Antimicrobial Resistance Gene Database Integration Toolkit

User manual

Contents

Overview

The Antimicrobial Resistance Gene Data Integration Toolkit (ARGDIT) consists of two main tools and three utilities for users to perform data validation and integration on antimicrobial resistance gene (ARG) databases. Basically it allows users to validate an ARG database against the coding sequence/protein information from NCBI databases, and to merge multiple validated databases into a single ARG database. It also supports re-annotating the output ARG sequences with NCBI sequence information, as well as predicting their ARG ontology class adopted from an existing ARG database (called schema database).

Main tools:

- ARG database validation tool (check_arg_db.py)
- ARG database integration tool (merge_arg_db.py)

Utilities:

- Database sequence replacement utility (replace_db_seqs.py)
- UniProt identifier to NCBI protein accession number conversion utility (convert_id_uniprot_to_ncbi.py)
- Version diff utility for ARG database (diff_with_old_ver.py)

Database eligibility

In order to use the data validation and integration tool, the ARG database (or other bacterial coding/protein sequence database) must be

1. In FASTA format
2. Every FASTA sequence header must contain an NCBI nucleotide/protein accession number. Uniprot ID is an alternative for protein accession number for protein sequence database (by converting the Uniprot IDs to protein NCBI accession numbers with the conversion utility provided)
3. ARG ontology class information, if any, must occupy at least one individual field in the sequence headers, in which all the fields are separated by the "|" symbol

Pre-requisites

The followings must be installed for the core ARGDIT operations:

1. Python version 3.5 or higher
2. BioPython version 1.70 or higher

If ARG ontology class prediction or class outlier sequence detection is required, then the followings must be installed:

1. MUSCLE version 3.8.31 or higher
2. OD-Seq
3. HMMER3 version 3.1b2 or higher

Installation

No installation is required. Make sure all the third-party software in pre-requisites are in the system path.

In order to access the NCBI databases, users must provide their own contact email addresses along with their access requests. Fill in your contact email address under the "Entrez" section in the configuration file (config.ini):

```
[Entrez]
Email = (your contact email address)
```

Important notice

All data retrieval of NCBI databases are performed through NCBI Entrez Programming Utilities, before using ARGDIT it is very important for every user to read its guidelines and requirements (https://www.ncbi.nlm.nih.gov/books/NBK25497/#chapter2.Usage_Guidelines_and_Requirement) and avoid overwhelming the NCBI servers. Based on these requirements, users are required to provide their contact email addresses (see the Installation section) so that NCBI may attempt contact before **blocking the abusing access**. Although this email address is intended for the software developers, it is more appropriate for the users to fill in their own so that they can be notified when situation happens.

Configurations

The configurable parameters for ARGDIT can be found in the configuration file "config.ini". These parameters are categorized into different sections listed in the table below:

<table>
<tr><th>Section</th><th>Parameter</th><th>Description</th><th>Default</th></tr>
<tr><td rowspan="2">ARGDIT</td><td>FastaHeaderFieldSeparator</td><td>Field separator in the FASTA sequence header</td><td>|</td></tr>
<tr><td>OperationalFieldSeparator</td><td>FASTA sequence header field separator to use during program execution; replaces original field separator (specified by FastaHeaderFieldSeparator) during operation</td><td>__</td></tr>
<tr><td rowspan="2">Ontology annotation check</td><td>MinSequenceCount</td><td>Minimum number of sequences for an ontology class to be validated or used for classification</td><td>3</td></tr>
<tr><td>BootstrapFactor</td><td>Determines the number of bootstrap iterations for sequence outlier detection according to the formula: No. of bootstrap iterations = No. of sequences in an ontology class × bootstrap factor</td><td>1000</td></tr>
<tr><td>Entrez</td><td>Email</td><td>User’s contact email address for the Entrez utilities</td><td></td></tr>
</table>

Sequence header field indexing

One-based indexing is applied to index the sequence fields. Assuming the use of the default FASTA sequence header field separator ("|"), for the sample header at the end of this section, the third field is "beta-lactamase_CTX-M-134", and the fourth field is empty string "". The fields can also be indexed from the last field back to the first field, with the last field indexed as -1, the second last field indexed as -2, and so on. For example, the field with index -4 in the sample header is "Escherichia_coli". Note that due to input limitation the negative sign "-" is replaced by "~" in the tool input argument.

