FastD: fast detection of insecticide target-site insensitive mutations and overexpressed detoxification genes in insect populations from RNA-Seq data

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Abstract

Target-site insensitive mutations and overexpression of detoxification genes are two major mechanisms conferring insecticide resistance. Many molecular assays were applied to detect these two kinds of resistance genetic markers in insect populations. Unfortunately, these assays are time-consuming and have high false-positive rates. RNA-Seq data, which contains information on the variation within expressed regions of the genome and expression information of detoxification genes, provides us a valuable resource to detect resistance-associated markers. At present, there is no corresponding method at present. Here, we collected 66 reported resistance mutations of four main insecticide targets (AChE, VGSC, RyR, and nAChR) of 82 insect species. Next, we obtained 403 sequences of the four target genes and 12,665 sequences of three kinds of detoxification genes including P450, GST, and CCE. Here, we developed a Perl program, FastD, to detect insecticide target-site insensitive mutations and overexpressed detoxification genes from RNA-Seq data, and constructed a web server for FastD (http://www.insect-genome.com/fastd). FastD program was then applied to detect two kinds of resistant markers in five populations of two insects, Plutella xylostella and Aphis gossypii. Results showed that RyR mutation G4946E was detected in all P. xylostella populations, with higher frequencies in two resistant populations, ZZ (66.1%) and CHR (94.55%), than a susceptible population CHS (2.32%). CYP6a2 was overexpressed 10.82-fold in ZZ population. As to A. gossypii, nAChR mutation R81T was detected in resistant population KR with 49.85% frequency, but not in susceptible population NS. CYP6CY22 and CYP6CY13 were overexpressed 39.61- and 22.04-fold respectively in KR population. FastD is a program using RNA-Seq data to detect two types of resistance markers to estimate resistance level of insect populations. Generally, resistance level estimated by FastD were consistent with previous reports, confirming the reliability of this program in predicting population resistance at omics-level.

Introduction

Insect pests have a great impact on many aspects of human life. Among all of these aspects, the harm to human health and the yield loss in agricultural production are the most concerning. To make matters worse, some insects serve as medium of pathogens, spreading diseases and causing damage simultaneously. For example, *Anopheles gambiae*spread malaria and caused millions of deaths annually in Africa (Consortium, 2017). As for agricultural production, the estimated yield loss of crops due to *insect pests* is over 18% globally (Oerke, 2005).

Although there are many *insect pest* control methods available, application of insecticides is still one of the most frequently used method. Chemical insecticides were first introduced to control*insect pests* in the 1940s. Since then, thousands of insecticides have been developed to protect human health and crops. Unfortunately, long-term mismanagement of insecticide application led to the development of insecticide resistance within *insect pest*populations. So far, more than 553 insect species have been reported to have developed resistance

to approximately 331 insecticides (Gould, Brown, & Kuzma, 2018). The development of insecticide resistance necessitates the application of higher dosages of said insecticide for controlling *insect pests* , which in turn causes more serious threats to human and environmental health (Kim, Kabir, & Jahan, 2017; Tang et al., 2018). Insecticide resistance has become one of the most formidable obstacles in *insect pest* control (Gould, Brown, & Kuzma, 2018).

Insecticide target-site insensitive mutations and overexpression of detoxification gene(s) are two major mechanisms conferring insecticide resistance (Ffrench-Constant, 2013). Due to long-term selection by insecticides, the individuals containing resistance associated genotypes rapidly accumulate within populations. Generally, insecticide resistance of *insect pest* populations can be predicted according to the prevalence of target insensitive mutations and overexpression of detoxification genes (Sonoda, 2010). To date, most resistance cases occurred within five classes of insecticides: organophosphates, pyrethroids, carbamates, neonicotinoids and diamides (Thomas & Ralf, 2015). According to the modes of action listed by the Insecticide Resistance Action Committee (IRAC), organophosphates and carbamates target acetylcholinesterases (AChE), pyrethroids target voltage gated sodium channels (VGSC), diamides target ryanodine receptors (RyR), and neonicotinoids target nicotinic acetylcholine receptor (nAChR). In addition, metabolic resistances of these five classes of insecticides are mainly associated with three important detoxification gene families: cytochrome P450 (P450), glutathione S-transferase (GST) and carboxyl/cholinesterases (CCE) (L. Yan et al., 2012).

