PRAP

A Python Tool for Pan Resistome Analyze Pipeline

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Contents

1. Introduction

PRAP is a platform independent Python3 tool used to analyze pan-resistome characteristics for multiple genomes. It accepts various format of sequence files as input files. The identification of antibiotic resistance genes is mainly based on the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder database.

2. What can PRAP do

1) Antibiotic resistance genes (ARGs) identification
2) Pan-resistome feature analysis
3) Classifying and analyzing for identified ARGs
4) Analysis of ARGs associated with given antibiotics

3. Installation

[1] Install Python3 v3.6+ for Win/Mac/Linux

(https://www.python.org/)

[2] Install Python3 accessory packages:

For PRAP, several Python3 packages are required. Therefore, PIP, (https://pypi.org/project/pip/) the PyPA tool for installing and managing Python packages, is recommended to install firstly if you don't have other packages management tools. For details of pip documentations, please see https://pip.pypa.io/en/stable/.

The packages required:

a) Biopython v1.7+ (https://biopython.org/)
b) NumPy v1.15+ (http://www.numpy.org/)
c) Pandas v0.23+ (http://pandas.pydata.org/)
d) SciPy v1.1+ (https://www.scipy.org/)
e) Matplotlib v3.0+ (https://matplotlib.org/)
f) Seaborn v0.9+ (http://seaborn.pydata.org/)
g) Scikit-learn v0.19+ (https://scikit-learn.org/stable/)

If you choose pip to install these packages, use "pip install XXX" for each module to install in command line interface. For example, to install Biopython module, try:

```
pip install biopython
```

You can view all packages and their versions by:

```
pip list
```

[3] Install blast v2.7.1+ for Win/Mac/Linux

Blast+ is available at https://blast.ncbi.nlm.nih.gov/Blast.cgi, you need to modify the directory of where you install blast. For example, if the blastn, blastp and makeblastdb programs are in C:/blast+/bin, please change the directory of "blast+" in "settings.txt" like:

```
[blast+=C:/blast+/bin/] #please use "/" to separate the directory
```

4. Modules of PRAP

Note: Module named with a prefix of "Ar" (example: ArKmer) refers to analyzing based on CARD database, and named with a prefix of "Res" (example: ResKmer) refers to analyzing based on ResFinder database.
Abbreviations: ARGs (antibiotic resistance genes)

[1] Preprocessing module

1) CDSex.py: extracts coding sequences from GenBank files (only for files with a ".gb" extension), forming both protein and nucleotide fasta files (files with a ".faa" and a ".fna" extension).

[2] Gene identification modules

#these modules accept sequence files as input files

1) ArKmer.py/ResKmer.py: find resistance genes from raw reads FASTQ files (files with a ".fastq" extension).
2) ArBlastn.py/ResBlastn.py: find resistance genes from nucleotide sequence FASTA nucleic acid files (files with a ".fna" extension)
3) ArBlastp.py/ResBlastp.py: find resistance genes from protein sequence FASTA amino acid files (files with a ".faa" extension)

[3] Analysis modules

#these modules accept annotation csv files genetated by "gene identification modules" as input files.

1) Pangenome.py: analyze pan-resistome features, including analysis of ARGs distribution and pan-resistome curve fitting.
2) PanAccess.py: classification and statistical analysis for all ARGs
3) ArMatrix.py/ResMatrix.py: analyze associated ARGs for each kind of antibiotics which are given in the input file "ar_phenotype.csv" or "res_phenotype.csv" for different database.

5. Input files

[1] Sequence files:

1) raw reads sequence: FASTQ files (example: A.fastq)
   a) single-end reads, the filenames should end with ".fastq"
      (If one of the input files is "A.fastq", the output filename of this genome will be written with a prefix "A")
   b) pair-end reads, the filenames end with ".1.fastq" and ".2.fastq"
      (The input filenames of strain A should be "A.1.fastq" and "A.2.fastq", the output filename of this genome will be written with a prefix "A")

   Data format (a unit of a fastq file contains four lines):

```
@READ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTT
+ READ_INFO
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC6
```

2) protein sequence: FASTA amino acid files (example: A.faa)
   (If one of the input files is "A.faa", the output filename of this genome will be written with a prefix "A")
   Data format (a unit of a fasta amino acid file):

```
>PROTEIN_ID
MKAYFIAILTLFTCIATVVRAQQMSELENRIDSLLNGKKATVGIAVWTDKGDMLRYNDH
```

3) nucleotide sequence: FASTA nucleic acid files (example: A.fna)
   (If one of the input files is "A.fna", the output filename of this genome will be written with a prefix "A")
   Data format (a unit of a fasta nuleic acid file):

```
>NUCLEOTIDE_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTT
```

4) GenBank annotation files (example: A.gb)
   (If one of the input files is "A.gb", the output filename of this genome will be written with a prefix "A")
   Data format (a unit of a GenBank file):

```
gene             complement(<1..102)
                 /locus_tag="DXN26_00005"
CDS               complement(<1..102)
                 /locus_tag="DXN26_00005"
                 /inference="COORDINATES: similar to AA
                 sequence:RefSeq:NP_460822.1"
                 /product="phage virulence factor"
                 /protein_id="PRJNA484101:DXN26_00005"
                 /translation="MKHVKSVFLAMVLILPSSLYPALTIAADSQDHKK"
```

[2] Setting file

The setting file in PRAP package (/PRAP/settings.txt) is used to set parameters for individual modules.

