Instruction manual of MiPRIME platform

1. Introduction

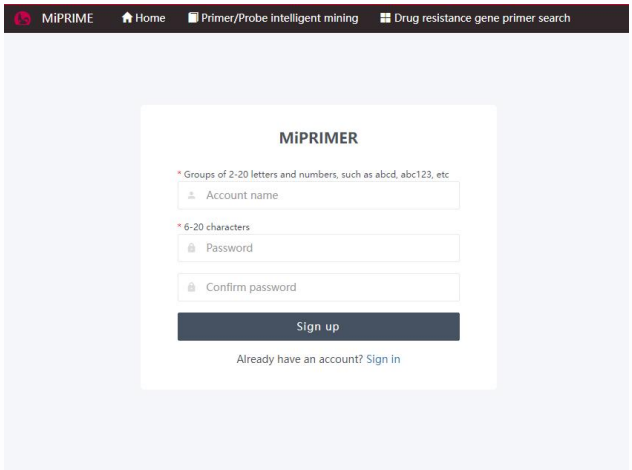
MiPRIME(https://www.ai-bt.com), a real-time Microbial Primer Mining platform for primer/probe sequences extraction of pathogenic microorganisms, reducing time and trial-and-error costs, and filling a gap in the pan-microbial field of literature-sourced primer platforms. The Platform instruction manual is designed to help users get familiar with and master the basic functions and use methods of this website. This website provides users with rich information and convenient interactive experience, through this manual, you will be able to easily get started, make full use of the various functions of the website.

The platform can be visited by computers and mobile phones, and supporting international common browsers, such as chrom, safari, firefox, edge and so on.

2. Register and Login

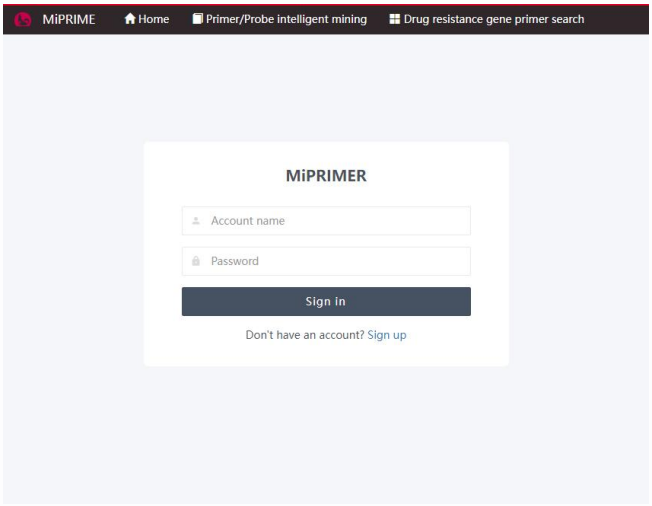
2.1 Register

Click the "Sign up" button on the homepage of the website and fill in the necessary personal information, including Account name and password.



