuged at 13000 rpm for 10 min, supernatant was divided into the 5 μL and 100 μL. Each portions were used to non-targeted metabolomics by LC-orbitrap-HRMS and targeted-metabolomics by GC-MS/MS, respectively. The former protion was directly injected to LC-orbitrap-HRMS, and the latter portion of 100 μL was dried using a speed vacuum concentrator (Hanil Modulspin 40) for TMS derivatization. The dried residue was reacted with 50 μL of methoxyamination reagent (20 mg/mL of methoxyamine hydrochloride in pyridine) at 37°C for 90 min while being shaken continuously, and then treated with 50 μL of MSTFA reagent (MSTFA + 1% TMCS) at 37°C for 30 min.

Targeted profiling and identification of metabolites by GC-MS/MS

Metabolites were analyzed with a GC-MS/MS (Shimadzu GCMS-TQ8040) using MRM ions for a total of 396 metabolites (331 metabolites from the Smart Metabolites Database (Shimadzu) and 65 metabolites from an in-house library made by our group). Both split (30:1) and split (500:1) modes were used on injection of a 1.0 μL aliquot sample into BPX-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, TRAJAN). The GC-MS/MS parameters were set to optimal conditions using an injector temperature of 250°C, and ion source and transfer lines temperatures of 230°C and 280°C, respectively. The initial oven temperature was 60°C (2 min), and the temperature was then increased to 320°C (10°C/min) and held there for 15 min. The flow rate of the helium carrier gas was 1 mL/min, and the collision gas was argon. The electron ionization energy was 70 eV. Data processing was performed with GCMS Solution software (version 4.3, Shimadzu). Peak reconfirmation was performed manually and the relative area of individual metabolites was calculated by comparing peak areas of the metabolites and the ribitol internal standard.

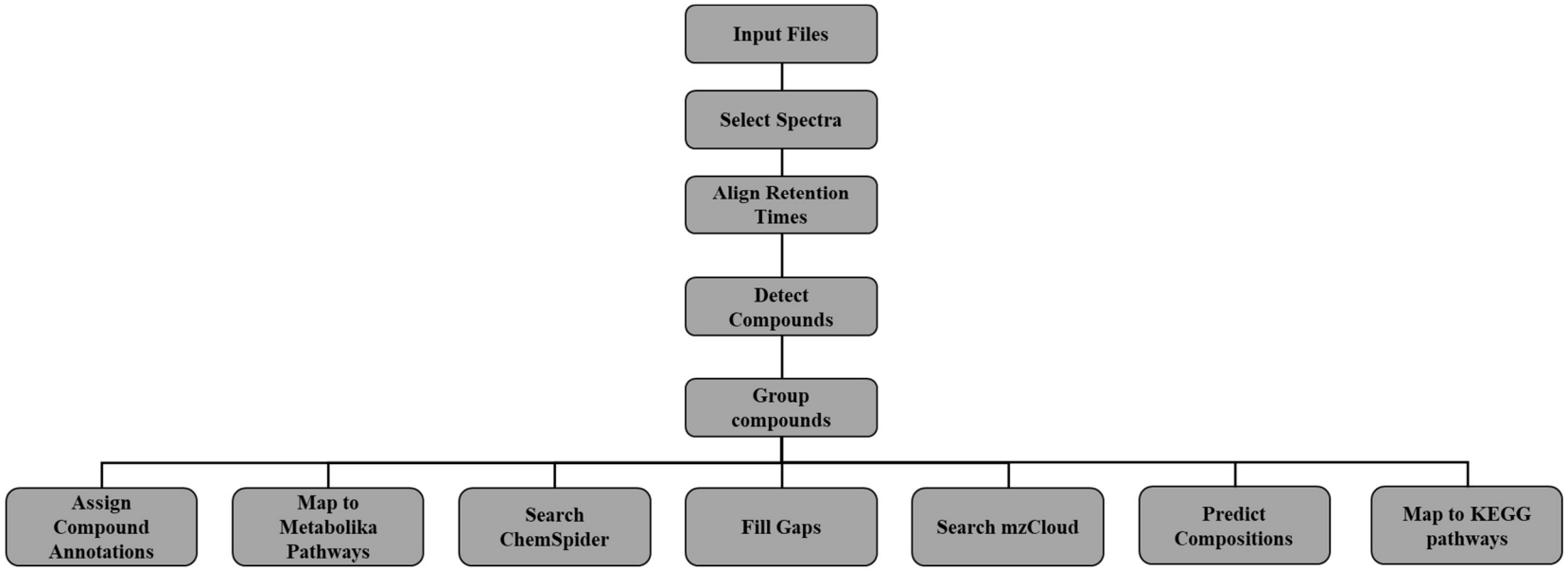
Non-targeted profiling and identification of metabolites by LC-Orbitrap-HRMS

An Accela HPLC system coupled with a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) was used in the present study. An aliquot (5 μL) was injected and chromatographic separation was performed on a HSS T3 C18 (2.1 x 100 mm, 1.8 μm, Waters, Milford, MA, USA) using gradient elution at the flow rate of 0.2 mL/min with 0.1% formic acid (A) and methanol including 0.1% formic acid (B) as the mobile phases. The following gradient was used: 10% B for 0-0.2 min, 10 to 55% B for 0.2-15 min, 55 to 98% for 15-20 min, 98% B for 20-35 min, 98 to 10% B for 35-35.1 min and 10% B for 35.1-40 min. The column temperature was 50°C, and the autosampler temperature was maintained at 4°C.

Mass spectra were obtained using a HESI source operating in both positive and negative mode. The HESI source conditions for metabolite profiling were optimized as follows: sheath gas flow rate, 35 (arbitrary units); auxiliary gas flow rate, 10 (arbitrary units); spray voltage, 4 kV; and heater temperature, 350°C. The MS was set to full scan with $ddMS^2$. Full scan MS data were obtained from m/z 100 to 1500 at a resolution of 70000, AGC target of 100000, and IT of 100 ms, while $ddMS^2$ spectra were acquired at a resolution of 17500, AGC target of 100000, and maximum IT of 50 ms using various collision energies. The criteria for $ddMS^2$ acquisition were minimum AGC target of 8000, intensity threshold of 160000, loop count of 5, and topN of 10. The calibration of Q-Exactive Orbitrap mass spectrometer was performed weekly using Pierce™ ESI Positive and Negative Ion Calibration Solution (Thermo Fisher Scientific Inc.).

MS spectral data were obtained using Xcalibur software (Thermo Fisher Scientific Inc.) and processed by Compound Discoverer 3.1 (CD 3.1, Thermo Fisher Scientific Inc.) for initial data processing, including peak detection, alignment, integration and peak identification. Workflow tree from the CD 3.1 shown in **Fig. 2.** displayed select data processing nodes and the associated workflow connections. Metabolites were identified by searching mass spectral data of metabolite peak from both ChemSpider chemical structure database and mzCloud spectral library. In mzCloud, a matching score of 80% or more was set as the estimated identification criterion (Li et al., 2018; Plassmann et al., 2018; Ng et al., 2020), and the metabolites identified were confirmed by Human Metabolome Database (HMDB) and KEGG.

Fig. 2. Workflow tree from the CD 3.1 software displaying select data processing nodes and the associated workflow connections.

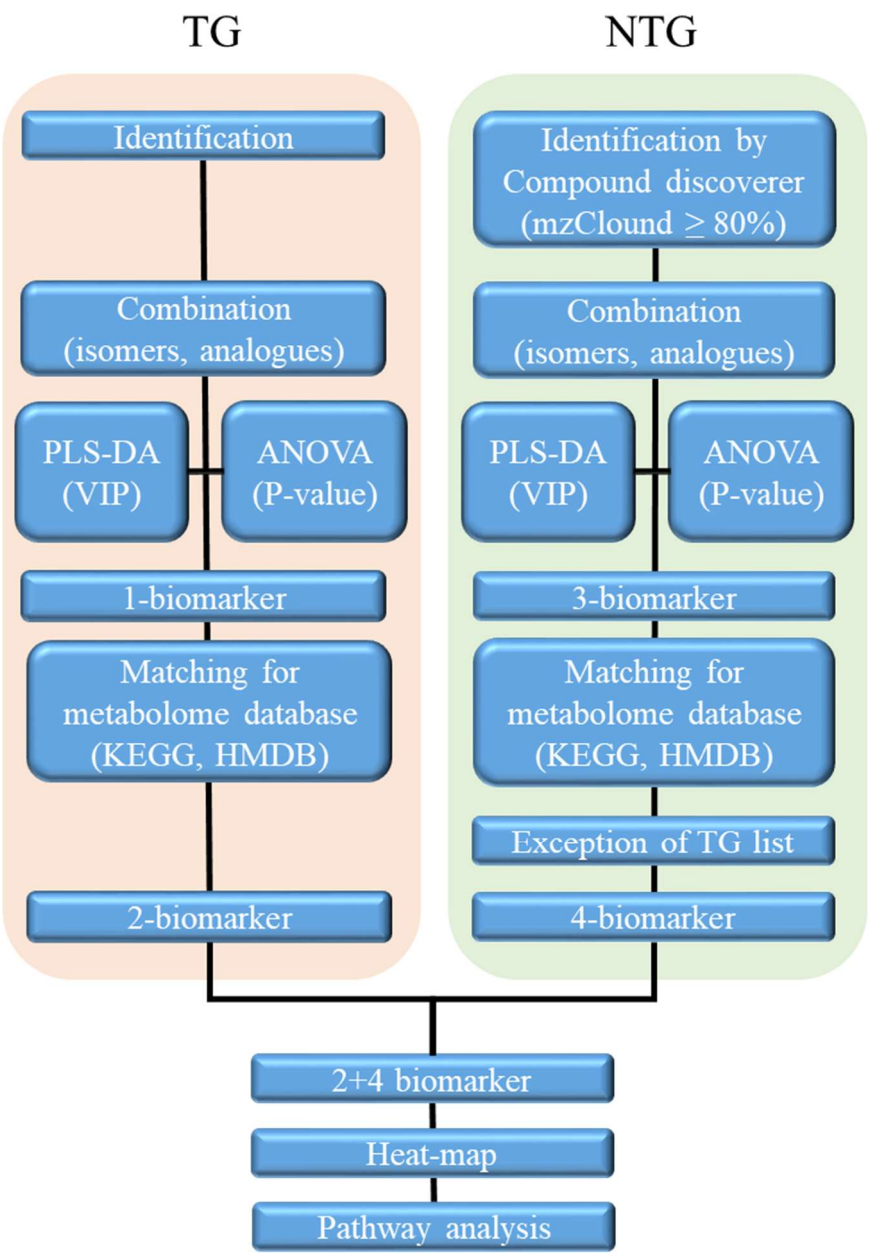

Input Files
Select Spectra
Align Retention Times
Detect Compounds
Group compounds
Assign Compound Annotations
Map to Metabolika Pathways
Search ChemSpider
Fill Gaps
Search mzCloud
Predict Compositions
Map to KEGG pathways

Statistical analysis and biomarker selection

The statistical analysis of targeted profiling (TG) and non-targeted profiling (NTG) were separately conducted to find biomarker. To find the biomarker by exposure of abametin in organism, the PLS-DA widely used for supervised pattern recognition method, was used by SIMCA-P+ software (version 12.0.1, Umetrics, Sweden) and calculated the variable important in the projection (VIP) score. Furthermore, ANOVA was performed by MetaboAnalyst 5.0 (www.metaboanalyst.ca) and p-value was deduced through the normalization including transformation and scaling. With the two factors VIP scores > 1 derived by PLS-DA and $p < 0.05$ from the ANOVA, biomarkers were selected.

In case of non-targeted metabolomics, biomarkers that were overlaped with the targeted-metabolomics database including 396 metabolites were excluded after statistical analysis in the same manner as above. And then, both biomarker grouops derived from TG and NTG profiling were combined and matched to the metabolome database (HMDB, KEGG) to filter out metabolomes related to the pathway. The schem of selecting biomarkers was shown in **Figure. 3.**

Figure. 3. Scheme of biomarker combined targeted profiling (TG) and non-targeted profiling (NTG).


TG
NTG
Identification
Combination (isomers, analogues)
PLS-DA (VIP)
ANOVA (P-value)
1-biomarker
Matching for metabolome database (KEGG, HMDB)
2-biomarker
Identification by Compound discoverer (mzClound ≥ 80%)
Combination (isomers, analogues)
PLS-DA (VIP)
ANOVA (P-value)
3-biomarker
Matching for metabolome database (KEGG, HMDB)
Exception of TG list
4-biomarker
2+4 biomarker
Heat-map
Pathway analysis

Metabolic pathway analysis

For the metabolic pathway analysis, the biomarkers matched to the metabolome database (HMDB, KEGG) were selected as the final biomarker used for metabolic pathway analysis that was performed by MetaboAnalyst 5.0.

Measurement of ROS and MDA contents

The reactive oxygen species (ROS) content was determined using fluorescent probe, DCFH-DiOxyQ (OxiSelect™ *In vitro* ROS/RNS assay kit, Cell Biolabs, Inc., CA, USA). A 20 mg of the zebrafish fine powder was homogenized in 500 μL of phosphate buffered saline (PBS), and the homogenates were centrifuged at 10,000g for 5 min. A 50 μL of the supernatant was mixed with 50 μL of catalyst and incubated for 5 min at room temperature. The DCFH solution 100 μL was added into the mixture and incubated at room temperature for 40 min. The fluorescence was measured at 480 nm exctation / 530 nm emission using a fluorescent spectrophotometer (SpectraMax i3, Molecular Devices).

The quantitative determination of malondialdehyde (MDA) was performed using thiobarbituric acid (TBA) assay method (OxiSelect™ TBARS assay kit, Cell Biolabs, Inc., CA, USA). A 30 mg of the zebrafish fine powder was homogenized in 500 μL of 1X butylated hydroxytoluene (BHT) solution, and the homogenates were centrifuged at 10,000g for 5 min. A 100 μL of the supernatant was mixed with 100 μL of SDS Lysis solution and incubated for 5 min at room temperature. The TBA reagent 250 μL was added into the mixture and incubated at 95℃ for 60 min. After incubation, sample tubes were cooled to room temperature and centrifuged at 3000 rpm for 15 min. The supernatant (300 μL) was extracted with butanol (300 μL) to prevent the interference of hemoglobin and its derivatives. The fluorescence of butanol solution was measured at 540 nm exctation / 590 nm emission using a fluorescent spectrophotometer (SpectraMax i3, Molecular Devices).

The protein concentrations of ROS and MDA samples were determined according to the Bradford method (Quick Start™ Bradford Protein Assay Kit, Bio-Rad, Inc., CA, USA). The absorbance was detected at 595 nm using spectrophotometer (SpectraMax i3, Molecular Devices).

Results and Discussion

Chemical exposure and sample preparation

In order to profiling living organisms exposed to abamectin, the exposure concentrations were set based on the contact half-lethal dose (LD_{50}) and half-lethal concentration (LC_{50}). The honeybees were exposed to two levels of abamectin which were 1.15 (0.5 LD_{50}) and 0.115 μg/mL (0.05 LD_{50}) with 1 μL of microapplicator because honeybee is known to have LD_{50} (0.0023 μg/bee) for abamectin (Agriculure, 2021). Meanwhile, zebrafish is known to have LC_{50} (59 μg/L) (Sanches et al., 2017) and 6 μg/L and 60 μg/L were set as low and high exposure concentration levels, respectively.

To efficiently extract metabolites from the honeybee and zebrafish, 80% methanol solution was used for honeybee (Shi et al., 2018) and zebrafish was extracted by 50% methanol (Lee et al., 2020). The targeted and non-targeted profiling were used to complement the analysis range. The targeted profiling by GC-MS/MS performed trimethylsilation (TMS) derivatization process to increase volatility and convert it into relative non-polar metabolites which were easily retained in GC separation column. Additionally, non-targeted profiling was conducted by direct injection of extracts to detect polar metabolites using LC-orbitrap-HRMS that can analyze broad range of metabolites without derivatization process.

Targeted profiling and identification of metabolites by GC-MS/MS

Targeted metabolomics analysis using GC-MS/MS is increasingly being used in medical and toxicology arenas to achieve better selectivity and sensitivity (Tsugawa et al., 2014a; Hirata et al., 2017a; Zaitsu et al., 2018). Commercially available GC-MS/MS-based targeted metabolome platforms can contribute significantly to metabolomics studies in a variety of areas (Zaitsu and Hayashi, 2019). In this study,

a total of 396 metabolites (331 metabolites from the Smart Metabolites Database and 65 metabolites from our in-house library) were analyzed using MRM mode. These metabolites included 18 alcohols, 10 amides, 10 amines, 75 amino acids, 78 carbohydrates, 3 esters, 41 fatty acids, 1 glycerides, 4 indoles, 1 inorganic compound, 9 nucleosides, 102 organic acids, 9 phenols, 11 purines, 7 pyridines, 4 pyrimidines, 6 steroids, 2 tocopherols, 2 nucleotides, 2 terpenoids, 1 azoles. The split-mode (30:1) injection was conducted for detection in both organism sample (honeybee and zebrafish). And the split-mode (500:1) injection was conducted for higher intensity peaks showing saturation phenomena in mass detector. After the GC-MS/MS analyses, 237 and 241 metabolites were confirmed in honeybee and zebrafish, respectively (**Table 3-4**). The area of identified metabolites were devided by the area of internal standard (ribitol), and this process could correct any deviations due to the instrumental analysis.

Table 3. Metabolites detected in whole-body honeybee samples by targeted metabolite analyses using GC-MS/MS with MRM mode.

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
alcohols	1	1-Hexadecanol	carbohydrates	81	Galactosamine	organic acids	161	2-Methyl-3-hydroxybutyric acid
	2	2-Aminoethanol		82	Galactose		162	3-Aminoglutaric acid
	3	Batyl alcohol		83	Galacturonic acid		163	3-Hydroxy-3-methylglutaric acid
	4	eicosanol		84	Glucaric acid		164	3-Hydroxybutyric acid
	5	ethylene glycol		85	Gluconic acid		165	3-Hydroxyglutaric acid
	6	Octadecanol		86	Glucono-1,4-lactone		166	3-Hydroxyisobutyric acid
	7	Octopamine		87	Glucosamine		167	3-Hydroxyisovaleric acid
amides	8	Allantoin		88	Glucose 6-phosphate		168	3-Hydroxypropionic acid
	9	Glycyl-Glycine		89	Glucuronic acid		169	3-Methoxy-4-hydroxybenzoic acid
	10	Oleamide		90	Glyceric acid		170	3-Methyl-2-oxovaleric acid
	11	oxamic acid		91	Glycerol		171	3-Phenyllactic acid
	12	Pantothenic acid		92	Glycerol 2-phosphate		172	3-Phosphoglyceric acid
	13	Urea		93	Glycerol 3-phosphate		173	4-Hydroxybenzoic acid
amines	14	Cadaverine		94	Inositol		174	4-Hydroxyphenylacetic acid
	15	Cystamine		95	Lactitol		175	4-Hydroxyphenyllactic acid
	16	Histamine		96	Lyxose		176	4-Hydroxyphenylpyruvic acid
	17	homoglutamine		97	Maltose		177	5-Dehydroquinic acid
	18	Putrescine		98	Mannitol		178	5-Hydroxymethyl-2-furoic acid
	19	Spermidine		99	Mannose		179	5-Oxoproline

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
amino acids	20	2-Aminoadipic acid		100	Mannose 6-phosphate		180	Aconitic acid
	21	2-Aminopimelic acid		101	melibiose		181	Adipic acid
	22	3-Aminoisobutyric acid		102	meso-Erythritol		182	Azelaic acid
	23	3-Aminopropanoic acid		103	N-Acetylmannosamine		183	Benzoic acid
	24	3-Sulfinoalanine		104	Psicose		184	Citramalic acid
	25	4-Aminobutyric acid		105	Ribonolactone		185	Citric acid
	26	4-Hydroxyproline		106	Ribose 5-phosphate		186	Dihydroorotic acid
	27	5-Aminolevulinic acid		107	Ribose		187	Dodecanedioic acid
	28	5-Hydroxy-tryptophan		108	Ribulose		188	ferulic acid
	29	Alanine		109	Sedoheptulose 7-phosphate		189	Fumaric acid
	30	Anthranilic acid		110	Sorbitol		190	Glutaric acid
	31	Arginine		111	Sorbose		191	Glycolic acid
	32	Asparagine		112	Sucrose		192	Hippuric acid
	33	Aspartic acid		113	Tagatose		193	Homovanillic acid
	34	Cystathionine		114	Threitol		194	Kynurenic acid
	35	Cysteine		115	Threonic acid		195	Lactic acid
	36	Dopa		116	Turanose		196	Malic acid
	37	Glutamic acid		117	Xylitol		197	Methylmalonic acid
	38	Glutamic acid 5		118	Xylose		198	Methylsuccinic acid

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
		-methylester						
	39	Glutamine		119	Xylulose		199	Nicotinic acid
	40	Glycine	esters	120	O-Phosphoethanolamine		200	Orotic acid
	41	Histidine		121	11-eicosaenoic acid		201	Oxalic acid
	42	Homocysteine		122	11-eicosenoic acid		202	Phenylacetic acid
	43	Homoserine		123	5-Aminovaleric acid		203	Porphobilinogen
	44	Isobutyrylglycine		124	alpha-linolenic acid		204	Protocatechuic acid
	45	Isoleucine		125	arachidic acid		205	pyroglutamic acid
	46	Isovalerylglycine		126	Caproic acid		206	Pyruvic acid
	47	Kynurenine		127	cis-10-heptadecenoic acid		207	Ribonic acid
	48	Leucine		128	Decanoic acid		208	Salicylic acid
	49	Lysine	fatty acids	129	Docosahexaenoic acid		209	Sebacic acid
	50	Methionine		130	Elaidic acid		210	Suberic acid
	51	Methionine sulfone		131	heptadecanoic acid		211	Succinic acid
	52	methyl cysteine		132	lauric acid		212	Tartaric acid
	53	methyl serine		133	Linoleic acid		213	Phosphoric acid
	54	N6-Acetyllysine		134	Margaric acid		214	Dopamine
	55	N-Acetylaspartic acid		135	Myristic acid	phenols	215	Epinephrine
	56	N-Acetylglutamine		136	nonadecanoic acid		216	Hydroquinone
	57	N-Acetyl-Lysine		137	Nonanoic acid		217	Norepinephrine

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
	58	N-Acetylserine		138	Octanoic acid		218	Tyramine
	59	N-Acetyltyrosine		139	Oleic acid		219	7-Methylguanine
	60	Ornithine		140	Palmitic acid		220	Adenine
	61	Phenylalanine		141	Palmitoleic acid		221	Adenosine
	62	Proline		142	pentadecanoic acid		222	Guanine
	63	Sarcosine		143	Stearic acid	purines	223	Guanosine
	64	Serine	glycerides	144	Monostearin		224	Hypoxanthine
	65	Threonine		145	5-Methoxytryptamine		225	Inosine
	66	Tryptophan	Indoles	146	Indol-3-acetic acid		226	Uric acid
	67	Tyrosine		147	Tryptamine		227	Xanthine
	68	Ureidopropionic acid	inorganic compounds	148	Hydroxylamine		228	3-hydroxypyridine
	69	Valine		149	5'-Methylthioadenosine		229	Niacinamide
	70	1,6-Anhydroglucose		150	Cytidine	pyridines	230	Pyridoxal
	71	2,3-Bisphosphoglyceric acid	nucleosides	151	Uridine		231	Pyridoxamine
carbohydrates	72	2-Deoxy-glucose		152	Uridine monophosphate		232	Cytosine
	73	6-Phosphogluconic acid		153	2-Hydroxyglutaric acid		233	Dihydrouracil
	74	Allose	organic acids	154	2-Hydroxyisobutyric acid	pyrimidines	234	Thymine
	75	Arabinose		155	2-Hydroxyisocaproic acid		235	Uracil

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
	76	Arabitol		156	2-Hydroxyisovaleric acid	steroids	236	Sitosterol
	77	Dihydroxyacetone phosphate		157	2-Isopropylmalic acid	tocopherols	237	alpha-tocopherol
	78	Fructose		158	2-Ketoadipic acid			
	79	Fucose		159	2-Ketoglutaric acid			
	80	Galactitol		160	2-Ketoisocaproic acid			

Table 4. Metabolites detected in whole-body zebrafish samples by targeted metabolite analyses using GC-MS/MS with MRM mode.

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
alcohols	1	1-Hexadecanol	carbohydrates	82	Erythrulose	organic acids	163	2-Hydroxyisobutyric acid
	2	2-Aminoethanol		83	Fructose		164	2-Hydroxyisocaproic acid
	3	Batyl alcohol		84	Fructose 1-phosphate		165	2-Hydroxyisovaleric acid
	4	diethanolamine		85	Fructose 6-phosphate		166	2-Ketoglutaric acid
	5	eicosanol		86	Fucose		167	2-Ketoisocaproic acid
	6	ethylene glycol		87	Galactitol		168	2-Keto-isovaleric acid
	7	Histidinol		88	Galactosamine		169	2-Methylhippuric acid
	8	Octadecanol		89	Galactose		170	2-Phosphoglyceric acid
	9	threoninol		90	Galacturonic acid		171	3-Aminoglutaric acid
	10	Triethanolamine		91	Glucaric acid		172	3-Hydroxybutyric acid
amides	11	Allantoin		92	Glucono-1,5-lactone		173	3-Hydroxyisobutyric acid
	12	Creatinine		93	Glucosamine		174	3-Hydroxyisovaleric acid
	13	Oleamide		94	Glucose 6-phosphate		175	3-Hydroxypropionic acid
	14	oxamic acid		95	Glucuronic acid		176	3-Hydroxypyruvic acid
	15	Pantothenic acid		96	Glyceraldehyde		177	3-Methyl-2-oxovaleric acid
	16	Urea		97	Glyceraldehyde 3-phosphate		178	3-Phosphoglyceric acid
	17	Cadaverine		98	Glyceric acid		179	4-Hydroxyphenyllactic acid
	18	Cystamine		99	Glycerol		180	4-Hydroxyphenylpyruvic acid

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
	19	Histamine		100	Glycerol 3-phosphate		181	5-Dehydroquinic acid
	20	homoglutamine		101	Inositol		182	5-Oxoproline
	21	Putrescine		102	Lactitol		183	Aconitic acid
	22	Spermidine		103	Lyxose		184	Adipic acid
	23	2-Aminoadipic acid		104	Mannitol		185	Azelaic acid
	24	2-Aminobutyric acid		105	Mannose		186	Benzoic acid
	25	2-Aminoisobutyric acid		106	Mannose 6-phosphate		187	Citramalic acid
	26	2-Aminooctanoic acid		107	meso-Erythritol		188	Citric acid
	27	2-Aminopimelic acid		108	N-Acetylmannosamine		189	Ethylmalonic acid
	28	3-Aminoisobutyric acid		109	Psicose		190	Fumaric acid
	29	3-Aminopropanoic acid		110	raffinose		191	Glutaric acid
amino acids	30	3-Sulfinoalanine		111	Rhamnose		192	Glycolic acid
	31	4-Aminobutyric acid		112	Ribonolactone		193	Glyoxylic acid
	32	4-Hydroxyproline		113	Ribose 5-phosphate		194	Hippuric acid
	33	5-Aminolevulinic acid		114	Ribose		195	Hypotaurine
	34	5-Hydroxy-tryptophan		115	Ribulose 5-phosphate		196	Isocitric acid
	35	Acetylglycine		116	Ribulose		197	Lactic acid
	36	Alanine		117	Sedoheptulose 7-phosphate		198	Malic acid
	37	Arginine		118	Sorbitol		199	Methylsuccinic acid

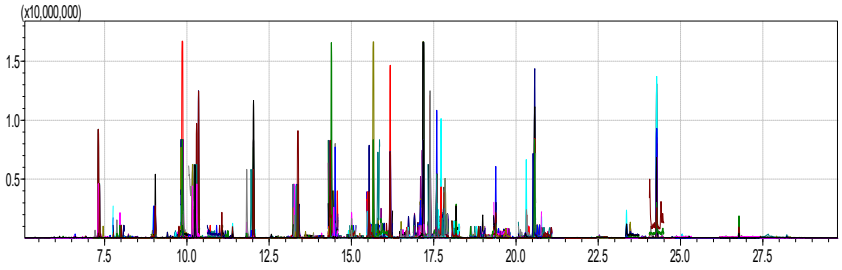
Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
	38	Asparagine		119	Sorbose		200	Nicotinic acid
	39	Aspartic acid		120	Sucrose		201	Orotic acid
	40	Cystathionine		121	Tagatose		202	Oxalic acid
	41	Cysteine		122	Threitol		203	Phenylacetic acid
	42	Glutamic acid		123	Threonic acid		204	Phosphoenolpyruvic acid
	43	Glutamic acid 5-methylester		124	Turanose		205	pyroglutamic acid
	44	Glutamine		125	Xylitol		206	Pyruvic acid
	45	Glycine		126	Xylose		207	quinic acid
	46	Histidine		127	Xylulose		208	Ribonic acid
	47	Homocysteine	esters	128	Mevalonic lactone		209	Sebacic acid
	48	Homoserine		129	O-Phosphoethanolamine		210	Shikimic acid
	49	Isoleucine		130	11-eicosaenoic acid		211	Suberic acid
	50	Kynurenine		131	11-eicosenoic acid		212	Succinic acid
	51	Leucine		132	5-Aminovaleric acid		213	Taurine
	52	Lysine		133	8, 11, 14-eicosatrienoic acid		214	Urocanic acid
	53	Methionine	fatty acids	134	Acetoacetic acid		215	Vanilmandelic acid
	54	Methionine sulfone		135	arachidic acid		216	Phosphoric acid
	55	methyl serine		136	Arachidonic acid		217	Dopamine
	56	N-Acetylaspartic acid		137	Caproic acid	phenols	218	Hydroquinone
	57	N-Acetyl-Lysine		138	Decanoic acid		219	Tyramine

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
	58	N-Acetylserine		139	Docosahexaenoic acid		220	7-Methylguanine
	59	Norvaline		140	Docosapentaenoic acid		221	Adenine
	60	Ornithine		141	Eicosapentaenoic acid		222	Adenosine
	61	Phenylalanine		142	Elaidic acid		223	Guanine
	62	Proline		143	heptadecanoic acid	purines	224	Guanosine
	63	Sarcosine		144	lauric acid		225	Hypoxanthine
	64	S-Benzyl-Cysteine		145	Linoleic acid		226	Inosine
	65	Serine		146	Margaric acid		227	Uric acid
	66	Threonine		147	Myristic acid		228	Xanthine
	67	Tiglylglycine		148	myristoleic acid		229	3-hydroxypyridine
	68	Tryptophan		149	Nonanoic acid		230	Niacinamide
	69	Tyrosine		150	Octanoic acid	pyridines	231	Pyridoxal
	70	Ureidopropionic acid		151	Oleic acid		232	Pyridoxamine
	71	Valine		152	Palmitic acid		233	Quinolinic acid
	72	1,6-Anhydroglucose		153	Palmitoleic acid		234	Cytosine
	73	2,3-Bisphosphoglyceric acid		154	pentadecanoic acid	pyrimidines	235	Dihydrouracil
carbohydrates	74	2-Deoxy-glucose		155	Stearic acid		236	Thymine
	75	6-Phosphogluconic acid	glycerides	156	Monostearin		237	Uracil
	76	Allose	Indoles	157	5-Methoxytryptamine	steroids	238	Cholestanol

Classification	No.	Metabolites	Classification	No.	Metabolites	Classification	No.	Metabolites
	77	Arabinose		158	Tryptamine		239	Cholesterol
	78	Arabitol	inorganic compounds	159	Hydroxylamine	terpenoids	240	Methoprene acid
	79	Dihydroxyacetone	nucleosides	160	Cytidine	tocopherols	241	alpha-tocopherol
	80	Dihydroxyacetone phosphate	organic acids	161	2-Hydroxybutyric acid			
	81	Erythorse		162	2-Hydroxyglutaric acid			

Fig. 4. TIC in whole-body honeybee samples (1.15 ng/µL) of abamectin exposure conditions (A) and individual chromatograms of some metabolites (B)

(A)


(x10,000,000)
1.5
1.0
0.5
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5

(B)

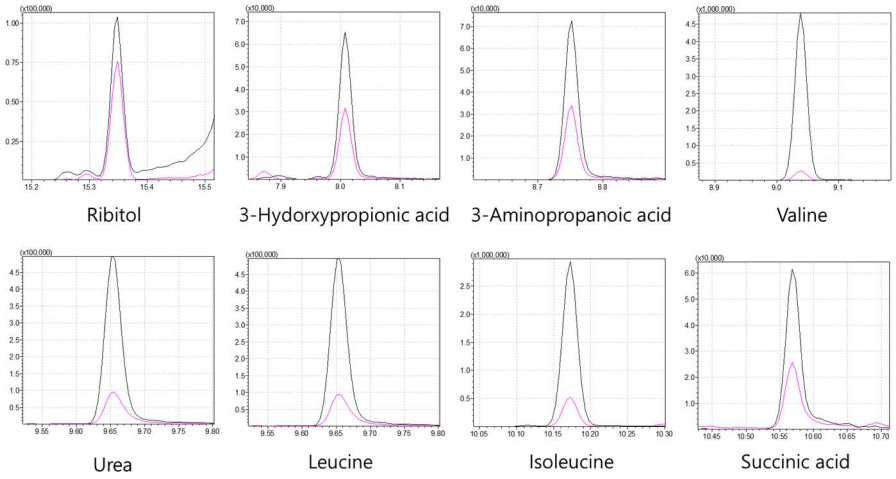
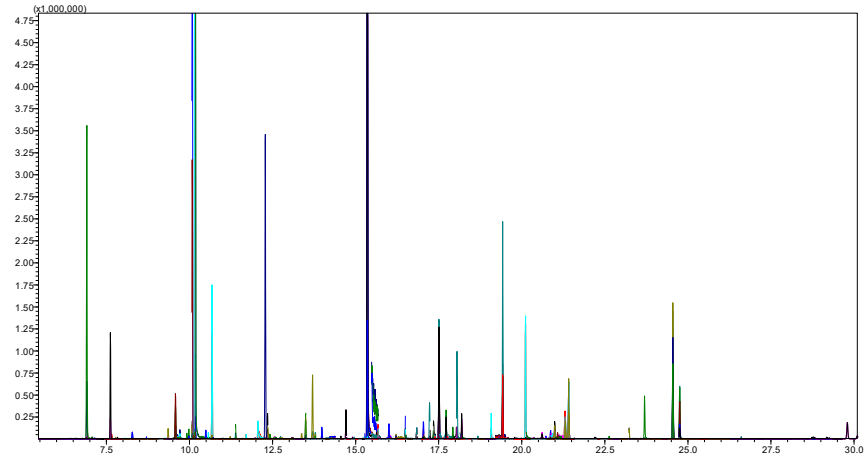
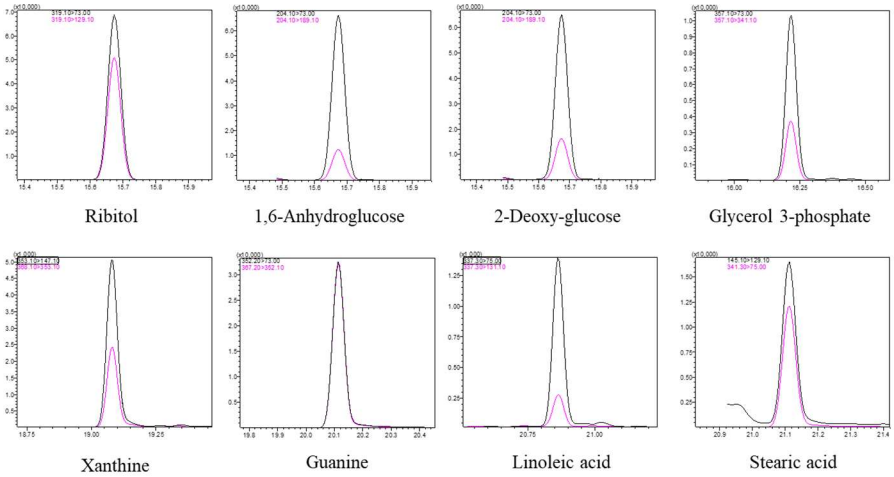

Ribitol
3-Hydorxypropionic acid
3-Aminopropanoic acid
Valine
Urea
Leucine
Isoleucine
Succinic acid

Fig. 5. TIC in whole-body zebrafish samples (60 μg/L) of abamectin exposure conditions (A) and individual chromatograms of some metabolites (B)

(A)



(B)

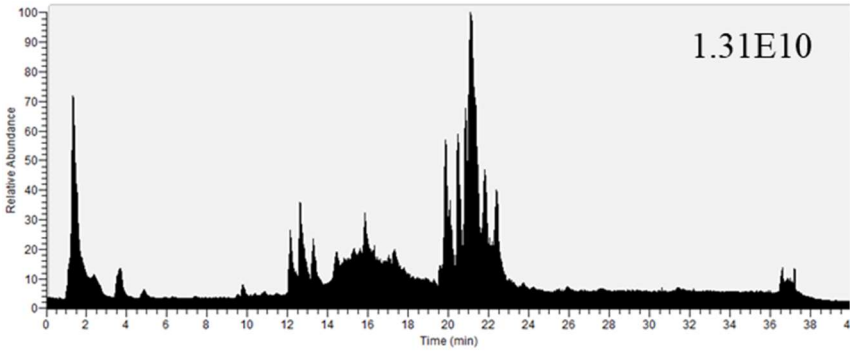

Ribitol
1,6-Anhydroglucose
2-Deoxy-glucose
Glycerol 3-phosphate
Xanthine
Guanine
Linoleic acid
Stearic acid

Non-targeted profiling and identification of metabolites by LC-Orbitrap-HRMS

Due to ultra-high resolution and accuracy (Huang et al., 2013; Gomez-Canela et al., 2017; Gomez-Canela et al., 2018; Simonato et al., 2019), LC-Orbitrap-HRMS is widely used in the field of medicine, environment, and toxicology. In this study, reverse phase condition with C18 column was used to separate metabolites peak in LC-Orbitrap-HRMS. LC-MS chromatograms belonging to the honeybee and zebrafish extract in positive and negative ESI modes are given in **Fig. 6-7**. After LC-Orbitrap-HRMS analysis and data processing with CD 3.1, the total of 500 and 212 metabolite peaks were detected and identified in honeybee and zebrafish, respectively. Then, isomers and analogues were combined and became to 367 and 169 metabolites in honeybee and zebrafish, respectively. These identified metabolites were performed statistical analysis and matching for metabolomes database to find biomarker.