1) Settings for identification modules
   a) blast+ directory (e.g. [blast+=C:/blast+/bin])
   b) blast identity threshold (e.g. [identity=95])
   c) blast coverage=query length/subject length
      (e.g. [query_coverage=0.80])
   d) k value for kmer analysis (e.g. [k=25])
      #k value must be an odd number
   e) number of kernels for kmer analysis (e.g. [kernel=2])
   f) depth of k bp length reads (e.g. [depth=20])
      #only time repeats higher than depth will be calculated
   g) threshold of score of area under curve (e.g. [area_score=100])

2) Settings for analysis modules

   Graph parameters:
   #different graphs have individual parameters
   a) length of the graph (e.g. [page_length=15])
   b) width of the graph (e.g. [page_width=15])
   c) dots per inch (DPI) of the graph (e.g. [dpi=200])
   d) the font type (e.g. [font_type=Times New Roman])
   e) the font size (e.g. [font_size=20])
   f) the size of dots in graph (e.g. [dot_size=30])
      #only for the correlation matrix graph
   g) the font size of labels of genomes (e.g. [genomelabel_fsize=auto])
      #because the number of genomes may change, “auto” can change the font size of labels of genomes according to genome amounts, and you can also use an itegre to modify the parameter.

Pangenome parameters:

h) fitting coverage of pan-resistome curve (e.g. [fit_coverage=0.8])
#only the use-specified portion will be fitted, because some models exhibit better fitting performance for latter part of the curve.

i) fitting model of the curve (e.g. [fit_model=False])
#three models are provided: power_law (power law model), polyfit (polynomial model), pangp (a model used by tool "PanGP").

j) fitting order of polynomial model (e.g. [fit_order=6])
# the highest order of the polynomial model

Cluster graph parameters:

k) cluster for data in each row (e.g. [row_cluster=True])
#if "True", cluster each row; if "False", no clustering

l) cluster for data in each column (e.g. [column_cluster=True])
#if "True", cluster each column; if "False", no clustering

m) cluster method (e.g. [cluster_method=average])
#the method of cluster, please see:
https://docs.scipy.org/doc/scipy/reference/generated/scipy.cluster.hierarchy.linkage.html

Phenotype analysis parameters:

n) the name of column (e.g. [column_name=allele])
#if "allele", clustering for each antibiotic associated genes is based on gene allele; if "detail", it is based on each gene.

o) whether to show phenotype (e.g. [show_phenotype=False])
#require "res_phenotype.csv" or "ar_phenotype.csv" as an input file.
#if "True", phenotype will be shown in the front of the first column; if "False", it will not be shown.

[3] Phenotype file:

This file is needed when you want to show associated ARGs for each kind of antibiotics given in this file.

And if you want to show your experimental results of antibiotic phenotypic traits together with their associated ARGs, more information needs to be added. See below for details.

1) "ar_phenotype.csv" is needed if you want to analyze based on the CARD database. An example file is given in PRAP package. The first row of the input file list all the antibiotics in the database. You can only remain the antibiotics you want to analyze and then copy the file to the

directory of your sequence files.

An example of “ar_phenotype.csv”:

(if open the file using a text document, each item is separated by a comma “,”; if using Microsoft Excel, each item will in an individual cell):

Using a text document:

```
#name,glycopeptide antibiotic,fluoroquinolone antibiotic, tetracycline antibiotic,penam
```

Using Excel:

#name	glycopeptide antibiotic	fluoroquinolone antibiotic	tetracycline antibiotic	penam

If you want to add phenotypic traits, just write down the number of antibiotics which the strain is resistant to for each kind. If a cell is left blank, PRAP will recognize it as value “0”. An example:

Using a text document:

```
#name,glycopeptide antibiotic,fluoroquinolone antibiotic, tetracycline antibiotic,penam
A,1,,0,2
B,0,1,,3
C,,1,1,2
```

Using Excel:

#name	glycopeptide antibiotic	fluoroquinolone antibiotic	tetracycline antibiotic	penam
A	1		0	2
B	0	1		3
C		1	1	2

2) “res_phenotype.csv” is needed if you want to analyze based on the ResFinder database. The main differences are different drug classes. Modification method of it is similar to “ar_phenoty.csv”.

Please note that the proper input file is used for each module!

[4] Annotation csv files:

These files are output files of “gene identification modules”, which are name with a suffix “_ar.csv”, and they are also input files of “analysis modules”.

For example, if you input a genome file “A.fasta”, and use “gene identification module-ArBlastn.py, an output file named “A_ar.csv” will be generated, and it is the input file for three analysis modules.

6. Usage and output files

Note:

1) For one run, only files with the same extension will be analyzed. For example, if ".gb" files and ".fna" files are in one folder simultaneously, and you choose "G1-1" to analyze them, only the ".gb" files will be analyzed and ".fna" files will be ignored.
2) If you have different type of input files, and want to analyze for all of them, please select corresponding "gene identification modules" to generate all annotation csv files. And after processing all the input sequence files, select "analysis modules" to analyze all the annotation csv files (all the annotation files should also be put in the same folder).

[1] Before running

1) All sequence files should be put in the same folder.

2) If you want to analyze associate genes and their antibiotic phenotypic traits, "ar_phenotype.csv" or "res_phenotype.csv" is also needed to be put in the same folder with sequence files. Please see "5. Input files-[3] Phenotype file" for more details.

3) Modify the settings. Especially for blast directory. Please see "5. Input files-[2] Setting file" for more details.