2.2 Login

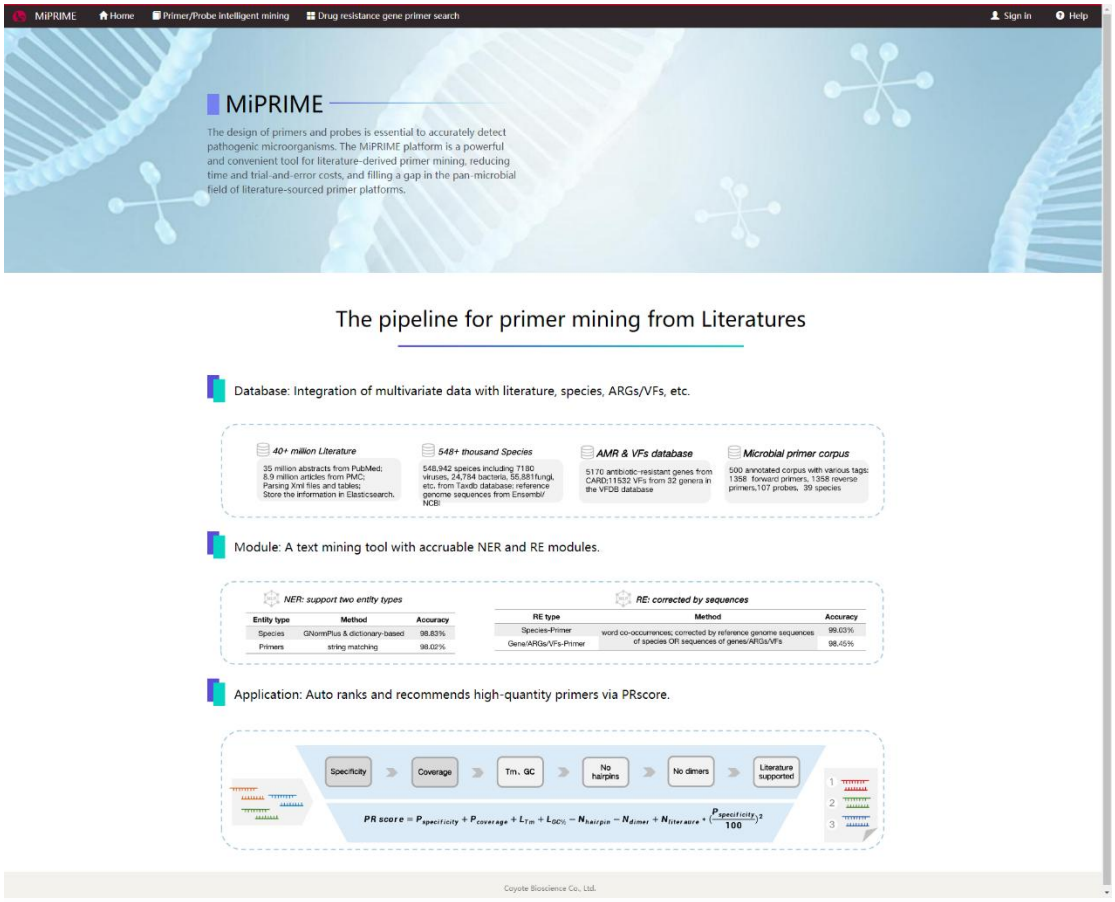
Please enter the account name and password that you fill in during registration into the "Sign In" box on the homepage of the website and click on the "Sign In" button. After successfully login, you will be able to access and use all the functions of the website.



3. Main Functions of MiPRIME

3.1 Home Page

The MiPRIME homepage mainly provides a summary of the platform overview and design highlights.



The pipeline of the MiPRIME platform consists of three major steps.

First, database construction, to construct a Pan-species primer/probe mining platform we built a comprehensive in-house database as data sources for worldwide primer/probe information, which including 3 databases of i) a biomedical literature database with 40 million open-access articles that provide sources of worldwide published pan-species microbial primer/probe sequences; ii) a pan-species sequence database with 548,942 microorganism reference genomes used to validate the accuracy of discovered primer/probe sequences; and iii) AMR and virulence factors (VFs) database for the annotation the resistance and toxin genes of target microbial species.

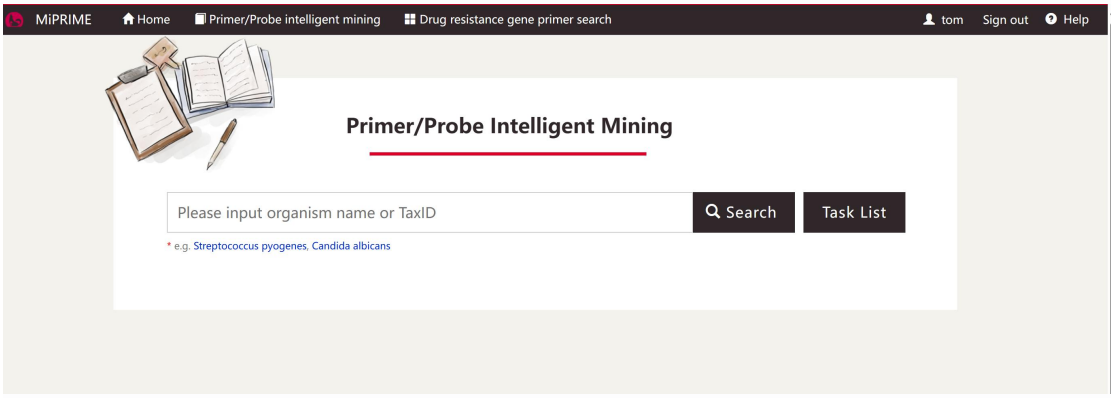
Secondly, primer/probe extraction model, to accurately identify targeting primer/probe information from massive literatures, we developed an BioBERT based text mining model that was fine-tuned using our in-house manually annotated corpus. In this model, entity recognition methods such as regular expression matching, dictionary-based matching, and GNormPlus were used for the identification of primers and species. And the association between them were supported and confirmed by both word co-occurrence analysis and sequence alignment.