Fig. 6. TIC in whole-body honeybee samples under high-concentration (1.15 ng/μL) of abamectin exposure conditions (A: positive ion mode, B: negative ion mode)

(A)


1.31E10
Relative Abundance
Time (min)

(B)

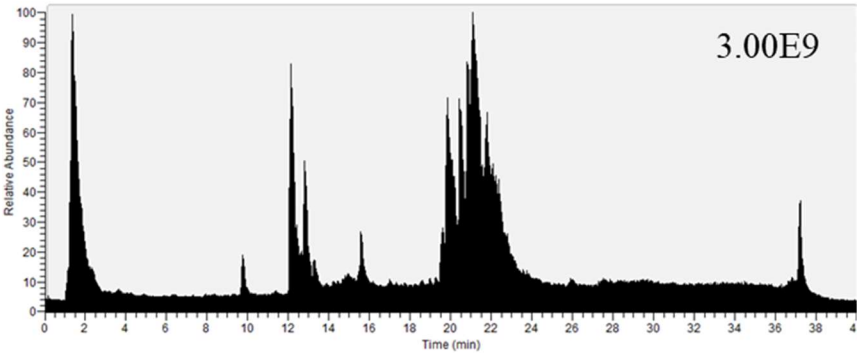
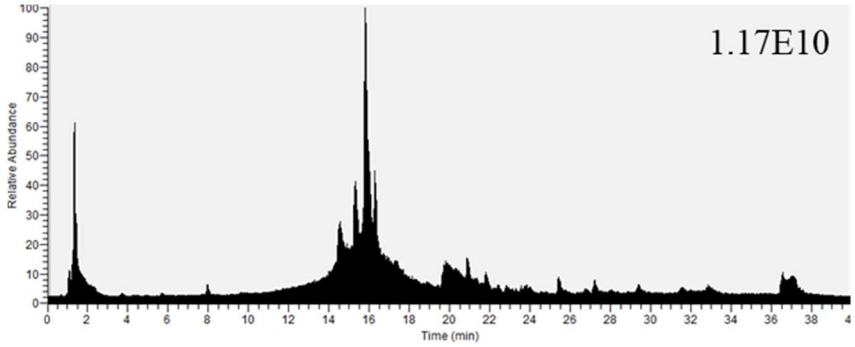
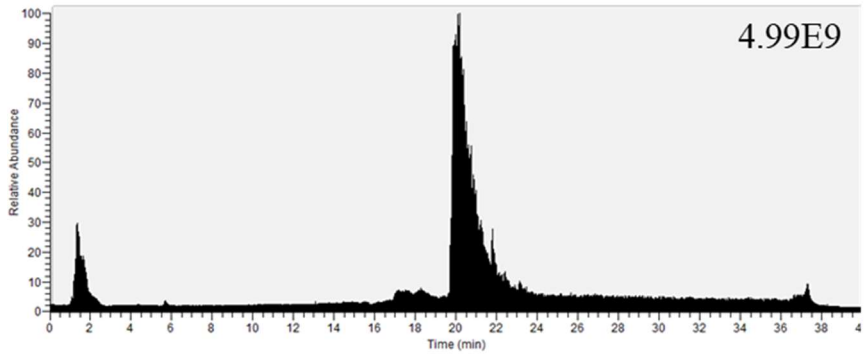

3.00E9
Relative Abundance
Time (min)

Fig. 7. TIC in whole-body zebrafish samples under high-concentration (0.06 mg/L) of abamectin exposure conditions (A: positive ion mode, B: negative ion mode)

(A)


1.17E10
Relative Abundance
Time (min)

(B)

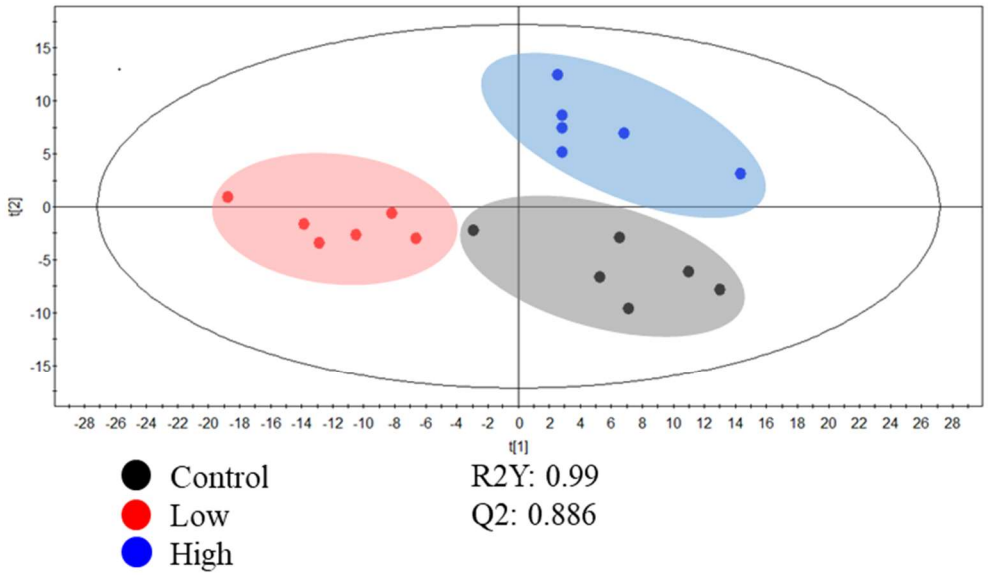

4.99E9
Relative Abundance
Time (min)

Statistical analysis and biomarker selection

The PLS-DA, which is a supervised multidimensional statistical model, was performed to identify the segregation between the control and two treatment groups at 48 h (**Fig. 8-9.**). PLS-DA demonstrated a significant difference in the metabolic profiles of the control and two treatment groups depending on the exposure concentrations. All samples were located within the 95% confidence interval, as indicated by the Hotelling's T2 ellipse, without anomalies. The quality of the PLS-DA model could be confirmed by the R^2Y and Q^2 values (Yoon et al., 2016a). R^2Y, the contribution rate of the supervised model, was calculated as 0.911 (honeybee) and 0.974 (zebrafish), indicating differences between the sample groups are reliable in the PLS-DA model. Q^2, which represents the predictability of the PLS-DA model, was calculated to be 0.763 (honeybee) and 0.647 (zebrafish) at 48 h, proving the predictive power of this model: in general, a robust mode has $Q^2 > 0.4$ (McCombie et al., 2009). Since the PLS-DA had good fitness, VIP analyses were carried out to select the significant metabolites according to the treatment concentration. The cutoff value for the VIP results was set at 1.0 (Xia and Wishart, 2010b). After ANOVA ($P < 0.05$) analyses of the honeybee and zebrafish metabolites, 102 (honeybee) and 43 (zebrafish) metabolites were recognized as biomarkers that significantly contributed to distinguish metabolic profiles between the sample groups in targeted profiling. Additionally, 19 (honeybee) and 13 (zebrafish) biomarker also were added by non-targeted profiling which was performed the same statistical analysis above. Finally, total of 116 (honeyee) and 54 (zerafish) biomarkers were determined by statistical analysis (**Table 5-6**).

The heat map with dendrograms of the biomarker were produced using MetaboAnalyst 5.0 software to intuitively identify trends in the alterations in metabolite levels between the control group and exposure groups (**Fig. 10-11.**). The color indicates the levels of metabolites from highest (red) to lowest (green).

Fig. 8. 2D and 3D PLS-DA (partial least squares-discriminant analysis) score plot based on the GC-MS/MS and LC-Orbitrap-HRMS from the honeybee exposed to abamectin at concentrations of 0 ng/μL (control), 0.115 ng/μL (low), 1.15 ng/μL (high) for 48 h.


t[1]
t[2]
Control
Low
High
R2Y: 0.99
Q2: 0.886

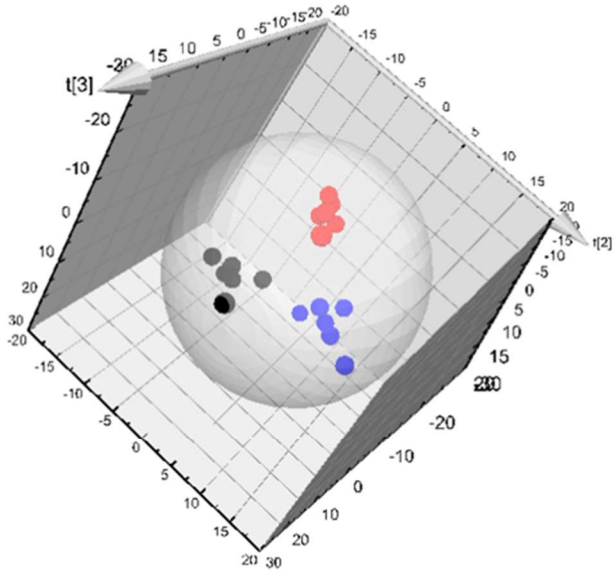
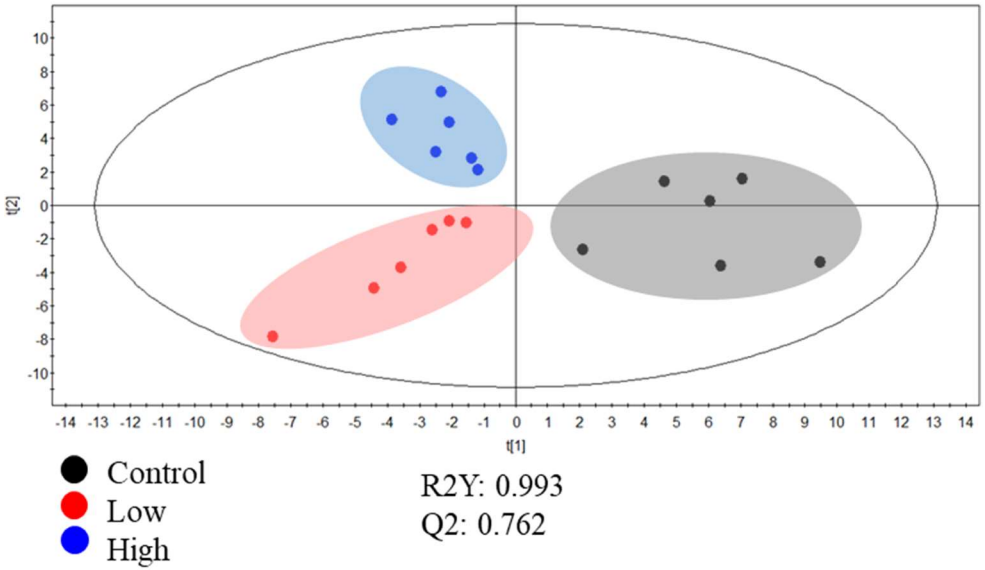

t[3]
t[2]

Fig. 9. 2D and 3D PLS-DA (partial least squares-discriminant analysis) score plot based on the GC-MS/MS and LC-Orbitrap-HRMS from the zebrafish exposed to abamectin at concentrations of 0 mg/L (control), 0.006 mg/L (low), 0.06 mg/L (high) for 48 h.


t[2]
t[1]
Control
Low
High
R2Y: 0.993
Q2: 0.762

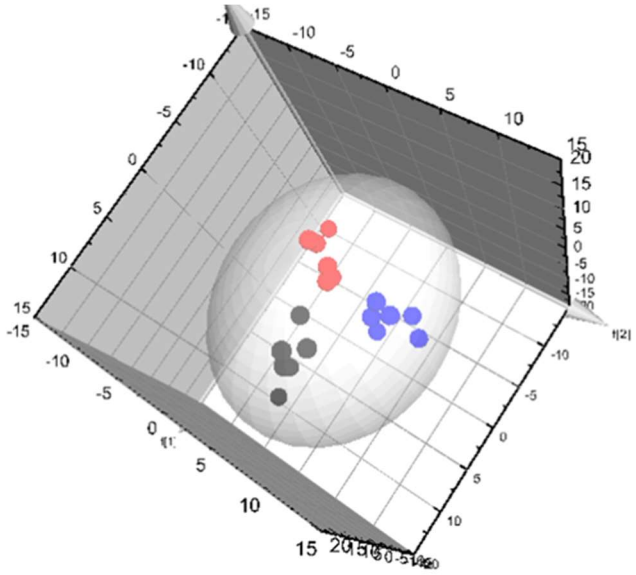

t[2]

Table 5. Significantly altered metabolites in honeybee exposed to abamectin (VIP > 1.0, p-value < 0.05).

Compound	VIP	p-value
(R)-3-Hydroxy-tetradecanoic acid	1.5	3.64E-04
11-eicosenoic acid	1.3	2.75E-04
15-Keto-13,14-dihydroprostaglandin A2	1.3	9.66E-03
2-Aminoadipic acid	1.1	3.38E-02
2-Aminopimelic acid	1.5	4.19E-06
2-Hydroxyisobutyric acid	1.5	5.63E-05
2-Hydroxymyristic acid	1.6	8.38E-05
3-Aminoisobutyric acid	1.0	1.33E-02
3-Methyl-2-oxovaleric acid	1.0	8.20E-03
3-Phosphoglyceric acid	1.3	3.21E-04
4a-Carbinolamine tetrahydrobiopterin	1.1	1.16E-03
4-Hydroxycoumarin	1.7	7.27E-03
4-Hydroxyphenylpyruvic acid	1.4	8.59E-06
4-Oxoproline	1.2	4.45E-02
5-Aminolevulinic acid	1.2	1.12E-03
5-Hydroxymethyl-2-furoic acid	1.1	9.15E-03
5-Hydroxy-tryptophan	1.2	8.97E-03
5-Methoxytryptamine	1.3	3.47E-04
6-Methylquinoline	1.4	3.26E-02
6-Phosphogluconic acid	1.2	2.62E-04
7alpha-Hydroxytestosterone	1.1	2.52E-02
Aconitic acid	1.2	1.80E-02
Adenine	1.0	5.34E-03
Alanine	1.0	6.22E-03
Allose	1.2	5.46E-04
alpha-linolenic acid	1.2	2.35E-04
alpha-tocopherol	1.2	2.67E-03
Arabitol	1.1	3.80E-02
arachidic acid	1.1	4.13E-03
Arginine	1.1	6.10E-04
Batyl alcohol	1.2	7.25E-03
Benzoic acid	1.1	8.35E-03
Citraconic acid	1.5	8.41E-05

Compound	VIP	p-value
Citric acid	1.5	4.74E-06
Cyclohexanecarboxylic acid	1.1	2.77E-03
Cytosine	1.2	5.56E-03
Daidzein	1.1	4.55E-02
Diacetoxyscirpenol	1.2	3.23E-03
Dihydroxyacetone phosphate	1.1	4.14E-03
Diisobutyl phthalate	1.9	1.45E-04
Docosahexaenoic acid	1.2	2.65E-04
Dopamine	1.2	3.27E-03
eicosanol	1.2	4.58E-02
Elaidic acid	1.0	3.09E-03
Epinephrine	1.2	1.05E-03
Fructose	1.3	6.79E-04
Galactitol	1.4	1.02E-05
Galactose	1.1	2.93E-03
Galacturonic acid	1.0	2.70E-03
Glucaric acid	1.0	1.84E-02
Gluconic acid	1.0	2.47E-02
Glucosamine	1.3	5.39E-04
Glucuronic acid	1.0	3.94E-03
Glutamic acid	1.3	1.46E-03
Glutaric acid	1.1	1.21E-02
Glycerol 3-phosphate	1.1	3.61E-03
Hippuric acid	1.1	2.31E-03
Homoserine	1.0	7.82E-03
Homovanillic acid	1.1	1.04E-03
Hydroquinone	1.2	4.62E-03
Hydroxylamine	1.3	8.83E-05
Hypoxanthine	1.3	2.75E-06
Indol-3-acetic acid	1.2	3.22E-03
Indoleacrylic acid	1.3	6.82E-04
Inosine	1.2	3.49E-04
Isoleucine	1.1	9.76E-04
Kynurenic acid	1.2	6.60E-03
Leucine	1.1	9.54E-04

Compound	VIP	p-value
Leucylproline	1.3	1.20E-02
Levamisole	1.0	7.47E-04
Lysine	1.0	8.23E-03
Malic acid	1.1	9.59E-03
Mannose	1.1	3.17E-03
melibiose	1.2	2.64E-03
meso-Erythritol	1.3	5.55E-03
Methylmalonic acid	1.3	8.96E-04
Methylsuccinic acid	1.4	1.35E-03
Myristic acid	1.0	3.53E-02
N6-Acetyllysine	1.1	1.40E-02
N-Acetylaspartic acid	1.2	5.34E-03
N-Acetyl-Lysine	1.1	2.52E-03
N-Acetylmannosamine	1.2	3.38E-03
Nicotinic acid	1.1	9.68E-04
nonadecanoic acid	1.2	3.55E-03
Norepinephrine	1.0	4.13E-02
Oleic acid	1.0	3.07E-03
Orotic acid	1.1	3.81E-03
Oxidized glutathione	1.4	1.17E-06
pentadecanoic acid	1.2	3.39E-04
Phenylalanine	1.1	9.04E-03
Porphobilinogen	1.4	1.03E-04
Protopine	1.0	9.69E-03
Pyridoxal	1.4	6.32E-05
Pyruvic acid	1.0	2.95E-02
Ribonolactone	1.3	5.77E-05
Ribose	1.1	9.54E-04
Ribose 5-phosphate	1.0	4.95E-03
Salicylic acid	1.2	5.81E-03
Sebacic acid	1.0	4.48E-03
Sedoheptulose 7-phosphate	1.1	1.15E-03
Serine	1.1	2.82E-02
Sitosterol	1.3	2.15E-03
Sorbitol	1.4	9.32E-06

Compound	VIP	p-value
Sorbose	1.5	4.02E-07
Stearic acid	1.1	2.62E-03
Succinic acid	1.4	9.82E-05
Tagatose	1.3	5.58E-04
Tetradecanedioic acid	1.0	2.46E-02
Thymine	1.3	3.52E-04
Tryptophan	1.2	2.64E-04
Tyramine	1.0	1.27E-02
Uracil	1.3	6.17E-05
Uridine monophosphate	1.2	5.43E-03
Valine	1.1	9.21E-04
Xanthine	1.0	2.83E-03
Xylitol	1.3	4.82E-05

Table 6. Significantly altered metabolites in zebrafish exposed to abamectin (VIP > 1.0, p-value < 0.05).

Compound	VIP	p-value
Erucic acid	1.00	4.47E-02
Linoleic acid	1.06	5.87E-03
8Z,11Z,14Z-Eicosatrienoic acid	1.34	3.03E-03
Taurine	1.38	2.73E-02
Oleic acid	1.39	3.19E-03
Citric acid	1.42	4.00E-03
Benzoic acid	1.48	4.26E-04
Spermine	1.57	4.01E-02
Stearamide	1.58	2.13E-03
Acetyl-L-carnitine	1.62	8.24E-04
Monobutyl phthalate	1.70	1.30E-03
2-Aminoethanol	1.33	6.07E-03
2-Aminopimelic acid	1.47	2.75E-02
2-Keto-isovaleric acid	2.09	4.23E-06
5-Hydroxy-tryptophan	1.50	2.76E-02
6-Phosphogluconic acid	1.49	4.32E-04
7-Methylguanine	1.19	4.09E-02
Acetoacetic acid	1.30	2.55E-02
Aconitic acid	1.57	4.24E-04
Adenine	1.43	1.45E-02
Arginine	1.16	2.62E-02
Cholestanol	1.37	6.25E-03
Cholesterol	1.35	6.16E-03
Cytidine	1.12	3.88E-02
D-Pipecolic acid	1.40	5.37E-03
eicosanol	1.35	9.77E-03
Glyceric acid	1.28	1.83E-02
Guanosine	1.46	6.60E-03
Histamine	1.09	4.54E-02
Histidinol	1.25	2.28E-02
Inosine	1.69	1.78E-03
Isoleucine	1.25	4.46E-02

Compound	VIP	p-value
Kynurenine	1.38	8.66E-03
Leucine	1.37	1.50E-02
Oxidized glutathione	1.48	4.96E-03
Monoethylhexyl phthalic acid	1.20	9.09E-05
N-Acetylaspartic acid	1.76	3.24E-03
Octadecanol	1.62	4.05E-04
O-Phosphoethanolamine	1.79	2.42E-04
Proline	1.20	3.19E-02
raffinose	1.25	4.06E-02
Sebacic acid	1.48	1.64E-02
Serine	1.20	2.74E-02
Spermidine	1.23	4.44E-02
Stearic acid	1.57	5.65E-03
Succinic acid	1.21	4.38E-02
Threitol	1.34	1.60E-02
Threonine	1.50	2.69E-03
Thymine	1.17	2.37E-02
Tryptamine	1.24	4.88E-02
Tryptophan	1.58	3.81E-04
Turanose	1.11	4.70E-02
Vanilmandelic acid	1.26	9.89E-03
Xanthine	1.25	1.58E-02

Fig. 10. Heat map of differentially expressed metabolites in the control and exposure groups in honeybee.

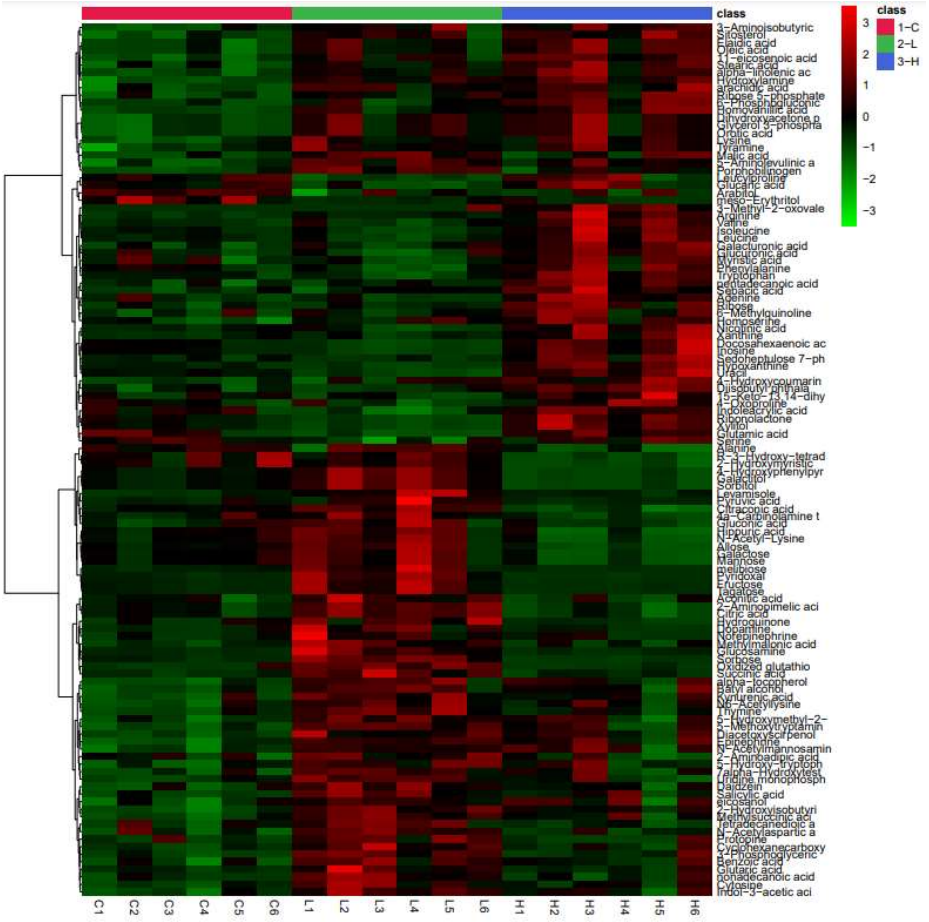
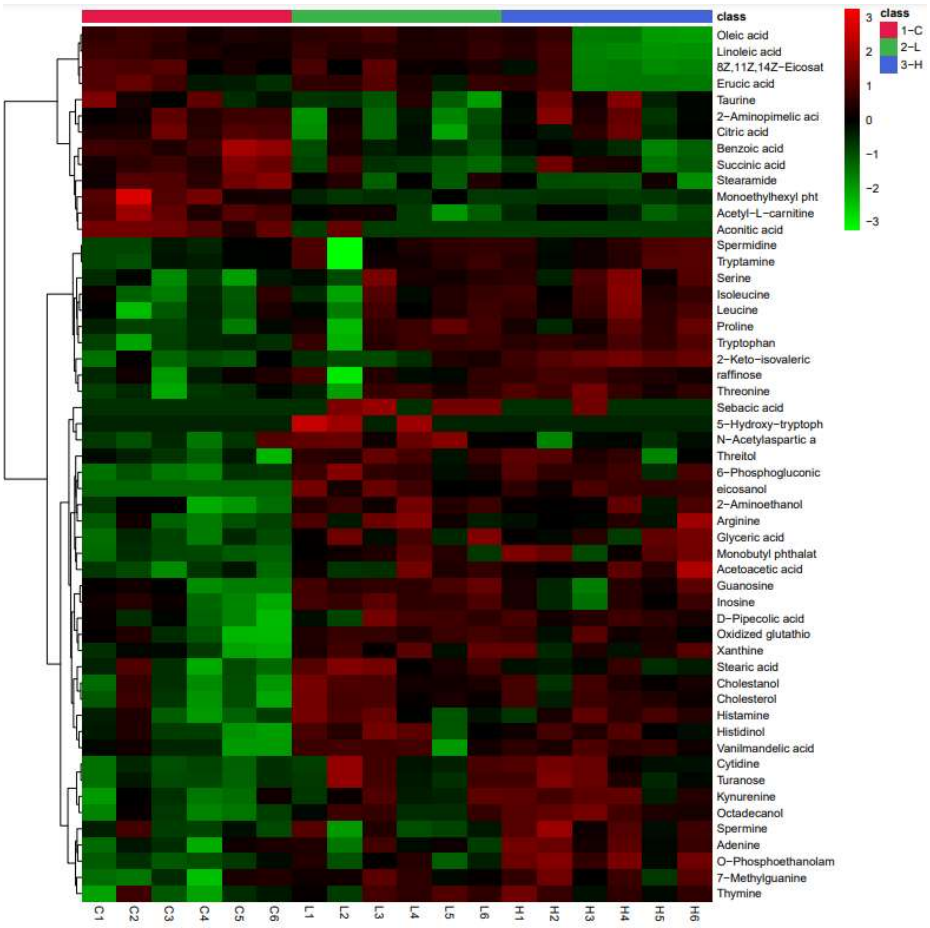

class
1−C
2−L
3−H
3
2
1
0
−1
−2
−3
C1
C2
C3
C4
C5
C6
L1
L2
L3
L4
L5
L6
H1
H2
H3
H4
H5
H6

Fig. 11. Heat map of differentially expressed metabolites in the control and exposure groups in zebrafish.


class
Oleic acid
Linoleic acid
8Z,11Z,14Z−Eicosat
Erucic acid
Taurine
2−Aminopimelic aci
Citric acid
Benzoic acid
Succinic acid
Stearamide
Monoethylhexyl pht
Acetyl−L−carnitine
Aconitic acid
Spermidine
Tryptamine
Serine
Isoleucine
Leucine
Proline
Tryptophan
2−Keto−isovaleric
raffinose
Threonine
Sebacic acid
5−Hydroxy−tryptoph
N−Acetylaspartic a
Threitol
6−Phosphogluconic
eicosanol
2−Aminoethanol
Arginine
Glyceric acid
Monobutyl phthalat
Acetoacetic acid
Guanosine
Inosine
D−Pipecolic acid
Oxidized glutathio
Xanthine
Stearic acid
Cholestanol
Cholesterol
Histamine
Histidinol
Vanilmandelic acid
Cytidine
Turanose
Kynurenine
Octadecanol
Spermine
Adenine
O−Phosphoethanolam
7−Methylguanine
Thymine
C1
C2
C3
C4
C5
C6
L1
L2
L3
L4
L5
L6
H1
H2
H3
H4
H5
H6
3
2
1
0
−1
−2
−3
class
1−C
2−L
3−H

Metabolic pathway analysis

The toxic xenobiotics can effect metabolic process (Zhang et al., 2017) which represents the alterations in the level of each metabolite. The MetaboAnalyst 5.0 contains pathway analysis module including algorithms and concepts from high-quality KEGG (Wang et al., 2016). As shown in **Table 5-6.** 116 (honeybee) and 54 (zebrafish) of biomarker significantly altered in each metabolic pathway. Considering the pathway impact (X-axis) and –log10(p) (Y-axis), Amino acid metabolism (tyrosine metabolism/phenylalanine, tyrosine, and tryptophan biosynthesis/alanine, aspartate, and glutamate metabolism) and carbohydrate metabolism (citrate cycle/ascorbate and aldarate metabolism) were identified as major perturbed pathways in honeybee. In case of zebrafish, translation (aminoacyl-tRNA biosynthesis), carbohydrate metabolism (citrate cycle, glyoxylate/dicarboxylate metabolism), and amino acid metabolism (tryptophan metabolism/arginine and proline metabolism) pathways were mainly changed by abamectin exposure. The disturbed pathways and relevant metabolites were summarized from the results of the metabolic pathway analyses and KEGG map (**Fig. 12-15.**).

Fig. 12. Summary of pathway analyses of honeybee with MetaboAnalyst 5.0, as visualized by bubble plots. Bubble size is proportional to the impact of each pathway and bubble color denotes the degree of significance, from the highest (red) to the lowest (white).

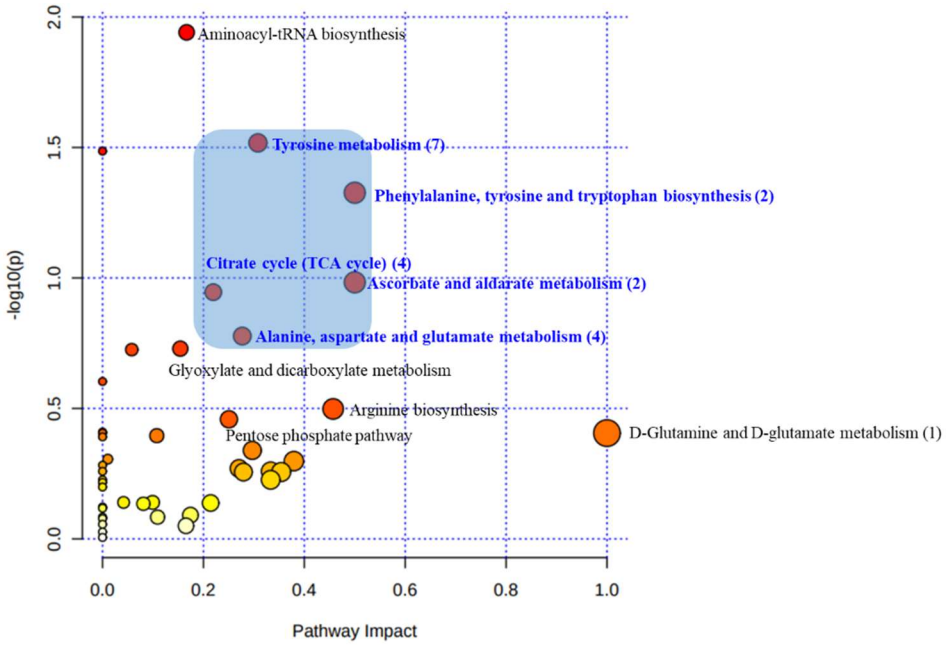

Aminoacyl-tRNA biosynthesis
Tyrosine metabolism (7)
Phenylalanine, tyrosine and tryptophan biosynthesis (2)
Citrate cycle (TCA cycle) (4)
Ascorbate and aldarate metabolism (2)
Alanine, aspartate and glutamate metabolism (4)
Glyoxylate and dicarboxylate metabolism
Arginine biosynthesis
Pentose phosphate pathway
D-Glutamine and D-glutamate metabolism (1)
-log10(p)
Pathway Impact
0.0
0.5
1.0
1.5
2.0
0.0
0.2
0.4
0.6
0.8
1.0

Fig. 13. Perturbed pathways and fluctuating metabolites in honeybee whole body induced by abamectin exposure. The colors of the metabolites represent significant increases or reductions in metabolite levels of abamectin exposure groups compared with the control group. The graphs represent the average relative areas of each metabolite and significantly different changes of $p < 0.05$ and $p < 0.005$ in relative areas are labeled by "*" and "", respectively.**

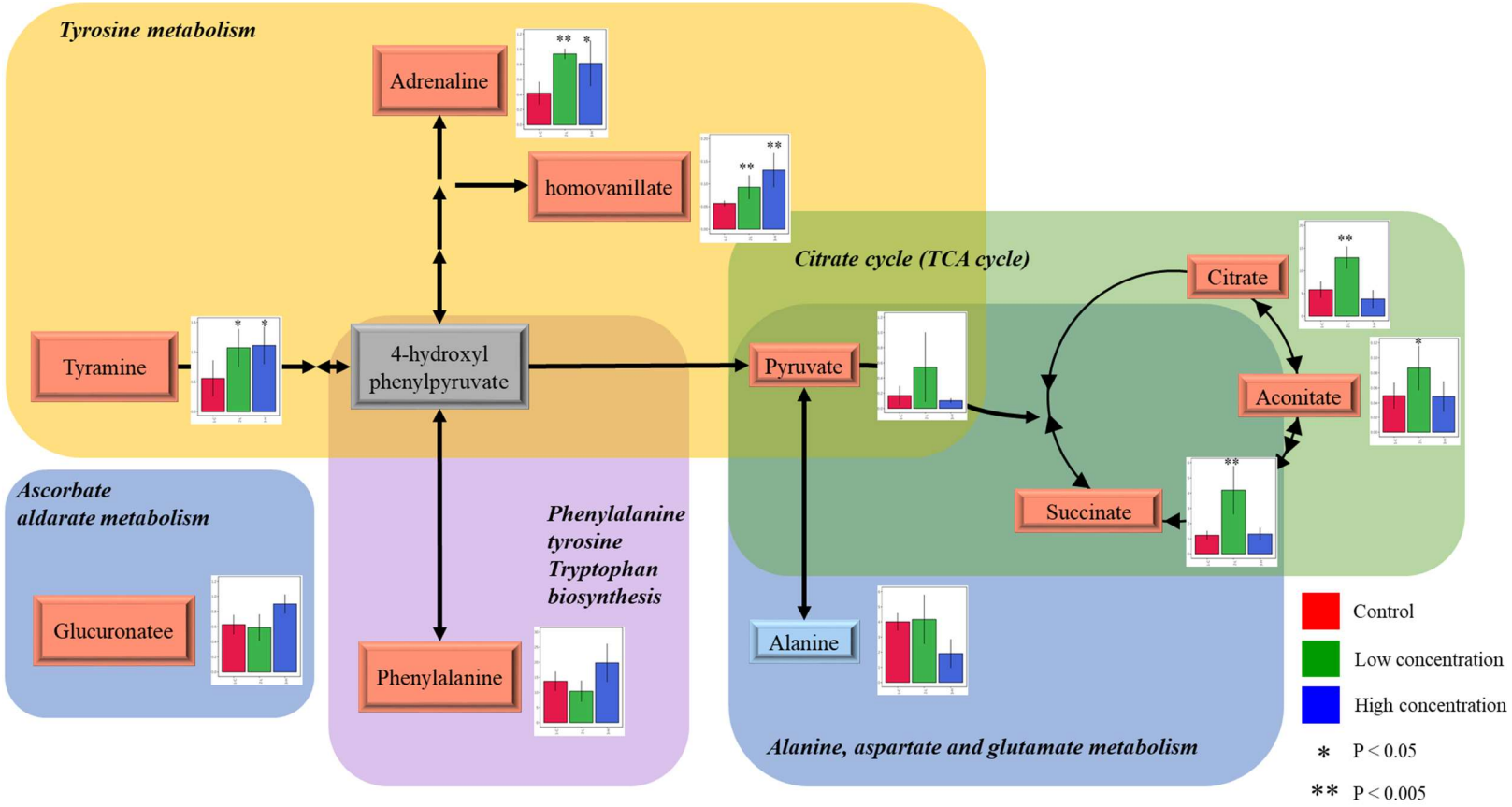

Tyrosine metabolism
Adrenaline
homovanillate
Tyramine
4-hydroxyl
phenylpyruvate
Pyruvate
Citrate cycle (TCA cycle)
Citrate
Aconitate
Succinate
Ascorbate
aldarate metabolism
Glucuronatee
Phenylalanine
tyrosine
Tryptophan
biosynthesis
Phenylalanine
Alanine
Alanine, aspartate and glutamate metabolism
Control
Low concentration
High concentration
* P < 0.05
** P < 0.005

Fig. 14. Summary of pathway analyses of zebrafish with MetaboAnalyst 5.0, as visualized by bubble plots. Bubble size is proportional to the impact of each pathway and bubble color denotes the degree of significance, from the highest (red) to the lowest (white).