[2] Running PRAP

1) Move to the installation directory. e.g.:

```
cd C:/PRAP/
```

2) Run PRAP

```
python PRAP.py
```

And then it will print:

```
==================================================================
=================Pan Resistome Analysis Pipeline==================
==================================================================
If you choose one of RUN ALL MODULES,
you don't need to RUN SEPARATE MODULE;
If you just want to run one module,
please see RUN SEPARATE MODULE
------------------------------------------------------------
```

```
==================================================================
RUN ALL MODULES:
------------------------------------------------------------------
# Raw reads as input files (files with a ".fastq" extension):
------------------------------------------------------------------
[R1] analysis with CARD nucleotide database
[R2] analysis with ResFinder nucleotide database
------------------------------------------------------------------
# Genbank files as input files (files with a ".gb" extension):
------------------------------------------------------------------
[G1-1] analysis with CARD nucleotide database
[G1-2] analysis with CARD protein database
[G2-1] analysis with ResFinder nucleotide database
[G2-2] analysis with ResFinder protein database
------------------------------------------------------------------
# Nucleotide seq as input files (files with a ".fna" extension):
------------------------------------------------------------------
[N1] analysis with CARD nucleotide database
[N2] analysis with ResFinder nucleotide database
------------------------------------------------------------------
# Protein seq as input files (files with a ".faa" extension):
------------------------------------------------------------------
[P1] analysis with CARD protein database
[P2] analysis with ResFinder protein database
------------------------------------------------------------------
==================================================================

==================================================================
RUN SEPARATE MODULE:
------------------------------------------------------------------
# Preprocessing module:
------------------------------------------------------------------
[a] "CDSex.py": extract coding sequence from genbank files;
      form both protein and nucleotide fasta files;
------------------------------------------------------------------
# Gene identification modules:
------------------------------------------------------------------
## Modules using CARD database------------------------------------
[b-1] "ArKmer.py": find resistance genes from raw reads;
[b-2] "ArBlastn.py": find resistance genes from ".fna" files;
[b-3] "ArBlastp.py": find resistance genes from ".faa" files;
```

```
## Modules using ResFinder database------------------------------
[c-1] "ResKmer.py": find resistance genes from raw reads;
[c-2] "ResBlastn.py": find resistance genes from ".fna" files;
[c-3] "ResBlastn.py": find resistance genes from ".fna" files;
-------------------------------------------------------------
# Analysis modules:
# Input files of Analysis modules are annotation files (files
# with "_ar.csv" suffixes) formed by gene identification modules
-------------------------------------------------------------
[d] "Pangenome.py": pan-resistome analysis
      (mainly analyze for pan-genome features)
[e] "PanAccess.py": pan & accessory resistome analysis
      (mainly classify and statistical analysis for ARGs)
## Module using CARD database------------------------------------
[f-1] "ArMatrix.py": analysis associated genes for each kind of
         antibiotics in CARD database
## Module using ResFinder database-------------------------------
[f-2] "ResMatrix.py": analysis associated ARGs for each kind of
         antibiotics in ResFinder database
==================================================================
```

3) run all modules

If you choose to run all modules, for example, there are dozens of GenBank annotation files in C:/data/, and you want to analyze them based on ResFinder nucleotide database, just type “G2-1” after “Your choice:”, like:

```
Your choice: G2-1
```

And then type the directory of input files:

```
please input the genebank files directory: C:/data/
```

All the annotation results and analysis output files will be generated in the input directory C:/data/.

4) run separate module

If you choose to run just one module, for example, to identify ARGs from dozens of raw reads files in C:/data/, and you want to analyze them based on CARD nucleotide databases, just type “b-1” after “Your choice:”, like:

```
Your choice: b-1
```

And then type the directory of input files:

```
please input the fastq files directory: C:/data/
```

Only the annotation csv files will be generated in the same folder C:/data, and for further analysis, for example, to study pan-resistome features, just run PRAP again and type "d" after "Your choice:", like:

```
Your choice: d
```

And then type the directory of annotation csv files:

```
please input the annotation csv files directory: C:/data/
```

The pangenome output files will be generated in C:/data/pangenome/.

[3] Output files

1) Outputs of gene identification modules.

   a) ArKmer/ResKmer:
   Input: C:/data/A.fastq or C:/data/A.1.fastq+C:/data/A.2.fastq

   Output_1: C:/data/kmer/A_25mer_countVSar_nucl.csv
   or C:/data/kmer/A_25mer_countVSres_nucl.csv

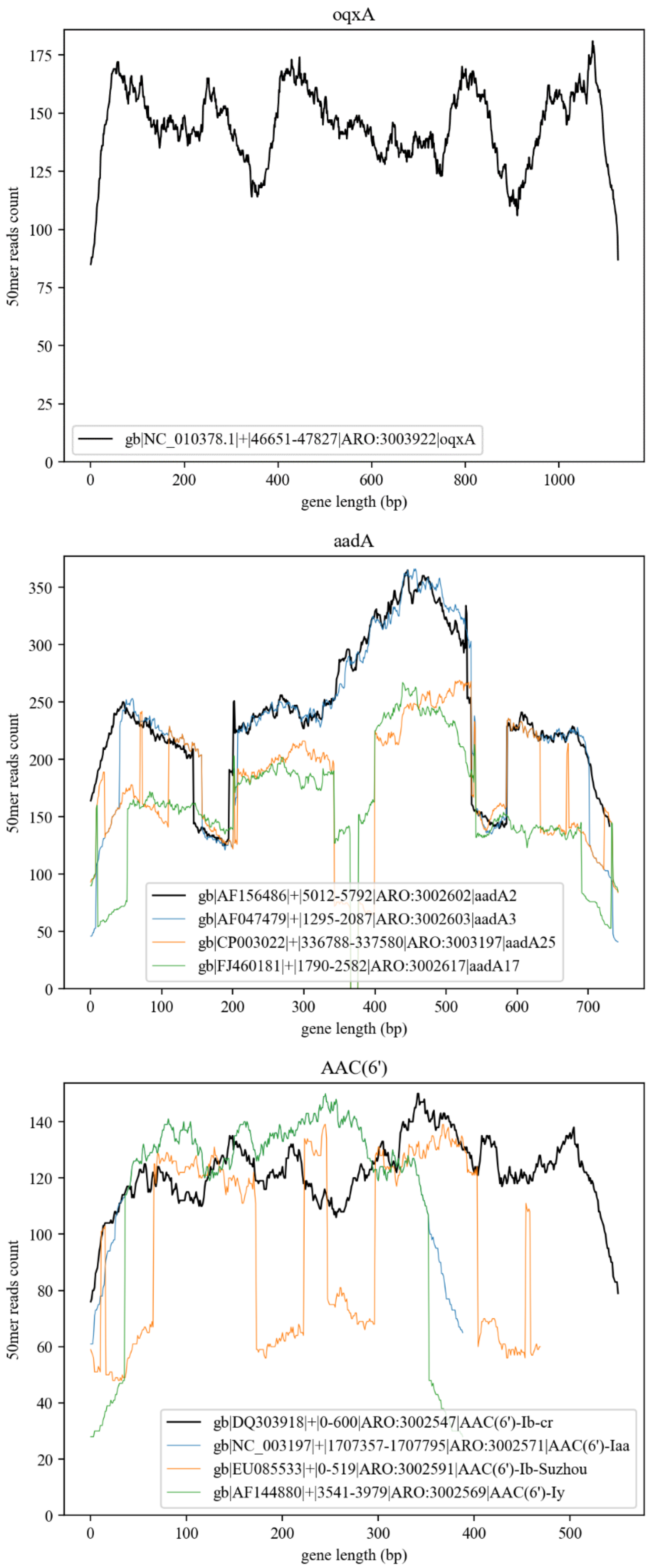
   "25" in "A_25mer_count" is the k value in the setting file, all the genes in the database beyond the threshold would be given in the file, including the gene name, the score (area under the curve of the corresponding gene in Output_2) and the coverage. The table below shows a part of the output file (Using CARD database).

