ARG-ANNOT:

ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) is a new tool that was created to detect existing and putative new antibiotic resistance (AR) genes in bacterial genomes. ARG-ANNOT uses a local blast program in Bio-Edit software that allows the user to analyze sequences without web interface.

ARG-ANNOT database consists of a single file covering nucleotide sequences in FASTA format from all antibiotics classes. The nucleotide sequences included in this database from different antibiotics classes are abbreviated as AGly: aminoglycosides, Bla: beta-lactamases, Fos: fosfomycin, Flq: fluoroquinolones, Gly: glycopeptides, MLS: macrolide-lincosamide-streptogramin, Phe: phenicols, Rif: rifampicin, Sul: sulfonamides, Tet: tetracyclines and Tmt: trimethoprim. A unified nomenclature system was followed in which the name contains all of the information regarding gene class, gene name, accession number, gene location in the sequence and gene size. For example, (AGly) AadA1:M95287:3320-4111:792 tells the researcher that the class of antibiotics is AGly: aminoglycosides, the gene name is AadA1, the accession number is M95287, the gene location is 3320-4111, and the gene size is 792.

The nucleotide sequence file in FASTA format can be used to create a local database for blast using BioEdit. It currently uses basic sequence annotation steps such as Blast. BioEdit uses the same Blast program as NCBI on the virtual interface. The required files for a local Blast database can be created in BioEdit, based on the sequences provided in the FASTA format.

Things needed to create local database are:

- Sequence file in FASTA
- BioEdit plateform (http://www.mbio.ncsu.edu/bioedit/ bioedit.html).

Click the link to open ARG-ANNOT:

http://www.mediterranee-infection.com/article.php?laref=282&titre=arg-annot

The Page has

1)- Screen shot for help

2)- Database sequences file

3)- Link to download BioEdit

4)-Tutorial

*Download the database from the page by clicking: Database sequence file

*Open the link to download BioEdit and follow the steps:

A) S**et up a local BLAST** database with BioEdit

1. Unzip functional copy of BioEdit on a desktop PC from the following link http://www.mbio.ncsu.edu/bioedit/ bioedit.html)
2. Follow the instruction to set up BioEdit on the PC.
3. Use the downloaded database sequence file from the link to create reference database.
4. For creating local database, open BioEdit interface> select Accessory Application> Blast> Create Database> Local Nucleotide/Protein Database.
5. Navigate database sequence file and select 'Open' BioEdit will create the files necessary for a local Blast database, based on the sequences
6. The file will save to the Location of BioEdit in hard drive:\BioEdit\database folder as default installation
    - ARGANNOT
    - ARGANNOT.nhr
    - ARGANNOT.nin
    - ARGANNOT.nsq

B) - For **Blast analysis** open the BioEdit window and follow the steps below:

1. BioEdit >Accessory Application>Blast> Local Blast> select blast type (blastn,tblastn, blastp and blastx)> select nucleotide database/protein database> upload or paste query sequences in the dialogue box and hit the search button after selecting the e-value and format for the blast.
2. Selecting 'Do Search' produces a text file with much of the information of an Internet BLAST report.
3. BioEdit has an option to filter the search by selecting a different e - value, limit the search by selecting the number of hits to show and number of alignments to display and there is an additional option (go to additional parameter -n for global alignment, -m9 for tabular with details and -m8 tabular form without details).
4. There is a tremendous amount of redundancy in the BLAST output, reflecting many variants of each gene and to reduce it we can use Microsoft XL sheet, in which the blast output (-m8 tabular form without details) can be pasted and filtered using different XL functions (8).

1)- The duplicates can be removed manually through XL function
2)- Go to Data and select removal of duplicates after pasting the blast output on XL sheet obataone using –m8 option in additional parameters.
3)- The duplicates can be removed from QSS (query sequence start) and QSE (query sequence ends) lines
4)- After duplicates removal the redundancy reduced to more than 95 % , which can be further reduced by filtering the smaller length sequences and sequences with poor e-value.

* The readers can further take help from Screen shot for help to create a database and to perform Blast.

C)- The database for the chromosomal mutation genes can be constructed in a similar way using the Mutational_Gene_Database file provided in the Database sequence files and blast can be performed at higher stringent conditions (e-value:1.0E-100).