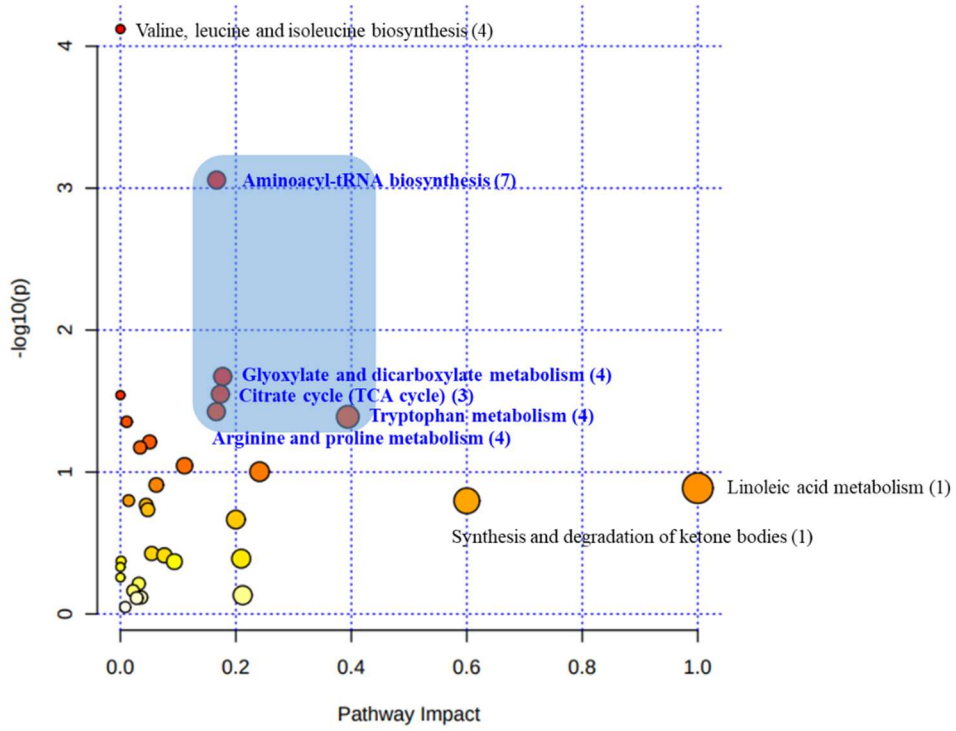
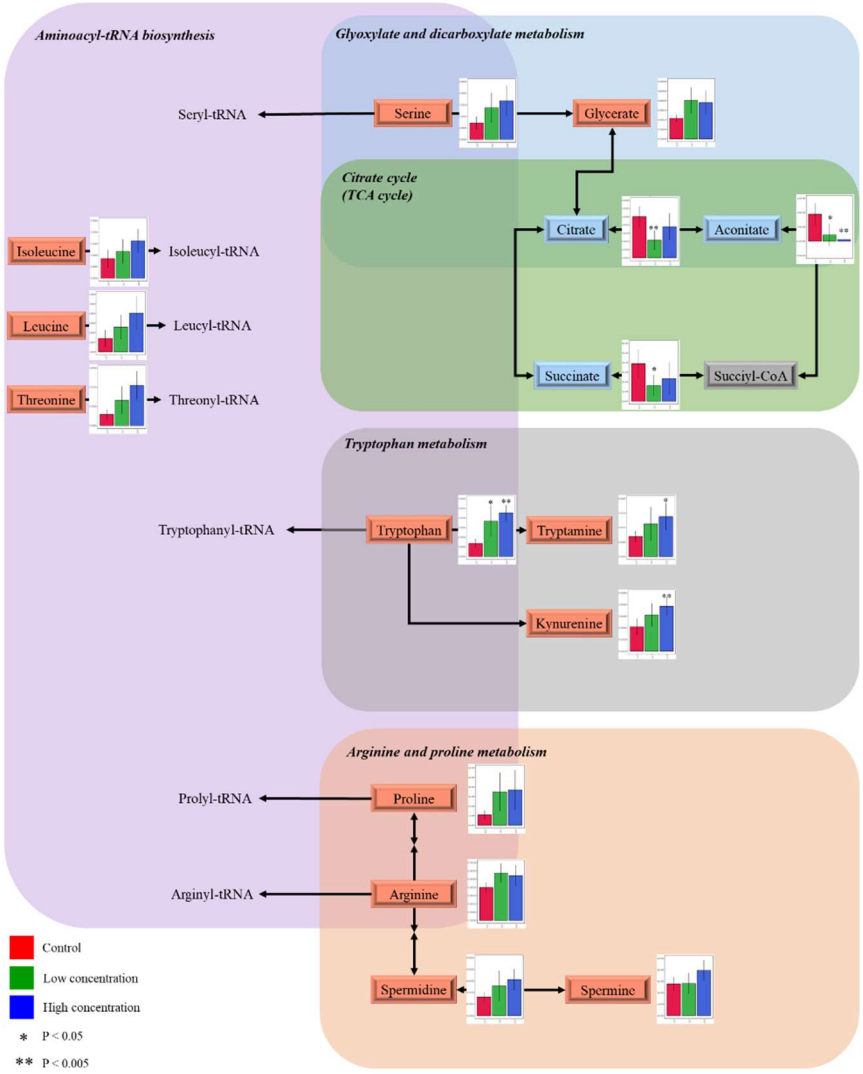

Valine, leucine and isoleucine biosynthesis (4)
Aminoacyl-tRNA biosynthesis (7)
Glyoxylate and dicarboxylate metabolism (4)
Citrate cycle (TCA cycle) (3)
Tryptophan metabolism (4)
Arginine and proline metabolism (4)
Linoleic acid metabolism (1)
Synthesis and degradation of ketone bodies (1)
-log10(p)
0
1
2
3
4
0.0
0.2
0.4
0.6
0.8
1.0
Pathway Impact

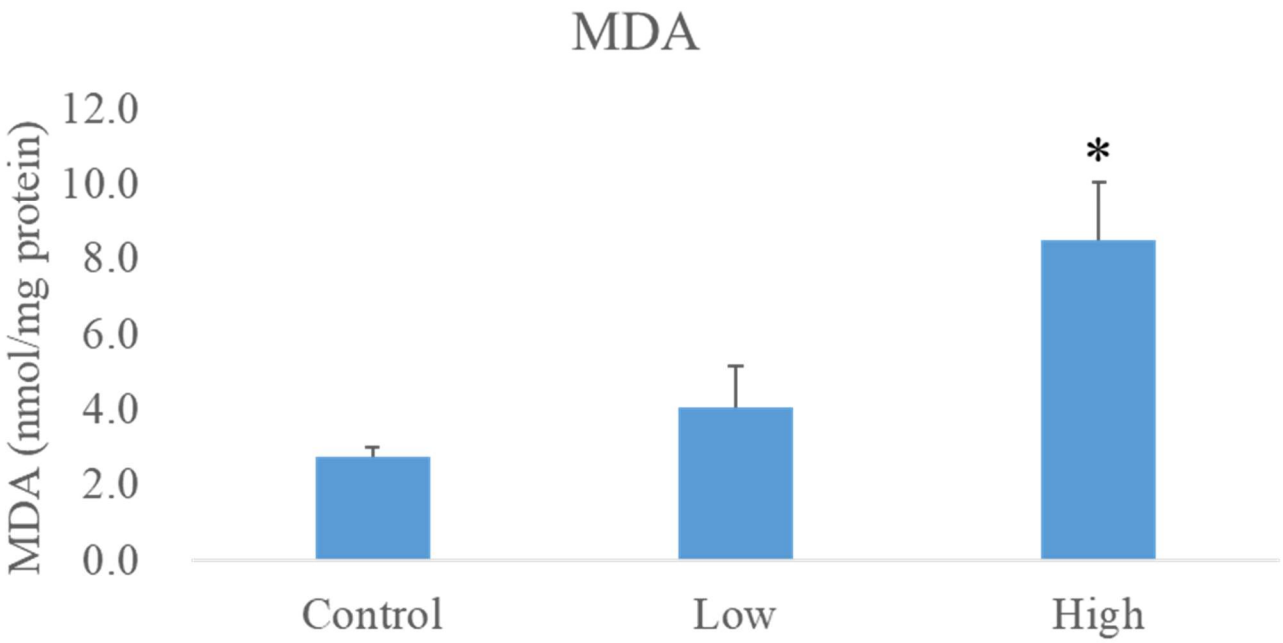
Fig. 15. Perturbed pathways and fluctuating metabolites in zebrafish whole body induced by abamectin exposure. The colors of the metabolites represent significant increases or reductions in metabolite levels of abamectin exposure groups compared with the control group. The graphs represent the average relative areas of each metabolite and significantly different changes of $p < 0.05$ and $p < 0.005$ in relative areas are labeled by "*" and "", respectively.**


Aminoacyl-tRNA biosynthesis
Glyoxylate and dicarboxylate metabolism
Seryl-tRNA
Serine
Glycerate
Citrate cycle
(TCA cycle)
Citrate
Aconitate
Isoleucine
Isoleucyl-tRNA
Leucine
Leucyl-tRNA
Threonine
Threonyl-tRNA
Succinate
Succinyl-CoA
Tryptophan metabolism
Tryptophanyl-tRNA
Tryptophan
Tryptamine
Kynurenine
Arginine and proline metabolism
Prolyl-tRNA
Proline
Arginyl-tRNA
Arginine
Spermidine
Spermine
Control
Low concentration
High concentration
* P < 0.05
** P < 0.005

Abamectin effects on MDA in zebrafish

MDA is the end product to evaluate the extent of oxidative stress, therefore, it can be a biomarker to evaluate the toxicity of abamectin to zebrafish. In the production of MDA in zebrafish, there were significant increase ($p < 0.05$) compared with that of the control group after 48 (**Fig. 16**). These result suggested that abamectin induced lipid peroxidation in cell membranes of zebrafish (Zhang et al., 2013). Similar results have been observed in several studies of zebrafish exposed to xenobiotics (Jin et al., 2010; Han et al., 2016).

Fig. 16. Levels of MDA of zebrafish exposed to abamectin. The significant changes between exposed samples and the controls ($p < 0.05$) are labeled by "*".


MDA
MDA (nmol/mg protein)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
*
Control
Low
High

Conclusions

In this study, the toxicological effects of abamectin in honeybee and adult zebrafish were determined using a GC-MS/MS-based targeted metabolomics approach with MRM for detection of the metabolites. Additionally, non-targeted metabolomics approach also performed by LC-Orbitrap-HRMS. The honeybee and zebrafish were exposed to abamectin at concentrations of 0.5 LD_{50}, 0.05 LD_{50} (honeybee) and LC_{50}, 0.1 LC_{50} (zebrafish) for 48 hours. After exposure, the organism samples were homogenized, and the metabolites were extracted before derivatization with TMS. On GC-MS/MS, MRM of 396 metabolites was used to detect 237 metabolites (honeybee) and 241 metabolites (zebrafish) with high sensitivity and selectivity. Based on VIP and ANOVA analyses, 97 (honeybee) and 41 (zebrafish) metabolites were selected as biomarkers by targeted profiling. From non-targeted profiling, 367 (honeybee) and 169 (zebrafish) metabolites also detected in LC-Orbitrap-HRMS and extracted to 19 (honeybee) and 13 (zebrafish) biomarker metabolites by statistical analysis. The combined targeted and non-targeted biomarker showed for their significant contributions to the differences in metabolite profiles as shown on the heat map with dendrograms. Pathway analyses of the biomarkers using MetaboAnalyst 5.0 and KEGG identified alterations in several metabolic pathways associated with amino acid metabolism and carbohydrate metabolism involved in both honeybee and zebrafish. Additionally in zebrafish, aminoacyl-tRNA biosynthesis pathway related to translation showed purterbed. To evaluate the biochemical toxicity of abamectin to zebrafish, MDA were additionally assessed, resulting in the MDA levels were significantly increased in the zebrafish exposed to abamectin. These results suggest that these pathways underwent significant perturbations during the exposure period. Such alteration and disturbance of

important metabolites of these pathways by abamectin could result in a biologically deleterious effect in honeybee and zebrafish.

Chapter II

Toxicoprotemomics of Abamectin in Honeybee (*Apis mellifera*) and Zebrafish (*Danio rerio*)

Introduction

Proteomics

The proteome is known as the composition of all proteins expressed by an organism's genome (Wasinger et al., 1995). n order to perform proteomic analysis, it is necessary to study cellular metabolic pathways and to identify possible drug targets. As cellular proteins are very complex and consist of thousands of proteins, analysis efforts beyond the capacity of standard laboratory equipment are required (Westermeier and Naven, 2002).

Proteomics is the study of simultaneous analysis of protein mixtures such as cell lysates and tissue extracts to find quantitative changes in expression levels. It extends to the fields of drug discovery, treatment, microbiology, biochemistry and plant research. Recent advances in mass spectrometry and genomic information enable protein and peptide analysis, and online peptide fragmentation enables rapid amino acid sequence analysis for a small amount of peptides at low operating costs. Also, due to the development of high throughput, genome databases of other organisms have been established in a short time. Genomic sequence data grows at a tremendous rate, but for most genes their function is unknown. Most proteins are modified by complex genetic interactions, cellular events, and environmental influences that lead to post-translational modifications. In particular, knowledge of the DNA sequence of an organism is very important for protein identification and characterization using mass spectrometry (Westermeier and Naven, 2002).

Protein expression has been widely used for 30 years to measure the exposure of an organism to contaminants or to evaluate its toxic properties (Gandar et al., 2017). However, some questions have been raised about the specificity of the classification of pollutants using biomarkers and the reproducibility between organism models (Benninghoff, 2007). Using toxicoproteomics, it is possible to

explore proteins and physiological pathways affected by chemicals without prior knowledge (Wetmore and Merrick, 2004; Benninghoff, 2007). It also helps to identify potential impact biomarkers and predict the impact of contaminants in larger tissues. In fish, it has been used to study the effects of pesticides (Sanchez et al., 2009; Biales et al., 2011; Chen and Huang, 2011; Laldinsangi et al., 2014), heavy metals (Ling et al., 2009; Dorts et al., 2011; Karlsen et al., 2011; Wang et al., 2013), perfluorooctane sulfonate (Roland et al., 2014), or brominated flame retardants (Kling et al., 2008; Kling and Förlin, 2009). This technique may also be used to study the effects of other environmental factor (e.g., hypoxia (Douxfils et al., 2012); osmotic stress (Kumar et al., 2009)) or multiple stressors in fish (Wang et al., 2008).

Methodology of proteomics

Proteomics is much more complex than the genome, but it is an important way to understand gene function (Lander et al., 2001). In order to differentiate between the two biological states of cells, changes in the level of gene expression can be identified through precarriage or proteomics analysis (Canales et al., 2006). Proteins, as effectors of biological functions, depend not only on the mRNA level, but also on host translation control and regulation. Therefore, proteomics is considered a suitable method for characterizing biological systems (Cox and Mann, 2007).

Protein purification techniques are traditionally based on ion exchange chromatography (IEC), size exclusion chromatography (SEC) and affinity chromatography (Jungbauer and Hahn, 2009; Hage et al., 2012). The ELISA (enzyme-linked immunosorbent assay) and western plotting can be used to selectively analyze proteins. However, it is limited to the analysis of several individual proteins and is insufficient to define the level of protein expression (Lequin, 2005; Kurien and Scofield, 2006). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DE) and

two-dimensional differential gel electrophoresis (2D-DIGE) techniques are used for separation of complex protein samples (Marouga et al., 2005; Issaq and Veenstra, 2008).

Although microarray was developed for high throughput and rapid expression analysis, it has limitations in understanding the function of the whole genome (Sutandy et al., 2013). On the other hand, mass spectrometry is used to analyze protein mixtures with the advantage of higher sensitivity in proteomics (McLafferty, 2011). In addition, Edman digestion was developed to determine the amino acid sequence of a protein (Thakkar et al., 2006). Isotope coding affinity tag (ICAT) labeling, stable isotope labeling using amino acids in cell culture (SILAC), and isotope tag for relative and absolute quantification (iTRAQ) technologies have recently been developed for quantitative proteomics (Shiio and Aebersold, 2006; Zhang et al., 2006; Wiese et al., 2007; Kroksveen et al., 2015). X-ray crystallography and NRM are used to provide the three-dimensional structure of proteins to understand biological functions (Smyth and Martin, 2000; Wiese et al., 2007).

Proteomics consists of dynamic characterization of functional proteins using biological mass spectrometry, and high accuracy and high resolution are essential to improve the robustness of complex sample studies (Vinh, 2019).

Rapid-throughput technology advances collect huge amounts of proteomics data, and bioinformatics databases are set up to process vast amounts of data (**Table 7**). Various bioinformatics tools are being developed for structural prediction of proteins, domain and motif analysis, rapid analysis of interactions between proteins, and data analysis of MS (Vihinen, 2001; Perez-Riverol et al., 2015). Proteomic analysis can use multiple analytical techniques to provide a complete description of the cellular functional information as well as the mechanisms of a cell's response to various types of stress and drugs (Aslam et al., 2017).

Pathway and network analysis technology is a method used to interpret results, and proteomic data analysis at the level of the path is increasingly popular. In the case of pathway analysis, it aims to identify activated pathway modules, and biological pathways can be viewed as signaling pathways, gene regulatory pathways, and metabolic pathways. Pathway analysis expands fragmentary protein pathways, making it possible to easily interpret the molecular mechanisms underlying expression (Khatri et al., 2012). For network analysis, refer to data analysis to build, visualize, and infer protein interaction networks. Unlike path analysis, this aims to use a comprehensive network derived from previous experimental sources and new in silico predictions to obtain biological meaning. (Wu et al., 2014). Many large knowledge bases on biological pathways and protein networks have been published, e.g., BioGRID (Chatr-Aryamontri et al., 2012), STRING (Franceschini et al., 2012), KEGG (Kanehisa and Goto, 2000), Reactome (Matthews et al., 2009), PID (Schaefer et al., 2009), HAPPI (Chen et al., 2009a), HPD (Chowbina et al., 2009), and PAGED (Huang et al., 2012) databases (Wu et al., 2014).

Fig. 17. Overview of proteomic approaches.

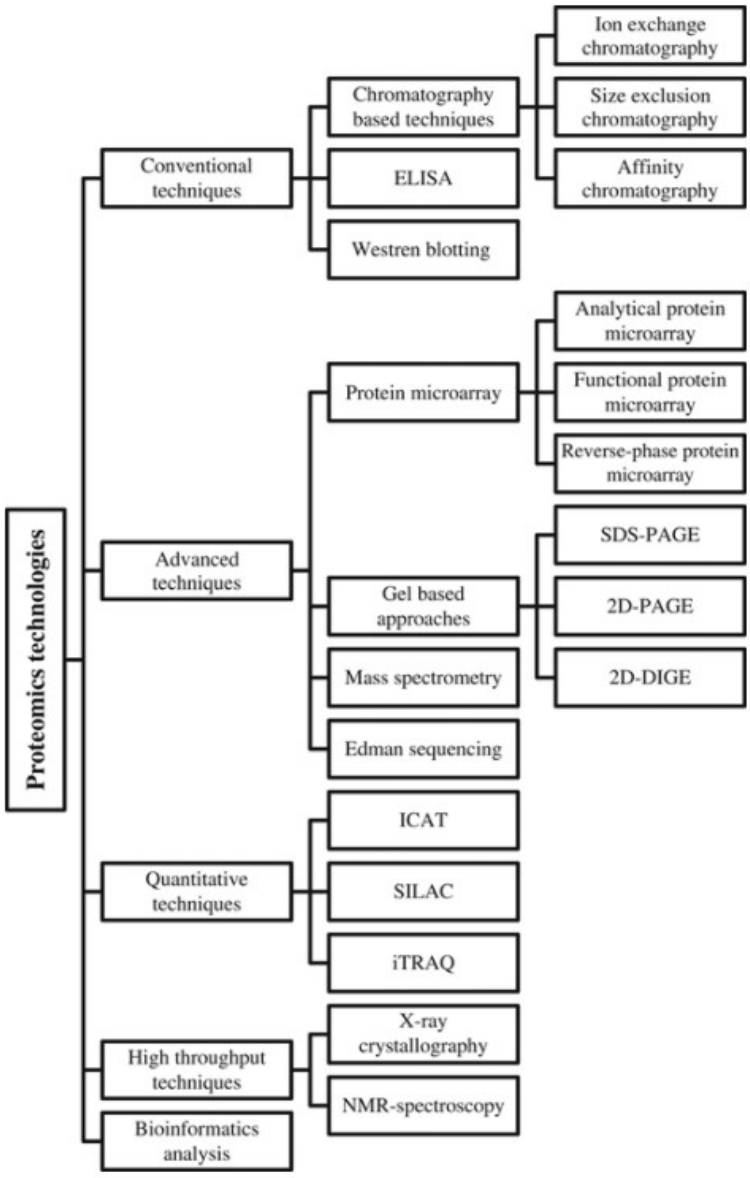

Proteomics technologies
Conventional techniques
Chromatography based techniques
Ion exchange chromatography
Size exclusion chromatography
Affinity chromatography
ELISA
Westren blotting
Advanced techniques
Protein microarray
Analytical protein microarray
Functional protein microarray
Reverse-phase protein microarray
Gel based approaches
SDS-PAGE
2D-PAGE
2D-DIGE
Mass spectrometry
Edman sequencing
Quantitative techniques
ICAT
SILAC
iTRAQ
High throughput techniques
X-ray crystallography
NMR-spectroscopy
Bioinformatics analysis

Table 7. General protein sequence databases, sequence similarity search, alignment tools and structural analysis and prediction servers (Aslam et al., 2017).

Name	Type	Link
GenBank	Database	http://www.ncbi.nih.gov/entrez/query.fcgi?db=protein
RefSeq	Database	https://www.ncbi.nlm.nih.gov/refseq/
nr	Database	http://www.ncbi.nlm.nih.gov/BLAST/
UniProt	Database	http://www.pir.uniprot.org/
UniRef	Database	http://www.pir.uniprot.org/database/nref.shtml
UniParc	Database	http://www.pir.uniprot.org/database/archive.shtml
TrEMBL	Database	http://kr.expasy.org/sprot/
SwissProt	Database	http://kr.expasy.org/sprot/
PIR	Database	http://pir.georgetown.edu/
OWL	Database	http://www.bioinf.man.ac.uk/dbbrowser/OWL/
BLASTP	BLAST	http://blast.ncbi.nlm.nih.gov
TBLASTN	BLAST	https://blast.ncbi.nlm.nih.gov
PSI-BLAST	Position Specific Iterated BLAST	https://blast.ncbi.nlm.nih.gov
PHI-BLAST	Pattern Hit Initiated BLAST	https://blast.ncbi.nlm.nih.gov
DELTA-BLAST	Domain Enhanced Lookup Time Accelerated BLAST	https://blast.ncbi.nlm.nih.gov
InterProScan	Protein domain servers	http://www.ebi.ac.uk/lnterProScan/

Name	Type	Link
CD server	Protein domain servers	http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml
ProWleScan	Protein domain servers	http://hits.isb-sib.ch/cgi-bin/PFSCAN
ScanProsite	Protein domain servers	http://us.expasy.org/tools/scanprosite/
PATTINPROT	Protein motif search tools	http://pbil.ibcp.fr/html/pbiljndex.html
SIRW	Protein motif search tools	http://sirw.embl.de/index.html
Match Box	Motif based alignment server	http://www.sciences.fundp.ac.be/biologie/bms/help.html
MEME	Motif based alignment server	http://meme.sdsc.edu/meme/website/meme.html
Gibbs	Motif based alignment server	http://bayesweb.wadsworth.org/gibbs/gibbs.html
Dialign	Motif based alignment server	http://bibiserv.techfak.uni-bielefeld.de/dialign/
BlockMakei	Motif based alignment server	http://blocks.fhcrc.org/make_blocks.html
PDB	Protein structure databases	http://www.rcsb.org/pdb/
SwissModel	Protein structure databases	http://swissmodel.expasy.org/repository/
SCOP	Protein structure databases	http://scop.mrc-lmb.cam.ac.uk/scop/
ModBase	Protein structure databases	http://alto.compbio.ucsf.edu/modbase-cgi/index.cgi
CATH	Protein structure databases	http://www.biochem.ucl.ac.uk/bsm/cath/
MMDD	Protein structure databases	http://www.ncbi.nlm.nih.gov/Structure/
ConSurf	Protein structure analysis server	http://consurf.tau.ac.il/
CASTp	Protein structure analysis server	http://sts.bioe.uic.edu/castp/index.php
ProtSkin	Protein structure analysis server	http://www.mcgnmr.ca/ProtSkin/intro/

Name	Type	Link
LigandProtein	Protein structure analysis server	http://bip.weizmann.ac.il/oca-bin/lpccsu
PredictProtein	Protein structure prediction sever	http://www.embl-heidelberg.de/predictprotein
O-GlycoBase	Protein structure prediction sever	http://www.cbs.dtu.dk/services/NetOGIyc/
PhosphoBase	Protein structure prediction sever	http://www.cbs.dtu.dk/services/NetPhos
SwissModel	Protein structure modeling server	http://www.expasy.org/swissmod
Whatlf	Protein structure modeling server	http://www.cmbi.kun.nl/gv/servers/WIWWWI
ESyPred3D	Protein structure modeling server	http://www.fundp.ac.be/urbm/bioinfo/esypred
EBI	Protein structure modeling server	http://biotech.ebi.ac.uk:8400/

Table 8. Pathway/network analysis resource that can benefit proteomics data analysis (Wu et al., 2014).

Name	Description	Link
GoMiner	Gene Ontology (GO) analysis for Omic data	http://discover.nci.nih.gov/gominer/
KEGG	Kyoto Encyclopedia of Genes and Genomes	http://www.genome.jp/kegg/
DAVID	The Database for Annotation, Visualization and Integrated Discovery	http://david.abcc.ncifcrf.gov/
PID	Pathway Interaction Database	http://pid.nci.nih.gov/
HPD	Human Pathway Database	http://bio.informatics.iupui.edu/HPD
GESA	Gene Set Enrichment Analysis	http://www.broadinstitute.org/gsea/
IPA	Ingenuity pathway analysis	http://www.ingenuity.com/
MetaCore	Thomson Reuters pathway analysis and knowledge mining	http://thomsonreuters.com/metacore/
Pathway-Express	A systems biology approach for pathway level impact analysis	http://vortex.cs.wayne.edu/projects.htm
SPIA	Signaling Pathway Impact Analysis	http://www.bioconductor.org/packages/2.12/bioc/html/SPIA.html
PAGED	An integrated Pathway And Gene Enrichment Database	http://bio.informatics.iupui.edu/PAGED
HAPPI	Human Annotated and Predicted Protein Interaction database	http://bio.informatics.iupui.edu/HAPPI
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins	http://string.embl.de/

CytoScape	An open source platform for complex network analysis and visualization	http://www.cytoscape.org/
WebGestalt	Web-based gene set analysis toolkit	http://webgestalt.org
MetaboAnalyst	Joint pathway analysis of gene/proteins and metabolites	https://www.metaboanalyst.ca/

Application of proteomics

Proteomics originated from pharmaceutical studies for the identification of new drug protein targets from initially modified cell lines or diseased tissues. In addition, validation of detected targets, in vitro and in vivo toxicity studies, and side effects testing can be used as approaches. Proteomics is widely used to compare normal samples, disease samples, and treatment samples, and to find molecular markers in body fluids for diagnosis. (Westermeier and Naven, 2002).

Another field where you can enter is microbiology. It can be applied to plant research for various purposes, such as increasing crop yield by growing plants with high stress resistance such as bacteria, heat, cold, and drought (Westermeier and Naven, 2002).

Proteomics is also used to compare species to analyze evolutionary relationships, and genomic studies in particular can be used to establish evolutionary relationships between different strains and species (Lovric, 2011).

Proteomics is used to analyze changes in organisms and cells, and it is possible to check differences according to various environmental factors such as culture conditions, stress on various food sources, temperature, oxygen, osmotic pressure, and toxins (Lovric, 2011).

Proteomics can also be used in some very straightforward commercial activities, for example for the improvement of bio-processing (Wang et al., 2003) and hence the rapid optimization of the production and processing of biomaterials by microorganisms (Lovric, 2011).

Examples of these applications with a rough classification are shown in **Table 9.** (Lovric, 2011).

Table 9. Common applications of proteomics (Lovric, 2011).

Fundamental biological process	
Which genes are expressed into proteins?	(Zougman et al., 2008) (De Godoy et al., 2008)
Relation between genome, trascriptome and proteome	(Kislinger et al., 2006) (Ambrósio et al., 2009)
Study of medel organism	(Washburn et al., 2001)
Study of certain compartments/organs	(Anderson et al., 2004)
Study of parasites	(Nett et al., 2009)
Molecular mechanism of cellular processes	
Physiological adaptations	(Hecker et al., 2008)
Correlation of composition and function of organelles	(Batrakou et al., 2009)
Study of signal transduction events	(Lovrić et al., 1998) (Casey et al., 2010)
Protein structure and function analysis	
Study of the associations of proteins	(Paul et al., 2009) (De Bodt et al., 2009)
Analysis of posttranslational modifications	(Ho et al., 2002) (Shu et al., 2004) (Choudhary et al., 2009)
Analyzing the effects of protein KO/suppression	(LaCourse et al., 2008) (Chen et al., 2009b)
Product analysis	
Detection of food contaminations	(Mamone et al., 2009)
Analysis of seeds	(Guo et al., 2008)
Optimization of products	(Lücker et al., 2009) (Wang et al., 2003)
Comparison of strains and species	
Evolutionary studies	(Arnesen et al., 2009) (Roth et al., 2009) (Dworzanski et al., 2006) (Pe'er et al., 2004)
Breeding	(Davoli and Braglia, 2007)
Biomarker discovery	
Diagnostic markers for cancers	(Sodek et al., 2008) (Lau et al., 2010) (Kussmann et al., 2006)

Biomarkers for a variety of deseases, for example cardiovascular or infections	(Cuesta et al., 2009) (Mini et al., 2006) (Cummins et al., 2010)
Biomarkers for the function of organs, for example kidneys	(Okano et al., 2007)
Markers for drug response	
System analysis	
Drug development/toxicity	(Gao et al., 2009) (Rix and Superti-Furga, 2009)
Development of drug targets	
Personalized medicine	(Marko-Varga et al., 2007)

Purpose of study

In the present study, a LC-orbitrap-HRNS-based non-targeted proteomics were used for a more comprehensive assessment of metabolic changes at the protein level to elucidate the toxicological effects of abamectin in honeybee and zebrafish. Furthermore, LC-orbitrap-HRMS based label-free quantitative proteome analysis was carried out to confirm the toxicological effects of abamectin in honeybee and zebrafish by the protein level.

Materials and Methods

Chemicals and reagents

Abamectin was purchased from Dr. Ehrenstorfer Gmb. (96.4%; Augsburg, Germany). Ammonium bicarbonate (≥ 99.0%), DL-dithiothreitol (≥ 99%), iodoacetamide (≥ 99%), urea (≥ 99.0%), was purchased from Sigma–Aldrich (St. Louis, MO, USA). Sequencing grade modified trypsin was obtained from Promega Corporation (Madison, WI USA). Sep-Pak C18 cartridge (1 cc, 100 mg) was purchased from Waters Corporation (Milford, MA, USA). Acetonitrile and water were of liquid chromatography-mass spectrometry grade and purchased from Fisher Scientific (Fair Lawn, NJ). Other reagents used in the experiments were of the highest available grade.

Prediction of metabolite-protein interactions

Metabolite-protein interaction network were predicted using Metabolites module of OmicsNet and metabolite to protein interaction data were based on all KEGG reactions.

Experimental animals and chemical exposure

The control and high exposure (for 48h) group samples of the previous study, 'Chapter I, Part 1. toxicometabolomics of abamectin in honeybee (*Apis mellifera*) using GC-MS/MS in multiple reaction monitoring mode', were used for proteomics study.

Sample preparation

Each honeybee and zebrafish samples was frozen in liquid N_2 and pulverized by a ball-mill instrument (MM 400, Retsch, GmbH, Haan, Germany) in 2 mL tube. Each

4 mg of the ground powder from the individuals of control and exposed groups were pooled as one replicate (n=6), and the pooled samples were homogenized in 500 μL of 0.1M phosphate buffer (pH7.4). The homogenates were filtered with cell strainer and then centrifuged at 1,000g at 4°C for 20 min. The protein concentrations of supernatant were degermind according to the Bradford method (Quick Start™ Bradford Protein Assay Kit, Bio-Rad, Inc., CA, USA). The absorbance was detected at 595 nm using UV/Vis spectrophotometer (Optizen POP, Mecasys Co.,Ltd, Korea). A 200 μg equivalent fo protein in the supernatant was used for label-free quantitative proteomic analysis.

Label-free quantitative proteomic analysis of samples was carried out as described previously (Min et al., 2017; Gupta et al., 2018). In brief, the extracted protein samples were used for the in-solution trypsin digestion by filter-aided sample preparation (FASP) method (Wisniewski et al., 2009). The extract solution equivalent to 200 μg of protein was evaporated, and the proteins were denaturated for 3 h at room temperature by 30.0 μL of 6 M urea in 50 mM ammonium bicarbonate. The solution was treated with 100 mM dithiothreitol (3.0 μL) and incubated for 3 h at room temperature, and further treated with 100 mM dithiothreitol (6.8 μL), 0.55 M iodoacetamide (1.2 μL) and incubated for 1 h under dark conditions. The sample was diluted with 164.2 μL of 50 mM ammonium bicarbonate to bring the urea concentration to 1 M, and treated with 4 μg trypsin in 40 μL buffer, followed by shaking for 18 h at 37℃. The digested protein solution was acidified by adding 9.8 μL of 10% formic acid. In the SPE step, a Sep-Pak C18 cartridge was conditioned with 5 mL of Solvent A (0.1% formic acid in 2% acetonitrile) followed by 10 mL of Solvent B (0.1% formic acid in 65% acetonitrile). The digested protein solution was loaded onto the cartridge, and 10 mL of Solvent A was added to wash the cartridge. The sample was eluted after adding 1 mL of Solvent B twice. The eluate was dried in a speed-vacuum and reconstituted in 50 μL of Solvent A.

Profiling and identification of proteins by LC-HRMS (Orbitrap)

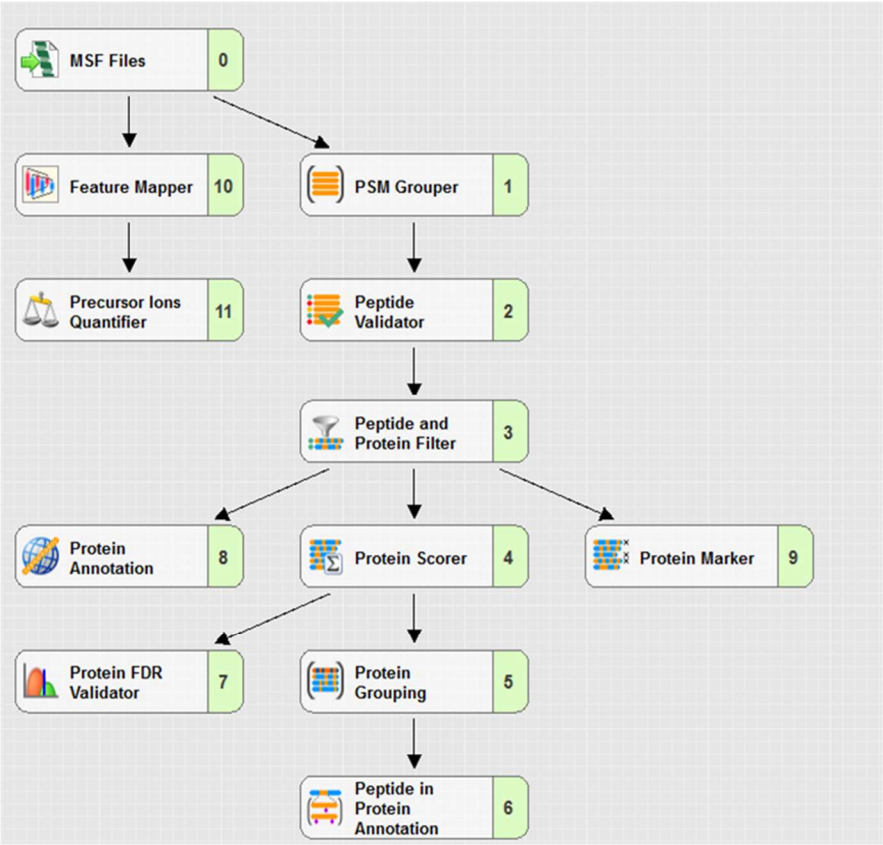
A 3 µL of aliquot was separated by reversed-phase chromatography using an ultra-high-performance liquid chromatography (UHPLC) Dionex UltiMate 3000 (Thermo Fisher Scientific, USA) instrument. For trapping the sample, the UHPLC instrument was equipped with an Acclaim PepMap 100 trap column (100 µm x 2 cm, nanoViper C18, 5 µm, 100 Å) and subsequently washed with 98% Solvent A for 10 min at a flow rate for 4 µL/min. The sample was continuously separated on a PepMap RSLC capillary column (75 µm x 50 cm, C18, 2 µm, 100 Å) using gradient elution at a flow rate of 300 nL/min with water in 0.1% formic acid (A) and acetonitrile in 0.1% formic acid (B) as the mobile phases (all v/v). The following gradient was used: 5% B for 0-5 min, 5 to 10% B for 5-10 min, 10 to 40% B for 10-150 min, 40 to 95% B for 150-152 min, 95% B for 152-162 min, 95 to 5% B for 162-165 min and 5% B for 165-180 min.

LC-MS/MS was coupled with an electrospray ionization source to the quadrupole-based mass spectrometer QExactive Orbitrap HRMS (Thermo Fisher Scientific, USA). Peptied samples were electrosprayed through a coated silica-emitted tip (PicoTip emitter, New Objective, USA) at an ion spray voltage of 2200 eV. The MS spectra were acquired at a resolution of 70,000 in a mass range of 350-1,800 m/z, and the MS/MS spectra were measured in a data-dependent mode at a resolution of 17,500. A maximum injection time was set to 20 ms for full mass and 80 ms for $ddMS^2$.