A_25mer_countVSar_nucl.csv

#gene_name	area_score	coverage
gb|AM261837|+|73-865|ARO:3002619|aadA22	97763	100.00%
gb|AF047479|+|1295-2087|ARO:3002603|aadA3	118409	100.00%
gb|DQ677333|+|0-780|ARO:3002621|aadA24	106672	100.00%

   Output_2: C:/data/kmer/A/ar_nucl_25/***(allele name).png

   "25" in "ar_nucl_25" is the k value in the setting file, and for each possible gene allele in Output_1, a kmer count graph will be drawn. In each allele, if the number of genes exceeds four, only the four genes with higher scores will be drawn, and the highest one will be drawn in black thickening line. The picture below shows several typical examples (allele of oqxA, aadA and aac(6')).



***(allele name).png

Output_3: C:/data/A_ar.csv

This is the final output file. Only one gene in each allele with highest score and coverage will be present in the final annotation file. Together with information in CARD/ResFinder database, the final elements for each gene in the annotation file includes: gene_num (the gene name formed by PRAP), gene_name (the gene name in database), coverage, area_score, ARO_num (the ARO accession in CARD), accession num (the accession of related gene in NCBI), ar_gene_allele (allele of the gene), drug_class (the kind of antibiotics the gene conferring according to the database) and ar_machanism (the mechanism of the gene recorded in the database). Note that only CARD has information of AR0_num and ar_mechanism. The table below shows a part of the annotation csv file.

A_ar.csv

#gene_num	gene_name	coverage	area_score	ARO_num	accession_num	ar_gene_allele	drug_class	ar_mechanism
A_AMRgene_0	OXA-1	100	113192	ARO:3001396	JN420336.1	OXA	cephalosporin; penam	antibiotic inactivation
A_AMRgene_1	APH(3')-Ia	99.47849	68005	ARO:3002641	BX664015.1	APH(3')	aminoglycoside antibiotic	antibiotic inactivation
A_AMRgene_2	AAC(3)-IV	100	90553	ARO:3002539	DQ241380.1	AAC(3)	aminoglycoside antibiotic	antibiotic inactivation

b) ArBlastn/ResBlastn

Input: C:/data/A.fna

Output_1: C:/data/AVSar_nucl.xls or C:/data/AVSres_nucl.xls

The output of blast+ using command "blastn -query A.fna -db ar_nucl/res_nucl -out AVSar_nucl/res_nucl.xls -outfmt "6 std slen" -evalue 1e-20 -perc_identity identity (in the setting file)".

Output_2: C:/data/arg/A_ar.fna

The sequence of ARGs identified from the input files will be written into a new fasta nucleic acid file. The below shows part of the file.

```
>A_AMRgene_1_from_Scaffold32 [oqxA|identity:100.000|ARO:3003922|
NC_010378.1|position:1-1176]
TCAGTTAAGGGTGGCGCTG......CCAGGTTTTTGCAGGCTCAT
>A_AMRgene_2_from_ Scaffold45 [AAC(6')-Ib-cr|identity:100.000|ARO:3002547|
DQ303918|position:1-555]
GTGACCAACAGCAACGATT......GAACACGCAGTGATGCCTAA
```

Output_3: C:/data/A_ar.csv

A similar annotation csv file to kmer output_3. The differences are replacing "coverage" with "identity" and "area_score" with "e_value". In order to locate the gene in the scaffolds, the origin of the gene together with start and end positions are added to the annotation file. The table below shows a part of the annotation csv file.

A_ar.csv

#gene_num	gene_name	identity	e_value	ARO_num	accession_num	ar_gene_allele	drug_class	ar_mechanism	origin	start	end
A_AMRgene_1_from_Scaffold32	oqxA	100	0	ARO:3003922	NC_010378.1	oqxA	fluoroquinolone…	antibiotic efflux	Scaffold32	1	1176
A_AMRgene_2_from_Scaffold45	AAC(6')-Ib-cr	100	0	ARO:3002547	DQ303918	AAC(6')	Fluoroquinolone…	antibiotic inactivation	Scaffold45	1	555

c) ArBlastp/ResBlastp

Input: C:/data/A.faa

Output_1: C:/data/AVSar_prot.xls or C:/data/AVSres_prot.xls

The output of blast+ using command "blastp -query A.faa -db ar_prot/res_prot -out AVSar_prot/res_prot.xls -outfmt "6 std slen" -evalue 1e-20 -num_alignments 6".