Thirdly, best primer/probe recommendation, for auto-recommendation of species-specific primers with best coverage and high PCR success rates, we proposed a primer evaluation metric, PRscore, which is a weighted sum of primer specificity, coverage, GC content, Tm, hairpin, dimer, etc. Usually, primers with the highest PRscores were preferred.

3.2 Primer Probe Intelligent Mining

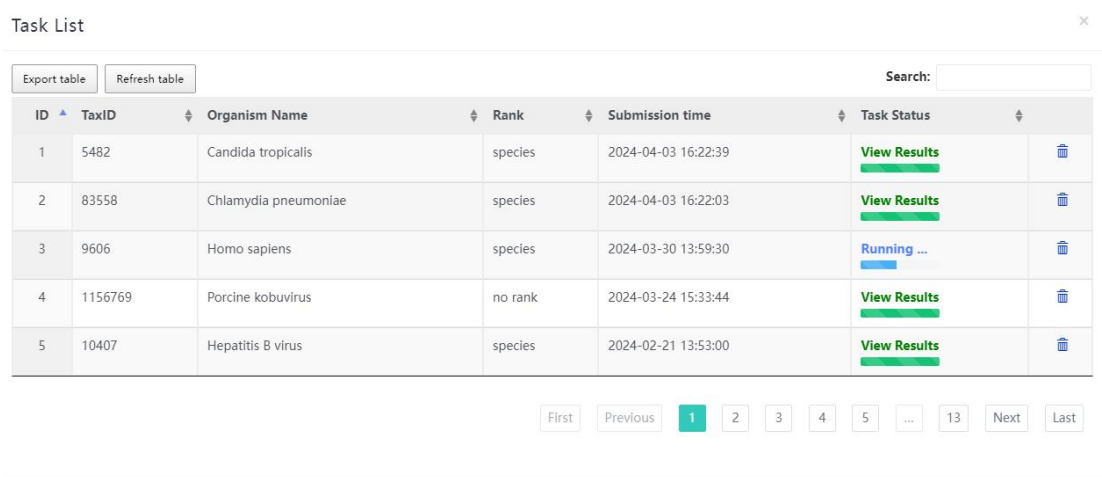
3.2.1 Search interface of MiPRIME

Users can enter the species name (eg. *Streptococcus pyogenes*) or species TaxID (eg. 1314) in search box, and the search supports auto-completion function.