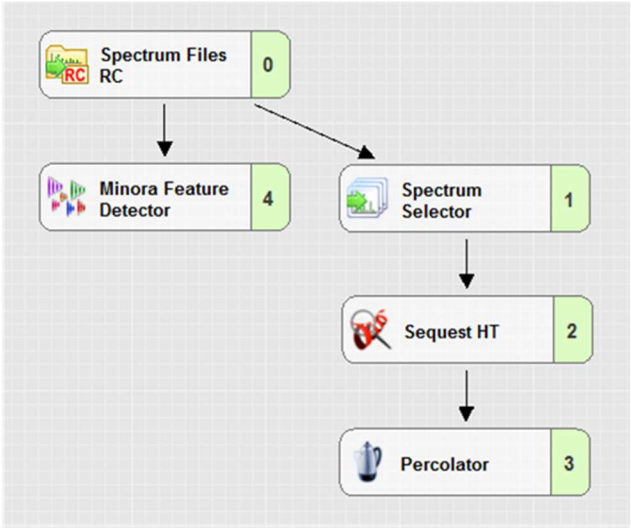
MS spectral data were obtained using Xcalibur software (Thermo Fisher Scientific Inc.) and the proteins were identified using Proteome Discoverer 2.4 (PD 2.4, Thermo Fisher Scientific Inc.) with selected *Apis mellifera* FASTA database from National Center for Biotechnology Information (NCBI). Workflow tree from the PD 2.4 shown in **Fig. 18.** displayed select data processing nodes and the associated workflow connections.

Fig. 18. Workflow tree from the PD 2.4 software displaying select data processing nodes and the associated workflow connections: consensus workflow (A) and processing workflow (B).

(A)


MSF Files
0
Feature Mapper
10
PSM Grouper
1
Precursor Ions Quantifier
11
Peptide Validator
2
Peptide and Protein Filter
3
Protein Annotation
8
Protein Scorer
4
Protein Marker
9
Protein FDR Validator
7
Protein Grouping
5
Peptide in Protein Annotation
6

(B)


Spectrum Files RC
0
Minora Feature Detector
4
Spectrum Selector
1
Sequest HT
2
Percolator
3

Statistical analysis

According to the abundance level, the comparison test of volcano plots was performed using MetaboAnayst 5.0. The proteins with an intergroup p-value < 0.05 and fold change > 2 were considered as the differential biomarker proteins.

Enrichment analysis

The online WEB-based DAVID (bioinformatics resources 6.8, https://david.ncifcrf.gov/) was used for the gene ontology enrichment analysis and KEGG pathway enrichment analysis of biomarker proteins.

Results and Discussion

Protein profiling using LC-orbitrap-HRMS

Recently, there has been a case study of the degree of impact on polycyclic aromatic hydrocarbons, pharmaceuticals and personal care products (PPCPs), PCBs, and contaminated sediments. A total of 652 (honeybee) and 2225 (zebrafish) proteins were identified by label-free quantitative proteomic analysis using LC-orbitrap-HRMS (**Fig. 19.**). The analytical data processing using PD 2.4 led to the identification and quantification of 650 and 648 proteins in the control and exposed groups in honeybee, respectively. While, 2155 and 2007 proteins were identified and quantification in the control and exposed groups in zebrafish, respectively. The Venn diagram showed 646 (honeybee), 1937 (zebrafish) common proteins among the two group samples (**Fig. 20.**).

Fig. 19. TIC of proteins of (A) honeybee samples and (B) zebrafish samples in control group and abamectin exposed group, respectively

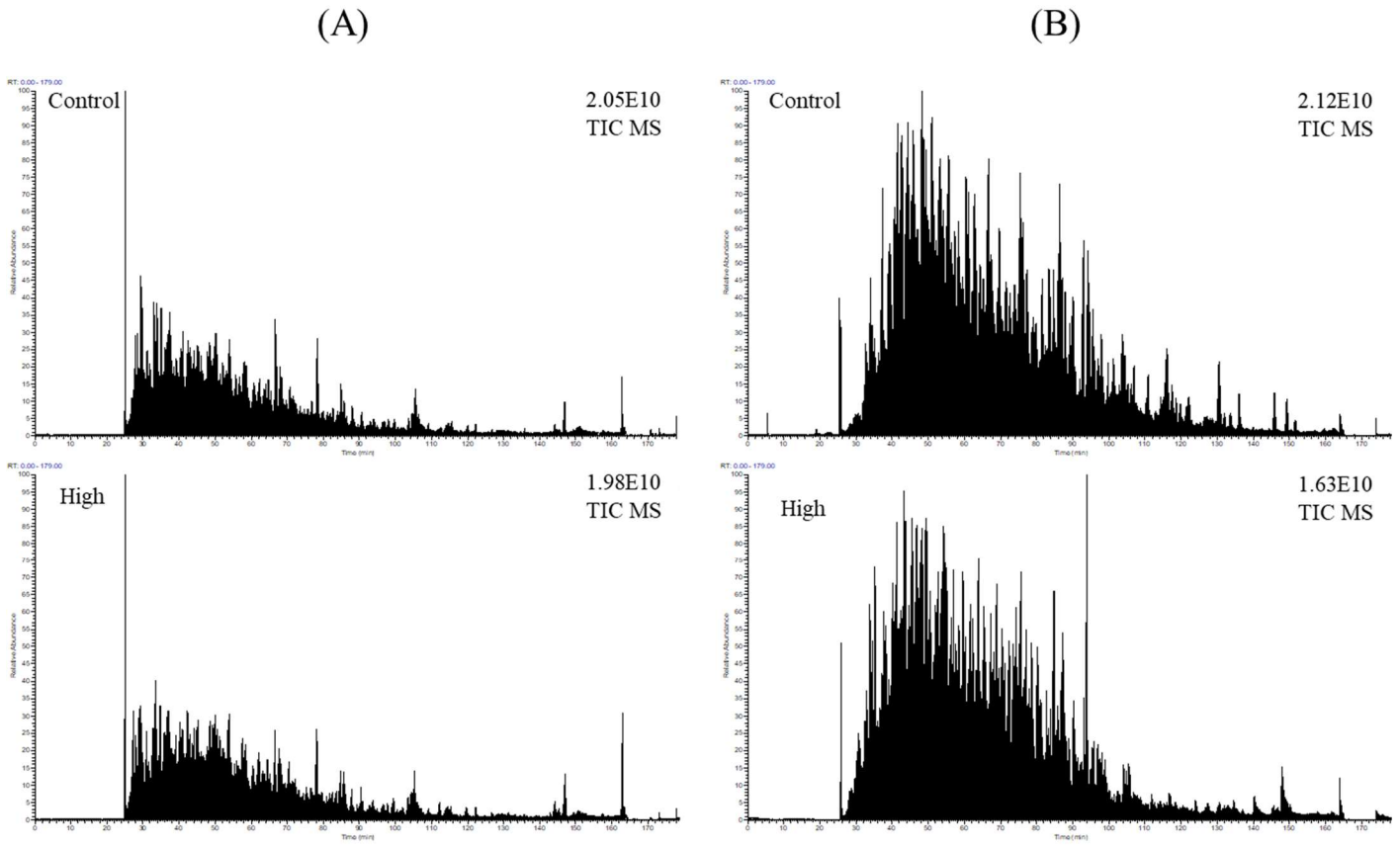
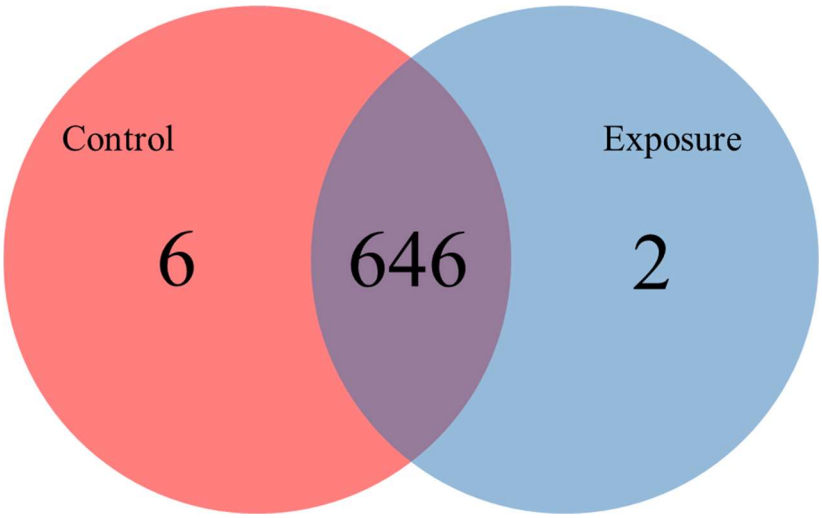
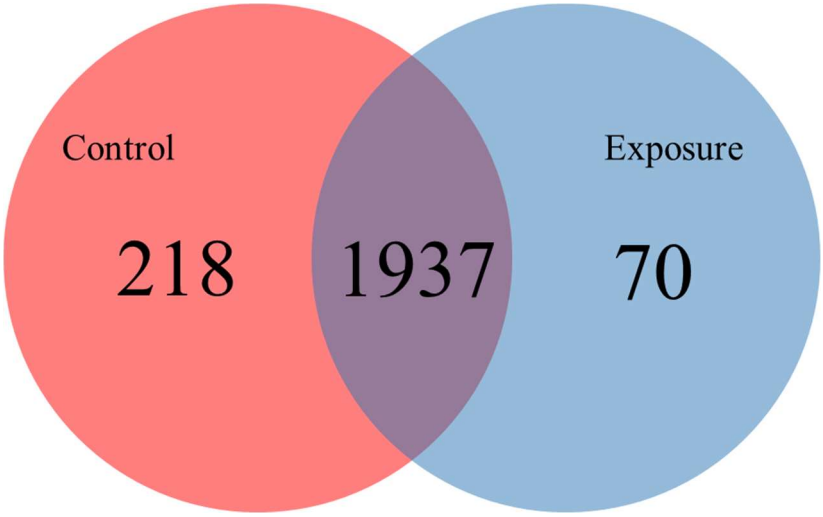

(A)
(B)
Control
2.05E10
TIC MS
Control
2.12E10
TIC MS
High
1.98E10
TIC MS
High
1.63E10
TIC MS

Fig. 20. Venn diagram of total identified proteins in (A) honeybee and (B) zebrafish.

(A)



(B)



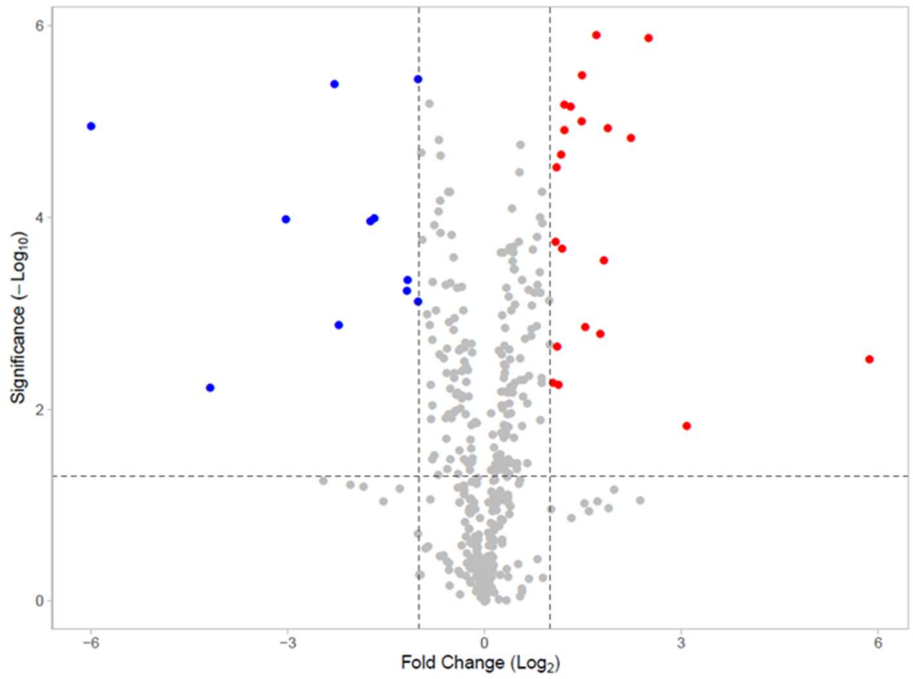
Proteomic alteration induced by abamectin exposure

In case of honebee 32 proteins were significantly changed in exposed group compared to the control (Volcano analysis, p-value < 0.05, fold change > 2.0) (**Fig. 21., Table 10.**). The Venn diagram showed that 32 and 31 proteins were detected in the control and exposed groups, respectively, and 31 proteins were commonly detected in both groups (**Fig. 22**). Among those, 32 proteins were up regulated by exposure of abamectin.

In zebrafish, 1607 (zebrafish) proteins were significantly changed in exposed group compared to the control (Volcano analysis, p-value < 0.05, fold change > 2.0) (**Fig. 21., Table 11.**). The Venn diagram showed that 1020 and 911 proteins were detected in the control and exposed groups, respectively, and 864 proteins were commonly detected in both groups (**Fig. 22**). Among those, 668 proteins were up regulated and 399 proteins were down regulated by exposure of abamectin.

Fig. 21. Volcano plots of total identified proteins. Blue (down-regulated) and red (up-regulated) dots indicate biomarker proteins with differences between groups selected according to the threshold (p-value < 0.05 and fold change > 2.0). in (A) honeybee and (B) zebrafish.

(A)


Significance (−Log10)
6
4
2
0
−6
−3
0
3
6
Fold Change (Log2)

(B)

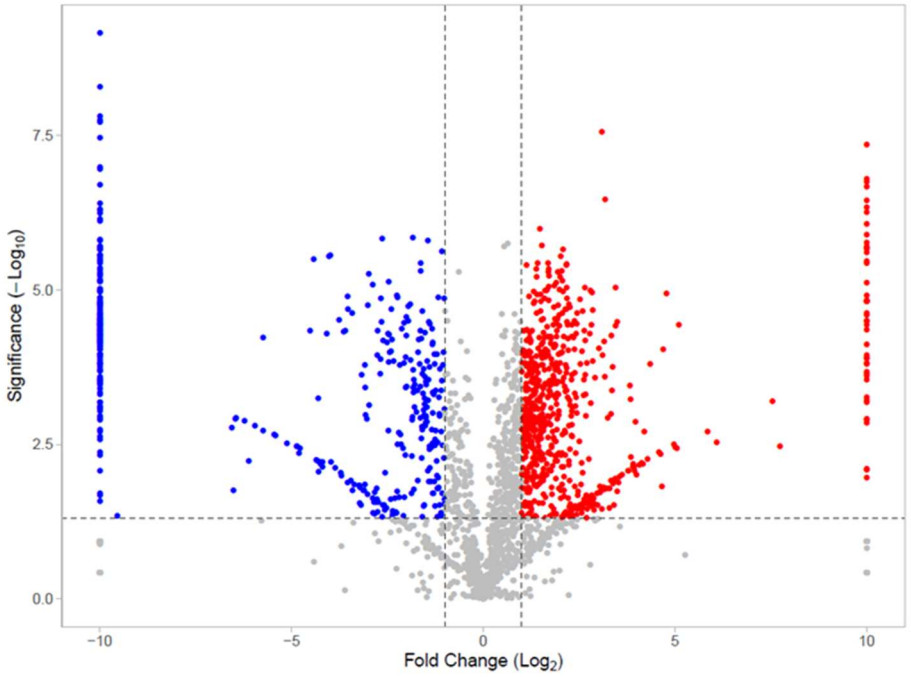

Significance (−Log10)
7.5
5.0
2.5
0.0
−10
−5
0
5
10
Fold Change (Log2)

Table 10. Significantly altered biomarker proteins in honeybee exposed to abamectin which were selected from proteomics approach.

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_392104.4	retinal dehydrogenase 1 [Apis mellifera]	LOC408559	1.25E-06	3.26	1.71
XP_006568212.1	fatty acid binding protein isoform X1 [Apis mellifera]	Fabp	1.33E-06	5.66	2.50
XP_395659.1	protein lethal(2)essential for life [Apis mellifera]	LOC412197	3.23E-06	2.80	1.49
NP_001229442.1	pyruvate dehydrogenase E1 component subunit beta, mitochondrial [Apis mellifera]	PDHB	3.63E-06	0.50	-1.01
XP_392722.1	26S proteasome regulatory subunit 6A-B [Apis mellifera]	Tbp-1	4.08E-06	0.20	-2.29
XP_006569857.1	myosin heavy chain, muscle isoform X1 [Apis mellifera]	Mhc1	6.55E-06	2.32	1.22
XP_016766933.1	protein artichoke [Apis mellifera]	LOC724779	6.87E-06	2.48	1.31
ABR45906.1	high Glx storage protein [Apis mellifera]	Hex110	9.82E-06	2.79	1.48
XP_016768414.1	UDP-glucuronosyltransferase 2C1 [Apis mellifera]	LOC725997	1.11E-05	0.19	-6.00
XP_006566311.1	troponin C type I isoform X1 [Apis mellifera]	TpnCI	1.17E-05	3.68	1.88
NP_001165874.1	cytochrome c oxidase subunit VIb polypeptide 1 [Apis mellifera]	Cox6b1	1.23E-05	2.32	1.21
ABD92639.1	OBP3 [Apis mellifera]	Obp3	1.46E-05	4.69	2.23
XP_006561863.1	beta-hexosaminidase subunit beta [Apis mellifera]	LOC726818	2.18E-05	2.25	1.17
XP_006557994.1	CDGSH iron-sulfur domain-containing protein 2 homolog isoform X1 [Apis mellifera]	LOC410636	2.97E-05	2.14	1.10
NP_001011652.1	troponin C type IIIb [Apis mellifera]	TpnCIIIb	1.02E-04	0.31	-1.68
XP_624487.1	adrenodoxin-like protein, mitochondrial [Apis mellifera]	Fdxh	1.05E-04	0.12	-3.03
XP_623128.1	thioredoxin-like protein 1 [Apis mellifera]	Trx1-like1	1.08E-04	0.30	-1.74
XP_006569359.1	protein croquemort [Apis mellifera]	SCR-B6	1.78E-04	2.12	1.08

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_006563494.1	uncharacterized protein LOC102653894 [Apis mellifera]	LOC102653894	2.09E-04	2.27	1.18
XP_395614.3	endoplasmin [Apis mellifera]	LOC412150	2.80E-04	3.53	1.82
XP_006569974.1	venom acid phosphatase Acph-1 [Apis mellifera]	LOC726737	4.47E-04	0.44	-1.18
XP_006559184.1	methylosome subunit pICln [Apis mellifera]	LOC551690	5.75E-04	0.44	-1.19
XP_624933.3	RNA 3'-terminal phosphate cyclase isoform X1 [Apis mellifera]	LOC552554	7.58E-04	0.49	-1.02
XP_625073.3	pyruvate dehydrogenase E1 component subunit beta, mitochondrial isoform X1 [Apis mellifera]	LOC412522	1.32E-03	0.21	-2.23
AAV90959.1	major royal jelly protein 3 [Apis mellifera carnica]	Mrjp3	1.37E-03	2.90	1.54
XP_395729.3	dynactin subunit 2 [Apis mellifera]	Dctn2	1.63E-03	3.39	1.76
ABD92645.1	OBP13 [Apis mellifera]	Obp13	2.20E-03	2.15	1.11
XP_623685.1	uncharacterized protein LOC408421 [Apis mellifera]	LOC408421	3.04E-03	41.72	5.87
XP_006558879.1	ras-related protein Rap1 [Apis mellifera]	R	5.31E-03	2.06	1.04
XP_001121007.2	uncharacterized protein LOC725122 isoform X1 [Apis mellifera]	LOC725122	5.56E-03	2.19	1.13
XP_016767446.1	60S ribosomal protein L7 [Apis mellifera]	RpL7	5.93E-03	0.08	-4.18
XP_394637.2	N-alpha-acetyltransferase 15, NatA auxiliary subunit [Apis mellifera]	LOC411163	1.48E-02	7.74	3.08

Table 11. Significantly altered biomarker proteins in zebrafish exposed to abamectin which were selected from proteomics approach.

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
Q6PI52.3	RecName: Full=Calmodulin; Short=CaM	calm1a	4.61E-05	0.33	-1.62
ABB29996.1	V-FBPL precursor [Danio rerio]	LOC100000126	3.53E-02	5.35	2.42
XP_021324351.1	afadin isoform X1 [Danio rerio]	mllt4	7.77E-04	5.16	2.37
XP_001338037.1	apolipoprotein A-I-like [Danio rerio]	LOC100004607	1.71E-02	2.31	1.21
XP_021330602.1	non-muscle caldesmon isoform X1 [Danio rerio]	cald1l	1.87E-03	0.40	-1.33
XP_021332753.1	collagen alpha-1(XVIII) chain isoform X1 [Danio rerio]	LOC100334893	4.90E-04	2.51	1.33
AAI55802.1	C14orf159 protein, partial [Danio rerio]	c14orf159	1.84E-03	2.82	1.50
XP_003199094.1	dihydropyrimidinase-related protein 2 [Danio rerio]	LOC798555	1.65E-04	4.34	2.12
XP_001341669.4	EMILIN-2 [Danio rerio]	LOC100001725	4.93E-04	0.00	-7.65
XP_003199702.2	NADPH:adrenodoxin oxidoreductase, mitochondrial [Danio rerio]	LOC100148371	1.61E-04	2.54	1.34
XP_021330210.1	filamin-A isoform X3 [Danio rerio]	si:ch211-222g5.4	1.84E-02	10.68	3.42
XP_002664631.3	integrin alpha-E-like isoform X1 [Danio rerio]	LOC100333951	2.59E-04	0.35	-1.50
NP_001093526.1	uncharacterized protein LOC796447 precursor [Danio rerio]	LOC796447	2.26E-04	0.34	-1.57
XP_704272.2	galectin-3 isoform X1 [Danio rerio]	si:ch211-67f24.5	9.74E-03	2.08	1.06
XP_009289535.1	neutral alpha-glucosidase AB [Danio rerio]	LOC561509	5.29E-03	0.33	-1.59
A5WVX0.1	RecName: Full=Inosine triphosphate pyrophosphatase; Short=ITPase; Short=Inosine triphosphatase; AltName: Full=Non-canonical purine NTP pyrophosphatase; AltName: Full=Non-standard purine NTP pyrophosphatase; AltName: Full=Nucleoside-triphosphate diphosphatase; AltName: Full=Nucleoside-triphosphate pyrophosphatase; Short=NTPase	itpa	1.48E-05	201.00	7.65

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_001313376.1	syntaxin-10 [Danio rerio]	LOC100151249	2.22E-05	5.87	2.55
XP_005171299.1	microtubule-associated protein 4 isoform X1 [Danio rerio]	LOC100537884	2.08E-03	2.81	1.49
XP_021326297.1	mucin-2 isoform X1 [Danio rerio]	LOC100148804	1.57E-03	2.91	1.54
XP_003198611.2	myosin regulatory light chain 2, ventricular/cardiac muscle isoform [Danio rerio]	LOC100333760	2.22E-03	0.03	-5.17
XP_687541.4	poly(ADP-ribose) glycohydrolase [Danio rerio]	LOC559134	4.93E-02	4.08	2.03
XP_009289494.1	protocadherin-1 isoform X1 [Danio rerio]	pcdh1	1.30E-04	201.00	7.65
AAH95793.1	Zgc:112374 [Danio rerio]	pon3	8.51E-03	14.34	3.84
AAH77125.1	Im:6903726 protein, partial [Danio rerio]	im:6903726	1.50E-02	5.12	2.36
XP_009302182.1	neurogenic locus notch homolog protein 2-like [Danio rerio]	LOC100535382	1.96E-08	0.00	-7.65
XP_009306032.1	autophagy-related protein 2 homolog A isoform X1 [Danio rerio]	LOC101883461	1.95E-04	2.28	1.19
XP_021323109.1	spectrin beta chain, non-erythrocytic 5 isoform X3 [Danio rerio]	LOC569585	6.21E-06	3.95	1.98
XP_021324257.1	band 4.1-like protein 2 [Danio rerio]	epb41l2	1.56E-03	3.01	1.59
AAC96114.1	Orb/CPEB-related RNA-binding protein [Danio rerio]	cepb1b	2.23E-03	2.77	1.47
XP_001345541.6	alpha-2-macroglobulin-like isoform X2 [Danio rerio]	sb:cb37	8.79E-04	2.07	1.05
XP_686234.7	kynurenine/alpha-aminoadipate aminotransferase, mitochondrial [Danio rerio]	LOC557979	2.51E-03	2.59	1.37
XP_009303055.1	target of Nesh-SH3 isoform X1 [Danio rerio]	LOC101884149	1.99E-03	0.00	-7.65
AAI10104.1	Actinin, alpha 2 [Danio rerio]	actn2	3.16E-03	27.19	4.76
NP_001311338.1	adipocyte enhancer-binding protein 1 precursor [Danio rerio]	LOC793308	4.50E-08	201.00	7.65
XP_021325504.1	agrin isoform X1 [Danio rerio]	hm:gc12	3.29E-03	0.48	-1.05

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI24813.1	Si:ch73-252g14.4 protein, partial [Danio rerio]	si:ch73-252g14.4	1.05E-04	0.49	-1.02
XP_009305061.1	delta-1-pyrroline-5-carboxylate synthase isoform X1 [Danio rerio]	LOC100332705	4.79E-03	3.73	1.90
XP_005169103.1	AMP deaminase 3-like isoform X2 [Danio rerio]	LOC556578	6.25E-04	201.00	7.65
XP_005165704.1	ankyrin 1, erythrocytic a isoform X20 [Danio rerio]	ank1	1.57E-05	201.00	7.65
NP_001071066.1	amiloride-sensitive amine oxidase [copper-containing] precursor [Danio rerio]	abp1	1.26E-02	2.15	1.11
NP_001013573.3	amine oxidase copper containing 2 [Danio rerio]	zgc:113006	7.26E-05	2.90	1.54
XP_021322407.1	apolipoprotein A-IV-like isoform X1 [Danio rerio]	zgc:194131	1.43E-06	0.28	-1.81
XP_002665852.3	apolipoprotein B-100 [Danio rerio]	LOC100330435	7.40E-05	3.34	1.74
AAI62151.1	Hypothetical LOC570390 [Danio rerio]	zgc:193682	2.83E-04	0.00	-7.65
XP_694888.4	rho GTPase-activating protein 27 isoform X1 [Danio rerio]	LOC566523	3.21E-03	2.38	1.25
NP_956316.1	ubiquitin-like-conjugating enzyme ATG3 [Danio rerio]	apg3l	4.75E-05	0.00	-7.65
AAF98358.1	Na+/K+ ATPase alpha subunit isoform 1 [Danio rerio]	atp1a1	1.40E-03	2.27	1.19
XP_688502.5	filensin [Danio rerio]	LOC560018	2.64E-03	2.19	1.13
NP_001314899.1	bridging integrator 1b isoform 1 [Danio rerio]	bin1	1.59E-02	2.24	1.17
AAH45899.1	LOC402804 protein, partial [Danio rerio]	LOC402804	3.14E-03	0.22	-2.21
XP_009294664.1	complement component c3b, tandem duplicate 1 isoform X1 [Danio rerio]	si:dkey-21e7.2	6.49E-03	0.06	-4.11
AAH68336.1	Calr protein [Danio rerio]	calr	4.64E-05	2.08	1.05
AAF13700.1	calreticulin [Danio rerio]	calr	7.39E-04	0.13	-2.92
AAH46906.1	Calrl protein [Danio rerio]	calrl	1.54E-04	2.03	1.02

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_001263999.1	serum deprivation-response protein [Danio rerio]	sdpra	1.28E-05	0.09	-3.46
AAI29235.1	Zgc:158446 protein [Danio rerio]	zgc:158446	3.65E-02	0.17	-2.53
XP_021325272.1	complement factor H-related protein 2 [Danio rerio]	LOC101884308	7.66E-04	2.56	1.35
AAQ97757.1	muscle cofilin 2 [Danio rerio]	cfl2l	1.09E-03	3.16	1.66
AAH95030.1	Zgc:112425 [Danio rerio]	zgc:112425	2.85E-04	0.29	-1.79
NP_001035411.2	cold-inducible RNA-binding protein isoform 1 [Danio rerio]	cirbp	1.58E-04	0.32	-1.64
AAH62860.1	Chloride intracellular channel a [Danio rerio]	clica	1.58E-04	18.55	4.21
Q5RGU1.2	RecName: Full=Atypical kinase COQ8A, mitochondrial; AltName: Full=Chaperone activity of bc1 complex-like; Short=Chaperone-ABC1-like; AltName: Full=Coenzyme Q protein 8A; AltName: Full=aarF domain-containing protein kinase 3; Flags: Precursor	adck3	1.01E-05	0.00	-7.65
XP_001333987.2	cytochrome c oxidase subunit 4 isoform 1, mitochondrial [Danio rerio]	LOC402880	6.19E-05	0.33	-1.60
NP_001019574.1	cytochrome c oxidase subunit Vaa [Danio rerio]	LOC100537689	7.78E-06	3.68	1.88
AAI33995.1	Cytochrome c oxidase, subunit VIIa 2 [Danio rerio]	cox7a2	4.48E-03	4.07	2.03
Q7SZC2.2	RecName: Full=Exportin-2; Short=Exp2; AltName: Full=Chromosome segregation 1-like protein; AltName: Full=Importin-alpha re-exporter	LOC100331234	8.57E-07	201.00	7.65
NP_571327.1	casein kinase 2, alpha 1 polypeptide [Danio rerio]	ck2a1	5.98E-05	2.73	1.45
XP_002667402.3	cysteine/serine-rich nuclear protein 3 [Danio rerio]	LOC100332048	4.46E-02	3.63	1.86
NP_001307020.1	cysteine and glycine-rich protein 3 isoform 1 [Danio rerio]	LOC100150192	1.71E-02	8.54	3.09
XP_009302008.1	homeobox protein cut-like 2 [Danio rerio]	cux2	1.40E-05	0.00	-7.65
XP_003198851.2	ubiquitin carboxyl-terminal hydrolase CYLD-like [Danio rerio]	LOC561108	1.24E-02	2.88	1.52
AAH76033.1	Cyp46a1 protein, partial [Danio rerio]	cyp46a1	5.25E-05	5.24	2.39

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_998441.1	N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 [Danio rerio]	zgc:85829	1.64E-04	0.48	-1.05
AAH67555.1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39b [Danio rerio]	ddx39b	2.93E-05	0.00	-7.65
XP_021334401.1	ATP-dependent RNA helicase DDX3X isoform X1 [Danio rerio]	ddx3	3.03E-04	3.23	1.69
XP_009290539.1	dynein heavy chain 11, axonemal [Danio rerio]	LOC567790	5.86E-07	0.00	-7.65
NP_001243566.1	desmoglein 2, tandem duplicate 1 precursor [Danio rerio]	dsg2	2.94E-04	3.28	1.71
XP_021327956.1	EH domain-binding protein 1-like protein 1 isoform X1 [Danio rerio]	LOC100331563	4.72E-02	4.69	2.23
AAH51785.1	Eukaryotic translation initiation factor 2, subunit 1 alpha [Danio rerio]	eif2s1	2.85E-02	5.90	2.56
NP_001313413.1	epiplakin 1 [Danio rerio]	LOC101885652	4.32E-02	5.27	2.40
XP_021323982.1	epidermal growth factor receptor substrate 15-like 1 isoform X1 [Danio rerio]	LOC100329948	1.26E-04	0.00	-7.65
NP_956500.2	endoplasmic reticulum aminopeptidase 1 precursor [Danio rerio]	zgc:56194	3.53E-02	8.21	3.04
AAI53984.1	Zgc:171591 protein [Danio rerio]	zgc:171591	6.80E-03	4.60	2.20
AAH55122.1	Zgc:63486 [Danio rerio]	agxt2l1	3.06E-04	2.42	1.28
XP_009289980.1	tumor necrosis factor alpha-induced protein 2 isoform X1 [Danio rerio]	LOC565839	1.60E-03	2.23	1.16
AAI64059.1	Fhla protein [Danio rerio]	fhl1a	8.68E-05	3.54	1.82
AKP49174.1	mitochondrial fission 1 protein [Danio rerio]	im:6905231	3.39E-02	0.18	-2.49
AAH50158.1	Fucosidase, alpha-L- 1, tissue [Danio rerio]	fuca1	2.00E-03	2.19	1.13
AAI64011.1	Gcdhl protein [Danio rerio]	gcdhl	3.71E-06	201.00	7.65
NP_997841.1	glutathione S-transferase mu, tandem duplicate 1 [Danio rerio]	gstm	1.18E-05	2.61	1.39
XP_021333974.1	glutathione S-transferase mu 3 isoform X1 [Danio rerio]	zgc:173994	1.37E-05	4.46	2.16

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_001156323.1	glutathione S-transferase mu tandem duplicate 3 [Danio rerio]	gstm3	1.91E-03	4.28	2.10
AAH91534.1	Si:xx-by187g17.5 [Danio rerio]	si:ch211-5k11.6	2.45E-03	3.52	1.81
XP_005165996.1	vigilin isoform X1 [Danio rerio]	hdlbp	4.01E-06	2.17	1.11
NP_001017763.1	beta-hexosaminidase subunit alpha precursor [Danio rerio]	zgc:112084	3.59E-02	4.96	2.31
XP_021335234.1	heterogeneous nuclear ribonucleoprotein A1 isoform X1 [Danio rerio]	zgc:66127	4.28E-02	3.47	1.79
AAH44442.1	Heterogeneous nuclear ribonucleoprotein A1 [Danio rerio]	hnrnpa1	2.01E-02	0.41	-1.29
XP_003198760.1	heterogeneous nuclear ribonucleoprotein U-like protein 1 isoform X1 [Danio rerio]	LOC100330645	2.51E-04	3.50	1.81
AAM51549.1	insulin-like growth factor binding protein 5 [Danio rerio]	igfbp5a	8.47E-03	0.00	-7.65
XP_009300927.1	immunoglobulin-like and fibronectin type III domain containing 1, tandem duplicate 1 isoform X4 [Danio rerio]	zgc:113358	1.95E-04	2.29	1.20
XP_009289517.1	lipopolysaccharide-responsive and beige-like anchor protein isoform X1 [Danio rerio]	lrba	3.86E-05	10.41	3.38
AAI10110.1	Zgc:123248 [Danio rerio]	zgc:123248	1.30E-04	201.03	7.65
XP_005163766.1	hematological and neurological expressed 1 protein isoform X1 [Danio rerio]	hn1b	3.39E-04	0.00	-7.65
AAQ94579.1	HN1-like protein [Danio rerio]	hn1l	1.59E-06	0.00	-7.65
CAQ53175.1	junction plakoglobin 1b [Danio rerio]	jup	1.25E-03	2.03	1.02
XP_021323761.1	kelch-like protein 38 isoform X1 [Danio rerio]	klhl38	1.99E-03	44.91	5.49
XP_001922967.1	kelch-like protein 41a [Danio rerio]	kbtbd10a	8.37E-04	2.88	1.53
F1QEG2.1	RecName: Full=Kelch-like protein 41b; AltName: Full=Kelch repeat and BTB domain-containing protein 10b	kbtbd10b	7.53E-04	4.71	2.24
AAI64525.1	Zgc:153629 protein [Danio rerio]	zgc:153629	8.84E-03	0.06	-4.16

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH75874.1	Zgc:92061 [Danio rerio]	zgc:92061	6.50E-03	2.36	1.24
AAI16530.1	Si:dkeyp-113d7.4 protein, partial [Danio rerio]	si:dkeyp-113d7.4	4.78E-03	0.00	-7.65
XP_009300357.1	kynureninase [Danio rerio]	LOC100334830	3.89E-02	0.21	-2.26
XP_003197932.2	laminin subunit gamma-2 [Danio rerio]	LOC567928	3.13E-04	0.00	-7.65
AAI33081.1	Zgc:158293 protein [Danio rerio]	zgc:158293	7.43E-04	2.95	1.56
NP_571395.2	plastin-2 [Danio rerio]	LOC100537803	8.83E-03	2.09	1.07
Q4QRF7.2	RecName: Full=Protein leg1b; Short=Leg1-B; AltName: Full=Liver-enriched gene protein 1-B; Flags: Precursor	zgc:77778	1.54E-03	2.27	1.19
XP_009291645.1	uncharacterized protein LOC100001846 isoform X3 [Danio rerio]	LOC100330401	7.10E-10	0.00	-7.65
XP_005156340.1	myozenin 1 isoform X1 [Danio rerio]	myoz1b	1.63E-05	0.00	-7.65
AAI63573.1	Cardiomyopathy associated 5 like [Danio rerio]	cmya5	4.82E-03	4.00	2.00
AAQ98016.1	RAD21 homolog [Danio rerio]	rad21a	8.22E-04	2.35	1.23
NP_001004682.1	fatty acid binding protein 4a [Danio rerio]	fabp11a	1.14E-03	3.28	1.71
AAH97115.1	Zgc:114051 [Danio rerio]	h2afy2	1.42E-04	0.37	-1.45
CAN88792.1	malic enzyme 2, NAD(+)-dependent, mitochondrial [Danio rerio]	me2	6.12E-04	4.67	2.22
CAH68861.1	reticulon 3 [Danio rerio]	rtn3	1.78E-04	0.38	-1.39
Q68EL3.1	RecName: Full=BET1-like protein	bet1l	5.61E-04	201.00	7.65
AAH67542.1	Pdlim7 protein [Danio rerio]	pdlim7	3.07E-05	2.84	1.50
XP_005161170.1	syntaxin-binding protein 1 isoform X1 [Danio rerio]	stxbp1a	1.14E-05	6.90	2.79
XP_684147.2	glucosamine-6-phosphate isomerase 2 [Danio rerio]	gnpda2	1.79E-04	3.63	1.86