Detecting the target insensitive mutations and overexpressed detoxification genes within an *insect pest* population has long been a useful method in monitoring resistance. Many methods have been developed to detect target mutations such as PCR amplification of specific alleles (PASA) (H. H. Yan et al., 2014) and random amplified polymorphic DNA (RAPD) (Ferguson & Pineda, 2010). DNA microarray has been used detecting overexpressed detoxification genes (Mavridis et al., 2019). *However, these methods are inefficient and time-consuming.*

RNA-Seq data contains information allowing detection of single nucleotide polymorphisms (SNPs) and gene expression levels (Costa, Angelini, De Feis, & Ciccodicola, 2010). Thus, RNA-Seq data can be used to detect target-site insensitive mutations and overexpressed detoxification genes (Bonizzoni et al., 2015; De Wit, Pespeni, & Palumbi, 2015). Here,to monitor the resistance of the aforementioned five classes of insecticides, we collected reported target insensitive mutations, target gene allelic sequences, three groups of detoxification gene sequences from 82 insect species, and then developed a program, FastD, to detect target insensitive mutations and overexpressed detoxification genes from RNA-Seq data. To validate the reliability, we applied FastD to detect target-site mutations and overexpressed detoxification genes in five populations of two notorious *insect pest* species, *P. xylostella* and *A. gossypii* .

Materials and methods

Target insensitive mutation collection by literature mining

To obtain the reported insensitive mutations in four targets associated with insecticide resistance, we first collected published literature from the NCBI PubMed database. For collection of literature relevant to target mutations in VGSC, we searched against NCBI PubMed with the term: ((“VGSC” [Abstract]) OR “voltage gated sodium channel” [Abstract]) AND “insecticide resistance” [Abstract]). For collection of literature relevant to target mutations in AChE, we searched against PubMed with the term: ((“AChE” [Abstract]) OR “acetylcholinesterase” [Abstract]) AND “insecticide resistance” [Abstract]). For collection of literature relevant to target mutations in RyR, we searched against PubMed with the term: ((“RyR” [Abstract]) OR “ryanodine receptor” [Abstract]) AND “insecticide resistance” [Abstract]). Finally, for collection of literature relevant to target mutations in nAChR, we searched against PubMed with the term: ((“nAChR” [Abstract]) OR “nicotinic acetylcholine receptor” [Abstract]) AND “insecticide resistance” [Abstract]).

Resistance associated gene sequences

We collected corresponding gene sequences from 82 insect species: 26 Hymenopterans, 21 Dipterans, 14 Lepidopterans, 10 Hemipterans, 6 Coleopterans, and 5 of other orders. According to the two main mechanisms of

insecticide resistance, resistance associated genes generally include two types: target genes and detoxification genes.

To collect target gene sequences, we downloaded confirmed the full cDNA sequences of VGSC, AChE, RyR, and nAChR from InsectBase (Yin et al., 2016). Next, these confirmed target gene sequences were used as queries to BLASTP against the NCBI GenBank for each target each species. The first search step obtained target sequences for species of most orders. Then, we selected the obtained target sequences from species with annotated genome as the secondary queries to search against other species within the same order. These two step searches yielded most sequences of four targets in the tested species. For species still without target sequences, we used the target sequences from the closely related species as the tertiary queries to search against the genome of this species.

To collect detoxification gene sequences of different species as comprehensively as possible, genome official gene set (OGS) files for the species were downloaded. Then, we selected all the sequences annotated as "cytochrome P450" or "glutathione S-transferase" or "carboxyl/cholinesterase". For some important insect species without published genome OGSs, the detoxification gene sequences were obtained by directly searching against NCBI nucleotide database with terms: (((cytochrome P450) OR glutathione S-transferase) OR carboxyl/cholinesterase) AND "species name" [Organism].

RNA-Seq data

We searched the NCBI SRA database with the term "insecticide resistance," yielding a total of 94 RNA-Seq datasets. We downloaded nine RNA-Seq datasets of three *P. xylostella* populations (CHS, ZZ, and CHR) and six RNA-Seq datasets from two *A. gossypii*populations (NS and KR) for further analysis. CHS is a sensitive population while ZZ and CHR populations are resistant to chlorantraniliprole with a resistance level 42-fold and 65-fold (Zhu, Xu, Shi, Gao, & Liang, 2017). NS is a sensitive population while KR is resistant to neonicotinoids with a resistance level 23.8- to 394-fold (K. Hirata, Jouraku, Kuwazaki, Shimomura, & Iwasa, 2017).