Output_2: C:/data/arg/A_ar.faa

The sequence of ARGs identified from the input files will be written into a new fasta amino acid file. The below shows part of the file.

```
>Protein_id1 [oqxA|identity:100.000|ARO:3003922|YP_001693237.1]
MSLQKTWGNIHLTALGAMM……GMPVNAKTVAMTSSATLN
>Protein_id2 [AAC(6')-Ib-cr|identity:99.457|ARO:3002547|ABC17627.1]
MTNSNDSVTLRLMTEHDLAM……AVYMVQTRQAFERTRSDA
```

Output_3: C:/data/A_ar.csv

A similar annotation csv file to kmer output_3. The differences are replacing "coverage" with "identity" and "area_score" with "e_value". Because there is no need to intercept amino acid sequences, so the origin, start and end positions are not added comparing with Ar/ResBlastn results, the original name of protein will remain. The table below shows a part of the annotation csv file.

A_ar.csv

#gene_num	gene_name	identity	e_value	ARO_num	accession_num	ar_gene_allele	drug_class	ar_mechanism
Protein_id1	oqxA	100	0	ARO:3003922	YP_001693237.1	oqxA	fluoroquinolone…	antibiotic efflux
Protein_id2	AAC(6')-Ib-cr	100	0	ARO:3002547	ABC17627.1	AAC(6')	Fluoroquinolone…	antibiotic inactivation

2) Outputs of analysis modules

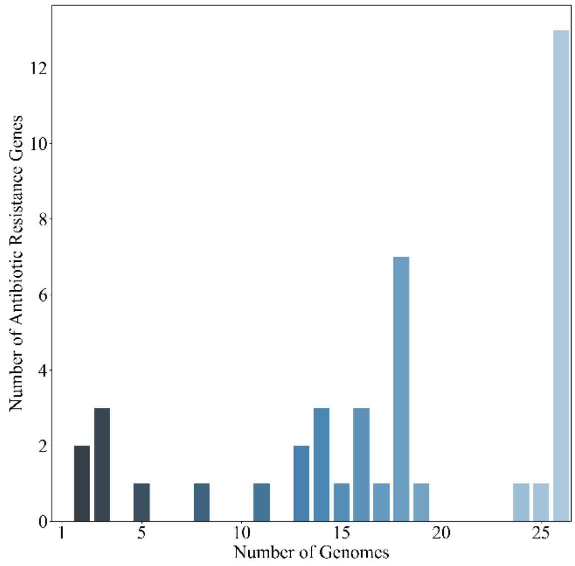
a) Pangenome

Input: A batch of annotation csv files (C:/data/A_ar.csv+B_ar.csv+C_ar.csv+……)

Output_1: C:/data/pangenome/ 4_ar_distribution.txt
& C:/data/pangenome/ 4_ar_distribution.png

Count the number of occurrences of each gene allele in each genome. For example, the alleles behind number "26" refers to the allele which has appeared in 26 genomes.

```
1
2    rmtB,QepA
3    rpoB,mphA,Mrx
…
25   ramR
26   parC,parE,soxR,soxS,MdtK,mdsC,mdsB,mdsA,golS,gyrA,gyrB,sdiA
```

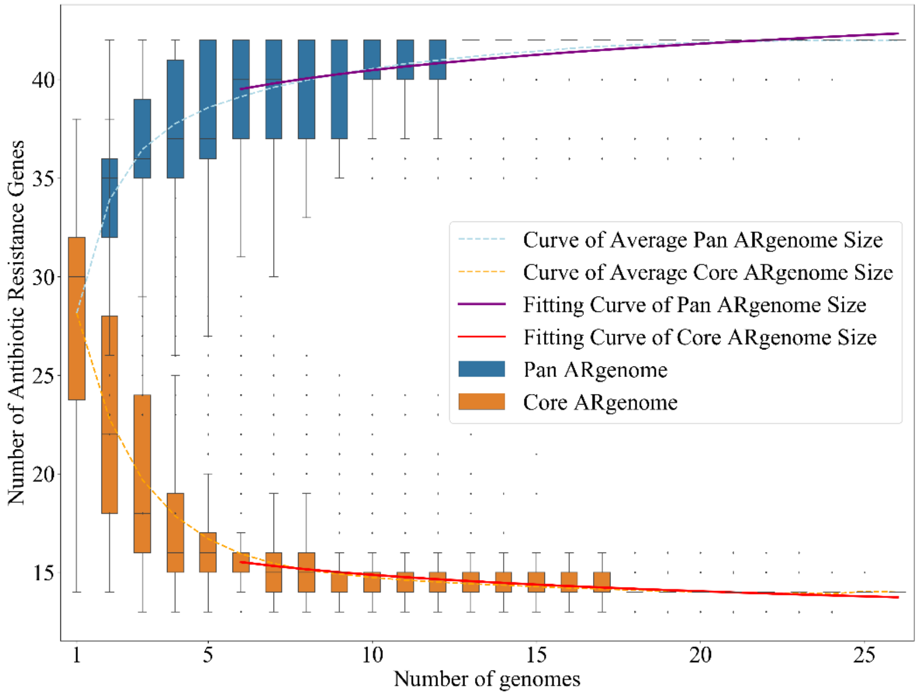


4_ar_distribution.png

Output_2: C:/data/pangenome/5_pangenome.txt
& C:/data/pangenome/5_pangenome.png
& C:/data/pangenome/5_pangenome _fitmodel.txt

Count the pan genome size and core genome size for antibiotic gene alleles. It traverses all combinations of given genomes and form a text document. A boxplot is drawn according to the data and the model in the setting file is applied for curve fitting. The final model together with R^2 value is also written into a text document.

```
Genome_Number      Pan_Genome_Size      Core_Genome_Size
      1                   30                   30
      1                   34                   34
      1                   31                   31
      …                   …                    …
      25                  41                   14
      25                  41                   15
      26                  41                   14
```