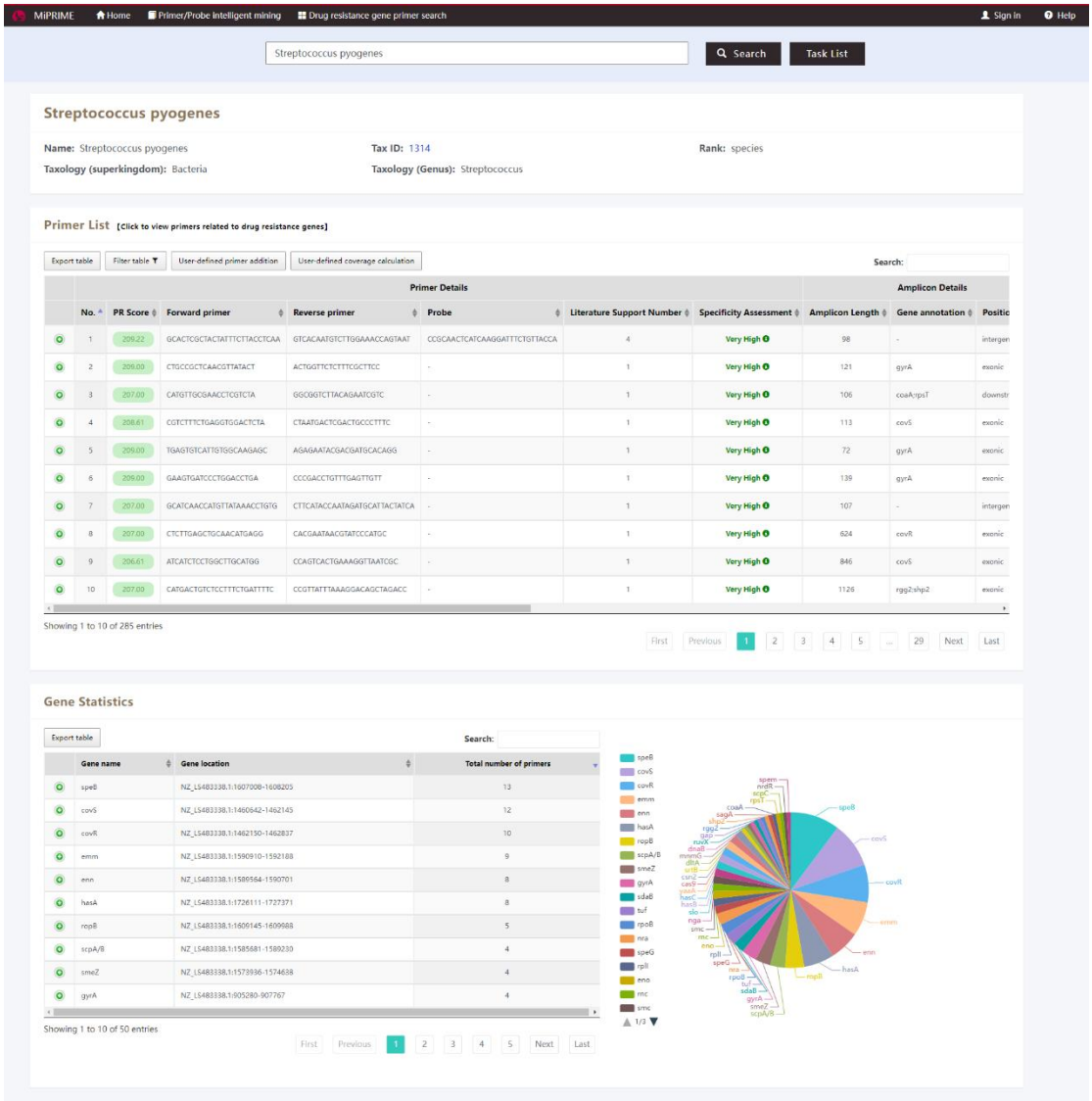
3.2.2 Task List

After the user submitted the query, the project enters the Task List, which records the task submission time and task status in detail. When Task Status displays "running", it means that the project is running; when Task Status displays "View Results", it means that the project results are
available.



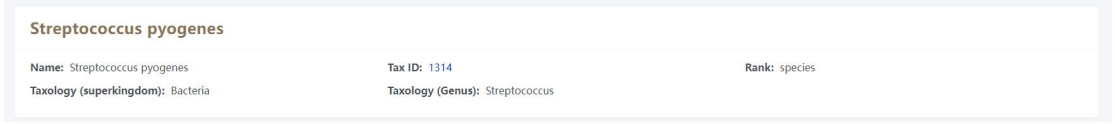
3.3 Platform results interface

The result page of MiPRIME is divided into 3 sections: species overview, primer list, and gene statistics.



3.3.1 Species overview

This section presents some basic information about the species, such as Name, Tax ID, Rank, Super kingdom, and Genus. In particular, the Tax id links to the NCBI website (eg. *Streptococcus pyogenes*, https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1314), which provides detailed information about the species.