Results

Target-site insensitive mutation profiles

By searching against the NCBI PubMed database, we obtained 440 articles reporting resistance to organophosphates and carbamates associated with insensitive mutations to AChE, 368 articles reporting resistance to pyrethroids associated with insensitive mutations to VGSC, 32 articles reporting resistance to diamides associated with insensitive mutations to RyR, and 81 articles reporting resistance to neonicotinoids associated with insensitive mutations to nAChR. Among these published insensitive mutations, 20 insensitive mutations at 17 sites on AChE were distributed amongst 36 insect species*(Supplementary, Table S1)* ; 46 insensitive mutations at 29 sites on VGSC were distributed amongst 39 insect species *(Supplementary, Table S2)* ; 6 insensitive mutations at 4 sites on RyR were distributed amongst 4 insect species*(Supplementary, Table S3)* ; 4 insensitive mutations at 4 sites on nAChR were distributed amongst 4 insect species*(Supplementary, Table S4)* . Due to the insecticide target gene sequence polymorphism among different insects, every type of target gene was aligned to the corresponding target gene of a specific insect, then the corresponding positions of all the mutations in the target gene were determined. Amino acid positions of all AChE mutations were aligned to the *AChE* in *Torpedo californica* ; amino acid positions of all VGSC mutations were aligned to the *VGSC* in *Musca domestica* ; amino acid positions of all *RyR* mutations were aligned to the *RyR* in *P. xylostella* ; amino acid positions of *nAChR* alpha1, alpha3, alpha6 and beta1 subunit mutations were aligned to the *nAChR* alpha1subunit, the alpha3 subunit in*Nilaparvata lugens* , the alpha6 subunit in *Frankliniella occidentalis* , and the beta1 subunit in *A. gossypii* , respectively (**Figure 1**).

Resistance-associated gene sequences

In total, we collected 403 insecticide target gene sequences, including 87 *AChE* sequences (41 ace1 gene sequences and 46 ace2 gene sequences), 71 *VGSC* sequences, 71 *RyR* sequences, and 174*nAChR* sequences (containing 69 alpha1 subunit sequences, 19 alpha3 subunit sequences, 15 alpha6 subunit sequences, and

71 beta1 subunit sequences). All of these gene sequences refer to 82 insect species with *ace* gene sequences belonging to 54 insect species, *VGSC* sequences belonging to 71 insect species, *RyR*sequences belonging to 71 species, and *nAChR* sequences belonging to 74 insect species.

Amongst the 82 insect species, the genome of 71 insects have been published and have annotated OGSs. In total, we extracted 11,356 detoxification gene sequences from the OGS files of 71 insect species. For the remaining 11 species, we obtained 1,309 detoxification gene sequences by searching against NCBI nucleotide database. In total, we obtained 12,665 detoxification gene sequences, including 9,260 P450 gene sequences, 2,188 GST gene sequences, and 1,217 CCE gene sequences.

The workflow of FastD program

There are two parts in the FastD program, FastD-TR (Fast Detection of Target-site Resistance) to detect target-site insensitive mutations and FastD-MR (Fast Detection of Metabolic Resistance) to detect overexpressed detoxification genes.

The workflow of FastD-TR consists of five main steps: pre-processing, mapping, mutation allele extraction, mutation allele frequency calculation, and visualization (**Figure 2**). Raw reads from RNA-Seq data should be processed by FastQC and trimmomatic (Bolger, Lohse, & Usadel, 2014) to filter out reads with low sequencing quality. The obtained clean reads are then mapped to the target gene sequence using Bowtie2 (Langdon, 2015) with additional option, –no-unal (filter out unaligned reads), to generate a Sequence Alignment/Map (SAM) file (H. Li et al., 2009). The mapped reads which contain insertions or deletions are deleted or marked with "N" respectively by parsing the CIGAR string in the SAM file. According to the mutation position in target gene and reads POS tag, mutation allele codons were extracted from mapped reads by a Perl script. Then, all the mutation allele codons are translated to amino acid residues. The reads containing the mutant amino acid residues were treated as resistant reads. *The mutation frequency can be estimated according to a formula (Després et al., 2014; D. Guo et al., 2017; Mackenzie-Impoinvil et al., 2019).* An R script called ggseqlogo (Wagih, 2017) was used to visualize the allele distribution in all of the mutation loci.