5_pangenome.png

```
Power Law Model of PanGenome:
P=(36.331034418648684)*x^(0.0469962382291344)
(R^2=0.9534322181666640470632029231)

Power Law Model of CoreGenome:
C=(18.021585517236634)*x^(-0.08327794834525747)
(R^2=0.9211436740795695974874930157)
```

b) PanAccess

Input: A batch of annotation csv files
(C:/data/A_ar.csv+B_ar.csv+C_ar.csv+……)

Output_1: C:/data/analysis/1_class_summary.csv
& C:/data/analysis/1_mech_summary.csv
& C:/data/analysis/1_class_summary.png
& C:/data/analysis/1_ar_cluster.png
& C:/data/analysis/1_ar_corr.png

Analyze all the ARGs for all genomes, and classify them into different drug classes and mechanism classes. A stacked bar graph, a cluster map and a comparison graph are drawn according to summary of drug classes.

1_class_summary.csv

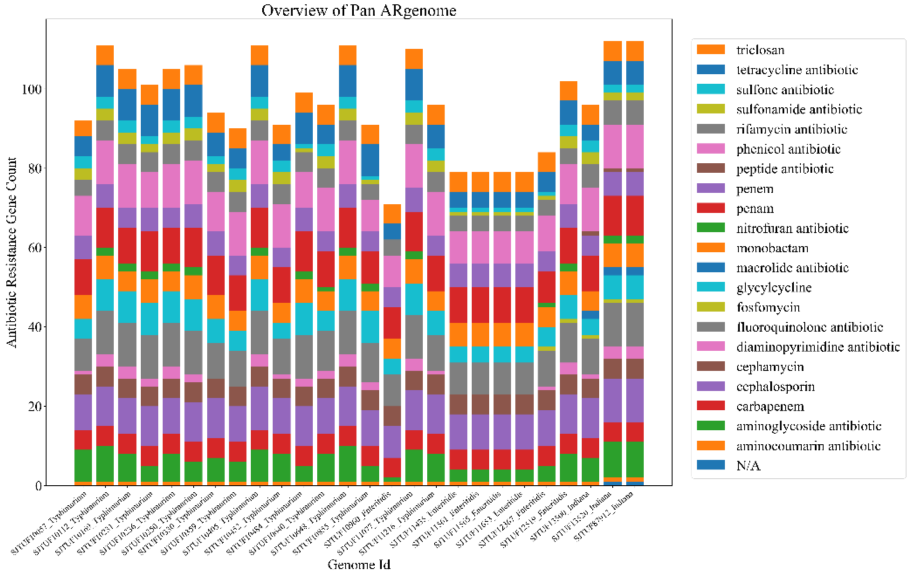
name	aminocoumarin antibiotic	aminoglycoside antibiotic	carbapenem	cephalosporin	…
A	1	8	5	9	…
B	1	9	5	10	…
C	1	4	5	10	…

#Each number in the cell represent the number of antibiotic associated ARGs in each genome.

1_mech_summary.csv

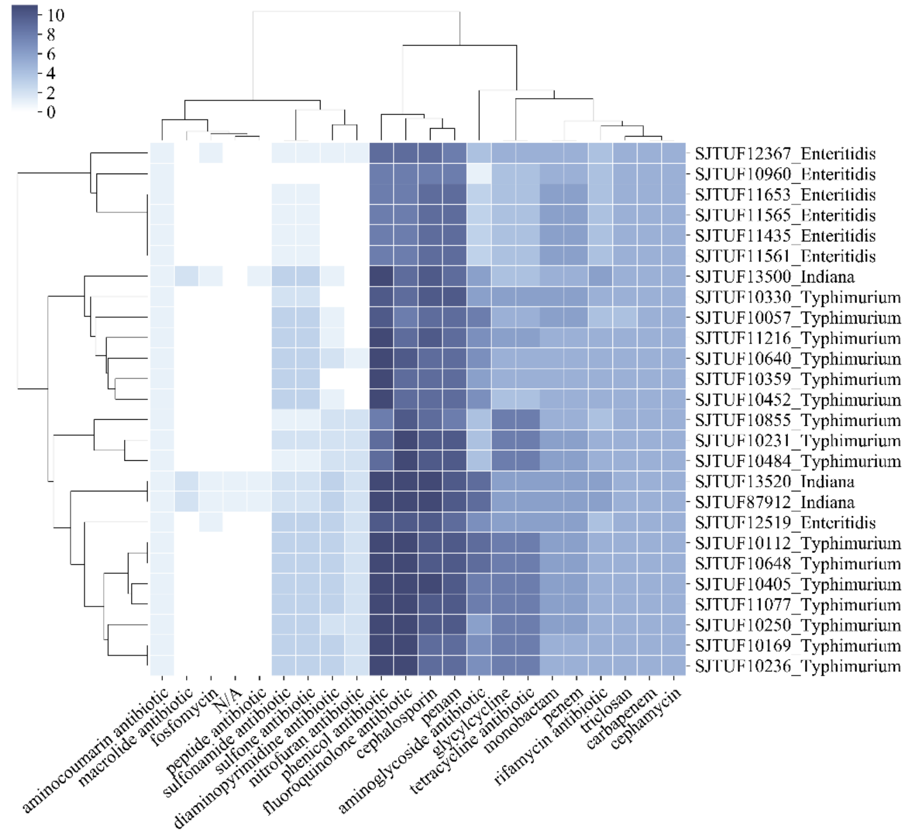
name	antibiotic efflux	antibiotic inactivation	antibiotic target alteration	antibiotic target replacement	…
A	12	12	7	4	…
B	15	13	8	4	…
C	15	10	8	4	…

#Each number in the cell represent the number of of ARGs related to each kind of antibiotic resistance mechanisms in each genome.