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_002664470.2	protein ATP1B4 [Danio rerio]	atp1b4	3.94E-04	2.91	1.54
XP_005167338.1	rho guanine nucleotide exchange factor 16 isoform X1 [Danio rerio]	arhgef16	3.84E-03	4.70	2.23
NP_001116769.1	charged multivesicular body protein 2b [Danio rerio]	chmp2ba	3.56E-03	27.73	4.79
XP_699952.2	ribosomal protein S6 kinase alpha-2 [Danio rerio]	rps6ka2	3.45E-03	2.68	1.42
XP_003201179.1	U6 snRNA-associated Sm-like protein LSm3 [Danio rerio]	LOC100535720	1.40E-04	0.27	-1.87
NP_001314782.1	membrane-associated guanylate kinase, WW and PDZ domain-containing protein 2 [Danio rerio]	magi2	3.02E-03	2.33	1.22
XP_021329227.1	electromotor neuron-associated protein 1 [Danio rerio]	si:dkey-37m8.10	7.47E-03	4.06	2.02
A1L243.2	RecName: Full=LRP chaperone MESD; AltName: Full=LDLR chaperone MESD; AltName: Full=Mesoderm development candidate 2; AltName: Full=Mesoderm development protein; Flags: Precursor	mesdc2	4.96E-03	3.62	1.86
Q7ZVJ8.3	RecName: Full=Methyltransferase-like 26	zgc:56719	2.36E-04	6.12	2.61
NP_001313322.1	39S ribosomal protein L47, mitochondrial [Danio rerio]	LOC557789	1.62E-02	9.41	3.23
AAI35021.1	Microsomal triglyceride transfer protein [Danio rerio]	mtp	6.37E-05	0.20	-2.35
XP_017210292.1	myosin-11 [Danio rerio]	myh11	2.87E-02	0.11	-3.16
XP_021332193.1	myosin heavy chain, fast skeletal muscle [Danio rerio]	LOC100149148	1.37E-05	0.50	-1.01
AAH62288.1	Zgc:66286 protein [Danio rerio]	zgc:66286	7.60E-03	0.38	-1.38
NP_996940.1	nicalin-1 precursor [Danio rerio]	ncl1	1.23E-05	201.03	7.65
AAI52140.1	Ndufa9 protein [Danio rerio]	ndufa9	7.37E-03	14.26	3.83
XP_021324735.1	neurofilament light polypeptide-like [Danio rerio]	LOC793912	3.69E-03	0.24	-2.07
Q9PU58.2	RecName: Full=Protein NipSnap homolog 2; Short=NipSnap2; AltName: Full=Glioblastoma-amplified sequence	gbas	4.47E-03	3.86	1.95

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
Q7T3C6.1	RecName: Full=Cleavage and polyadenylation specificity factor subunit 5; AltName: Full=Nudix hydrolase 21	cpsf5	2.90E-04	0.00	-7.65
XP_021326058.1	obscurin [Danio rerio]	LOC572412	4.31E-04	9.76	3.29
NP_001017897.1	phenazine biosynthesis-like protein domain containing 2 [Danio rerio]	zgc:113291	8.26E-05	0.00	-7.65
NP_001315285.1	pyruvate carboxylase a [Danio rerio]	pcl	1.97E-03	3.44	1.78
AAH52972.1	Solute carrier family 16 (monocarboxylic acid transporters), member 8 [Danio rerio]	slc16a8	5.48E-03	6.55	2.71
AAQ97983.1	programmed cell death 8 [Danio rerio]	aifm1	2.19E-03	2.11	1.08
XP_005170845.2	phosphatidylethanolamine-binding protein 4 [Danio rerio]	LOC101882266	3.76E-03	2.11	1.08
XP_698635.3	ATP-dependent 6-phosphofructokinase, liver type [Danio rerio]	LOC570106	8.23E-03	2.04	1.03
NP_001315318.1	phosphofructokinase, liver b [Danio rerio]	zgc:110298	4.91E-02	4.94	2.30
XP_021323733.1	ATP-dependent 6-phosphofructokinase, platelet type isoform X2 [Danio rerio]	LOC561416	1.05E-05	3.10	1.63
NP_001119868.1	phosphoglucomutase-like protein 5 [Danio rerio]	LOC560297	3.37E-03	2.27	1.19
NP_001299846.1	serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform [Danio rerio]	ppp2r1a	9.07E-03	5.86	2.55
XP_005155370.1	protein phosphatase 3, catalytic subunit, gamma isoform isoform X1 [Danio rerio]	LOC100005803	2.88E-05	0.00	-7.65
AAH45954.1	PRP19/PSO4 homolog (S. cerevisiae) [Danio rerio]	prp19	8.38E-03	3.36	1.75
AAH55625.1	Zgc:66382 [Danio rerio]	zgc:66382	2.18E-04	2.00	1.00
BAC06824.1	prostaglandin D synthase homolog [Danio rerio]	ptgdsb	1.17E-03	0.28	-1.82
NP_001038878.1	lipocalin-15 precursor [Danio rerio]	zgc:153154	4.54E-02	0.01	-7.31
AAH95345.1	Zgc:110639 [Danio rerio]	hdhd1	1.03E-03	9.60	3.26

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_017212888.1	serine/arginine repetitive matrix protein 2-like isoform X4 [Danio rerio]	LOC565130	8.38E-05	0.00	-7.65
XP_017212317.1	phosphorylase, glycogen, muscle b isoform X1 [Danio rerio]	LOC573841	1.23E-03	2.59	1.37
AAH50239.1	RAB1A, member RAS oncogene family [Danio rerio]	rab1a	2.41E-04	0.12	-3.11
AAQ94567.1	retinoblastoma binding protein 4 [Danio rerio]	rbb4	2.96E-04	3.35	1.74
NP_001313380.1	uncharacterized protein LOC569958 [Danio rerio]	LOC569958	2.79E-05	0.25	-1.98
XP_009290986.1	protein transport protein Sec16A isoform X1 [Danio rerio]	LOC567075	1.10E-03	2.23	1.16
AAO65272.1	15 kDa selenoprotein SeP15 [Danio rerio]	Sep15	4.87E-03	2.77	1.47
AAH97235.1	LOC100000597 protein, partial [Danio rerio]	hm:zeh0351	3.02E-02	7.62	2.93
NP_001002067.1	splicing factor 3B subunit 6 [Danio rerio]	sf3b14	7.08E-04	3.71	1.89
XP_001920670.3	NEDD8-conjugating enzyme Ubc12 [Danio rerio]	wu:fb58e05	9.93E-06	3.13	1.65
NP_001292504.1	si:ch1073-340i21.3 [Danio rerio]	wu:fb16f09	1.12E-07	0.00	-7.65
XP_002664527.1	uncharacterized protein si:ch211-113d11.5 [Danio rerio]	wu:fi74c02	9.39E-04	0.00	-7.65
XP_021328354.1	monoacylglycerol lipase ABHD12 [Danio rerio]	LOC555902	2.69E-04	4.21	2.07
XP_003199893.1	ES1 protein homolog, mitochondrial [Danio rerio]	sb:cb252	1.17E-03	0.02	-5.93
XP_017209619.2	olfactomedin-4-like [Danio rerio]	LOC101883669	1.25E-02	0.10	-3.38
XP_001919896.1	alanine aminotransferase 2-like isoform X1 [Danio rerio]	LOC100148522	1.45E-04	6.98	2.80
XP_021323674.1	apoptosis-associated speck-like protein containing a CARD [Danio rerio]	LOC100536808	9.27E-05	0.00	-7.65
XP_005168067.1	tripeptidyl-peptidase 2 isoform X1 [Danio rerio]	tpp2	5.11E-06	3.21	1.68
XP_005170187.1	uncharacterized protein LOC799904 isoform X1 [Danio rerio]	LOC799904	2.81E-06	0.00	-7.65
XP_009301181.2	uncharacterized protein si:ch73-368j24.3 [Danio rerio]	LOC103911339	1.67E-02	3.86	1.95

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_001345438.6	alpha-2-macroglobulin-like [Danio rerio]	LOC100006782	5.39E-05	0.00	-7.65
NP_001230250.1	calpain-3-like [Danio rerio]	capn3b	1.60E-04	201.00	7.65
XP_005173766.1	uncharacterized protein si:dkey-151j17.4 isoform X9 [Danio rerio]	LOC567193	9.07E-05	2.68	1.42
XP_017211145.1	NACHT, LRR and PYD domains-containing protein 3-like [Danio rerio]	LOC101884333	1.12E-04	0.00	-7.65
XP_688162.3	thread biopolymer filament subunit alpha [Danio rerio]	wu:fb15e04	1.12E-03	0.00	-7.65
XP_021335521.1	probable glutamate receptor [Danio rerio]	LOC100151589	1.38E-02	9.92	3.31
XP_002662020.3	heat shock protein beta-1-like [Danio rerio]	LOC100331249	4.29E-04	0.47	-1.09
XP_003199920.1	trypsin inhibitor ClTI-1 [Danio rerio]	LOC100537819	2.35E-04	0.16	-2.65
XP_009290120.1	GTPase IMAP family member 8 isoform X1 [Danio rerio]	wu:fd60c08	1.41E-02	4.08	2.03
NP_001313477.1	uncharacterized protein C7orf57 homolog [Danio rerio]	wu:fq41a10	5.18E-03	2.13	1.09
XP_001335256.1	uncharacterized protein si:dkey-30j10.5 [Danio rerio]	LOC795145	2.58E-05	0.00	-7.65
NP_001307331.1	si:dkey-57c15.4 precursor [Danio rerio]	LOC100535584	1.61E-04	3.41	1.77
XP_021324295.1	palladin isoform X1 [Danio rerio]	palld	9.51E-03	2.48	1.31
XP_001332452.2	natterin-like protein [Danio rerio]	LOC794319	5.57E-05	0.25	-1.98
XP_002666918.1	kunitz-type U19-barytoxin-Tl1a-like [Danio rerio]	LOC100333708	5.00E-02	4.92	2.30
XP_005159515.1	oxidation resistance protein 1 isoform X1 [Danio rerio]	oxr1b	1.41E-02	5.71	2.51
XP_017209015.1	cell surface A33 antigen isoform X1 [Danio rerio]	sc:d0144	1.12E-02	2.34	1.23
XP_001339837.3	microfibril-associated glycoprotein 4-like isoform X1 [Danio rerio]	LOC100007462	2.04E-05	4.33	2.11
BAL44665.1	monocarboxylate transporter 1a [Danio rerio]	slc16a1	1.50E-02	22.49	4.49
AAI64812.1	Slc9a3r2 protein [Danio rerio]	slc9a3r1	1.05E-02	2.97	1.57

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_017207094.1	synaptosomal-associated protein 23 isoform X1 [Danio rerio]	zgc:56072	1.44E-02	2.67	1.42
AAH46034.1	NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae) [Danio rerio]	nhp2l1b	2.10E-02	0.00	-7.65
AAH65677.1	Zgc:77147 [Danio rerio]	zgc:77147	3.34E-02	5.34	2.42
NP_001003984.1	signal transducer and activator of transcription 5b [Danio rerio]	stat5.2	5.55E-03	2.03	1.02
XP_017210012.2	synaptopodin isoform X2 [Danio rerio]	LOC100536545	4.10E-02	4.58	2.19
XP_005172875.1	thimet oligopeptidase isoform X1 [Danio rerio]	zgc:92139	5.76E-05	4.86	2.28
NP_001315189.1	si:ch73-206h6.3 precursor [Danio rerio]	si:ch73-206h6.3	4.07E-02	0.21	-2.23
AAP59458.1	transketolase [Danio rerio]	tkt	4.76E-03	0.25	-2.00
XP_009301498.1	talin-2 isoform X1 [Danio rerio]	tln2	2.32E-02	6.66	2.74
XP_021336901.1	thymosin beta [Danio rerio]	si:ch211-11c15.3	2.38E-03	0.00	-7.65
BAH97323.1	thymosin beta-11 [Danio rerio]	zgc:195154	2.81E-06	0.07	-3.88
AAH71518.1	Zgc:86895 [Danio rerio]	tnni1al	2.27E-03	0.03	-5.14
NP_001313368.1	uncharacterized protein LOC559490 [Danio rerio]	LOC559490	5.78E-04	0.06	-4.17
AAH98884.1	TRNA selenocysteine 1 associated protein 1 [Danio rerio]	trnau1apb	2.70E-02	0.15	-2.69
ABG48499.1	titin b [Danio rerio]	ttnb	2.37E-05	4.11	2.04
ABG48500.1	titin a [Danio rerio]	ttna	1.07E-05	4.16	2.06
XP_021334745.1	titin [Danio rerio]	ttna	3.38E-05	0.00	-7.65
XP_005155933.1	ubiquitin-like modifier-activating enzyme 1 [Danio rerio]	LOC100001302	9.14E-04	3.85	1.95
NP_001108154.1	ubiquitin-protein ligase E3B [Danio rerio]	LOC556864	3.22E-04	0.00	-7.65

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI34962.1	Zgc:162320 protein [Danio rerio]	zgc:162320	1.04E-02	0.08	-3.61
AAI62980.1	Vcl protein [Danio rerio]	vcl	8.88E-03	2.48	1.31
NP_001304681.1	vinculin b [Danio rerio]	LOC100334859	2.84E-04	6.00	2.58
XP_021335198.1	VIP peptides isoform X1 [Danio rerio]	vip2	6.93E-05	0.00	-7.65
Q5RGJ6.1	RecName: Full=WASH complex subunit 3; AltName: Full=Coiled-coil domain-containing protein 53	ccdc53	5.69E-04	0.35	-1.52
E7F1H9.1	RecName: Full=YTH domain-containing family protein 2	yth2	2.08E-02	3.41	1.77
NP_001007777.1	far upstream element-binding protein 3 [Danio rerio]	fubp3	1.08E-03	3.85	1.95
AAI65010.1	Zgc:109957 protein [Danio rerio]	sarnp	4.47E-02	4.47	2.16
AAH92829.1	Zgc:110256 [Danio rerio]	tmem256	4.63E-02	5.06	2.34
NP_001017593.1	epithelial cell adhesion molecule [Danio rerio]	epcam	3.43E-02	5.88	2.56
XP_017206442.1	eukaryotic translation initiation factor 3 subunit K isoform X1 [Danio rerio]	eif3k	9.20E-05	22.96	4.52
XP_005157433.1	serine/threonine-protein phosphatase 5 isoform X1 [Danio rerio]	ppp5c	1.51E-03	2.96	1.57
NP_001017745.3	CCAAT/enhancer-binding protein zeta [Danio rerio]	cebpz	2.90E-04	201.00	7.65
AAI65793.1	Zgc:112384 protein [Danio rerio]	wbp4	2.04E-07	0.00	-7.65
AAI00057.1	Zgc:112414 [Danio rerio]	psph	8.34E-04	3.35	1.74
AAI29306.1	Zgc:158580 [Danio rerio]	zgc:158580	1.13E-04	0.42	-1.27
NP_001032465.2	alpha-N-acetylgalactosaminidase precursor [Danio rerio]	naga	2.81E-04	0.00	-7.65
Q6NZS4.1	RecName: Full=tRNA-splicing ligase RtcB homolog	rtcb	2.43E-05	5.32	2.41
AAI52628.1	Zgc:92237 [Danio rerio]	rps6	3.07E-04	2.39	1.25

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH93295.1	Calpain 2, (m/II) large subunit, like [Danio rerio]	capn2l	1.89E-02	7.99	3.00
XP_021332406.1	cytosolic phospholipase A2 gamma [Danio rerio]	LOC100332119	2.01E-05	0.00	-7.65
AAH76321.1	Ribosomal protein L35a [Danio rerio]	rpl35a	5.34E-09	0.00	-7.65
AAI28792.1	Zgc:109885 protein [Danio rerio]	eef1a1l2	1.57E-08	0.00	-7.65
NP_001274012.1	desmocollin-1 [Danio rerio]	dsc2l	1.84E-08	0.00	-7.65
AAI65598.1	Zgc:154009 protein [Danio rerio]	desmb	2.78E-08	8.18	3.03
Q7ZUS1.1	RecName: Full=Serine/threonine-protein kinase VRK1; AltName: Full=Vaccinia-related kinase 1	vrk1	3.49E-08	0.00	-7.65
AAB61137.1	Allele: hi4 [Danio rerio]	nup93	1.06E-07	0.00	-7.65
NP_001025406.1	echinoderm microtubule-associated protein-like 2 [Danio rerio]	eml2	1.64E-07	201.00	7.65
AAI65577.1	Gtf2f1 protein [Danio rerio]	gtf2f1	1.82E-07	201.00	7.65
AAH85625.1	Phenylalanine-tRNA synthetase-like [Danio rerio]	farsb	2.20E-07	201.03	7.65
AAI65490.1	Zgc:109973 protein [Danio rerio]	sub1a	3.44E-07	8.70	3.12
NP_001191098.1	si:rp71-1c23.2 [Danio rerio]	isg15	3.57E-07	201.03	7.65
AAH44186.1	Splicing factor, arginine/serine-rich 11 [Danio rerio]	srsf11	4.12E-07	0.00	-7.65
XP_005163854.1	prohibitin isoform X1 [Danio rerio]	phb	4.66E-07	201.00	7.65
ACB72378.1	cellular retinaldehyde-binding protein b [Danio rerio]	rlbp1b	4.98E-07	0.00	-7.65
AAI54126.1	LOC100007703 protein [Danio rerio]	LOC100007703	5.24E-07	0.00	-7.65
NP_956210.1	protein phosphatase 1, catalytic subunit, beta isoform, like [Danio rerio]	ppp1cbl	5.64E-07	200.97	7.65
NP_001004575.1	ATP-dependent 6-phosphofructokinase, muscle type [Danio rerio]	pfkma	7.26E-07	0.00	-7.65

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH97019.1	Ewing sarcoma breakpoint region 1b [Danio rerio]	ewsr1b	7.68E-07	0.00	-7.65
AAZ08576.1	fatty acid binding protein 1b [Danio rerio]	fabp1b.1	1.05E-06	2.75	1.46
XP_005167793.1	probable ubiquitin carboxyl-terminal hydrolase FAF-X isoform X1 [Danio rerio]	usp9	1.30E-06	201.03	7.65
XP_005162980.2	microtubule-associated protein 4-like isoform X3 [Danio rerio]	LOC100001114	1.49E-06	0.17	-2.60
AAH66524.1	Importin 7 [Danio rerio]	ipo7	1.54E-06	0.00	-7.65
NP_001018321.1	myosin heavy chain 4 [Danio rerio]	myhc4	1.61E-06	0.37	-1.43
Q800G8.2	RecName: Full=Interferon-induced GTP-binding protein MxB; AltName: Full=IFN-inducible antiviral protein MxB; AltName: Full=Interferon-inducible MxB protein	mxb	1.74E-06	201.00	7.65
Q6DG22.2	RecName: Full=Adenosine deaminase; AltName: Full=Adenosine aminohydrolase	ada	1.95E-06	2.86	1.51
NP_997875.1	protein phosphatase 1, catalytic subunit, alpha-like [Danio rerio]	ppp1cab	2.01E-06	0.00	-7.65
AAH75749.1	Archain 1 like [Danio rerio]	arcn1b	2.01E-06	201.00	7.65
XP_021336237.1	cancer-related nucleoside-triphosphatase isoform X1 [Danio rerio]	ntpcr	2.04E-06	0.00	-7.65
AAC13314.1	beta-actin [Danio rerio]	actb1	2.14E-06	0.00	-7.65
AAG30275.1	Na+/K+ ATPase alpha subunit isoform 5 [Danio rerio]	atp1a3a	2.15E-06	201.03	7.65
AAI22406.1	Calcitonin gene-related peptide-receptor component protein [Danio rerio]	crcp	2.16E-06	201.00	7.65
XP_021322489.1	plectin isoform X1 [Danio rerio]	si:ch211-165e15.1	2.24E-06	4.15	2.05
AAH79521.1	Acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain [Danio rerio]	acads	2.25E-06	0.00	-7.65
AAH55159.1	Zgc:63600 [Danio rerio]	srm	2.26E-06	0.00	-7.65
XP_005165751.1	myosin-9 isoform X1 [Danio rerio]	myh9a	2.40E-06	0.48	-1.07

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH76224.1	Zgc:92746 [Danio rerio]	srprb	2.49E-06	201.00	7.65
NP_998275.1	glia maturation factor beta [Danio rerio]	gmfb	2.78E-06	0.00	-7.65
CAQ13820.1	novel protein similar to vertebrate polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa (POLR2I) [Danio rerio]	LOC560907	2.82E-06	0.00	-7.65
XP_021332609.1	catenin delta-1 isoform X1 [Danio rerio]	ctnnd1	2.93E-06	0.07	-3.91
AAH53233.1	Pyruvate dehydrogenase (lipoamide) beta [Danio rerio]	pdhb	2.94E-06	3.99	2.00
AAI63132.1	Zgc:194962 [Danio rerio]	gyg2	3.13E-06	0.00	-7.65
CAP08002.1	zgc:86661, partial [Danio rerio]	lum	3.28E-06	0.05	-4.28
AAH92825.1	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 4 [Danio rerio]	psmd4b	3.34E-06	0.00	-7.65
NP_001073416.1	sulfotransferase family 3, cytosolic sulfotransferase 2 [Danio rerio]	sult3st2	3.47E-06	0.00	-7.65
XP_021330897.1	filamin-C isoform X1 [Danio rerio]	flncb	3.48E-06	201.00	7.65
CAE30410.1	novel actinin [Danio rerio]	actn3b	3.74E-06	2.64	1.40
AAG27060.1	Na+/K+ ATPase alpha subunit isoform 8 [Danio rerio]	atp1a1a.4	3.76E-06	3.18	1.67
XP_021325144.1	myosin heavy chain, fast skeletal muscle [Danio rerio]	wu:fd14a01	3.77E-06	0.33	-1.61
XP_005162209.1	alpha-enolase isoform X1 [Danio rerio]	eno1a	3.79E-06	0.00	-7.65
ACZ28497.1	Actn3a [Danio rerio]	actn3a	3.95E-06	4.43	2.15
NP_958481.1	THO complex subunit 1 [Danio rerio]	thoc1	4.05E-06	4.40	2.14
XP_005171929.2	transcription factor BTF3 isoform X1 [Danio rerio]	btf3	4.54E-06	0.00	-7.65
AAT68052.1	40S ribosomal protein S3a [Danio rerio]	rps3a	4.60E-06	3.22	1.69
XP_005162833.1	eukaryotic translation initiation factor 5A-2 isoform X1 [Danio rerio]	eif5a	4.63E-06	2.58	1.37

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_005159981.1	CD99 molecule isoform X2 [Danio rerio]	cd99	4.91E-06	0.00	-7.65
AAB97964.1	beta actin [Danio rerio]	actb2	4.93E-06	0.33	-1.62
AAI65034.1	Zgc:101581 protein [Danio rerio]	eif4eb	4.95E-06	4.05	2.02
AAH76480.1	Zgc:92453 [Danio rerio]	uqcrc2a	5.12E-06	3.79	1.92
NP_001116082.3	vitellogenin 6 precursor [Danio rerio]	vtg6	5.31E-06	0.00	-7.65
AAH64291.1	Elongation factor 1-alpha [Danio rerio]	eef1a1l1	5.53E-06	0.13	-2.93
XP_005171255.1	coiled-coil and C2 domain-containing protein 1B isoform X1 [Danio rerio]	cc2d1b	5.58E-06	0.00	-7.65
NP_001071216.1	secretogranin-2 precursor [Danio rerio]	scg2b	5.99E-06	0.00	-7.65
AAH59454.1	Ribosomal protein L10a [Danio rerio]	rpl10a	6.11E-06	3.24	1.69
AAQ97849.1	myeloid leukemia-associated SET translocation protein [Danio rerio]	setb	6.19E-06	2.61	1.38
AAQ91245.1	karyopherin alpha 3 [Danio rerio]	kpna3	7.06E-06	0.00	-7.65
XP_005173159.1	ras GTPase-activating protein-binding protein 1 isoform X1 [Danio rerio]	g3bp1	7.15E-06	0.00	-7.65
XP_696748.4	asparagine--tRNA ligase, cytoplasmic [Danio rerio]	nars	7.19E-06	4.11	2.04
AAH59705.1	Ubiquinol-cytochrome c reductase core protein I [Danio rerio]	uqcrc1	7.22E-06	0.00	-7.65
XP_009292473.1	insulin-like growth factor 2 mRNA-binding protein 3 isoform X1 [Danio rerio]	igf2bp3	7.47E-06	0.19	-2.43
AAI42751.1	LOC100148802 protein, partial [Danio rerio]	myoz3a	7.49E-06	0.00	-7.65
NP_001020626.1	acyl-CoA-binding domain-containing protein 6 [Danio rerio]	acbd6	7.84E-06	200.97	7.65
XP_021328137.1	succinate--CoA ligase [GDP-forming] subunit beta, mitochondrial isoform X2 [Danio rerio]	suclg2	8.31E-06	0.14	-2.83
XP_005159941.1	inorganic pyrophosphatase 2, mitochondrial isoform X1 [Danio rerio]	ppa2	8.63E-06	3.55	1.83

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_009293521.1	complement C3 [Danio rerio]	LOC100331492	9.05E-06	3.22	1.69
AAI64159.1	Mhc1uba protein [Danio rerio]	mhc1uba	9.34E-06	4.41	2.14
NP_957270.2	pre-mRNA-processing-splicing factor 8 [Danio rerio]	prpf8	9.36E-06	6.10	2.61
AAH45909.1	Nuclear distribution gene C homolog [Danio rerio]	nudc	9.38E-06	10.37	3.37
XP_017209018.1	uncharacterized protein LOC393530 isoform X1 [Danio rerio]	mybpha	9.75E-06	0.00	-7.65
XP_005167974.1	uncharacterized protein LOC327557 isoform X1 [Danio rerio]	zgc:66484	1.00E-05	3.89	1.96
AAH54903.1	Zgc:63524 [Danio rerio]	canx	1.02E-05	3.02	1.60
AAH95296.1	Transforming growth factor, beta-induced [Danio rerio]	tgfbi	1.04E-05	0.00	-7.65
AFS66692.1	mitochondrial calcium uniporter [Danio rerio]	mcu	1.05E-05	6.71	2.75
CAM46852.1	novel protein similar to vertebrate Ras family [Danio rerio]	rab11a	1.06E-05	2.47	1.31
NP_997744.2	collagen alpha-2(IX) chain precursor [Danio rerio]	col9a2	1.08E-05	0.00	-7.65
NP_001116846.1	serine hydroxymethyltransferase, mitochondrial [Danio rerio]	shmt2	1.08E-05	0.00	-7.65
NP_001108613.1	pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15 [Danio rerio]	dhx15	1.17E-05	24.15	4.59
AAF79948.1	brain-type fatty-acid binding protein [Danio rerio]	fabp7a	1.20E-05	2.79	1.48
XP_021326089.1	myosin-7-like [Danio rerio]	LOC100329748	1.25E-05	0.21	-2.22
XP_005161215.1	complement component C6 isoform X1 [Danio rerio]	c6	1.30E-05	2.28	1.19
XP_690189.3	extracellular matrix protein 2 [Danio rerio]	si:dkey-32e6.6	1.32E-05	0.00	-7.65
AAH67633.1	Ighm protein, partial [Danio rerio]	ighm	1.35E-05	0.45	-1.16
AAH68191.1	Taldo1 protein [Danio rerio]	taldo1	1.37E-05	0.22	-2.20

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAQ97741.1	mitochondrial aldehyde dehydrogenase 2 family [Danio rerio]	aldh2.2	1.38E-05	2.98	1.58
NP_981966.1	periostin isoform 2 [Danio rerio]	postnb	1.40E-05	0.16	-2.63
NP_001002673.1	U4/U6.U5 tri-snRNP-associated protein 1 [Danio rerio]	sart1	1.43E-05	5.62	2.49
AAH50167.1	Aldolase b, fructose-bisphosphate [Danio rerio]	aldob	1.50E-05	3.21	1.68
NP_001001944.2	5'-3' exoribonuclease 2 [Danio rerio]	xrn2	1.52E-05	201.00	7.65
NP_001108153.2	cleavage and polyadenylation specificity factor subunit 1 [Danio rerio]	cpsf1	1.53E-05	201.00	7.65
AAH65468.1	Voltage-dependent anion channel 3 [Danio rerio]	vdac3	1.53E-05	2.51	1.33
AAI65870.1	Zgc:56036 protein [Danio rerio]	acaa2	1.55E-05	0.00	-7.65
AAO24759.1	cyclase-associated protein-1 [Danio rerio]	cap1	1.57E-05	2.79	1.48
AAH95013.1	Preproinsulin [Danio rerio]	ins	1.58E-05	0.00	-7.65
CAQ13816.1	novel protein similar to vertebrate small nuclear ribonucleoprotein D2 polypeptide 16.5kDa (SNRPD2) [Danio rerio]	snrpd2	1.65E-05	0.00	-7.65
AAI53609.1	Voltage-dependent anion channel 3 [Danio rerio]	vdac3	1.67E-05	0.00	-7.65
XP_005157792.1	calpain, small subunit 1 b isoform X1 [Danio rerio]	capns1b	1.67E-05	2.47	1.30
AAI42778.1	LOC572200 protein, partial [Danio rerio]	LOC572200	1.70E-05	0.27	-1.89
AAH66767.1	Txndc4 protein, partial [Danio rerio]	erp44	1.71E-05	0.00	-7.65
XP_009298560.1	voltage-dependent calcium channel subunit alpha-2/delta-1 isoform X1 [Danio rerio]	cacna2d1a	1.72E-05	4.61	2.21
XP_021329305.1	troponin C, skeletal muscle [Danio rerio]	si:rp71-17i16.4	1.75E-05	0.00	-7.65
XP_005169739.1	SH3 domain-binding glutamic acid-rich protein isoform X1 [Danio rerio]	sh3bgr	1.77E-05	0.13	-2.95
XP_021337011.1	alpha-actinin-4 isoform X3 [Danio rerio]	actn4	1.77E-05	3.81	1.93

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH90697.1	Crystallin, zeta (quinone reductase) [Danio rerio]	cryz	1.78E-05	0.00	-7.65
XP_009298457.1	thymidine phosphorylase [Danio rerio]	tymp	1.88E-05	0.00	-7.65
AAH45520.1	Myosin, light polypeptide 2, skeletal muscle [Danio rerio]	mylpfa	1.90E-05	0.26	-1.93
AAI54660.1	Zgc:111961 [Danio rerio]	atp5b	1.95E-05	4.68	2.23
AAI24801.1	Zgc:154056 [Danio rerio]	dars	1.96E-05	3.53	1.82
XP_009295034.2	myosin-7 isoform X1 [Danio rerio]	myh7bb	2.08E-05	0.09	-3.45
CAE30440.1	novel alpha-globin [Danio rerio]	hbaa1	2.08E-05	4.39	2.13
AAH62855.1	Pvalb3 protein [Danio rerio]	pvalb3	2.11E-05	0.19	-2.36
XP_688661.4	protein ERGIC-53 [Danio rerio]	lman1	2.13E-05	7.01	2.81
NP_001018434.1	thymopoietin b [Danio rerio]	tmpob	2.14E-05	0.00	-7.65
XP_005156129.1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 like isoform X1 [Danio rerio]	atp2a1l	2.23E-05	2.79	1.48
NP_001314855.1	complement component C9 precursor [Danio rerio]	c9	2.27E-05	0.34	-1.57
AAH68220.1	Myosin, light polypeptide 9, regulatory [Danio rerio]	myl9b	2.33E-05	0.00	-7.65
AAH68436.1	Heterogeneous nuclear ribonucleoprotein U-like 1 [Danio rerio]	hnrnpul1	2.37E-05	201.00	7.65
AAH66386.1	H1 histone family, member X [Danio rerio]	h1fx	2.37E-05	0.10	-3.35
NP_001013529.2	myosin-binding protein C, fast-type [Danio rerio]	mybpc2b	2.40E-05	2.60	1.38
AAI65581.1	Zgc:110766 protein [Danio rerio]	tufm	2.51E-05	0.00	-7.65
AAH81638.1	Chymotrypsin-like [Danio rerio]	ctrl	2.52E-05	0.00	-7.65
Q4H4B6.1	RecName: Full=Protein scribble homolog; AltName: Full=Scribble1	scrib	2.52E-05	0.00	-7.65

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_001003566.1	14 kDa phosphohistidine phosphatase [Danio rerio]	phpt1	2.60E-05	3.88	1.95
NP_001032505.2	UDP glycosyltransferase 1 family, polypeptide A1 precursor [Danio rerio]	ugt1a1	2.62E-05	201.03	7.65
NP_957193.1	myozenin 2a [Danio rerio]	myoz2a	2.80E-05	0.00	-7.65
XP_005163170.3	pyruvate kinase isozymes M1/M2 isoform X1 [Danio rerio]	pkmb	2.94E-05	3.76	1.91
AAH55649.1	Eif4e1b protein [Danio rerio]	eif4e1b	2.95E-05	2.92	1.55
AAQ94564.1	ribosomal protein S3 [Danio rerio]	rps3	2.97E-05	3.59	1.84
NP_955834.2	apoptosis inhibitor 5 [Danio rerio]	api5	2.97E-05	0.00	-7.65
NP_001032185.1	cytochrome b-c1 complex subunit 6, mitochondrial [Danio rerio]	uqcrh	3.06E-05	0.08	-3.67
NP_001122234.1	uncharacterized protein LOC571819 precursor [Danio rerio]	zgc:194887	3.11E-05	0.00	-7.65
XP_005165898.1	glycerol-3-phosphate dehydrogenase, mitochondrial isoform X1 [Danio rerio]	gpd2	3.12E-05	0.00	-7.65
AAI24090.1	Nenf protein [Danio rerio]	nenf	3.12E-05	0.00	-7.65
AAH85651.1	NADH dehydrogenase (ubiquinone) Fe-S protein 1 [Danio rerio]	ndufs1	3.17E-05	5.09	2.35
CAQ14649.1	novel protein similar to human and mouse SKB1 homolog (S. pombe) [Danio rerio]	prmt5	3.22E-05	201.00	7.65
XP_005167741.1	60S ribosomal protein L8 isoform X1 [Danio rerio]	rpl8	3.24E-05	0.00	-7.65
AAH92763.1	Stathmin 1/oncoprotein 18 [Danio rerio]	stmn1b	3.26E-05	0.26	-1.94
NP_001136064.1	leucine-rich PPR motif-containing protein, mitochondrial [Danio rerio]	lrpprc	3.27E-05	200.97	7.65
AAH74079.1	Zgc:91835 [Danio rerio]	dbnla	3.30E-05	4.45	2.15
AAA50026.1	ependymin [Danio rerio]	epd	3.32E-05	0.38	-1.41
XP_009303329.1	PDZ and LIM domain 5 isoform X1 [Danio rerio]	pdlim5b	3.36E-05	10.68	3.42

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
ABE66385.1	truncated integrin beta1 subunit-like protein 2 [Danio rerio]	itgb1b.2	3.39E-05	0.16	-2.61
NP_001292499.1	acyl carrier protein, mitochondrial [Danio rerio]	ndufab1b	3.41E-05	0.00	-7.65
AAI50441.1	Crygmx protein [Danio rerio]	crygmx	3.44E-05	0.24	-2.04
XP_005168047.1	ATP synthase-coupling factor 6, mitochondrial isoform X2 [Danio rerio]	atp5j	3.49E-05	0.00	-7.65
AAH67728.1	Zgc:85975 [Danio rerio]	zgc:85975	3.53E-05	0.00	-7.65
AAH59620.1	Atp5l protein [Danio rerio]	atp5l	3.54E-05	5.27	2.40
NP_956848.1	S-methyl-5'-thioadenosine phosphorylase [Danio rerio]	mtap	3.55E-05	3.72	1.89
NP_001038378.1	vitellogenin 2 isoform 1 [Danio rerio]	vtg2	3.57E-05	0.00	-7.65
XP_005165357.1	fructose-1,6-bisphosphatase isozyme 2 isoform X1 [Danio rerio]	fbp2	3.62E-05	0.38	-1.38
XP_009297510.1	uncharacterized protein LOC103910221 [Danio rerio]	LOC103910221	3.62E-05	0.00	-7.65
AAH81495.1	Si:ch211-258l4.7, partial [Danio rerio]	rbbp9	3.67E-05	4.86	2.28
NP_001296453.1	neuro-oncological ventral antigen 2 [Danio rerio]	nova2	3.69E-05	0.00	-7.65
NP_001073479.1	alpha-2-antiplasmin precursor [Danio rerio]	serpinf2b	3.70E-05	0.00	-7.65
AAH49014.1	B-cell receptor-associated protein 31 [Danio rerio]	bcap31	3.70E-05	201.00	7.65
XP_021333014.1	sciellin-like [Danio rerio]	LOC100330805	3.72E-05	0.00	-7.65
NP_956378.1	phosphomannomutase 2 [Danio rerio]	pmm2	3.73E-05	6.82	2.77
AAI65437.1	Zgc:110323 protein [Danio rerio]	bdh2	3.74E-05	29.30	4.87
AAH76470.1	Plastin 3 (T isoform) [Danio rerio]	pls3	3.77E-05	3.49	1.80
Q7SXP2.2	RecName: Full=NEDD8-activating enzyme E1 regulatory subunit; AltName: Full=APP-BP1; AltName: Full=Amyloid protein-binding protein 1	nae1	3.79E-05	4.50	2.17