$$Mutation\ frequency\ (\%) = \frac{\text{Number of resistant reads}}{\text{Number of all reads containing mutation loci}} \times 100\%$$

The workflow of FastD-MR consists of four main steps: pre-processing, mapping, read count calculation, and differential gene expression analysis (**Figure 2**). The pre-processing step of FastD-MR is the same as what is used for FastD-TR. The obtained clean reads are then mapped to the tested detoxification gene sequences using Bowtie2 with additional parameter, –no-unal, to generate a SAM file. Read counts per detoxification gene can be calculated by a Perl Script. To estimate the expression fold change, read counts per gene from different samples are processed by DESeq2 (Love, Huber, & Anders, 2014).

Webserver

A webserver (http://www.insect-genome.com/fastd) was constructed to provide online services. The Apache HTTP server (Version 2.4.6) runs on a CentOS Linux 7.4.1708 (core) system. The Web pages were written in HTML and Cascading Style Sheets (CSS). The cDNA sequences of four kinds of insecticide targets and three groups of detoxification genes were stored in a MySQL database (Version 5.7.17). A PHP script was used to call the FastD program when the HTTP server receives the request from a Web client. Both Linux and Windows versions of the FastD standalone software are available for download. The cDNA sequences of the target genes and detoxification genes can also be downloaded from the Webserver.

Insensitive mutations and overexpressed detoxification genes in diamondback moth

We used FastD-TR to detect insensitive RyR mutations in two resistant populations, ZZ and CHR, and a sensitive population, CHS, of *P. xylostella* . The results showed that the frequencies of the insensitive mutation G4946E were 2.32%, 66.1% and 94.55% in CHS, ZZ and CHR, respectively (**Table 1**) *(30 individuals in pool,one mutated allele can be detected in a pool containing 59 wild type alleles)* . However, FastD-MR searching showed that no detoxification gene was expressed more than two-fold ($|\log2FoldChange| > 1$, P-

value < 0.01) higher in the CHR population. In contrast, six detoxification genes were overexpressed in the ZZ population (**Table 2**). Among these genes, *CYP6a2* was overexpressed 10.82-fold in ZZ population. *CYP6BG1* had elevated expression levels by 3.3-fold in the ZZ population. These genes were reported to confer chlorantraniliprole resistance (X. Li, Li, Zhu, Gao, & Liang, 2018). Our results indicated that both the insensitive RyR mutation G4946E and six overexpressed detoxification genes contribute to the resistance to chlorantraniliprole in *P. xylostella* populations.

Insensitive nAChR mutations and overexpressed detoxification genes in cotton aphid

We applied FastD-TR to detect insensitive nAChR mutations in two*A. gossypii* populations showing that no insensitive nAChR mutations were detected in the sensitive population NS; while an insensitive mutation R81T on the beta1 subunit was detected in the resistant population KR, with a frequency of 49.85% (**Table 3**)*(15 individuals in pool, one mutated allele can be detected in a pool containing 29 wild type alleles)* . By using FastD-MR, nine detoxification genes were detected with elevated expression levels more than two-fold (|log2FoldChange| > 1, P-value < 0.01) in the KR population (**Table 4**). Among these genes, *CYP6CY22* and *CYP6CY13* , which were reported to be associated with neonicotinoid resistance (K. Hirata et al., 2017), had elevated expression levels by 39.61- and 22.04-fold in the resistant KR population, respectively. These results indicated that resistance to neonicotinoids in the KR population may be conferred by the mutation R81T and nine overexpressed detoxification genes.