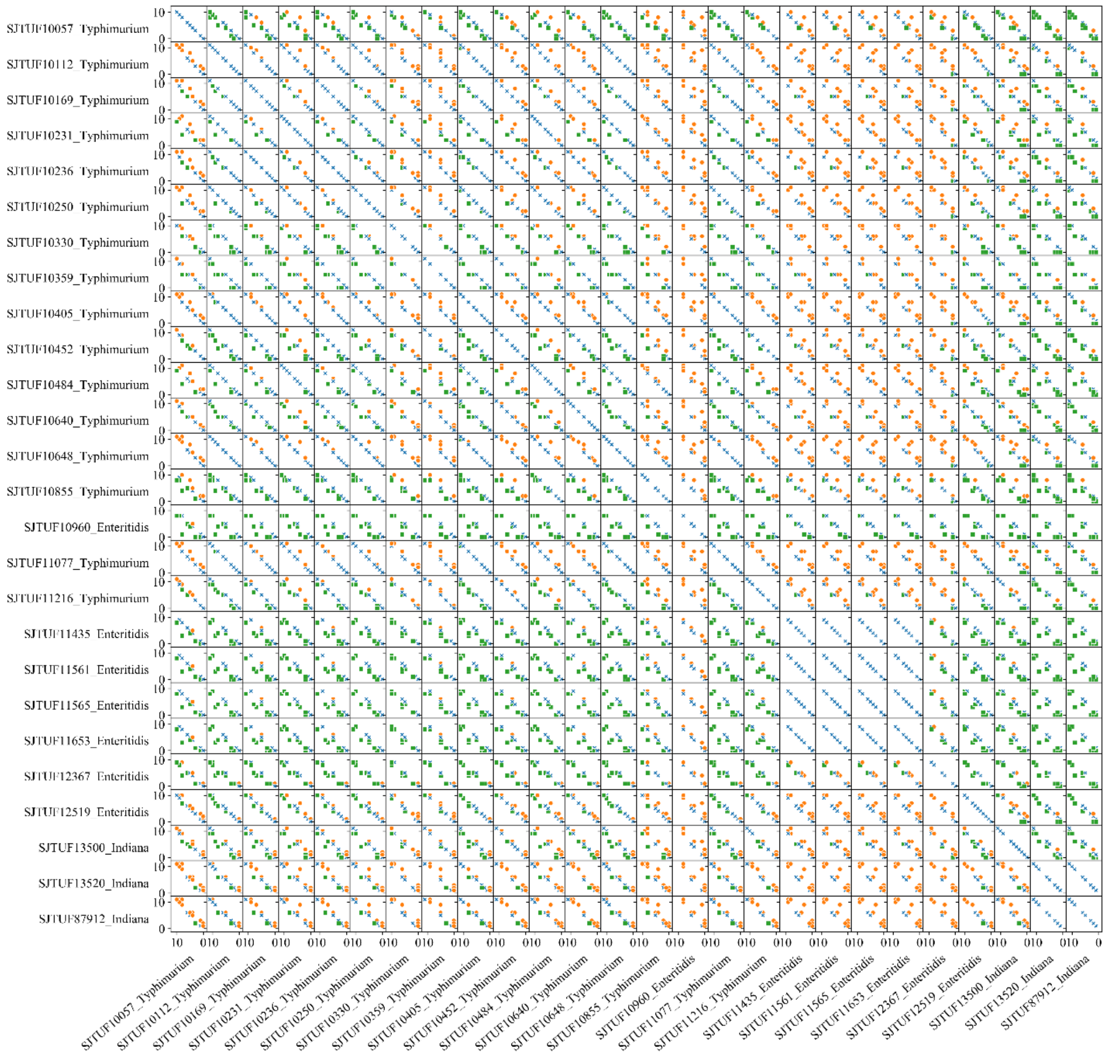
1_class_summary.png

#A stacked bar graph based on 1_class_summary.csv. Different colors represent different kind of antibiotics



1_ar_cluster.png

#A cluster map based on 1_class_summary.csv. The darker the color, the more the number of related genes



1_ar_corr.png

#A comparison matrix graph based on 1_class_summary.csv. Each subgraph represents a comparison of resistance genes in two genomes. Each dot represents an antibiotic, and dots in orange means the number of associated ARGs of the antibiotic in the genome in y axis is higher than that in x axis, while blue means equal and green mean lower.

Output_2: C:/data/A_ar_accessory.csv
& C:/data/analysis/2_accessory_class_summary.csv
& C:/data/analysis/2_accessory_class_summary.png
& C:/data/analysis/2_accessory_ar_cluster.png
& C:/data/analysis/2_accessory_ar_corr.png

The output_2 is similar to output_1, and the difference is that the data used is accessory ARgenome instead of pan-resistome. A_ar_accessory.csv is the annotation file that excludes all core ARGs from A_ar.csv, and their elements are all the same.

d) ArMatrix/ResMatrix

Input: A batch of annotation csv files
(C:/data/A_ar.csv+B_ar.csv+C_ar.csv+……)
& "C:/data/ar_phenotype.csv" or "C:/data/res_phenotype.csv"