Multiple fields can be extracted from the header by slice. For the sample header, by specifying 1-2 the extracted information is "JX896165|blaCTX-M-134", while "1-876|876|complete" is extracted with the slice ~1–~3.

```
JX896165|blaCTX-M-134| beta-lactamase_CTX-M-134|      |Escherichia_coli| 1-876| 876| complete
    1           2                  3               4          5            6     7      8
   -8          -7                 -6              -5         -4           -3    -2     -1
```

Usage

Database validation tool

Command

```
./check_arg_db.py [optional arguments] seq_db_path
```

Mandatory argument

`seq_db_path`	nucleotide/protein database FASTA file path

Optional arguments

`-f/--fields FIELD_NUMS`	specify the ontology label field numbers FIELD_NUMS to perform ontology class outlier sequence detection, e.g. -f 4-5, -f ~1-~3
`-r/--refine`	export refined DNA sequences
`-e/--exportlog`	export validation results and process log
`-h/--help`	show help message and exit

Description

check_arg_db.py performs ARG database validation. The --refine option allows the tool to trim at most two spurious bases before the start codon or after the stop codon, and export the trimmed sequences into an individual file specified by the tool. To perform ARG ontology class outlier sequence detection, specify the ARG ontology class fields after the --fields option. For example, the hierarchical ontology class can be extracted from MEGARes database by "-f ~1-~3". The validation results and process log are printed to stdout (i.e. screen) by default, and by specifying the --exportlog option they will be sent to a .log file in the same directory as the database file.

Database integration tool

Command

```
./merge_arg_db.py [optional arguments] -o OUTPUT_SEQ_DB_PATH seq_db_paths
```

Mandatory arguments

`-o OUTPUT_SEQ_DB_PATH`	specify the output database file path OUTPUT_SEQ_DB_PATH
`seq_db_paths`	nucleotide/protein database FASTA file paths

Optional arguments

`-s/--schema SCHEMA_DB_PATH FIELD_NUMS`	specify the schema database SCHEMA_DB_PATH and ontology label field numbers FIELD_NUMS to perform sequence ontology class prediction
`-a/--annotate`	perform automatic re-annotation using NCBI database information
`-p/--protein`	export protein sequences
`-r/--redundant`	allow redundant sequences
`-e/--exportlog`	export integration results and process log
`-h/--help`	show help message and exit

Description

merge_arg_db.py performs integration of multiple ARG databases. The --annotate option performs re-annotation of the sequences in the output database. By specifying the schema database file path and the ARG ontology class fields after the --schema option, the class labels of the output sequences will be predicted. However, note that the protein sequences of the schema database are not validated here, so it is advised to validate them using the validation tool. By default only non-redundant sequences are exported, but this can be overridden with the --redundant option. The tool provides the --protein option to translate all DNA sequences to protein sequences.

Sequence replacement utility

Command

```
./replace_db_seqs.py [optional argument] seq_db_path replace_seq_file_path
output_seq_db_path
```

Mandatory arguments

`seq_db_path`	nucleotide/protein database FASTA file path
`replace_seq_file_path`	FASTA file path for replacement sequences
`output_seq_db_path`	output database file path

Optional argument

`-h/--help`	show help message and exit

Description

By matching identical FASTA headers of the sequences, this utility replaces the sequences in the database FASTA file with those in the replacement sequence file. The database sequences, no matter replaced or not, are exported to the output database file specified by the user.

Uniprot ID conversion utility

Command

```
./convert_id_uniprot_to_ncbi.py [optional argument] seq_db_path
output_seq_db_path
```

Mandatory arguments

`seq_db_path`	FASTA file path for protein database with Uniprot IDs
`output_seq_db_path`	output file path for protein database with converted NCBI protein accession no.

Optional argument

`-h/--help`	show help message and exit

Description

This tool queries the UniProt database for the Uniprot ID to NCBI protein accession number mappings, and then replaces the UniProt IDs in the sequence headers by the mapped protein accession numbers. The processed sequences are exported to the output database file specified by the user.

Database version diff utility

Command

```
./diff_with_old_ver.py [optional argument] seq_db_path old_seq_db_path
```

Mandatory arguments

`seq_db_path`	nucleotide/protein database FASTA file path
`old_seq_db_path`	FASTA file path for previous database release

Optional argument

`-h/--help`	show help message and exit

Description

This utility compares the sequence database with its previous version, and generates a FASTA file storing all identical sequences, as well as another FASTA file storing those that appear in the current database only. Two sequences are said to be different when either their nucleotide/protein sequences or their sequence headers are different.

Known issues

It is sometimes (but not often) possible to have incomplete data retrieval from NCBI databases due to server-side issues such as heavy workload. This means information for some nucleotide/protein accession numbers cannot be retrieved at the moment; the outcome is like these accession numbers are not present in the NCBI databases. When many sequences are spuriously reported as having their accession numbers not found and/or sequence mismatches, it is advised to try using the database validation and the integration tools later.