Discussion

Insecticide resistance monitoring is the key to sustain insecticide-mediated control efficiency. Molecular detecting assays can be used to detect resistant markers accurately at early stages to avoid resistance evolution (Network, 2016). Target-site resistance, which is mainly caused by target insensitive mutations, and metabolic resistance, which is mainly caused by overexpressed detoxification genes, are the two main mechanisms of insecticide resistance (Ffrench-Constant, 2013). The detection of these two kinds of resistance can well reveal the mechanism of resistance of insect pest populations. PCR-based target-site mutation detection assays rely on genotyping individuals one by one within an insect pest population and are not only time-consuming, but also result in a high false-positive rate (Hirayama et al., 2010; Blais et al., 2015). The DNA microarray which used to detect differentially expressed detoxification genes are inefficient and complex, because of the demand for prerequisite knowledge of the reference sequences, low resolution of expression level, *and background signals (Kogenaru, Qing, Guo, & Wang, 2012; Mantione et al., 2014).* RNA-Seq sequences the transcription products of pooled samples of insect pest populations and can obtain the SNP information in gene expressed regions as well as provide gene expression level comparison (De Wit et al., 2015). More and more researchers have adopted RNA-Seq as a method to study resistance mechanisms and detect resistant markers (David et al., 2014; Faucon et al., 2017; Mamidala et al., 2012).

Here, we developed FastD to detect the target insensitive mutations and overexpressed detoxification genes. By collecting insensitive mutations on four kinds of insecticide targets and resistance-associated gene sequences of 82 insect species, the FastD program can be applied to detect resistant markers of a wide-range of species. The webserver of the FastD program uses SAM files as input and can analyze the samples more quickly than traditional methods such as PCR or microarrays. With these characteristics, FastD program offers a wide range of applications and great value.

As a proof of concept, FastD program was used to detect the resistance-associated markers of two insects, *P. xylostella* and*A. gossypii* . The resistance of insect populations can be well estimated by these resistant markers via FastD program. The *RyR*mutation G4946E and *CYP6BG1* gene overexpression have also been reported to be associated with resistance to chlorantraniliprole (Guo, Liang, Zhou, & Gao, 2014; X. Li et al., 2018). *Interestingly, The resistance level of CHR population with higher G4946E frequency (94.55%) is higher than ZZ population with lower G4946E frequency (66.1%) and six overexpressed detoxification genes. We speculated that G4946E may play a dominant role in resistance or there are other mechanisms conferring resistance in these resistant populations.* In addition, *40 resistant allele reads among 575 all allele reads were detected in susceptible CHS population. We speculated that there may be few resistant individuals*

in CHS population. The discrepancy need further investigation. The nAChR beta1 subunit mutation R81T (Koichi Hirata et al., 2015) and overexpression of *CYP6CY22* and*CYP6CY13* genes have been reported to be associated with resistance to neonicotinoids. Moreover, four genes overexpressed in the*P. xylostella* ZZ population and seven genes overexpressed in the*A. gossypii* KR population which were not reported before are worth further study, indicating the value of FastD as a tool for both confirmation of resistance and discovery of new resistance mechanisms.

As a tool to detect resistant markers to monitor the emergence and development of insecticide resistance from RNA-Seq data, there are still some limitations. We plan to improve the following areas in the future. First, insecticide resistance with the polygene inheritance model is also associated with other important mechanisms, especially the detoxification gene amplification. Due to the limitation of RNA-Seq technique, gene amplification can't be identified by FastD-MR. We plan to add new function to identify gene amplification based on genome resequencing data. *Second, the accuracy of mutation frequency calculated by FastD-TR is limited by the fact that RNA-Seq reads from pooled sample have potentially different levels of contribution from each insect sample and allele. Therefore, we recommend users to use larger number of individuals sampled in pool to get more accurate result.* Third, the resistance level is determined empirically based on detected resistant markers by the FastD program. More quantitative relationships between the resistant markers and resistance are critical and could be established with machine learning methods. Fourth, aside from insecticide resistance, resistance in other pests (herbicide resistance and fungicide resistance) are also associated with target insensitive mutations and overexpressed detoxification genes (Bohnert et al., 2019; Q. Li et al., 2013). Estimating the resistance to herbicide and fungicide will be added in the next version of FastD program.