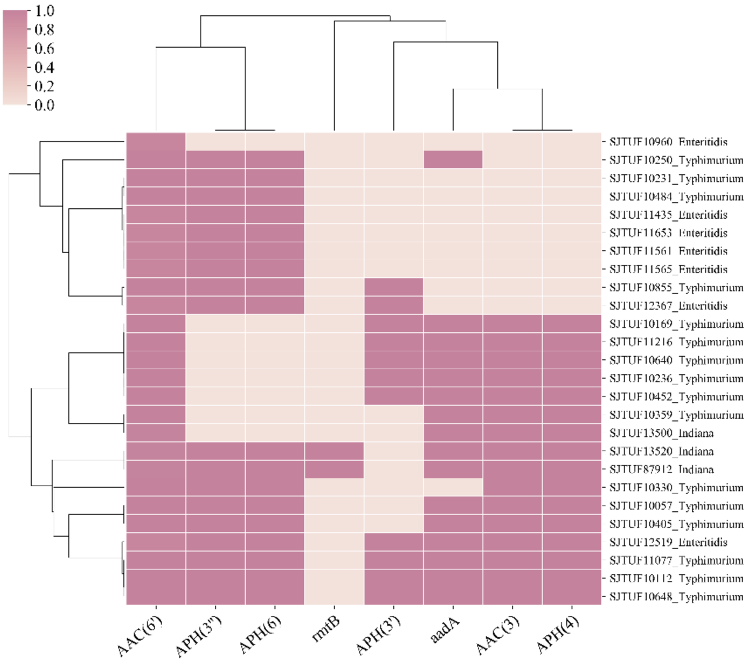
Output: C:/data/analysis/3_***_matrix.csv
& C:/data/analysis/3_***_matrix.png
& C:/data/analysis/3_***_contrivution.txt

"***" is the name of antibiotics given in the first row of "ar_phenotype.csv" or "res_phenotype.csv". For example, if one of the antibiotics given is "aminoglycoside antibiotic", the output name will be "3_aminoglycoside antibiotic_matrix". The details are shown below:

3_aminoglycoside antibiotic_matrix.csv

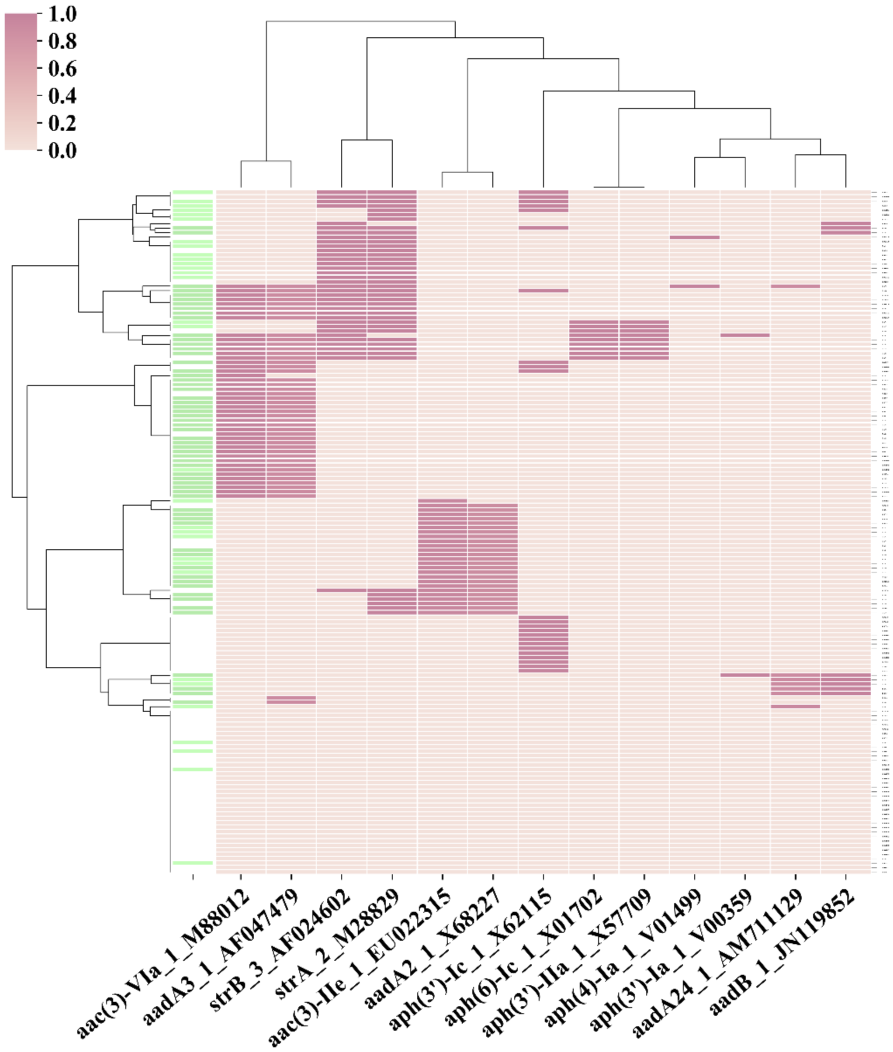
name	AAC(6')	aadA	AAC(3)	APH(4)	APH(3'')	APH(6)	APH(3')	rmtB	PHENOTYPE
A	1	0.99747	0.99871	1	0.99751	0.99881	0	0	N/A
B	1	0.99621	0.99871	1	0.99751	0.99881	0.99386	0	N/A

#Genes or alleles (according to settings [column_name=detali] or [column_name=allele]) in the first row are all genes or alleles identified from all genomes, which are related to the given antibiotic. The number in each cell is the identity or coverage of the gene in annotation files.



3_aminoglycoside antibiotic_matrix.png

#A cluster map based on the matrix file above ([column_name=allele]).



3_aminoglycoside antibiotic_matrix.png

#A cluster map for another hundreds of genomes using setting [column_name=details]. The phenotypic triats information are added (see "Input files for more details"), so a green column is drawn to represent antibiotic resistance phenotype.

3_aminoglycoside antibiotic_contribution.txt

```
aph(3')  0.14229004048910107
oqxA     0.07805108832073461
tet  0.0742476898364476
……
```

# The contribution of each allele to the antibiotic is predicted by the random forest method in the scikit-learn API. For example, the contribution of aph(3') is 14.23%, the highest one among all genes, indicating the strong correlation with aminoglycoside antibiotics resistance.