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_001007785.1	gamma-crystallin N-A [Danio rerio]	crygn1	3.84E-05	3.90	1.96
Q6P5L3.1	RecName: Full=60S ribosomal protein L19	rpl19	3.86E-05	0.00	-7.65
Q6NYS8.2	RecName: Full=Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit; Short=DDOST 48 kDa subunit; Short=Oligosaccharyl transferase 48 kDa subunit; Flags: Precursor	ddost	3.88E-05	0.00	-7.65
AAH67546.1	Rbb4l protein [Danio rerio]	rbb4l	4.03E-05	3.12	1.64
AAH67372.1	Oxidative-stress responsive 1b [Danio rerio]	oxsr1b	4.08E-05	0.00	-7.65
AAH85580.1	Proteasome (prosome, macropain) subunit, beta type, 1 [Danio rerio]	psmb1	4.21E-05	0.00	-7.65
NP_001019547.1	PDZ and LIM domain protein 3 [Danio rerio]	pdlim3a	4.24E-05	0.00	-7.65
AAH66491.1	Heat shock protein 8 [Danio rerio]	hspa8	4.29E-05	0.40	-1.32
NP_997830.1	DnaJ subfamily A member 2-like [Danio rerio]	dnaja2l	4.32E-05	0.23	-2.10
AAM94025.1	protein 4.1 [Danio rerio]	epb41b	4.42E-05	201.00	7.65
Q58EE9.2	RecName: Full=Glial fibrillary acidic protein; Short=GFAP	gfap	4.51E-05	2.18	1.13
XP_021330731.1	cartilage acidic protein 1a isoform X1 [Danio rerio]	crtac1a	4.53E-05	0.00	-7.65
AAI15317.1	Qdpra protein [Danio rerio]	qdpra	4.60E-05	0.00	-7.65
AAH67580.1	Ribosomal protein L4 [Danio rerio]	rpl4	4.61E-05	2.09	1.06
AAI64906.1	Zgc:77429 protein [Danio rerio]	eif5a2	4.61E-05	2.40	1.26
Q8JFV8.1	RecName: Full=Synaptic vesicle membrane protein VAT-1 homolog	vat1	4.62E-05	3.72	1.89
AAH59456.1	Interleukin enhancer binding factor 2 [Danio rerio]	ilf2	4.64E-05	6.04	2.59
AAH85451.1	Parvalbumin 8 [Danio rerio]	pvalb8	4.67E-05	0.05	-4.36
AAF81264.1	dopachrome tautomerase [Danio rerio]	dct	4.67E-05	0.00	-7.65

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH95647.1	Si:dkey-180p18.9 protein, partial [Danio rerio]	si:dkey-180p18.9	4.68E-05	0.15	-2.71
AAI24588.1	LOC558601 protein, partial [Danio rerio]	pipox	4.69E-05	0.00	-7.65
NP_957454.1	3-hydroxyisobutyrate dehydrogenase, mitochondrial [Danio rerio]	hibadhb	4.71E-05	0.09	-3.52
NP_001002072.1	ubiquitin-conjugating enzyme E2 L3 [Danio rerio]	ube2l3a	4.84E-05	0.09	-3.55
NP_957099.1	peroxiredoxin-6 [Danio rerio]	prdx6	4.92E-05	0.35	-1.50
AAQ97739.1	adenylosuccinate lyase [Danio rerio]	adsl	4.98E-05	2.94	1.56
NP_001076265.2	bifunctional purine biosynthesis protein PURH [Danio rerio]	atic	5.02E-05	2.30	1.20
XP_005157476.1	dehydrogenase/reductase SDR family member 11-like isoform X1 [Danio rerio]	zgc:92630	5.17E-05	0.00	-7.65
AAH85367.1	Zgc:101554 [Danio rerio]	cfhl2	5.21E-05	0.00	-7.65
AAH66536.1	Cbr1l protein [Danio rerio]	cbr1l	5.21E-05	0.06	-3.96
AAH98550.1	Ribosomal protein L23a [Danio rerio]	rpl23a	5.23E-05	0.00	-7.65
NP_001096096.2	slow myosin heavy chain 2 [Danio rerio]	smyhc2	5.23E-05	0.18	-2.45
AAI51953.1	Capns1b protein [Danio rerio]	capns1b	5.25E-05	6.64	2.73
CAE30438.1	novel protein similar to zebrafish epithelial cadherin 1 (cdh1), partial [Danio rerio]	si:busm1-71b9.3	5.28E-05	0.00	-7.65
AAH59677.1	Zgc:73367 [Danio rerio]	rps28	5.35E-05	0.19	-2.43
AAH76294.1	SUB1 homolog (S. cerevisiae) [Danio rerio]	sub1b	5.48E-05	9.65	3.27
AAI65236.1	Clint1 protein [Danio rerio]	clint1a	5.56E-05	0.00	-7.65
AAH92817.1	Zgc:110239 [Danio rerio]	zgc:110239	5.64E-05	0.00	-7.65
NP_001013544.1	six-cysteine containing astacin protease 3 precursor [Danio rerio]	c6ast3	5.69E-05	3.71	1.89

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH76020.1	Ubiquitin fusion degradation 1-like [Danio rerio]	ufd1l	5.79E-05	4.21	2.07
AAH55244.1	Aldehyde dehydrogenase 2 family (mitochondrial)a [Danio rerio]	aldh2.1	5.80E-05	3.16	1.66
Q7SXN5.1	RecName: Full=Dynamin-1-like protein	dnm1l	5.81E-05	0.00	-7.65
NP_571849.2	annexin A13 [Danio rerio]	anxa13	5.85E-05	3.33	1.74
Q4TVV3.1	RecName: Full=Probable ATP-dependent RNA helicase DDX46; AltName: Full=DEAD box protein 46	ddx46	5.92E-05	0.00	-7.65
NP_956511.1	voltage-dependent anion-selective channel protein 2-like [Danio rerio]	zgc:56235	5.95E-05	2.99	1.58
XP_005166847.1	integrin beta-4 isoform X1 [Danio rerio]	itgb4	5.96E-05	2.29	1.20
XP_005155551.1	tropomyosin beta chain isoform X1 [Danio rerio]	tpm2	5.97E-05	0.02	-5.40
AAI53638.1	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 6 [Danio rerio]	psmd6	6.04E-05	4.98	2.32
NP_998565.1	protein FAM136A [Danio rerio]	fam136a	6.11E-05	4.52	2.18
XP_005163453.1	inositol monophosphatase 1 isoform X1 [Danio rerio]	impa1	6.18E-05	0.00	-7.65
AAI25818.1	Zgc:152778 [Danio rerio]	znf185	6.20E-05	0.00	-7.65
AAI53664.1	Tubulin folding cofactor B [Danio rerio]	tbcb	6.29E-05	3.35	1.74
AAH79502.1	Prefoldin 5 [Danio rerio]	pfdn5	6.36E-05	0.00	-7.65
AAH96907.1	Zgc:113369 [Danio rerio]	rnaset2	6.37E-05	2.26	1.18
XP_001920098.4	myosin-9 isoform X1 [Danio rerio]	myh9b	6.49E-05	0.32	-1.66
NP_957319.1	phosphoglucomutase-1 [Danio rerio]	pgm1	6.57E-05	2.17	1.12
AAI39637.1	Anthrax toxin receptor 2a [Danio rerio]	antxr2a	6.65E-05	6.12	2.61
NP_001007063.2	myelin-associated glycoprotein isoform 1 precursor [Danio rerio]	mag	6.67E-05	0.00	-7.65

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_005156329.1	LIM domain binding 3 isoform X2 [Danio rerio]	ldb3b	6.69E-05	0.17	-2.57
AAI63717.1	Laminin, alpha 4 [Danio rerio]	lama4	6.73E-05	2.34	1.23
NP_998175.2	medium-chain specific acyl-CoA dehydrogenase, mitochondrial [Danio rerio]	acadm	6.76E-05	2.29	1.20
AAH45328.1	Protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1) [Danio rerio]	ptpn11a	6.84E-05	8.54	3.09
AAI65484.1	Zgc:110133 protein [Danio rerio]	sncga	6.94E-05	0.00	-7.65
NP_998488.1	phosphatidylethanolamine-binding protein 1 [Danio rerio]	pebp1	7.02E-05	0.00	-7.65
AAI16608.2	Scinderin like a [Danio rerio]	scinla	7.24E-05	0.41	-1.30
AAI65110.1	Zgc:110762 protein [Danio rerio]	apobec2a	7.27E-05	2.33	1.22
NP_001002058.1	SUMO-activating enzyme subunit 1 [Danio rerio]	sae1	7.30E-05	0.00	-7.65
AAI55113.1	Glutamic-oxaloacetic transaminase 1, soluble [Danio rerio]	got1	7.31E-05	0.18	-2.47
AAH93233.1	Zgc:112160 [Danio rerio]	zgc:112160	7.46E-05	0.00	-7.65
AAH76512.1	Zgc:92360 [Danio rerio]	zgc:92360	7.63E-05	201.00	7.65
XP_005163674.1	glycogen debranching enzyme isoform X2 [Danio rerio]	agla	7.63E-05	3.60	1.85
XP_009289839.1	fructose-bisphosphate aldolase C-A isoform X1 [Danio rerio]	aldoca	7.64E-05	0.40	-1.31
AAQ97832.1	hypothetical protein PRO2013 [Danio rerio]	abracl	7.67E-05	0.00	-7.65
AAH83504.1	Heat shock protein 9 [Danio rerio]	hspa9	7.73E-05	2.84	1.51
AAI07624.1	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 5 [Danio rerio]	ndufb5	8.04E-05	3.64	1.87
AAH64000.1	Ceruloplasmin [Danio rerio]	cp	8.26E-05	4.18	2.06
AAH44347.1	Cadherin 17, LI cadherin (liver-intestine) [Danio rerio]	cdh17	8.47E-05	3.54	1.82

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH91464.1	Crabp1a protein [Danio rerio]	crabp1a	8.78E-05	7.77	2.96
AAH95787.1	Zgc:112368 [Danio rerio]	zgc:112368	9.11E-05	5.09	2.35
AAH83275.1	Zgc:101768 [Danio rerio]	cdkn2aipnl	9.12E-05	3.04	1.60
NP_001292511.1	apoptotic chromatin condensation inducer in the nucleus [Danio rerio]	acin1b	9.25E-05	0.00	-7.65
AAH83505.1	Arginyl-tRNA synthetase [Danio rerio]	rars	9.45E-05	2.06	1.05
AAF19642.1	NCC receptor protein 1 [Danio rerio]	nccrp1	9.54E-05	4.55	2.18
AAI54795.1	UDP-glucose dehydrogenase [Danio rerio]	ugdh	9.63E-05	3.00	1.58
XP_003201021.1	uncharacterized protein si:rp71-36a1.3 [Danio rerio]	si:rp71-36a1.3	9.80E-05	2.27	1.18
XP_017208890.1	osteomodulin [Danio rerio]	omd	9.86E-05	0.19	-2.37
AAI65002.1	Ube2k protein [Danio rerio]	ube2kb	9.94E-05	5.80	2.54
AAL18005.1	RuvB-like DNA helicase reptin [Danio rerio]	ruvbl2	1.01E-04	3.17	1.67
XP_021327155.1	myosin-binding protein C, fast-type isoform X1 [Danio rerio]	mybpc2a	1.02E-04	2.33	1.22
NP_001128155.1	40S ribosomal protein S8 [Danio rerio]	rps8b	1.03E-04	4.08	2.03
AAH76251.1	S100 calcium binding protein, beta (neural) [Danio rerio]	s100b	1.04E-04	0.19	-2.40
AAH62838.1	Hydroxysteroid dehydrogenase like 2 [Danio rerio]	hsdl2	1.04E-04	0.31	-1.68
AAI39675.1	Hnrnpd protein [Danio rerio]	hnrnpd	1.04E-04	2.79	1.48
CAI79040.1	growth hormone [Danio rerio]	gh1	1.05E-04	2.16	1.11
AAQ91261.1	phosphogluconate dehydrogenase [Danio rerio]	pgd	1.06E-04	1.99	0.99
AAH65887.1	Cel.2 protein, partial [Danio rerio]	cel.2	1.07E-04	3.96	1.99
NP_001004634.2	glutathione peroxidase 1b [Danio rerio]	gpx1b	1.09E-04	3.98	1.99

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_001339206.5	myosin heavy chain, fast skeletal muscle [Danio rerio]	myhb	1.10E-04	0.44	-1.19
AAI22336.1	LOC563048 protein, partial [Danio rerio]	LOC563048	1.11E-04	0.00	-7.65
AAH42328.1	Ela2 protein, partial [Danio rerio]	ela2	1.12E-04	0.00	-7.65
AAH49049.1	Zgc:56576 [Danio rerio]	zgc:56576	1.12E-04	2.94	1.55
XP_009296313.1	harmonin isoform X1 [Danio rerio]	ush1c	1.13E-04	3.82	1.93
XP_005167394.1	drebrin-like protein isoform X1 [Danio rerio]	dbnlb	1.15E-04	2.21	1.14
AAI52671.1	Spint1a protein [Danio rerio]	spint1a	1.15E-04	2.81	1.49
XP_021332659.1	neurobeachin-like protein 1 isoform X1 [Danio rerio]	nbeal1	1.15E-04	8.29	3.05
AAI59208.1	Zgc:113194 protein [Danio rerio]	acss1	1.16E-04	0.15	-2.71
AAH44450.1	Glucose phosphate isomerase a [Danio rerio]	gpia	1.16E-04	3.21	1.68
AAH76412.1	Arginyl aminopeptidase (aminopeptidase B) [Danio rerio]	rnpep	1.16E-04	201.00	7.65
NP_957429.1	V-type proton ATPase catalytic subunit A [Danio rerio]	atp6v1aa	1.17E-04	3.11	1.64
AAZ08415.1	glycine cleavage system protein T [Danio rerio]	amt	1.19E-04	2.59	1.37
NP_001159594.1	Purkinje cell protein 4 [Danio rerio]	pcp4a	1.19E-04	2.11	1.07
XP_021331600.1	uncharacterized protein si:ch211-132g1.3 isoform X1 [Danio rerio]	si:ch211-132g1.3	1.20E-04	0.00	-7.65
AAI07639.1	Sorting nexin 3 [Danio rerio]	snx3	1.23E-04	3.02	1.60
NP_001013588.1	mimecan precursor [Danio rerio]	ogn	1.24E-04	0.25	-2.00
NP_957041.1	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial [Danio rerio]	ndufv2	1.28E-04	2.87	1.52
NP_001092696.1	methylmalonyl-CoA mutase, mitochondrial [Danio rerio]	mut	1.30E-04	2.36	1.24

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_001077285.2	uncharacterized protein LOC553299 [Danio rerio]	zgc:162509	1.31E-04	3.38	1.76
AAI50353.1	Si:dkey-208k4.2 protein [Danio rerio]	si:dkey-208k4.2	1.35E-04	0.16	-2.64
AAT68120.1	40S ribosomal protein s11 [Danio rerio]	rps11	1.38E-04	0.00	-7.65
XP_691138.1	reticulocalbin-1 [Danio rerio]	rcn1	1.46E-04	0.20	-2.31
AAH63979.1	Protein disulfide isomerase associated 4 [Danio rerio]	pdia4	1.46E-04	2.36	1.24
NP_001153056.1	intelectin 3 precursor [Danio rerio]	itln3	1.49E-04	0.19	-2.43
NP_958864.1	flotillin-1 [Danio rerio]	flot1b	1.52E-04	6.33	2.66
AAH76040.1	Zgc:92502 [Danio rerio]	akr7a3	1.52E-04	0.00	-7.65
AAI07504.1	Cytochrome c-1 [Danio rerio]	cyc1	1.53E-04	3.68	1.88
AAH81587.1	Zgc:92083 [Danio rerio]	acsl5	1.53E-04	2.49	1.31
XP_005169416.1	angiotensin-converting enzyme 2 isoform X2 [Danio rerio]	ace2	1.54E-04	201.00	7.65
NP_001035465.1	stathmin 1a [Danio rerio]	stmn1a	1.56E-04	0.24	-2.04
AAI53645.1	Dci protein, partial [Danio rerio]	eci1	1.56E-04	3.95	1.98
AAQ91234.1	eukaryotic translation elongation factor 2 [Danio rerio]	eef2b	1.57E-04	2.12	1.08
AAO64982.1	SULT2 sulfotransferase [Danio rerio]	sult2st1	1.57E-04	0.00	-7.65
AAH64304.1	Actin related protein 2/3 complex, subunit 2 [Danio rerio]	arpc2	1.59E-04	2.24	1.16
CAK04157.1	ribosomal protein L13a [Danio rerio]	rpl13a	1.59E-04	0.00	-7.65
NP_957412.2	protein AMBP precursor [Danio rerio]	ambp	1.59E-04	0.00	-7.65
AAH66706.1	Eukaryotic translation initiation factor 2, subunit 2 beta [Danio rerio]	eif2s2	1.60E-04	2.05	1.04
AAI15261.1	Zgc:136771 protein [Danio rerio]	anpepb	1.60E-04	5.63	2.49

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
CAQ13809.1	platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit [Danio rerio]	pafah1b3	1.60E-04	2.44	1.29
AAI52230.1	Etfb protein [Danio rerio]	etfb	1.61E-04	2.15	1.10
NP_001038362.3	vitellogenin 1 precursor [Danio rerio]	vtg1	1.63E-04	0.42	-1.26
AAO64983.1	SULT1 sulfotransferase isoform 1 [Danio rerio]	sult1st1	1.63E-04	0.12	-3.03
AAH58061.1	Ubiquitin-conjugating enzyme E2 variant 2 [Danio rerio]	ube2v2	1.64E-04	3.49	1.80
AAH59549.1	Acireductone dioxygenase 1 [Danio rerio]	adi1	1.64E-04	2.91	1.54
XP_021337009.1	alpha-actinin-4 isoform X1 [Danio rerio]	actn4	1.65E-04	2.54	1.35
AAF89686.1	catalase [Danio rerio]	cat	1.68E-04	0.23	-2.15
NP_001191151.2	copper chaperone for superoxide dismutase [Danio rerio]	ccs	1.68E-04	2.82	1.50
AAH63949.1	Major vault protein [Danio rerio]	mvp	1.75E-04	2.14	1.10
AAH76332.1	Zgc:92872 [Danio rerio]	rpl18	1.78E-04	0.00	-7.65
AAH70026.1	Zgc:85981 [Danio rerio]	cpe	1.78E-04	2.62	1.39
AAT97403.1	myosin VIa [Danio rerio]	myo6a	1.81E-04	9.86	3.30
Q9DGK4.1	RecName: Full=Translationally-controlled tumor protein homolog; Short=TCTP	tpt1	1.82E-04	2.54	1.34
AAI65092.1	Zgc:103619 protein [Danio rerio]	apip	1.85E-04	3.75	1.91
XP_017206833.1	uridine phosphorylase 1 isoform X1 [Danio rerio]	upp1	1.86E-04	3.74	1.90
NP_001007365.1	coatomer subunit epsilon [Danio rerio]	cope	1.87E-04	4.43	2.15
AAH76177.1	Arrestin 3, retinal (X-arrestin), like [Danio rerio]	arr3a	1.89E-04	0.46	-1.12
Q92051.2	RecName: Full=Carbonic anhydrase; AltName: Full=Carbonate dehydratase	cahz	1.89E-04	2.33	1.22

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH77140.1	Aldo-keto reductase family 1, member A1a (aldehyde reductase) [Danio rerio]	akr1a1a	1.91E-04	0.34	-1.54
NP_999958.1	40S ribosomal protein S8 [Danio rerio]	rps8a	1.92E-04	2.46	1.30
NP_001038407.1	poly [ADP-ribose] polymerase 1 [Danio rerio]	parp1	1.95E-04	3.24	1.70
AAI14307.1	Zgc:136908 [Danio rerio]	zgc:136908	1.96E-04	2.57	1.36
AAH92995.1	Troponin I, skeletal, fast 2b.1 [Danio rerio]	tnni2b.1	1.97E-04	0.00	-7.65
NP_899181.1	piwi-like protein 1 [Danio rerio]	piwil1	1.97E-04	2.99	1.58
AAC60261.1	es1 protein [Danio rerio]	es1	2.00E-04	0.28	-1.81
AAH85565.1	Zgc:92745 protein [Danio rerio]	zgc:92745	2.01E-04	3.61	1.85
AAI62644.1	Lysozyme [Danio rerio]	lyz	2.03E-04	0.00	-7.65
Q1JPX3.2	RecName: Full=Phenylalanine--tRNA ligase alpha subunit; AltName: Full=Phenylalanyl-tRNA synthetase alpha subunit; Short=PheRS	farsa	2.09E-04	0.00	-7.65
NP_998547.1	splicing factor, arginine/serine-rich 2 [Danio rerio]	srsf2a	2.17E-04	3.89	1.96
XP_009291485.1	bifunctional glutamate/proline--tRNA ligase isoform X1 [Danio rerio]	eprs	2.18E-04	201.00	7.65
XP_005169315.1	copine-1 isoform X2 [Danio rerio]	cpne1	2.19E-04	4.40	2.14
NP_001003875.1	U1 small nuclear ribonucleoprotein 70 kDa [Danio rerio]	snrnp70	2.20E-04	2.40	1.26
XP_021333215.1	myosin-binding protein C, cardiac-type isoform X3 [Danio rerio]	mybpc3	2.20E-04	5.12	2.36
NP_998521.2	ubiquilin-4 [Danio rerio]	ubqln4	2.22E-04	2.66	1.41
XP_021322272.1	pyruvate kinase PKLR isoform X1 [Danio rerio]	pklr	2.27E-04	2.24	1.16
AAH91794.1	RNA binding motif protein 39b [Danio rerio]	rbm39b	2.33E-04	201.00	7.65
NP_001007362.1	protein SGT1 homolog [Danio rerio]	sgut1	2.42E-04	4.84	2.27

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI29323.1	REST corepressor 1 [Danio rerio]	rcor1	2.45E-04	201.00	7.65
AAQ97859.1	tubulin, beta, 2 [Danio rerio]	tubb4b	2.52E-04	2.19	1.13
NP_001122157.1	UPF0587 protein C1orf123 homolog [Danio rerio]	si:ch211-284b7.3	2.55E-04	0.00	-7.65
AAH66628.1	Zgc:77115 [Danio rerio]	rcc2	2.60E-04	8.63	3.11
XP_021324341.1	triadin isoform X1 [Danio rerio]	trdn	2.65E-04	2.57	1.36
XP_021325780.1	aminoacyl tRNA synthase complex-interacting multifunctional protein 2 isoform X1 [Danio rerio]	aimp2	2.70E-04	2.40	1.27
AAH44154.1	Adenosine monophosphate deaminase 3 [Danio rerio]	ampd3b	2.72E-04	7.27	2.86
AAI53431.1	Zgc:101900 protein [Danio rerio]	pdxka	2.81E-04	3.53	1.82
XP_021336457.1	jeltraxin [Danio rerio]	si:ch211-270n8.1	2.86E-04	0.38	-1.41
NP_001082833.1	junctophilin-2 [Danio rerio]	jph2	2.91E-04	4.82	2.27
AAH59467.1	Calbindin 2, (calretinin) [Danio rerio]	calb2b	2.93E-04	0.48	-1.07
NP_954688.1	adenosylhomocysteinase [Danio rerio]	ahcy	2.96E-04	3.49	1.80
NP_001032791.3	importin subunit beta-1 [Danio rerio]	kpnb1	2.99E-04	2.71	1.44
Q6DGJ1.1	RecName: Full=Grifin; AltName: Full=DrGRIFIN; AltName: Full=Galectin-related inter-fiber protein	grifin	3.02E-04	0.00	-7.65
CAE17619.1	novel protein (zgc:100957) [Danio rerio]	casq1a	3.10E-04	4.11	2.04
AAH59564.1	Septin 5a [Danio rerio]	sept5a	3.13E-04	3.82	1.93
Q5RZ65.1	RecName: Full=Anterior gradient protein 2 homolog; Short=Zagr2; Flags: Precursor	agr2	3.13E-04	0.36	-1.48
Q6PCR7.1	RecName: Full=Eukaryotic translation initiation factor 3 subunit A; Short=eIF3a; AltName: Full=Eukaryotic translation initiation factor 3 subunit 10; AltName: Full=eIF-3-theta	eif3s10	3.14E-04	2.36	1.24

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH67640.1	Propionyl Coenzyme A carboxylase, beta polypeptide [Danio rerio]	pccb	3.16E-04	3.38	1.76
NP_999933.1	superkiller viralicidic activity 2-like 2 [Danio rerio]	skiv2l2	3.16E-04	2.42	1.28
AAH97041.1	Sept7a protein [Danio rerio]	sept7a	3.27E-04	3.69	1.89
AAS92647.1	karyopherin alpha 4 [Danio rerio]	kpna4	3.32E-04	3.02	1.60
AAI54627.1	Zgc:172270 protein [Danio rerio]	zgc:172270	3.34E-04	0.00	-7.65
XP_009301303.1	CD99 antigen-like protein 2 isoform X1 [Danio rerio]	cd99l2	3.35E-04	2.16	1.11
AAI71460.1	Phosphoglycerate mutase 2 (muscle) [Danio rerio]	pgam2	3.37E-04	2.53	1.34
AAM63548.1	Arp3 [Danio rerio]	actr3	3.38E-04	3.49	1.80
AAI51885.1	Nop58 protein [Danio rerio]	nop58	3.42E-04	3.33	1.74
NP_001006664.1	F-actin-capping protein subunit alpha-1 [Danio rerio]	capza1b	3.44E-04	0.36	-1.48
XP_684923.1	probable ATP-dependent RNA helicase DDX6 [Danio rerio]	ddx6	3.45E-04	0.29	-1.77
ABO92967.1	NEDD8 [Danio rerio]	nedd8l	3.53E-04	3.30	1.72
XP_021324991.1	elastase-like isoform X1 [Danio rerio]	zgc:92745	3.53E-04	2.71	1.44
XP_005161979.1	serine/arginine-rich splicing factor 6a isoform X1 [Danio rerio]	srsf6a	3.53E-04	13.26	3.73
AAN61915.1	N-cadherin [Danio rerio]	cdh2	3.59E-04	2.98	1.58
NP_001076266.1	ubiquitin-conjugating enzyme E2 L3 [Danio rerio]	ube2l3b	3.64E-04	0.26	-1.97
AAW78657.1	cystathionine beta-synthase [Danio rerio]	cbsb	3.66E-04	4.27	2.09
CAB64946.1	apolipoprotein E precursor protein [Danio rerio]	apoeb	3.67E-04	0.33	-1.62
BAF62161.1	insulin-degrading enzyme [Danio rerio]	ide	3.78E-04	0.25	-2.00
AAH62383.1	Glyoxalase 1 [Danio rerio]	glo1	3.80E-04	0.12	-3.02

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI65141.1	Rab2a protein [Danio rerio]	rab2a	3.84E-04	2.58	1.37
ABB89039.1	thioredoxin-like protein [Danio rerio]	txndc12	3.85E-04	0.29	-1.81
NP_001035341.1	uncharacterized protein LOC678524 [Danio rerio]	zgc:136872	3.87E-04	2.13	1.09
XP_021324687.1	E3 ubiquitin-protein ligase NEDD4-like isoform X1 [Danio rerio]	nedd4l	3.92E-04	0.00	-7.65
ABK91938.1	14-3-3 gamma 1 [Danio rerio]	ywhag1	3.94E-04	2.23	1.16
XP_009304960.1	TOM1-like protein 2 isoform X1 [Danio rerio]	tom1l2	4.25E-04	4.42	2.15
AAL26325.1	alcohol dehydrogenase [Danio rerio]	adh5	4.29E-04	2.43	1.28
AAH91851.1	Aldh16a1 protein, partial [Danio rerio]	aldh16a1	4.31E-04	2.15	1.10
NP_001180281.1	nascent polypeptide-associated complex subunit alpha isoform 1 [Danio rerio]	naca	4.32E-04	0.31	-1.70
XP_005161082.1	calpastatin isoform X2 [Danio rerio]	cast	4.32E-04	0.43	-1.23
AAH63322.1	Suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein) [Danio rerio]	st13	4.33E-04	2.19	1.13
AAH75779.1	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) [Danio rerio]	mthfd1b	4.36E-04	3.24	1.70
AAH45466.1	RAB5C, member RAS oncogene family [Danio rerio]	rab5c	4.41E-04	4.32	2.11
AAH44541.1	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 19 (DBP5 homolog, yeast) [Danio rerio]	ddx19	4.43E-04	3.29	1.72
NP_001295479.1	mucin 13b, cell surface associated precursor [Danio rerio]	muc13b	4.48E-04	2.55	1.35
XP_021336562.1	uncharacterized protein LOC563946 isoform X1 [Danio rerio]	zgc:136930	4.56E-04	0.00	-7.65
AAI63974.1	Zgc:103467 protein [Danio rerio]	myl9a	4.57E-04	0.29	-1.79
NP_001002547.2	proteasome inhibitor PI31 subunit [Danio rerio]	psmf1	4.69E-04	0.41	-1.30

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH78375.1	Proteasome (prosome, macropain) 26S subunit, ATPase, 5 [Danio rerio]	psmc5	4.70E-04	3.75	1.91
F1QGW6.1	RecName: Full=Eukaryotic translation initiation factor 2 subunit 3; AltName: Full=Eukaryotic translation initiation factor 2 subunit gamma; Short=eIF-2-gamma	eif2s3	4.83E-04	5.12	2.36
Q1L8L9.1	RecName: Full=Adenylate kinase 2, mitochondrial; Short=AK 2; AltName: Full=ATP-AMP transphosphorylase 2; AltName: Full=ATP:AMP phosphotransferase; AltName: Full=Adenylate monophosphate kinase	ak2	4.87E-04	0.34	-1.55
XP_017207446.1	ryanodine receptor 1 isoform X1 [Danio rerio]	ryr1b	4.89E-04	6.17	2.63
AAH59660.1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10 [Danio rerio]	ndufa10	4.91E-04	3.84	1.94
AAI64767.1	Zgc:153353 protein [Danio rerio]	agmat	4.97E-04	0.41	-1.28
XP_005159798.1	proteasome subunit beta type-4 isoform X1 [Danio rerio]	psmb4	4.98E-04	2.88	1.53
NP_001107090.1	malectin precursor [Danio rerio]	mlec	5.22E-04	5.97	2.58
AAH48042.2	DnaJ (Hsp40) homolog, subfamily A, member 2 [Danio rerio]	dnaja2	5.23E-04	3.51	1.81
XP_005162560.1	thioredoxin domain-containing protein 5 isoform X1 [Danio rerio]	txndc5	5.33E-04	2.37	1.25
AAH67570.1	Signal peptidase complex subunit 3 homolog (S. cerevisiae) [Danio rerio]	spcs3	5.35E-04	3.21	1.68
AAI25893.1	Gsna protein, partial [Danio rerio]	gsna	5.43E-04	0.45	-1.15
AAH67644.1	Gcat protein, partial [Danio rerio]	gcat	5.45E-04	3.17	1.67
NP_658910.2	glucose-6-phosphate isomerase [Danio rerio]	gpib	5.46E-04	4.51	2.17
AAI64751.1	Dhrs1 protein [Danio rerio]	dhrs1	5.49E-04	5.23	2.39
AAH53130.1	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide [Danio rerio]	ywhah	5.53E-04	2.77	1.47
AAI64493.1	Prkar2aa protein [Danio rerio]	prkar2aa	5.74E-04	3.65	1.87

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH57478.1	Glycine dehydrogenase (decarboxylating) [Danio rerio]	gldc	5.87E-04	13.43	3.75
NP_998215.1	chitinase, acidic.1 precursor [Danio rerio]	chia.1	5.88E-04	2.11	1.08
XP_009302555.1	heterogeneous nuclear ribonucleoprotein K isoform X1 [Danio rerio]	hnrnpk	5.89E-04	2.14	1.10
NP_001313616.1	dystrophin isoform 1 [Danio rerio]	dmd	5.95E-04	2.26	1.17
XP_005159433.1	tropomyosin alpha-3 chain isoform X2 [Danio rerio]	tpm3	6.12E-04	0.37	-1.43
AAH93321.1	Aminopeptidase-like 1 [Danio rerio]	npepl1	6.21E-04	2.61	1.38
AAI64061.1	Uox protein [Danio rerio]	uox	6.33E-04	2.17	1.12
AAI55151.1	Aldo-keto reductase family 1, member A1b (aldehyde reductase) [Danio rerio]	akr1a1b	6.46E-04	2.75	1.46
NP_001034716.1	cation-independent mannose-6-phosphate receptor precursor [Danio rerio]	igf2r	6.48E-04	96.76	6.60
AAH68395.1	Zgc:136493 protein, partial [Danio rerio]	zgc:136493	6.57E-04	2.23	1.15
AAH75983.1	Proteasome (prosome, macropain) subunit, beta type, 2 [Danio rerio]	psmb2	6.57E-04	201.00	7.65
NP_001002436.2	26S proteasome non-ATPase regulatory subunit 9 [Danio rerio]	psmd9	6.63E-04	0.00	-7.65
AAH56815.1	Pex19 protein, partial [Danio rerio]	pex19	6.66E-04	0.00	-7.65
AAI54202.1	Zgc:91912 [Danio rerio]	tha1	6.80E-04	2.25	1.17
AAI52672.1	Zgc:64031 [Danio rerio]	abhd14b	6.80E-04	2.41	1.27
NP_998458.1	serine/threonine-protein phosphatase 2A catalytic subunit beta isoform [Danio rerio]	ppp2cb	6.84E-04	2.20	1.14
AAH71498.1	Ribosomal protein L5b [Danio rerio]	rpl5b	7.01E-04	6.76	2.76
XP_009304444.1	collagen alpha-1(VI) chain [Danio rerio]	col6a1	7.15E-04	0.35	-1.51
AAH81481.1	Zgc:103495 [Danio rerio]	mrpl40	7.57E-04	0.00	-7.65

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH57412.1	Carbonic anhydrase II [Danio rerio]	ca2	7.63E-04	2.50	1.32
XP_005172323.1	uncharacterized protein LOC406277 isoform X1 [Danio rerio]	rbm4.3	7.84E-04	2.12	1.08
AAI65700.1	Pitpnb protein [Danio rerio]	pitpnb	7.84E-04	2.51	1.33
AAI55588.1	Zgc:172053 protein [Danio rerio]	zgc:172053	7.86E-04	0.29	-1.77
AAI53567.1	Anxa3b protein [Danio rerio]	anxa3b	7.89E-04	2.01	1.01
XP_005165525.1	endoplasmic reticulum aminopeptidase 2 isoform X1 [Danio rerio]	erap2	7.93E-04	4.91	2.30
AAH49060.1	Fatty acid binding protein 3, muscle and heart [Danio rerio]	fabp3	7.97E-04	0.36	-1.46
Q7SXW6.1	RecName: Full=Actin-related protein 2-A; AltName: Full=Actin-like protein 2-A	actr2a	8.20E-04	2.56	1.36
NP_001018582.1	uncharacterized protein LOC553782 [Danio rerio]	zgc:110425	8.22E-04	0.00	-7.65
NP_001070932.2	myosin heavy chain 7-like [Danio rerio]	vmhcl	8.29E-04	0.00	-7.65
XP_005160888.1	ralBP1-associated Eps domain-containing protein 1 isoform X1 [Danio rerio]	reps1	8.52E-04	2.52	1.33
AAY18965.1	beta A2-crystallin [Danio rerio]	cryba2a	8.52E-04	2.12	1.08
NP_001005950.1	glutaredoxin 3 [Danio rerio]	glrx3	8.53E-04	2.68	1.42
NP_958879.1	4-trimethylaminobutyraldehyde dehydrogenase A [Danio rerio]	aldh9a1a.1	8.58E-04	0.50	-1.00
AAI46704.1	Zgc:165344 protein [Danio rerio]	mybphb	8.77E-04	0.34	-1.54
AAF36403.1	syntaxin 1B [Danio rerio]	stx1b	8.95E-04	2.19	1.13
NP_999883.1	eukaryotic translation initiation factor 4B [Danio rerio]	eif4bb	9.17E-04	4.86	2.28
NP_997770.1	14-3-3 protein epsilon [Danio rerio]	ywhae1	9.46E-04	2.05	1.04
NP_001002343.1	guanylate binding protein 1 [Danio rerio]	gbp1	9.51E-04	0.30	-1.72

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_005157480.1	clathrin, heavy chain b (Hc) isoform X1 [Danio rerio]	cltcb	9.79E-04	5.29	2.40
AAH55247.1	Eukaryotic translation initiation factor 3, subunit J [Danio rerio]	eif3ja	9.82E-04	2.35	1.23
XP_017213161.1	tropomyosin alpha-4 chain isoform X2 [Danio rerio]	tpm4b	9.83E-04	0.40	-1.31
AAI35069.1	Krt1-19d protein, partial [Danio rerio]	krt1-19d	9.99E-04	2.09	1.06
AAH74082.1	HLA-DPA1 protein, partial [Danio rerio]	zgc:123107	1.02E-03	3.49	1.80
XP_021335899.1	ankyrin-3 isoform X1 [Danio rerio]	ank3b	1.03E-03	2.87	1.52
AAH66521.1	Tagln2 protein [Danio rerio]	tagln2	1.03E-03	0.45	-1.14
AAI25915.1	Zgc:153928 [Danio rerio]	cd99	1.05E-03	0.35	-1.52
AAI65897.1	Ubiquinol-cytochrome c reductase binding protein [Danio rerio]	uqcrb	1.07E-03	0.12	-3.01
Q9PV90.1	RecName: Full=60S acidic ribosomal protein P0; AltName: Full=60S ribosomal protein L10E	rplp0	1.09E-03	0.46	-1.13
AAH91470.1	Si:dkey-38l12.3 protein, partial [Danio rerio]	ces3	1.10E-03	0.35	-1.51
XP_005159794.1	programmed cell death 6-interacting protein isoform X1 [Danio rerio]	pdcd6ip	1.10E-03	2.36	1.24
AAD52042.1	type I cytokeratin [Danio rerio]	cyt1	1.11E-03	2.76	1.46
AAH86700.1	Zgc:101540 [Danio rerio]	zgc:101540	1.11E-03	0.36	-1.48
Q6NXA4.2	RecName: Full=Interleukin enhancer-binding factor 3 homolog	ilf3b	1.12E-03	2.09	1.07
AAH71519.1	Zgc:86896 [Danio rerio]	zgc:86896	1.12E-03	6.21	2.63
NP_997927.1	40S ribosomal protein S15a [Danio rerio]	rps15a	1.12E-03	4.81	2.27
XP_009302703.1	secernin-3 isoform X1 [Danio rerio]	scrn3	1.14E-03	2.50	1.32
AAI62206.1	Crystallin, alpha B, a [Danio rerio]	cryaba	1.14E-03	2.44	1.29