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Data Accessibility

The FastD program, the online version and standalone version, installation instructions, resistance related gene sequences, and a step-by-step example on how to use are freely available at the web server:*http://www.insect-genome.com/fastd . The RNA-Seq dataset used in this study is publicly available (NCBI SRA database): under project accession PRJNA359752 and PRJDB5458.*

Author contributions

F.L. conceived and designed the whole project; Z.H. improved the work; L. C. and K. L. wrote the program and did the assay with RNA-Seq data. Y. M. and Z. S. constructed the webserver of FastD; K. H. and H. X. participate the discussion; L. C drafted the manuscript. H.X. and F. L. improved and finalized the manuscript. All authors approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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Figures

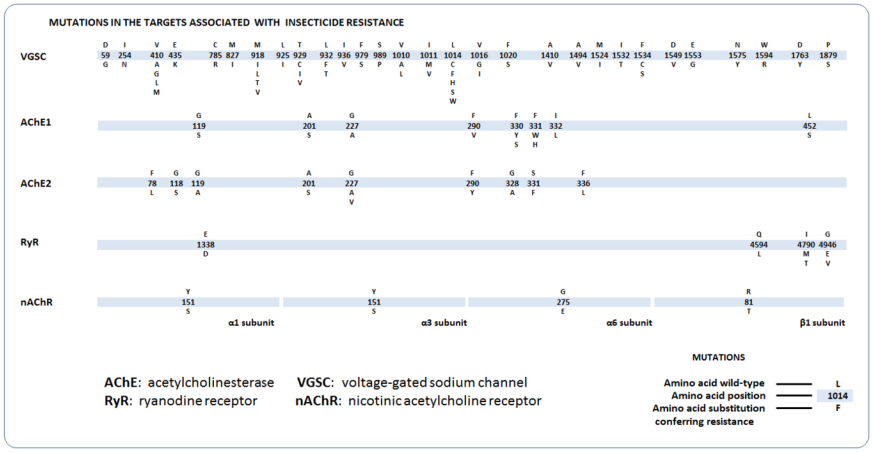

Figure 1. Mutation profiles of four insecticide targets collected by literature mining. The mutation positions in *VGSC* ,*AChE* and *RyR* were determined by alignment of corresponding cDNA sequences of *Musca domestica* , *Torpedo Californica* , and *P. xylostella* . In addition, the mutation positions in four subunits of *nAChR* , alpha1, alpha3, alpha6, and beta1, were determined by aligning the corresponding cDNA sequences of *N. lugens* , *N. lugens* , *Frankliniella occidentalis* , and*A. gossypii* , respectively.