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH92977.1	Zgc:110679 [Danio rerio]	myl10	1.17E-03	0.32	-1.62
XP_021329098.1	LOW QUALITY PROTEIN: succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial-like isoform X2 [Danio rerio]	LOC797311	1.18E-03	9.10	3.19
NP_001278305.1	collagen alpha-1(XXII) chain precursor [Danio rerio]	prp	1.18E-03	0.00	-7.65
AAH67628.1	Twinfilin, actin-binding protein, homolog 1a [Danio rerio]	twf1a	1.19E-03	2.18	1.12
XP_005163206.1	troponin I, skeletal, fast 2a.4 isoform X1 [Danio rerio]	tnni2a.4	1.20E-03	0.36	-1.48
AAH67143.1	Pkm2a protein [Danio rerio]	pkma	1.21E-03	2.09	1.06
NP_001007422.1	bridging integrator 2 [Danio rerio]	bin2a	1.22E-03	3.75	1.91
AAH44387.1	Glycogenin 1 [Danio rerio]	gyg1a	1.22E-03	2.51	1.33
AAH59522.1	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived); ribosomal protein S30 [Danio rerio]	faua	1.22E-03	0.02	-5.94
XP_021332463.1	60S acidic ribosomal protein P2 isoform X1 [Danio rerio]	rplp2	1.22E-03	0.13	-2.98
NP_958906.2	4-aminobutyrate aminotransferase, mitochondrial [Danio rerio]	abat	1.23E-03	2.32	1.22
AAH67603.1	Zgc:85752 [Danio rerio]	lrrc59	1.24E-03	201.03	7.65
NP_957226.1	5'-nucleotidase precursor [Danio rerio]	nt5e	1.25E-03	2.97	1.57
AAQ94576.1	regucalcin [Danio rerio]	rgn	1.25E-03	2.78	1.48
AAH71336.1	Hypoxanthine phosphoribosyltransferase 1, like [Danio rerio]	hprt1l	1.26E-03	2.73	1.45
AAH66611.1	Farsa protein, partial [Danio rerio]	farsa	1.28E-03	0.00	-7.65
AAI15350.1	LOC557059 protein, partial [Danio rerio]	zgc:152670	1.28E-03	201.00	7.65
AAI64034.1	Tcp1 protein [Danio rerio]	tcp1	1.28E-03	0.28	-1.83
AAH67330.1	Hexokinase 1 [Danio rerio]	hk1	1.30E-03	2.44	1.29

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAP57207.1	developmentally regulated GTP-binding protein 1 [Danio rerio]	drg1	1.32E-03	2.30	1.20
XP_691712.4	lon protease homolog, mitochondrial [Danio rerio]	lonp1	1.33E-03	2.11	1.08
AAQ91264.1	2-peptidylprolyl isomerase A [Danio rerio]	ppiaa	1.34E-03	0.02	-5.77
NP_956323.1	60S acidic ribosomal protein P1 [Danio rerio]	rplp1	1.36E-03	0.37	-1.45
NP_001191053.1	cytochrome c oxidase polypeptide VIc-like [Danio rerio]	cox6c	1.38E-03	14.58	3.87
Q6DRC4.1	RecName: Full=Eukaryotic translation initiation factor 3 subunit G; Short=eIF3g; AltName: Full=Eukaryotic translation initiation factor 3 RNA-binding subunit; Short=eIF-3 RNA-binding subunit; AltName: Full=Eukaryotic translation initiation factor 3 subunit 4	eif3g	1.39E-03	2.89	1.53
AAH71516.1	FK506 binding protein 4 [Danio rerio]	fkbp4	1.39E-03	2.64	1.40
XP_005164267.1	ubiquitin domain-containing protein UBFD1 isoform X1 [Danio rerio]	ubfd1	1.40E-03	2.45	1.29
XP_005171556.1	uncharacterized protein LOC100007431 isoform X2 [Danio rerio]	zgc:172051	1.40E-03	3.05	1.61
XP_005162619.1	protein CDV3 homolog isoform X1 [Danio rerio]	cdv3	1.41E-03	0.36	-1.49
BAU80756.1	n-myc downstream-regulated gene 1a-2 [Danio rerio]	ndrg1a	1.44E-03	2.05	1.03
AAH74071.1	Carboxypeptidase A4 [Danio rerio]	cpa4	1.44E-03	200.97	7.65
ABQ96277.1	immunoglobulin light chain, partial [Danio rerio]	LOC101884804	1.45E-03	3.22	1.69
BAK26516.1	S100A1 protein [Danio rerio]	s100a1	1.46E-03	2.39	1.26
XP_005155688.1	dihydropyrimidinase-related protein 2 isoform X1 [Danio rerio]	dpysl2b	1.49E-03	2.04	1.03
AFX74876.1	PSMD8 [Danio rerio]	psmd8	1.50E-03	3.11	1.64
XP_002666982.1	filamin-C isoform X1 [Danio rerio]	flnca	1.51E-03	2.46	1.30
NP_001007367.1	visinin-like 1 [Danio rerio]	vsnl1a	1.51E-03	2.13	1.09

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_021325568.1	protein phosphatase 1 regulatory subunit 12B-like isoform X1 [Danio rerio]	LOC101885790	1.53E-03	2.23	1.15
NP_001070210.2	mitochondrial enolase superfamily member 1 [Danio rerio]	enosf1	1.55E-03	3.33	1.74
AAI65880.1	Hpd protein [Danio rerio]	hpdb	1.56E-03	4.84	2.27
XP_017210784.1	interleukin-1 receptor-associated kinase 3 [Danio rerio]	irak3	1.56E-03	3.00	1.58
AAT68157.1	seryl-tRNA synthetase [Danio rerio]	sars	1.57E-03	0.35	-1.50
XP_021335547.1	collagen alpha-2(VI) chain isoform X1 [Danio rerio]	col6a2	1.57E-03	0.02	-5.57
XP_003201252.2	msx2-interacting protein isoform X1 [Danio rerio]	spen	1.60E-03	2.78	1.48
AAH59684.1	Zgc:73375 [Danio rerio]	ndufb6	1.62E-03	4.62	2.21
AAI27565.1	Coactosin-like 1 (Dictyostelium) [Danio rerio]	cotl1	1.63E-03	2.14	1.10
AAH71311.1	Hprt1 protein [Danio rerio]	hprt1	1.64E-03	2.37	1.25
XP_021326447.1	mucin-2-like, partial [Danio rerio]	LOC110438407	1.64E-03	0.33	-1.58
XP_005155715.1	SRSF protein kinase 1b isoform X1 [Danio rerio]	srpk1b	1.67E-03	2.27	1.18
AAI64029.1	Adka protein [Danio rerio]	adka	1.68E-03	2.97	1.57
AAH76221.1	Ribosomal protein S15 [Danio rerio]	rps15	1.69E-03	0.02	-6.00
NP_998507.1	aromatic-L-amino-acid decarboxylase [Danio rerio]	ddc	1.74E-03	2.36	1.24
AAH53174.1	Palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infantile) [Danio rerio]	ppt1	1.83E-03	2.10	1.07
CAA72925.1	Cu/Zn-superoxide dismutase [Danio rerio]	sod1	1.84E-03	0.34	-1.57
NP_001002105.1	CDK5 regulatory subunit-associated protein 3 [Danio rerio]	cdk5rap3	1.84E-03	2.55	1.35
AAH95163.1	Cytochrome c oxidase subunit IV isoform 1 [Danio rerio]	cox4i1	1.84E-03	2.00	1.00

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI65638.1	Zgc:55773 protein [Danio rerio]	trim25	1.85E-03	3.73	1.90
AAQ97857.1	TIA1 cytotoxic granule-associated RNA binding protein [Danio rerio]	tia1l	1.86E-03	2.15	1.11
XP_021335293.1	calponin-2 isoform X1 [Danio rerio]	cnn2	1.86E-03	0.00	-7.65
AAF01276.1	Sk-tropomodulin [Danio rerio]	tmod4	1.89E-03	0.18	-2.44
AAH47816.1	Zgc:56049 [Danio rerio]	rab1ba	1.90E-03	2.18	1.12
NP_956808.1	galectin-1 [Danio rerio]	lgals2b	1.90E-03	2.43	1.28
AAI64533.1	Gdi1 protein [Danio rerio]	gdi1	1.92E-03	2.94	1.55
NP_001313377.1	apolipoprotein C-II precursor [Danio rerio]	apoc2	1.92E-03	0.02	-5.40
AAI33833.1	Neuraminidase 1 [Danio rerio]	neu1	1.93E-03	2.53	1.34
NP_001002447.1	myozenin 1 [Danio rerio]	myoz1b	1.93E-03	0.43	-1.23
XP_005156056.1	eukaryotic translation initiation factor 3 subunit C isoform X1 [Danio rerio]	eif3c	1.97E-03	16.88	4.08
NP_001315316.1	pantothenate kinase 4 [Danio rerio]	pank4	1.98E-03	2.85	1.51
AAY27891.1	cypher/ZASP splice variant 2 gamma [Danio rerio]	ldb3a	2.00E-03	0.47	-1.09
XP_002661418.2	mitochondrial 10-formyltetrahydrofolate dehydrogenase [Danio rerio]	aldh1l2	2.04E-03	2.10	1.07
XP_005157053.1	alpha-actinin-1 isoform X5 [Danio rerio]	actn1	2.05E-03	4.34	2.12
AAH71363.1	Glycogenin, like [Danio rerio]	gyg1b	2.06E-03	0.22	-2.18
ABQ82135.1	dynamin 1 [Danio rerio]	dnm1a	2.21E-03	0.23	-2.13
AAH62865.1	Complement component 8, gamma polypeptide [Danio rerio]	c8g	2.21E-03	2.19	1.13
AAI65894.1	Cygb1 protein [Danio rerio]	cygb1	2.22E-03	4.11	2.04

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH66618.1	Chloride intracellular channel 1 [Danio rerio]	clic1	2.26E-03	2.10	1.07
XP_009297739.2	ubiquitin carboxyl-terminal hydrolase 7 isoform X1 [Danio rerio]	usp7	2.26E-03	4.83	2.27
NP_001025345.2	DNA ligase 3 [Danio rerio]	lig3	2.32E-03	2.28	1.19
AAP37040.1	zygote arrest 1 [Danio rerio]	zar1	2.35E-03	4.89	2.29
AAI24402.1	Zgc:153624 [Danio rerio]	prkar1b	2.36E-03	2.71	1.44
Q90474.3	RecName: Full=Heat shock protein HSP 90-alpha 1	hsp90aa1.1	2.39E-03	2.50	1.32
XP_021332540.1	eukaryotic translation initiation factor 4E transporter isoform X1 [Danio rerio]	eif4enif1	2.41E-03	2.10	1.07
NP_957451.1	rho GDP-dissociation inhibitor 2 [Danio rerio]	arhgdig	2.46E-03	2.76	1.46
AAH71323.1	Ywhai protein [Danio rerio]	ywhaz	2.51E-03	3.18	1.67
ABL10370.1	glucose-dependent insulinotropic polypeptide [Danio rerio]	gip	2.52E-03	0.33	-1.60
NP_001003449.1	ras-related protein Rab-18-B [Danio rerio]	rab18b	2.60E-03	2.81	1.49
NP_998058.1	dihydropyrimidine dehydrogenase [NADP(+)] [Danio rerio]	dpydb	2.61E-03	2.27	1.19
AAI33844.1	Si:ch211-240l19.5 protein [Danio rerio]	si:ch211-240l19.5	2.62E-03	2.06	1.04
NP_001313383.1	PR domain zinc finger protein 13 [Danio rerio]	prdm13	2.62E-03	0.00	-7.65
XP_005164871.1	splicing factor 45 isoform X1 [Danio rerio]	rbm17	2.68E-03	0.42	-1.24
NP_956722.1	zgc:66313 precursor [Danio rerio]	zgc:66313	2.69E-03	2.15	1.11
XP_017211202.1	NACHT, LRR and PYD domains-containing protein 12-like [Danio rerio]	si:dkeyp-15a6.2	2.70E-03	3.54	1.82
ACG75896.1	10-formyltetrahydrofolate dehydrogenase [Danio rerio]	aldh1l1	2.77E-03	2.03	1.02
NP_001019590.1	2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial [Danio rerio]	bckdha	2.81E-03	3.65	1.87

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH95790.1	LOC553479 protein, partial [Danio rerio]	si:ch211-133j6.3	2.84E-03	5.52	2.47
NP_001004527.2	serine/threonine-protein phosphatase PP1-beta catalytic subunit [Danio rerio]	ppp1cb	2.89E-03	2.66	1.41
AAH85636.1	Atp2a1 protein [Danio rerio]	atp2a1	2.91E-03	2.38	1.25
AAH56310.1	Stanniocalcin 1 [Danio rerio]	stc1l	2.99E-03	0.44	-1.18
AAH54606.1	Acyl-Coenzyme A dehydrogenase, very long chain [Danio rerio]	acadvl	2.99E-03	50.80	5.67
AAU14809.1	fibronectin 1b [Danio rerio]	fn1b	3.02E-03	0.03	-4.90
NP_999881.1	heat shock protein 4a [Danio rerio]	hspa4a	3.04E-03	2.32	1.21
NP_991138.2	troponin I, skeletal, fast [Danio rerio]	tnni2a.3	3.05E-03	0.40	-1.32
NP_001003453.1	ADP-ribosylarginine hydrolase [Danio rerio]	adprh	3.06E-03	2.06	1.04
NP_001013495.2	uncharacterized protein LOC541350 [Danio rerio]	zgc:110216	3.16E-03	0.23	-2.11
NP_001315492.1	thymopoietin a isoform 1 [Danio rerio]	tmpoa	3.31E-03	0.42	-1.24
AAH66678.1	Ribophorin II [Danio rerio]	rpn2	3.36E-03	3.39	1.76
NP_001020350.1	troponin T2d, cardiac [Danio rerio]	tnnt2d	3.40E-03	0.38	-1.39
XP_009290703.1	tyrosine-protein phosphatase non-receptor type 6 isoform X1 [Danio rerio]	ptpn6	3.41E-03	0.04	-4.67
AAH63975.1	Hedgehog acyltransferase-like, a [Danio rerio]	hhatla	3.41E-03	103.48	6.69
AAH71367.1	Alcohol dehydrogenase 8b [Danio rerio]	adh8b	3.43E-03	2.12	1.09
XP_001340316.2	myelin basic protein isoform X1 [Danio rerio]	mbpa	3.44E-03	0.04	-4.66
AAH45322.1	Thioredoxin-like 1 [Danio rerio]	txnl1	3.46E-03	2.40	1.26
ABG78033.1	glycerol-3-phosphate dehydrogenase 1h [Danio rerio]	gpd1b	3.50E-03	0.39	-1.34

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
Q5BLE8.1	RecName: Full=All-trans-retinol 13,14-reductase; AltName: Full=All-trans-13,14-dihydroretinol saturase A; Short=RetSat A; AltName: Full=All-trans-retinol 7,8-reductase; Flags: Precursor	retsat	3.56E-03	3.40	1.77
NP_001002444.2	2,4-dienoyl-CoA reductase, mitochondrial [Danio rerio]	decr1	3.64E-03	2.41	1.27
AAX48020.1	mitochondrial ATP synthase alpha chain, partial [Danio rerio]	atp5a1	3.68E-03	28.42	4.83
XP_021321989.1	uncharacterized protein LOC110437886 [Danio rerio]	LOC110437886	3.70E-03	0.04	-4.59
XP_009302754.1	myc box-dependent-interacting protein 1-like isoform X1 [Danio rerio]	LOC563561	3.83E-03	2.48	1.31
NP_001034927.1	neurofilament, light polypeptide b [Danio rerio]	neflb	3.87E-03	0.38	-1.39
XP_021331635.1	MOB kinase activator 1A isoform X1 [Danio rerio]	mob1a	4.07E-03	2.08	1.06
Q6IQE0.2	RecName: Full=Poly(U)-binding-splicing factor PUF60-B	puf60b	4.13E-03	2.75	1.46
AAH49011.1	Isocitrate dehydrogenase 3 (NAD+) alpha [Danio rerio]	idh3a	4.19E-03	2.16	1.11
NP_998191.1	succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial [Danio rerio]	sucla2	4.23E-03	3.23	1.69
NP_571647.1	death-associated protein 1 [Danio rerio]	dap	4.26E-03	0.00	-7.65
Q7ZW47.2	RecName: Full=Double-stranded RNA-binding protein Staufen homolog 2	stau2	4.26E-03	21.45	4.42
NP_001005974.1	uncharacterized protein LOC449801 [Danio rerio]	zgc:103601	4.34E-03	2.65	1.40
AAI63562.1	Myosin, heavy polypeptide 6, cardiac muscle, alpha [Danio rerio]	myh6	4.38E-03	0.04	-4.62
Q5U3U3.2	RecName: Full=Carnitine O-palmitoyltransferase 2, mitochondrial; AltName: Full=Carnitine palmitoyltransferase II; Short=CPT II; Flags: Precursor	cpt2	4.44E-03	5.20	2.38
AAS92629.1	brain glycogen phosphorylase Pygb [Danio rerio]	pygb	4.45E-03	2.52	1.33
XP_005156321.1	protein phosphatase 1A, magnesium dependent, alpha isoform X1 [Danio rerio]	ppm1bb	4.49E-03	3.20	1.68

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH96960.1	Scel protein [Danio rerio]	scel	4.56E-03	2.88	1.52
AAH59509.1	Zgc:73149 [Danio rerio]	rpl23	4.56E-03	22.05	4.46
AAH46082.1	SET translocation (myeloid leukemia-associated) A [Danio rerio]	seta	4.59E-03	3.34	1.74
NP_001002561.1	chloride intracellular channel protein 2 [Danio rerio]	clic2	4.69E-03	2.78	1.47
AAH47184.1	Cullin-associated and neddylation-dissociated 1 [Danio rerio]	cand1	4.77E-03	4.32	2.11
NP_001002607.1	renalase [Danio rerio]	rnls	4.79E-03	3.73	1.90
XP_021329493.1	myosin-7 [Danio rerio]	LOC100329813	4.86E-03	0.28	-1.84
NP_001277022.1	eukaryotic translation initiation factor 3 subunit B [Danio rerio]	eif3ba	5.19E-03	13.50	3.75
AAH45426.1	Isovaleryl Coenzyme A dehydrogenase [Danio rerio]	ivd	5.35E-03	2.44	1.28
AAI63986.1	Zgc:92869 protein [Danio rerio]	gstz1	5.36E-03	2.55	1.35
AAI65492.1	Snrpd3l protein [Danio rerio]	snrpd3l	5.41E-03	0.49	-1.02
NP_001008593.1	protein NDRG2 [Danio rerio]	ndrg2	5.41E-03	2.95	1.56
NP_001018481.2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial [Danio rerio]	ndufs2	5.50E-03	17.76	4.15
XP_694376.3	acyl-CoA synthetase short-chain family member 3, mitochondrial [Danio rerio]	acss3	5.62E-03	2.56	1.35
NP_999924.1	legumain precursor [Danio rerio]	lgmn	5.67E-03	0.05	-4.22
XP_001919901.3	desmoplakin isoform X1 [Danio rerio]	dspa	5.71E-03	2.81	1.49
AAH81522.1	Zgc:103752 [Danio rerio]	fkbp1ab	5.82E-03	4.61	2.21
NP_001138256.1	alpha-internexin [Danio rerio]	inaa	5.86E-03	0.02	-5.69
XP_009295237.1	E3 ubiquitin-protein ligase HUWE1 isoform X1 [Danio rerio]	huwe1	5.86E-03	3.74	1.90

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
CAP08010.1	dcn, partial [Danio rerio]	dcn	5.92E-03	0.06	-4.17
XP_017213957.1	interferon regulatory factor 3 isoform X1 [Danio rerio]	irf3	5.95E-03	3.62	1.86
XP_005172154.1	protein-arginine deiminase type-2 isoform X1 [Danio rerio]	padi2	5.99E-03	0.43	-1.20
XP_005162006.2	microtubule-associated protein RP/EB family member 1 isoform X1 [Danio rerio]	mapre1b	6.20E-03	0.06	-4.14
NP_571924.2	alcohol dehydrogenase class-3 [Danio rerio]	adh5	6.21E-03	0.06	-4.07
XP_021333882.1	peroxisomal multifunctional enzyme type 2 isoform X1 [Danio rerio]	hsd17b4	6.22E-03	0.07	-3.86
NP_001013362.1	prefoldin subunit 1 [Danio rerio]	pfdn1	6.33E-03	5.75	2.52
CAI11879.1	novel protein [Danio rerio]	si:ch211-240l19.6	6.33E-03	2.52	1.33
XP_009303637.1	rab5 GDP/GTP exchange factor isoform X1 [Danio rerio]	rabgef1	6.45E-03	16.12	4.01
NP_999936.1	epidermal retinol dehydrogenase 2 [Danio rerio]	sdr16c5b	6.52E-03	14.00	3.81
AAH46086.1	Deoxyhypusine hydroxylase/monooxygenase [Danio rerio]	dohh	6.53E-03	3.11	1.64
XP_021333077.1	transportin-2 [Danio rerio]	LOC100005536	6.65E-03	16.46	4.04
NP_957378.2	cell division cycle 5-like protein [Danio rerio]	cdc5l	6.68E-03	2.44	1.29
XP_017212786.1	AMP deaminase 1 isoform X1 [Danio rerio]	ampd1	6.81E-03	2.59	1.37
XP_005156696.1	eukaryotic translation initiation factor 4E family member 1c isoform X1 [Danio rerio]	eif4e1c	6.94E-03	15.56	3.96
AAH42325.1	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 12 [Danio rerio]	psmd12	7.22E-03	3.44	1.78
AAP82281.1	Coro1A [Danio rerio]	coro1a	7.28E-03	4.17	2.06
XP_005163134.1	protein NDRG4 isoform X1 [Danio rerio]	ndrg4	7.32E-03	2.27	1.18
NP_991125.1	barrier-to-autointegration factor [Danio rerio]	banf1	7.40E-03	0.06	-4.07

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI65557.1	Pofut1 protein [Danio rerio]	pofut1	7.43E-03	2.36	1.24
XP_005170948.1	arfaptin-1 isoform X1 [Danio rerio]	arfip1	7.50E-03	13.96	3.80
NP_001313411.1	uncharacterized protein LOC325758 [Danio rerio]	si:ch211-105c13.3	7.57E-03	0.07	-3.76
Q800G5.1	RecName: Full=Interferon-induced GTP-binding protein MxE; AltName: Full=IFN-inducible antiviral protein MxE; AltName: Full=Interferon-inducible MxE protein	mxe	7.67E-03	2.04	1.03
XP_005158614.1	la-related protein 1B isoform X1 [Danio rerio]	larp1b	7.82E-03	201.00	7.65
AAQ91275.1	sorting nexin 12, variation 2 [Danio rerio]	snx12	7.85E-03	2.94	1.55
NP_955939.1	eukaryotic translation initiation factor 4E-binding protein 1 [Danio rerio]	eif4ebp1	8.09E-03	201.00	7.65
AAH59553.1	Protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1 [Danio rerio]	pin1	8.09E-03	3.21	1.68
AAI64209.1	Cd2bp2 protein [Danio rerio]	cd2bp2	8.38E-03	0.37	-1.43
AAI00048.1	Zgc:112053 [Danio rerio]	ndufa12	8.38E-03	12.90	3.69
AAH72704.1	Mecr protein, partial [Danio rerio]	mecr	8.58E-03	12.87	3.69
AAH76408.1	Propionyl-Coenzyme A carboxylase, alpha polypeptide [Danio rerio]	pcca	8.60E-03	2.20	1.14
NP_938170.1	pre-mRNA-processing factor 40 homolog A [Danio rerio]	prpf40a	8.95E-03	2.11	1.07
NP_001315354.1	carboxypeptidase B isoform 1 precursor [Danio rerio]	cpb1	8.96E-03	2.02	1.01
AAH90901.1	Zgc:103568 [Danio rerio]	asrgl1	9.10E-03	2.91	1.54
XP_009300420.1	myosin-10 isoform X1 [Danio rerio]	myh10	9.16E-03	0.17	-2.52
NP_997901.1	methyl-CpG-binding protein 2 isoform 1 [Danio rerio]	mecp2	9.17E-03	0.08	-3.63
XP_002667598.5	prolyl endopeptidase-like, partial [Danio rerio]	LOC100334014	9.19E-03	2.20	1.14

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
Q6TGS6.2	RecName: Full=Tyrosine--tRNA ligase, cytoplasmic; AltName: Full=Tyrosyl-tRNA synthetase; Short=TyrRS	yars	9.74E-03	3.12	1.64
XP_005165755.1	peptidyl-prolyl cis-trans isomerase FKBP11 isoform X1 [Danio rerio]	fkbp11	9.82E-03	2.86	1.52
AAH46069.1	Zgc:56317 [Danio rerio]	actr1	9.82E-03	2.01	1.00
AAH59517.1	S-adenosylhomocysteine hydrolase-like 2 [Danio rerio]	ahcyl2	9.90E-03	14.82	3.89
XP_005156414.2	protein transport protein Sec24C isoform X1 [Danio rerio]	sec24c	9.93E-03	11.60	3.54
AAH78645.1	Protein phosphatase 2, regulatory subunit B (B56) 2 [Danio rerio]	ppp2r5ea	1.02E-02	2.09	1.07
CAD60636.1	novel flotillin [Danio rerio]	flot1a	1.04E-02	0.44	-1.19
XP_021329294.1	emerin isoform X1 [Danio rerio]	emd	1.04E-02	2.22	1.15
AAI42848.1	Ptmab protein [Danio rerio]	ptmab	1.05E-02	4.22	2.08
AAH75914.1	Aspartoacylase (aminocyclase) 3.2 [Danio rerio]	acy3.2	1.07E-02	2.62	1.39
AAH56300.1	Carboxypeptidase A5 [Danio rerio]	cpa5	1.07E-02	2.52	1.33
XP_009303467.1	tight junction protein ZO-2 isoform X1 [Danio rerio]	tjp2a	1.08E-02	11.07	3.47
AAC34932.1	plasticin [Danio rerio]	prph	1.09E-02	0.45	-1.16
XP_021325606.1	protein TASOR [Danio rerio]	fam208ab	1.09E-02	2.82	1.50
Q6GMH3.1	RecName: Full=Twinfilin-2; AltName: Full=Twinfilin-1-like protein	twf2	1.10E-02	201.00	7.65
AAO20273.1	annexin 5 [Danio rerio]	anxa5b	1.10E-02	2.85	1.51
AAH76168.1	OTU domain, ubiquitin aldehyde binding 1, like [Danio rerio]	otub1a	1.16E-02	10.35	3.37
AAH83193.1	Saccharopine dehydrogenase b [Danio rerio]	sccpdhb	1.16E-02	3.65	1.87
AAQ94603.1	RAD23 homolog B [Danio rerio]	rad23b	1.21E-02	2.42	1.27

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI63932.1	Solute carrier family 4, anion exchanger, member 1 [Danio rerio]	slc4a1a	1.22E-02	2.92	1.55
AAI62829.1	Heparan sulfate 6-O-sulfotransferase 3 [Danio rerio]	hs6st3b	1.22E-02	9.61	3.26
NP_955882.1	eukaryotic translation initiation factor 1B [Danio rerio]	eif1b	1.22E-02	0.31	-1.69
XP_005158972.1	fermitin family homolog 2 isoform X1 [Danio rerio]	fermt2	1.23E-02	4.66	2.22
XP_003201709.1	uncharacterized protein LOC100538255 [Danio rerio]	LOC100538255	1.23E-02	10.04	3.33
XP_005172430.1	cytoplasmic aconitate hydratase isoform X1 [Danio rerio]	aco1	1.26E-02	2.18	1.12
AAH44413.1	Arpc5a protein [Danio rerio]	arpc5a	1.27E-02	0.42	-1.27
XP_683018.3	uncharacterized protein si:ch211-242e8.1 isoform X1 [Danio rerio]	si:ch211-242e8.1	1.29E-02	0.38	-1.41
AAH71407.1	Proliferation-associated 2G4, a [Danio rerio]	pa2g4a	1.29E-02	0.39	-1.35
NP_001002401.1	probable 39S ribosomal protein L24, mitochondrial precursor [Danio rerio]	mrpl24	1.32E-02	10.91	3.45
Q7ZV34.1	RecName: Full=Malignant T-cell-amplified sequence 1; Short=MCT-1	mcts1	1.33E-02	2.78	1.47
XP_017208600.1	tropomyosin alpha-4 chain isoform X2 [Danio rerio]	tpm4a	1.34E-02	0.09	-3.46
NP_001167456.1	alpha-aminoadipic semialdehyde synthase, mitochondrial [Danio rerio]	aass	1.36E-02	2.72	1.44
NP_001314918.1	kalirin isoform 1 [Danio rerio]	kalrnb	1.40E-02	2.49	1.32
AAH78249.1	BCL2-associated athanogene 3 [Danio rerio]	bag3	1.41E-02	0.12	-3.03
AAI55253.1	Zgc:101621 protein [Danio rerio]	scp2b	1.41E-02	0.10	-3.30
NP_001004296.1	moesin [Danio rerio]	msna	1.48E-02	0.11	-3.17
XP_021334300.1	sciellin isoform X2 [Danio rerio]	scel	1.48E-02	2.51	1.33
AAH65589.1	Cathepsin B, a [Danio rerio]	ctsba	1.49E-02	0.11	-3.18

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAI64134.1	Dhdhl protein [Danio rerio]	dhdhl	1.52E-02	2.61	1.38
AAH71419.1	Heat shock 10 protein 1 (chaperonin 10) [Danio rerio]	hspe1	1.54E-02	7.91	2.98
XP_009304599.1	calcium activated nucleotidase 1b isoform X1 [Danio rerio]	cant1b	1.56E-02	3.38	1.76
Q1JPZ7.2	RecName: Full=Pre-mRNA-processing factor 39; AltName: Full=PRP39 homolog	prpf39	1.58E-02	8.22	3.04
AAI62366.1	Transforming growth factor, beta 1 [Danio rerio]	tgfb1a	1.60E-02	8.50	3.09
AAH56287.1	Mpx protein [Danio rerio]	mpx	1.61E-02	0.11	-3.15
XP_017207545.1	cadherin-13 isoform X1 [Danio rerio]	cdh13	1.61E-02	0.45	-1.15
AAI16552.1	Prefoldin subunit 2 [Danio rerio]	pfdn2	1.62E-02	2.11	1.08
XP_021324983.1	xaa-Pro aminopeptidase 1 isoform X1 [Danio rerio]	xpnpep1	1.62E-02	0.15	-2.73
NP_001003747.1	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial [Danio rerio]	ndufv1	1.64E-02	1.99	1.00
NP_998051.1	phosphoglucomutase-2 [Danio rerio]	pgm2	1.65E-02	0.45	-1.16
Q6DH42.1	RecName: Full=Transcription and mRNA export factor ENY2; AltName: Full=Enhancer of yellow 2 transcription factor homolog	eny2	1.66E-02	2.03	1.02
AAI64979.1	Crygs3 protein [Danio rerio]	crygs3	1.67E-02	0.44	-1.19
NP_001004003.1	transportin-2 [Danio rerio]	tnpo2	1.67E-02	8.95	3.16
XP_021331756.1	alpha-crystallin B chain isoform X1 [Danio rerio]	cryabb	1.72E-02	0.29	-1.79
XP_021327337.1	IgGFc-binding protein isoform X1 [Danio rerio]	si:dkey-65b12.6	1.74E-02	4.00	2.00
XP_005159484.1	S100 calcium binding protein S isoform X1 [Danio rerio]	s100s	1.75E-02	0.12	-3.07
XP_694691.5	heterogeneous nuclear ribonucleoprotein U isoform X1 [Danio rerio]	hnrnpua	1.75E-02	4.22	2.08
NP_001124122.1	uncharacterized protein LOC100170815 [Danio rerio]	zgc:193598	1.78E-02	0.10	-3.34

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
XP_009303963.1	beta-crystallin A4 isoform X1 [Danio rerio]	cryba4	1.78E-02	0.12	-3.04
NP_001071238.1	peptidyl-prolyl cis-trans isomerase FKBP3 [Danio rerio]	fkbp3	1.80E-02	0.02	-5.97
XP_003197844.3	keratin, type I cytoskeletal 47 kDa [Danio rerio]	wu:fk65c09	1.81E-02	0.22	-2.21
XP_021332688.1	xin actin-binding repeat-containing protein 2 isoform X1 [Danio rerio]	xirp2a	1.81E-02	5.41	2.43
XP_009292890.1	protein PRRC2C isoform X1 [Danio rerio]	prrc2c	1.88E-02	2.20	1.13
AAH49058.1	Rplp0 protein [Danio rerio]	rplp0	1.91E-02	2.28	1.19
Q7SXG4.2	RecName: Full=SUMO-activating enzyme subunit 2; AltName: Full=Ubiquitin-like 1-activating enzyme E1B; AltName: Full=Ubiquitin-like modifier-activating enzyme 2	uba2	1.91E-02	7.83	2.97
NP_958453.1	polyadenylate-binding protein 4 [Danio rerio]	pabpc4	1.93E-02	0.14	-2.79
AAI42762.1	Sult2st2 protein [Danio rerio]	sult2st2	1.95E-02	0.00	-7.65
AAH56783.1	Zgc:63561 [Danio rerio]	cisd1	1.95E-02	7.52	2.91
XP_009293048.1	ryanodine receptor 3 isoform X1 [Danio rerio]	ryr3	1.96E-02	0.48	-1.06
AAH66384.1	Collagen, type I, alpha 3 [Danio rerio]	col1a1b	1.96E-02	0.28	-1.85
AAH76451.1	Zgc:91854 [Danio rerio]	blvrb	1.98E-02	0.40	-1.30
XP_017209697.2	von Willebrand factor A domain-containing protein 5A-like isoform X1 [Danio rerio]	LOC100535191	2.06E-02	2.93	1.55
AAM61767.1	laminin beta 1 [Danio rerio]	lamb1a	2.08E-02	0.13	-2.97
AAI24417.1	Atp5i protein [Danio rerio]	atp5ib	2.13E-02	5.39	2.43
AAI64367.1	Phyh protein [Danio rerio]	phyh	2.15E-02	6.24	2.64
XP_021326622.1	cullin-9 [Danio rerio]	cul9	2.16E-02	6.87	2.78

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
Q803D2.3	RecName: Full=Lissencephaly-1 homolog B; AltName: Full=Platelet-activating factor acetylhydrolase IB subunit alpha b	pafah1b1b	2.17E-02	4.23	2.08
AAH71412.1	Tmed9 protein, partial [Danio rerio]	tmed9	2.26E-02	6.67	2.74
AAI15052.1	Si:dkey-51e6.1 protein [Danio rerio]	si:dkey-51e6.1	2.28E-02	0.25	-2.03
AAH63230.1	Sterol carrier protein 2 [Danio rerio]	scp2a	2.30E-02	0.22	-2.17
NP_001002139.1	60S ribosomal protein L11 [Danio rerio]	rpl11	2.32E-02	3.04	1.60
AAH55183.1	Zgc:63647 [Danio rerio]	ftcd	2.33E-02	4.55	2.19
CAI21060.1	novel elastase protein (zgc:637440), partial [Danio rerio]	ela2l	2.34E-02	0.21	-2.27
Q803F5.1	RecName: Full=Adipocyte plasma membrane-associated protein	apmap	2.34E-02	7.13	2.83
NP_001005587.1	carnitine O-acetyltransferase [Danio rerio]	crata	2.39E-02	4.00	2.00
AAH83471.1	Multiple coagulation factor deficiency 2 [Danio rerio]	mcfd2	2.39E-02	0.14	-2.83
AER12034.1	laminin alpha 2 chain [Danio rerio]	lama2	2.44E-02	0.15	-2.73
XP_021331868.1	acetyl-CoA carboxylase 1 isoform X1 [Danio rerio]	acaca	2.44E-02	6.03	2.59
AAH68037.1	Chaperonin containing TCP1, subunit 5 (epsilon) [Danio rerio]	cct5	2.45E-02	6.73	2.75
XP_005168739.1	adenylate kinase isoenzyme 1 isoform X1 [Danio rerio]	ak1	2.47E-02	0.50	-1.00
AAH81523.1	Pvalb6 protein [Danio rerio]	pvalb6	2.49E-02	0.19	-2.38
NP_955909.2	serine hydrolase-like protein [Danio rerio]	serhl	2.53E-02	6.95	2.80
AAI39579.1	Si:dkey-222f8.3 protein [Danio rerio]	si:dkey-222f8.3	2.61E-02	2.50	1.32
XP_009297554.1	voltage-dependent L-type calcium channel subunit beta-1 isoform X5 [Danio rerio]	cacnb1	2.65E-02	0.00	-7.65
NP_001004674.1	high mobility group box 2b [Danio rerio]	hmgb2b	2.72E-02	0.14	-2.80

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAQ94580.1	actin related protein 2/3 complex subunit 5, 16 kDa [Danio rerio]	arpc5b	2.73E-02	2.12	1.08
AAP55637.1	SULT1 sulfotransferase isoform 3 [Danio rerio]	sult1st3	2.75E-02	0.15	-2.74
XP_021324396.1	desmoplakin isoform X1 [Danio rerio]	wu:fi04e12	2.77E-02	6.27	2.65
NP_001121836.1	sulfhydryl oxidase 1 [Danio rerio]	qsox1	2.82E-02	2.89	1.53
NP_001003501.1	calpain-9 [Danio rerio]	capn9	2.85E-02	6.15	2.62
XP_017207875.1	ectonucleotide pyrophosphatase/phosphodiesterase family member 2-like [Danio rerio]	LOC101886447	2.88E-02	2.28	1.19
NP_001074133.1	sideroflexin-3 [Danio rerio]	sfxn3	2.88E-02	2.71	1.44
AAI64001.1	Twf1b protein [Danio rerio]	twf1b	2.88E-02	6.41	2.68
AAQ98002.1	dystrobrevin, beta [Danio rerio]	dtnba	2.89E-02	6.56	2.71
AAI64419.1	Zgc:56116 protein [Danio rerio]	pdlim5b	2.92E-02	2.27	1.18
ACB10574.1	aquaporin-3a [Danio rerio]	aqp3a	2.93E-02	5.93	2.57
XP_021333133.1	dynactin 1a isoform X1 [Danio rerio]	dctn1a	2.93E-02	0.50	-1.01
AAI63876.1	Chaperonin containing TCP1, subunit 7 (eta) [Danio rerio]	cct7	2.93E-02	7.23	2.85
NP_001007366.1	troponin I type 2a (skeletal, fast), tandem duplicate 1 [Danio rerio]	tnni2a.1	2.94E-02	2.25	1.17
XP_017209987.2	basal cell adhesion molecule-like [Danio rerio]	LOC100536698	2.98E-02	4.71	2.23
NP_001003564.1	DNA-directed RNA polymerases I, II, and III subunit RPABC1 [Danio rerio]	polr2eb	3.01E-02	0.17	-2.58
XP_009304711.1	APOBEC1 complementation factor [Danio rerio]	a1cf	3.01E-02	0.11	-3.13
AAH74086.1	Zgc:91874 [Danio rerio]	anks4b	3.04E-02	0.17	-2.54
NP_001007391.1	3-hydroxyanthranilate 3,4-dioxygenase [Danio rerio]	haao	3.04E-02	2.01	1.01