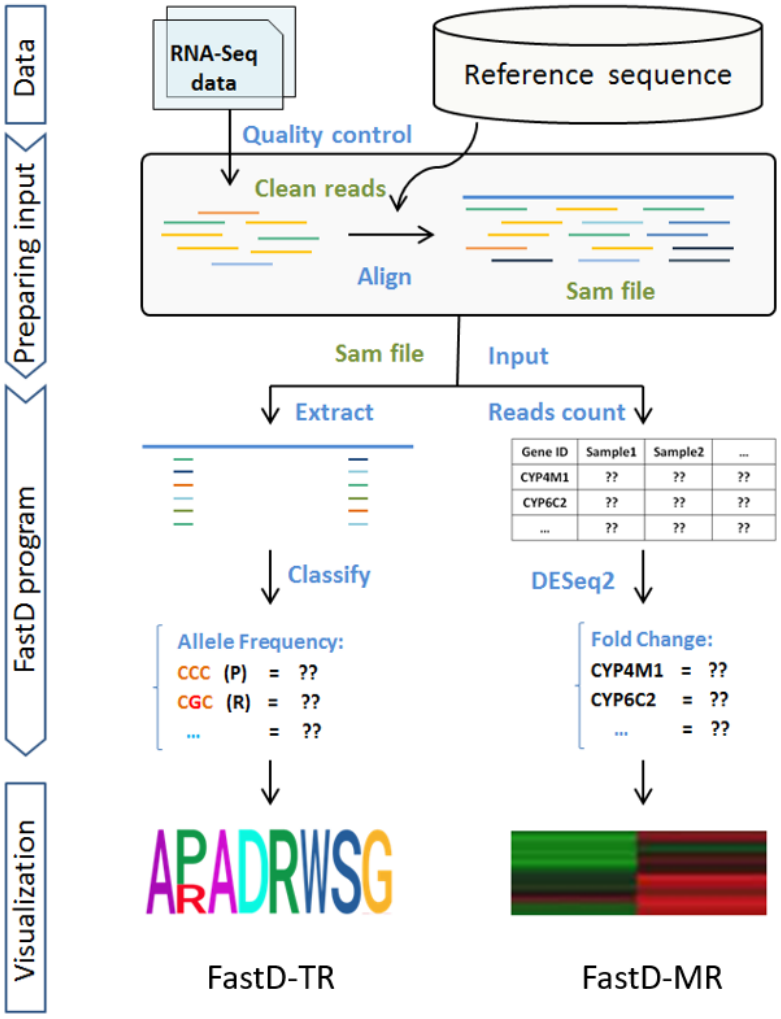

Figure 2. Workflow of the two FastD pipelines, FastD-TR and FastD-MR.The FastD program includes two pipelines, FastD-TR and FastD-MR, which detect target-site insensitive mutations and overexpressed detoxification genes, respectively. The workflow of FastD-TR is illustrated in the left part of the figure and the workflow of FastD-MR is illustrated in the right part.

Tables

Table 1 The mutation frequency of G4946E on the *RyR* gene in three *Plutella xylostella* populations

Population	Resistance level	SRA accession	Mutation R81T in beta1 subunit of *nAChR* Number of resistant reads	Mutation R8 Number of al
CHS	S	SRR5171274	0	655
		SRR5171275	0	652
		SRR5171277	40	575
ZZ	42-fold	SRR5171455	413	662
		SRR5171456	538	749
		SRR5171457	437	682
CHR	65-fold	SRR5171278	465	508

	SRR5171279	619	642
	SRR5171453	757	791

Table 2 The overexpressed detoxification genes in the*Plutella xylostella* ZZ population compared to the CHS population

Gene ID	**Annotation**	**FC**	**Log_2FC**	**SE**	*95% CI*	*P* **value**
XM_011563531.1	*CYP6a2*	10.82	3.44	0.27	*2.90-3.97*	1.45E-35
XM_011550722.1	*CYP4c21*	4.38	2.13	0.60	*0.94-3.32*	1.87E-03
XM_011567276.1	*CYP6B6*	3.73	1.90	0.13	*1.63-2.16*	2.52E-44
XM_011557040.1	*CYP6BG1*	3.27	1.71	0.14	*1.43-1.99*	1.16E-32
XM_011569337.1	*CYP6k1*	2.35	1.23	0.13	*0.97-1.49*	3.56E-20
XM_011566337.1	*CYP4C1*	2.08	1.06	0.22	*0.61-1.50*	1.68E-05

FC fold change, SE standard error, CI confidence interval.

Table 3 The mutation frequencies of R81T on the beta1 subunit of the *nAChR* gene in three *Plutella xylostella* populations

Population	**Resistance level**	**SRA accession**	**Mutation R81T in beta1 subunit of *nAChR*** **Number of resistant reads**	**Mutation R8** **Number of al**
NS	S	DRR083631	0	33
		DRR083632	0	77
		DRR083633	0	45
KR	23.8 to 394-fold	DRR083625	24	47
		DRR083626	16	31
s		DRR083627	15	32

Table 4 The overexpressed detoxification genes in the*Aphis gossypii* KR population compared to the NS population

Gene ID	**Annotation**	**FC**	**Log_2FC**	**SE**	*95% CI*	*P* **value**
XM_027986087.1	*CYP6CY22*	39.61	5.31	0.63	*4.05-6.57*	2.39E-15
XM_027986082.1	*CYP6CY13*	22.04	4.46	0.87	*2.73-6.20*	2.01E-06
XM_027998534.1	*CYP6a13*	7.00	2.81	0.45	*1.91-3.70*	1.24E-08
XM_027998535.1	*CYP6a13*	6.61	2.72	0.46	*1.80-3.65*	5.44E-08
XM_027998540.1	*CYP6a13*	4.85	2.28	0.47	*1.34-3.22*	8.14E-06
XM_027983025.1	*CYP6a14*	3.92	1.97	0.34	*1.30-2.64*	5.90E-08
XM_027985966.1	*CYP4C1*	3.21	1.68	0.28	*1.12-2.25*	4.48E-08
XM_027993601.1	*CYP6a13*	2.88	1.53	0.29	*0.95-2.11*	1.25E-06
XM_027987563.1	*CYP6a13*	2.77	1.47	0.26	*0.95-1.99*	1.47E-07

FC fold change, SE standard error, CI confidence interval.