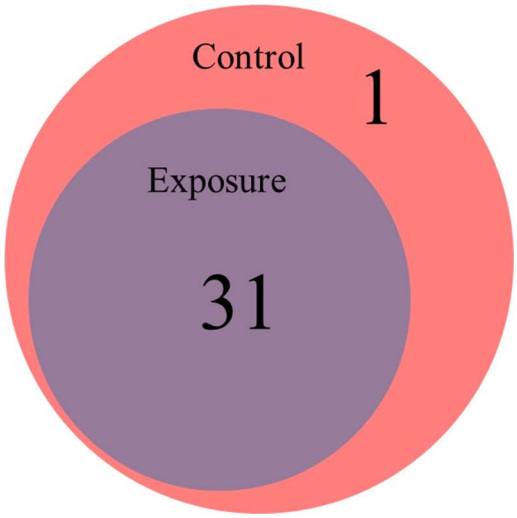
Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
ABE66384.1	truncated integrin beta1 subunit-like protein 1 [Danio rerio]	itgb1b.2	3.05E-02	10.10	3.34
AAX59994.1	Sult1 isoform 6 [Danio rerio]	sult1st6	3.06E-02	0.35	-1.52
ACB45514.1	plasma membrane calcium ATPase 4 [Danio rerio]	atp2b4	3.07E-02	2.34	1.23
AAU85774.1	gammaM2b-crystallin [Danio rerio]	crygm2b	3.07E-02	0.46	-1.13
NP_001019624.1	RNA polymerase II-associated factor 1 homolog [Danio rerio]	paf1	3.10E-02	5.56	2.47
XP_009295045.1	filamin-A isoform X1 [Danio rerio]	flna	3.11E-02	2.16	1.11
AAI59235.1	Zgc:111960 protein [Danio rerio]	homer2	3.12E-02	2.22	1.15
AAI52261.1	Proteasome (prosome, macropain) 26S subunit, ATPase, 6 [Danio rerio]	psmc6	3.14E-02	2.10	1.07
NP_001003860.1	protein BUD31 homolog [Danio rerio]	bud31	3.14E-02	6.85	2.78
AAH71527.1	Phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase [Danio rerio]	paics	3.15E-02	5.15	2.37
XP_021324673.1	collagen alpha-1(V) chain isoform X1 [Danio rerio]	col5a1	3.15E-02	0.34	-1.54
XP_691535.5	complexin-1 [Danio rerio]	LOC563082	3.18E-02	0.42	-1.25
NP_001028282.1	mannose-6-phosphate isomerase [Danio rerio]	mpi	3.19E-02	3.37	1.75
NP_001116258.1	threonine--tRNA ligase, cytoplasmic isoform 1 [Danio rerio]	tars	3.28E-02	4.63	2.21
AAI62610.1	Dihydropyrimidinase-like 5b [Danio rerio]	dpysl5b	3.40E-02	5.52	2.46
NP_001014327.1	uncharacterized protein LOC541492 [Danio rerio]	zgc:110843	3.43E-02	9.34	3.22
AAF05847.1	type II cytokeratin [Danio rerio]	krt5	3.49E-02	3.51	1.81
XP_005159867.1	tropomodulin-1 isoform X1 [Danio rerio]	tmod1	3.57E-02	2.37	1.24
AAH81629.1	Ganglioside induced differentiation associated protein 2 [Danio rerio]	gdap2	3.60E-02	6.36	2.67

Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
AAH52476.1	HLA-B-associated transcript 3 [Danio rerio]	bag6	3.66E-02	7.15	2.84
AAI52167.1	Zgc:109940 [Danio rerio]	cfd	3.76E-02	0.20	-2.33
AAH54941.1	Calpain 1, (mu/I) large subunit a [Danio rerio]	capn1a	3.85E-02	4.78	2.26
AAH47852.1	Nucleobindin 2b [Danio rerio]	nucb2b	3.88E-02	2.36	1.24
AAH67561.1	Capping protein (actin filament) muscle Z-line, alpha 1 [Danio rerio]	capza1a	3.94E-02	0.15	-2.71
XP_021336193.1	protein phosphatase 1A isoform X1 [Danio rerio]	ppm1aa	3.98E-02	4.34	2.12
AAI63537.1	Myosin, heavy polypeptide 1, skeletal muscle [Danio rerio]	myhz1.1	4.03E-02	0.14	-2.83
NP_001007054.1	phosphoacetylglucosamine mutase [Danio rerio]	pgm3	4.05E-02	1.99	0.99
AAI64432.1	Zgc:77734 protein [Danio rerio]	dbi	4.08E-02	0.47	-1.10
AAH57421.1	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein [Danio rerio]	apbb1ip	4.16E-02	5.77	2.53
AAH76216.1	Succinate-CoA ligase, GDP-forming, alpha subunit [Danio rerio]	suclg1	4.18E-02	4.22	2.08
XP_005159305.1	ADP-ribosylation factor GTPase-activating protein 2 isoform X1 [Danio rerio]	arfgap2	4.18E-02	0.20	-2.32
Q6NSN2.1	RecName: Full=Amine oxidase [flavin-containing]; AltName: Full=Monoamine oxidase; Short=MAO; Short=Z-MAO	mao	4.20E-02	4.48	2.16
NP_001001404.1	voltage-dependent anion-selective channel protein 1 [Danio rerio]	vdac1	4.22E-02	3.68	1.88
NP_956228.1	V-type proton ATPase subunit G 1 [Danio rerio]	atp6v1g1	4.23E-02	0.14	-2.79
XP_001923457.2	chondroitin sulfate proteoglycan 4 [Danio rerio]	cspg4	4.25E-02	4.81	2.26
AAH68342.1	Zgc:85644 [Danio rerio]	fam213b	4.26E-02	2.24	1.16
XP_009290813.1	succinate-semialdehyde dehydrogenase, mitochondrial isoform X1 [Danio rerio]	aldh5a1	4.44E-02	0.20	-2.33

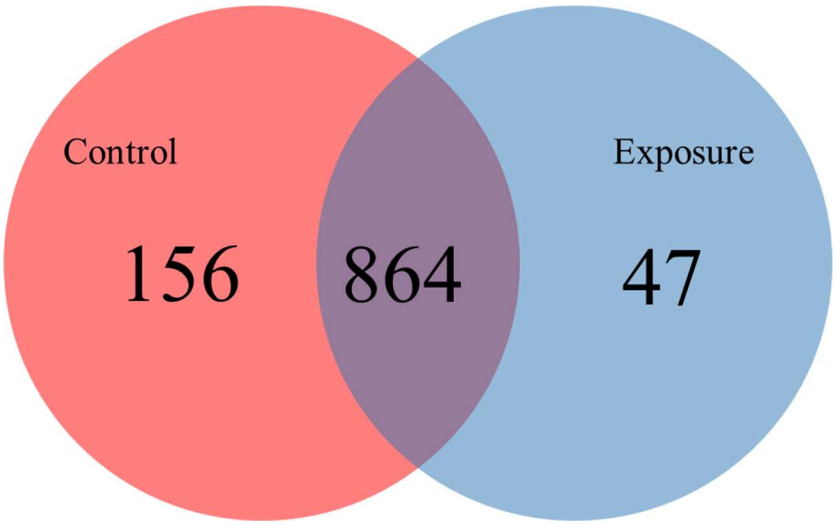
Accession	Description	Gene symbol	p-value	Fold change	log2(FC)
NP_001186657.1	apolipoprotein M precursor [Danio rerio]	apom	4.44E-02	0.47	-1.08
XP_005171631.1	growth factor receptor-bound protein 2 isoform X1 [Danio rerio]	grb2b	4.49E-02	0.24	-2.06
NP_001032468.2	dual specificity mitogen-activated protein kinase kinase 2 [Danio rerio]	map2k2a	4.49E-02	3.30	1.72
AAH57438.1	Inner membrane protein, mitochondrial (mitofilin) [Danio rerio]	immt	4.60E-02	0.47	-1.10
NP_001159728.1	junctophilin-4 [Danio rerio]	jph1a	4.63E-02	2.54	1.35
NP_957147.1	WD repeat-containing protein 61 [Danio rerio]	wdr61	4.67E-02	4.09	2.03
NP_001036210.1	cytoplasmic dynein 1 heavy chain 1 [Danio rerio]	dync1h1	4.69E-02	0.17	-2.60
XP_005169562.1	mitotic checkpoint protein BUB3 isoform X1 [Danio rerio]	bub3	4.71E-02	2.32	1.21
NP_001070183.1	tubulin beta-4B chain-like [Danio rerio]	zgc:153426	4.71E-02	0.44	-1.18
NP_001032501.1	high mobility group protein B2 [Danio rerio]	hmgb2a	4.72E-02	2.58	1.37
AAI71423.1	Calpain 2, (m/II) large subunit b [Danio rerio]	capn2b	4.75E-02	2.10	1.07
NP_001243652.1	complement component C8 beta chain precursor [Danio rerio]	c8b	4.77E-02	0.34	-1.57
AAI62706.1	Itgb1b protein [Danio rerio]	itgb1b	4.78E-02	4.10	2.04
AAH58047.1	Ras-related nuclear protein [Danio rerio]	ran	4.94E-02	3.03	1.60
AAI53400.1	Zgc:153978 protein [Danio rerio]	cox5b2	4.95E-02	6.28	2.65
XP_005161033.1	phytanoyl-CoA dioxygenase domain-containing protein 1 isoform X2 [Danio rerio]	phyhd1	4.98E-02	0.20	-2.34

Fig. 22. Venn diagram of biomarker proteins in (A) honeybee and (B) zebrafish.

(A)



(B)



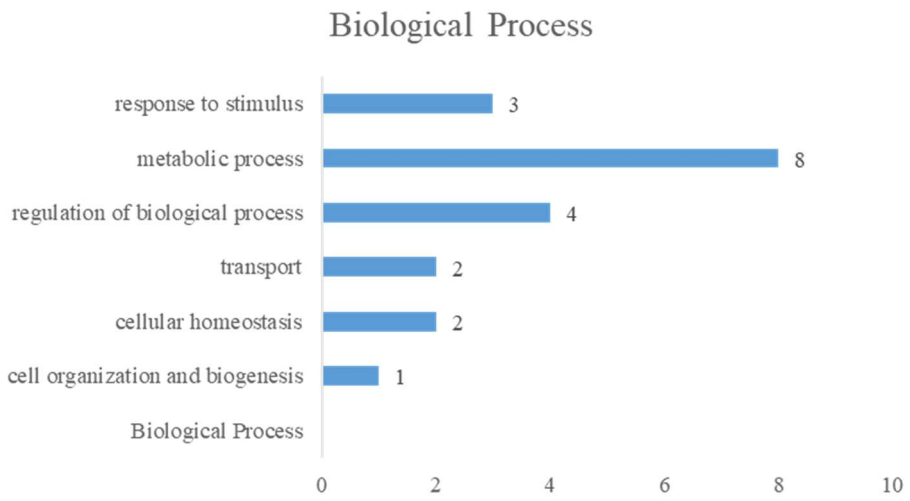
Enrichment analysis

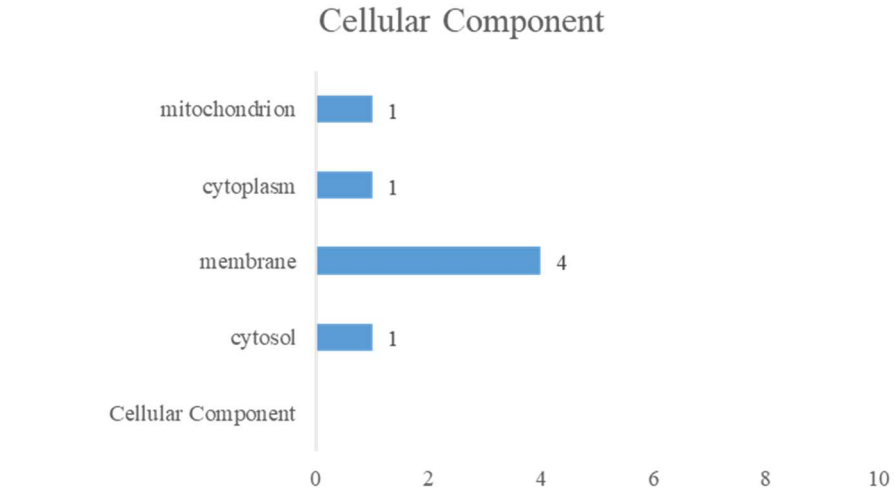
The "Gene ontology" is defined in structured and controlled terms (Ashburner et al., 2000). The Gene ontology is known as one of the major sources of biological information by providing a specific definition of protein function (Milano, 2019). These methods are divided into molecular functions, biological processes and non-overlapping ontology of cellular components (Milano, 2019). The web-based "DAVID" was used to predict the ontology of proteins that become biomarkers in honeybees and zebrafish. The DAVID is a suite of tools for functional enhancement analysis in various biological contexts. The gene ontology classifications were based on biological process, cellular component and molecular function (**Fig. 23.**). In honeybee sample, when classified according to biological process, the biomarker proteins were mostly involved in metabolic process that included 8 proteins. In cellular component categories, four of proteins related in membrane was showed. Lastly, the proteins involved in catalytic activity were nine.

In zebrafish, the highest number of proteins were related in oxidation-reduction process in biological process classification. Meanwhile, the proteins involved in cytoplasm and action binding were major proteins in cellular component and molecular function, respectively (**Fig. 24-26**).

In addition, using the proteins as biomarker, the corresponding metabolic pathway could be predicted using enrichment analysis. The pyruvate metabolism and glycolysis/gluconeogenesis pathways were significantly perturbed in honeybee (**Table 12.**). While, biosynthesis of antibiotics, carbon metabolism, and glycolysis/gluconeogenesis pathways were major perturbed pathway in protein levels of zebrafish. (**Table 13.**)

Fig. 23. Gene ontology classification of biomarker proteins in honeybee.


Biological Process
response to stimulus
metabolic process
regulation of biological process
transport
cellular homeostasis
cell organization and biogenesis
Biological Process
3
8
4
2
2
1
0
2
4
6
8
10


Cellular Component
mitochondrion
cytoplasm
membrane
cytosol
Cellular Component
1
1
4
1
0
2
4
6
8
10

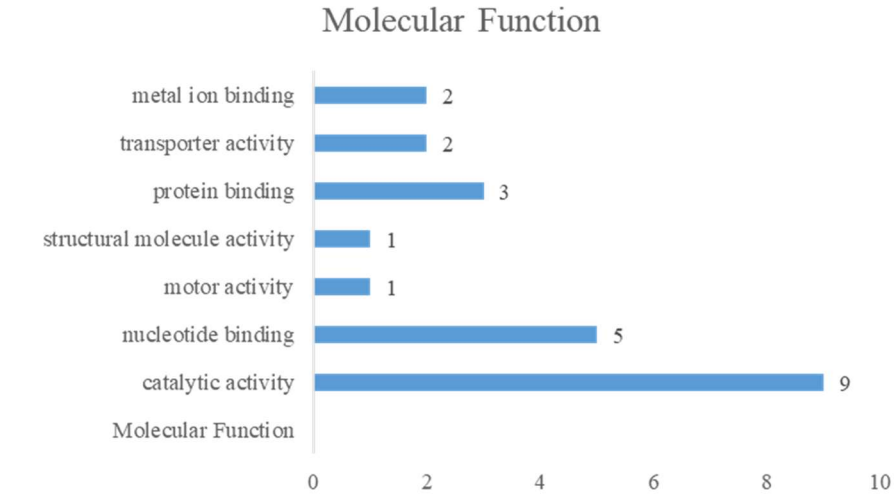

Molecular Function
metal ion binding
transporter activity
protein binding
structural molecule activity
motor activity
nucleotide binding
catalytic activity
Molecular Function
2
2
3
1
1
5
9
0
2
4
6
8
10

Fig. 24. Gene ontology classification (biological process) of biomarker proteins in zebrafish.

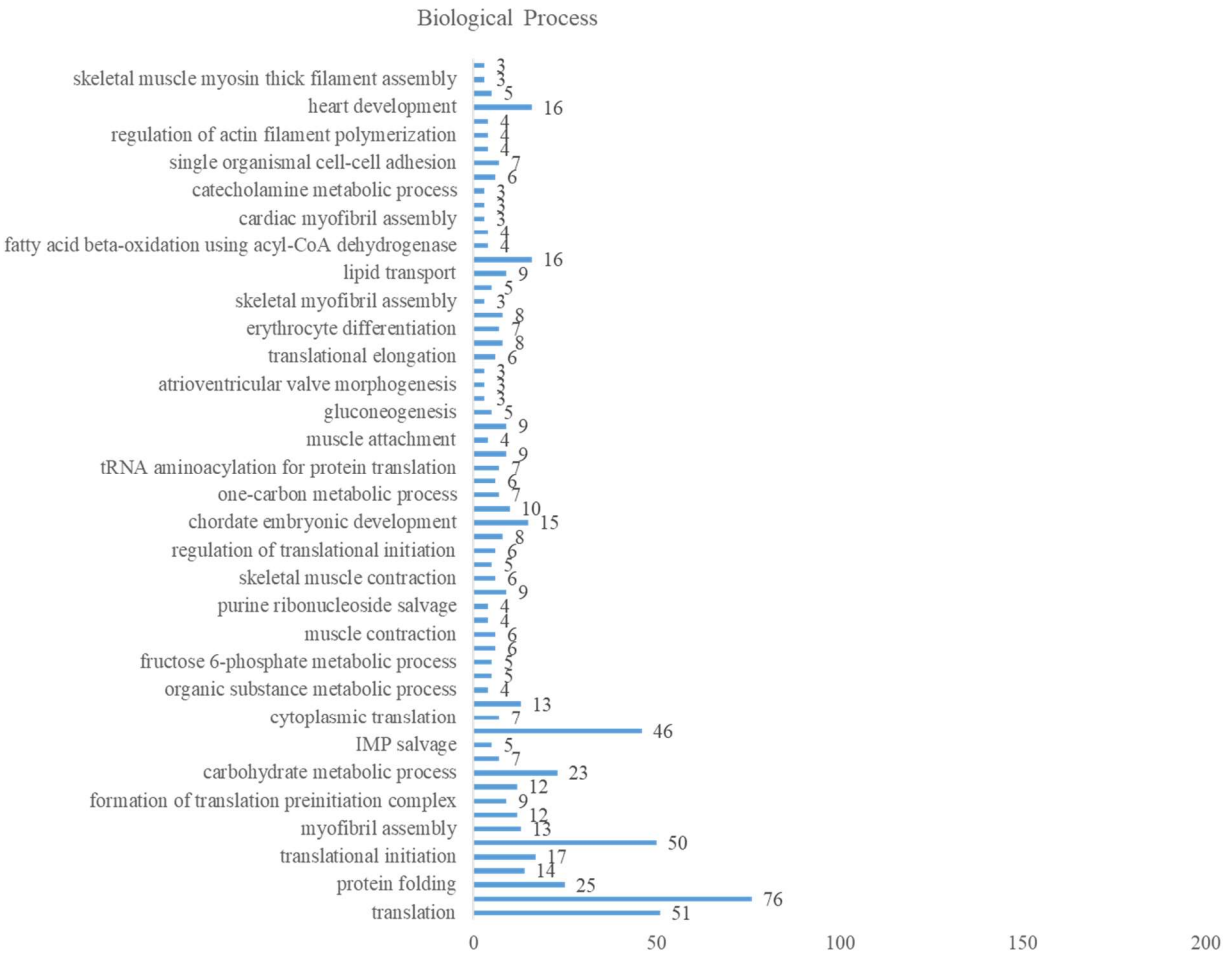

Biological Process
skeletal muscle myosin thick filament assembly
heart development
regulation of actin filament polymerization
single organismal cell-cell adhesion
catecholamine metabolic process
cardiac myofibril assembly
fatty acid beta-oxidation using acyl-CoA dehydrogenase
lipid transport
skeletal myofibril assembly
erythrocyte differentiation
translational elongation
atrioventricular valve morphogenesis
gluconeogenesis
muscle attachment
tRNA aminoacylation for protein translation
one-carbon metabolic process
chordate embryonic development
regulation of translational initiation
skeletal muscle contraction
purine ribonucleoside salvage
muscle contraction
fructose 6-phosphate metabolic process
organic substance metabolic process
cytoplasmic translation
IMP salvage
carbohydrate metabolic process
formation of translation preinitiation complex
myofibril assembly
translational initiation
protein folding
translation
0
50
100
150
200

Fig. 25. Gene ontology classification (cellular component) of biomarker proteins in zebrafish.

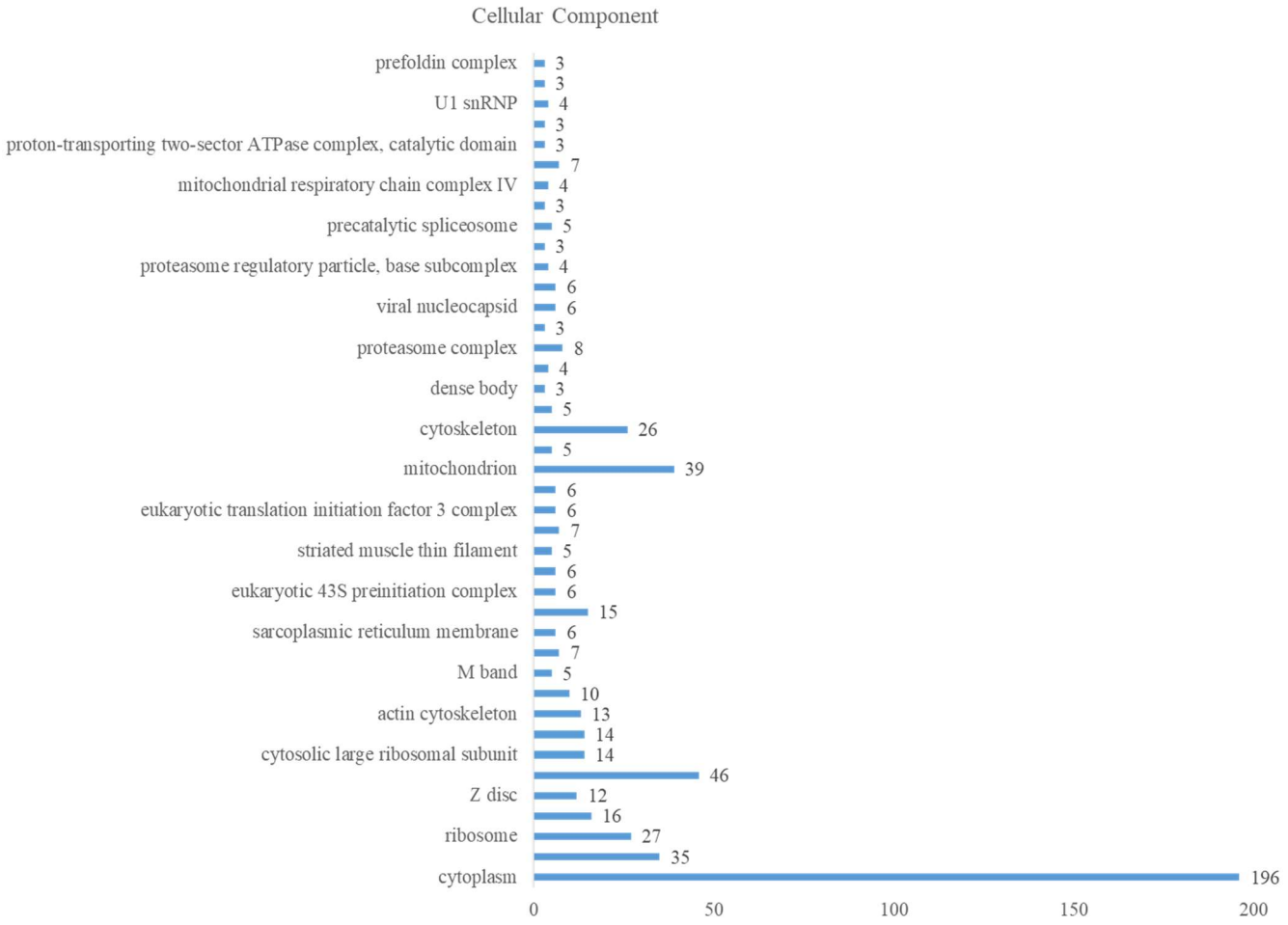

Cellular Component
prefoldin complex
U1 snRNP
proton-transporting two-sector ATPase complex, catalytic domain
mitochondrial respiratory chain complex IV
precatalytic spliceosome
proteasome regulatory particle, base subcomplex
viral nucleocapsid
proteasome complex
dense body
cytoskeleton
mitochondrion
eukaryotic translation initiation factor 3 complex
striated muscle thin filament
eukaryotic 43S preinitiation complex
sarcoplasmic reticulum membrane
M band
actin cytoskeleton
cytosolic large ribosomal subunit
Z disc
ribosome
cytoplasm
3
3
4
3
3
7
4
3
5
3
4
6
6
3
8
4
3
5
26
5
39
6
6
7
5
6
6
15
6
7
5
10
13
14
14
46
12
16
27
35
196
0
50
100
150
200

Fig. 26. Gene ontology classification (molecular function) of biomarker proteins in zebrafish

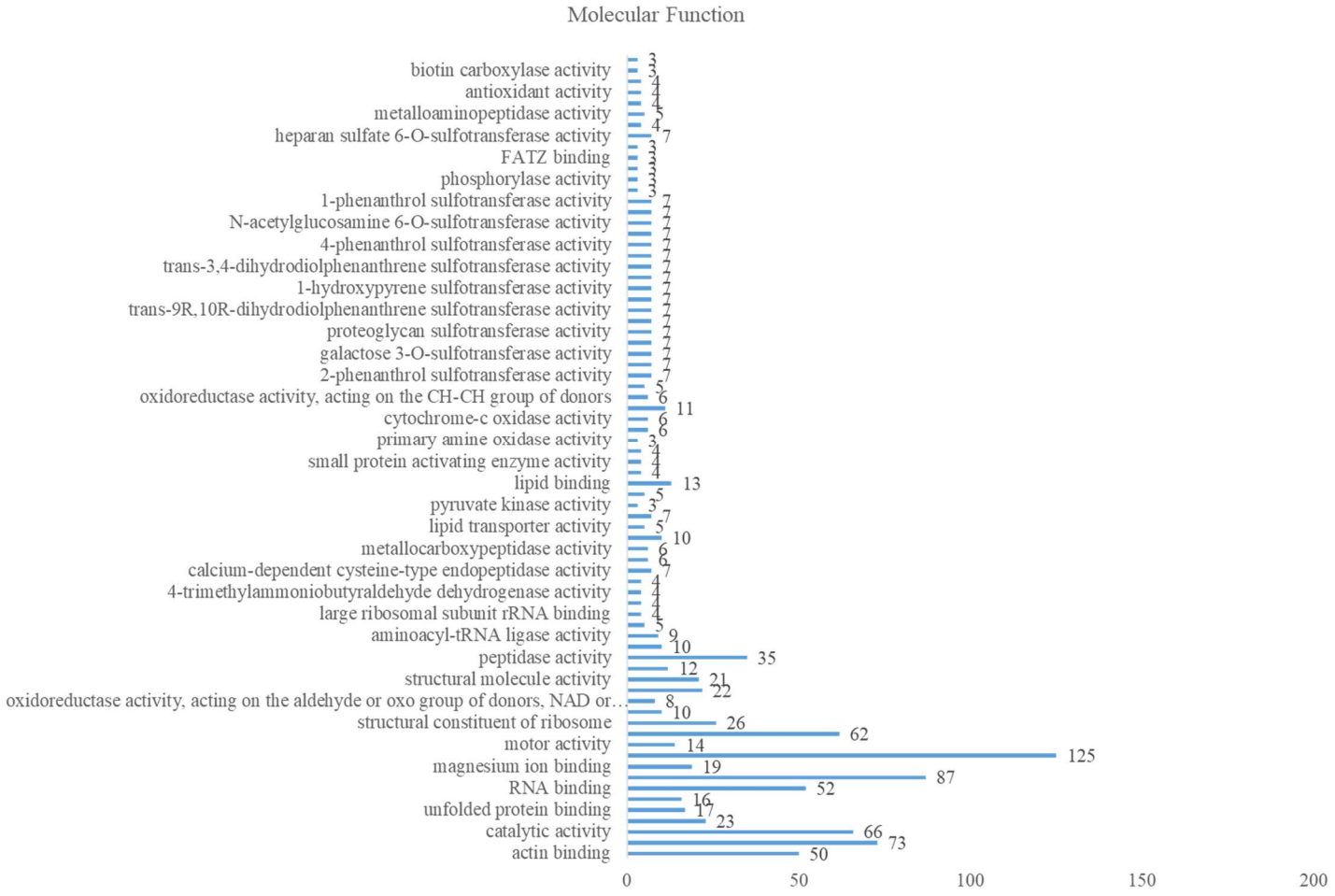

Molecular Function
biotin carboxylase activity
antioxidant activity
metalloaminopeptidase activity
heparan sulfate 6-O-sulfotransferase activity
FATZ binding
phosphorylase activity
1-phenanthrol sulfotransferase activity
N-acetylglucosamine 6-O-sulfotransferase activity
4-phenanthrol sulfotransferase activity
trans-3,4-dihydrodiolphenanthrene sulfotransferase activity
1-hydroxypyrene sulfotransferase activity
trans-9R,10R-dihydrodiolphenanthrene sulfotransferase activity
proteoglycan sulfotransferase activity
galactose 3-O-sulfotransferase activity
2-phenanthrol sulfotransferase activity
oxidoreductase activity, acting on the CH-CH group of donors
cytochrome-c oxidase activity
primary amine oxidase activity
small protein activating enzyme activity
lipid binding
pyruvate kinase activity
lipid transporter activity
metallocarboxypeptidase activity
calcium-dependent cysteine-type endopeptidase activity
4-trimethylammoniobutyraldehyde dehydrogenase activity
large ribosomal subunit rRNA binding
aminoacyl-tRNA ligase activity
peptidase activity
structural molecule activity
oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or…
structural constituent of ribosome
motor activity
magnesium ion binding
RNA binding
unfolded protein binding
catalytic activity
actin binding
0
50
100
150
200

Table 12. The specially altered metabolic pathways by protein biomarkers in honeybee.

KEGG pathways	Related protein count	Involved genes/total genes (%)	p-value
Pyruvate metabolism	3	9.1	4.50E-03
Glycolysis / Gluconeogenesis	3	9.1	7.30E-03

Table 13. The specially altered metabolic pathways by protein biomarkers in zebrafish.

KEGG pathways	Related protein count	Involved genes/total genes (%)	p-value
Biosynthesis of antibiotics	59	5.6	6.30E-14
Carbon metabolism	39	3.7	2.60E-12
Glycolysis / Gluconeogenesis	25	2.4	5.50E-09
Metabolic pathways	160	15.2	9.10E-08
Pentose phosphate pathway	14	1.3	7.80E-07
Cardiac muscle contraction	24	2.3	6.30E-06
Biosynthesis of amino acids	21	2	2.30E-05
Oxidative phosphorylation	29	2.8	2.60E-05
Propanoate metabolism	11	1	7.90E-05
Fatty acid degradation	13	1.2	2.30E-04
Valine, leucine and isoleucine degradation	14	1.3	4.30E-04
Tight junction	22	2.1	4.50E-04
Ribosome	25	2.4	5.60E-04
Pyruvate metabolism	12	1.1	9.50E-04
Spliceosome	24	2.3	1.20E-03
Fructose and mannose metabolism	11	1	2.40E-03
Degradation of aromatic compounds	4	0.4	2.60E-03
One carbon pool by folate	7	0.7	3.40E-03
Ascorbate and aldarate metabolism	7	0.7	3.40E-03

KEGG pathways	Related protein count	Involved genes/total genes (%)	p-value
Glycine, serine and threonine metabolism	11	1	4.90E-03
beta-Alanine metabolism	9	0.9	5.50E-03
Focal adhesion	35	3.3	5.80E-03
Tryptophan metabolism	11	1	6.80E-03
Histidine metabolism	7	0.7	1.00E-02
Starch and sucrose metabolism	8	0.8	1.90E-02
Glyoxylate and dicarboxylate metabolism	8	0.8	2.30E-02
Tyrosine metabolism	8	0.8	2.30E-02
Amino sugar and nucleotide sugar metabolism	11	1	2.30E-02
RNA transport	22	2.1	3.40E-02
Pentose and glucuronate interconversions	6	0.6	3.90E-02
Phenylalanine metabolism	5	0.5	4.60E-02
Proteasome	10	0.9	5.00E-02

Conclusions

In this study, the toxicological effects of abamectin in honey bees and zebrafish were confirmed using a label-free quantitative proteomic approach using LC-orbitrap-HRMS.

The honeybee and zebrafish samples exposed to 0-1.15 ng/bee (honeybee), 0-60 μg/L of abamectin for 48h, which were the same sample of the previous study (Chapter I), were homogenized, and the proteins were extracted with 0.1M phosphate buffer (pH 7.4). 200 μg of proteins were used for the in-solution trypsin digestion by FASP method. After LC-HRMS analysis and data processing with PD 2.4, a total of 652, 2155 proteins were detected and identified in honeybee and zebrafish, respectively. Through volcano analysis, 32, 1020 proteins were selected as biomarkers significantly changed after exposure to abamectin in honeybee and zebrafish, respectivley. Pathway analysis of the 32 biomarkers using DAVID and KEGG identified that pyruvate metabolism and glycolysis/gluconeogenesis were discovered in honeybee. In zebrafish, 1067 biomarkers were showed as biomarkers which were involved in serveral metabolic pathways that included carbon metabolism, biosynthesis of antibiotics, and glycolysis/gluconeogenesis etc. Based on these results, it was confirmed that when bees and zebrafish were exposed to abamectin, a protein selected as a biomarker involved in an important metabolic pathway was disturbed.

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초 록

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Abamectin은 자연적으로 발생하는 토양 방선균인 *Streptomyces avermitilis*의 발효에서 분리 된 것으로 알려진 살충제다. 꿀벌과 어류의 독성이 높기 때문에 본 연구는 가스크로마토그래피-탠덤 질량 분석법 (GC-MS/MS) 및 액체크로마토그래피-고분해능 질량 분석법(LC-orbitrap-HRMS)에 의한 비표적 및 표적 대사 체학 접근법을 사용하여 꿀벌(*Apis mellifera*) 및 제브라피시 (*Danio rerio*)에서 abamectin의 독성 메커니즘을 조사했다. 모든 균질화 된 샘플을 80 % 메탄올(꿀벌) 또는 50 % 메탄올(제브라피쉬)로 추출하고 GC-MS/MS 분석을 위해 추출액을 TMS로 유도체화반응을 적용하였다. 표적 대사체학 접근법에서는 396개의 대사산물에 대한 GC-MS/MS의 다중 반응 모니터링(MRM) 모드를 사용하여 꿀벌에서 239개의 대사산물과 제브라피시에서 243개의 대사산물을 검출했다. 각 샘플에서 검출 된 대사 산물의 결과를 바탕으로 PLS-DA 패턴분석, VIP score 및 ANOVA 분산 분석과 같은 통계 분석을 수행하여 중요한 바이오마커를 식별했다. 이러한 바이오 마커와 관련된 대사경로는 MetaboAnalyst 5.0을 사용하여 확인 할 수 있었다. GC-MS/MS를 사용하는 표적 대사체와 LC-orbitrap-HRMS를 사용하는 비표적 대사체학을 통하여 꿀벌의 노출 실험에서 tyrosine metabolism, phenylalanine/tyrosine/tryptophan biosynthesis, citrate cycle, ascorbate/aldarate metabolism, and alanine/aspartate/glutamate metabolism가 상당히 교란 된 것으로 확인되었다. 반면, 제브라피쉬는 aminoacyl tRNA biosynthesis, glyoxylate/dicarboxylate metabolism, citrate cycle, and tryptophan metabolism이 상당히 변화함을 확인하였다. Abamectin에 의한 중요한 대사산물의 심각한 교란은 꿀벌과 제브라피쉬에 생물학적으로 위험한 영향을 미칠 수 있음을 확인 할 수 있었다. 한편, 독성 단백질체학 연구에서는 꿀벌과 제브라피쉬에서 LC-HRMS를 통하여 비표지 정량을 활용한 단백질체학적 접근방식을 사용하였다. 꿀벌과 제브라피시 샘플의 단백질은 0.1M

phosphate buffer (pH 7.4)로 추출하고 200 μg의 단백질은 in-solution filter-aided sample preparation (FASP) 방법을 사용하여 트립신으로 분해하였다. LC-HRMS 분석 후 총 670개의 단백질이 확인되었고, 화산 분석을 통해 32개의 단백질이 바이오 마커로 선정되었다. 제브라 피쉬에서는 2189개의 단백질이 확인되었고 통계 분석을 통해 1050개의 단백질이 바이오 마커로 선택되었다.

주요어 : abamectin, 대사, 대사체학, 단백질체학, LC-HRMS, GC-MS/MS, 꿀벌, 제브라피쉬

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