3.3.2 The summary list of literature source primers

3.3.2.1 Primer table Details

	Primer Details						
	No.	PR Score	Forward primer	Reverse primer	Probe	Literature Support Number	Specificity Assessment
	1	209.22	GCACTCGCTACTATTTCTTACCTCAA	GTCACAATGTCTTGGAAACCAGTAAT	CCGCAACTCATCAAGGATTTCTGTTACCA	4	Very High
	2	209.00	CTGCCGCTCAACGTTATACT	ACTGGTTCTCTTTCGCTTCC	-	1	Very High
	3	207.00	CATGTTGCGAACCTCGTCTA	GGCGGTCTTACAGAATCGTC	-	1	Very High
	4	208.61	CGTCTTTCTGAGGTGGACTCTA	CTAATGACTCGACTGCCCTTTC	-	1	Very High
	5	209.00	TGAGTGTCATTGTGGCAAGAGC	AGAGAATACGACGATGCACAGG	-	1	Very High
	6	209.00	GAAGTGATCCCTGGACCTGA	CCCGACCTGTTTGAGTTGTT	-	1	Very High
	7	207.00	GCATCAACCATGTTATAAACCTGTG	CTTCATACCAATAGATGCATTACTATCA	-	1	Very High
	8	207.00	CTCTTGAGCTGCAACATGAGG	CACGAATAACGTATCCCATGC	-	1	Very High
	9	206.61	ATCATCTCCTGGCTTGCATGG	CCAGTCACTGAAAGGTTAATCGC	-	1	Very High
	10	207.00	CATGACTGTCTCCTTTCTGATTTTC	CCGTTATTTAAAGGACAGCTAGACC	-	1	Very High

(1) PRscore

Primer Ranking score, a weighted primer evaluation metric to assess the performance of a primer. The table is sorted by default in descending order of the PRscore, which was calculated using the following formula:

$$PRscore = P_{specificity} + P_{coverage} + L_{Tm} + L_{GC\%} - N_{hairpin} - N_{dimer} + N_{literaure} * (\frac{P_{specificity}}{100})^2$$

where "$P_{specificity}$" refers to the percentage of the primers successfully mapped to the reference genome; "$P_{coverage}$" refers to the percentage of the sequences successfully aligned; "L_{TM}" refers to whether the value of Tm is between 50 -80℃, where 1 is yes, 0 is no; "$L_{GC\%}$" refers to whether the GC content is between 40-60%, also 1 is yes, 0 is no; "$N_{hairpin}$" refers to the sum of forward primer hairpin score level and reverse primer hairpin score level; the primer hairpin score leverl refers to whether the ΔG of hairpin calculated by MFEprimer is less than 4, when 1 is yes, 0 is no. "N_{dimer}" refers to the sum of forward primer self-dimer score level, reverse primer self-dimer score level, and cross-dimer score level of forward/reverse primer score; the dimer score level refers to whether the ΔG of hairpin calculated by MFEprimer is less than 4, when 1 is yes, 0 is no. "$N_{literaure}$" refers to the number of supported articles. Here we modify the literature weights by the specificity of the primers, since primers with lower specificity usually have higher numbers of supported references.

Among these variables, the $P_{specificity}$ of the primer was computed as follows, and divided into four levels: (1) Very High: 100. (2) High: ⩾90. (3) Moderate: ⩾50. (4) Low: <50.

$$P_{specificity} = \frac{Num_{genome\ numbers\ that\ successfully\ mapped\ to\ at\ the\ query\ species\ level}}{Num_{genome\ numbers\ that\ successfully\ mapped\ to\ on\ spieces-wide\ genomes}} \times 100$$

(2) Literature number

The number of supported articles

(3) Specificity Assessment

Primer specificity scores are obtained as follows: a) comparing primer sequences (F/R) against the whole species reference genomes by a software, blastn. b) counting the number of species or subspecies reference genomes that primers successfully mapped to. c) calculating the percentage of successful sequence alignment.

The primer specificity scores are graded into four levels: a) Very High: the specificity score is 100. b) High: the specificity score ⩾ 90. c） Moderate: the specificity score ⩾ 50. d) Low: the specificity score <50.

Primer specificity assessment

Result of primer specificity assessment

Forward primer	GCACTCGCTACTATTTCTTACCTCAA	Reverse primer	GTCACAATGTCTTGGAAACCAGTAAT
Primer specificity score	100	Primer specificity grade	Very High

Details of the primer alignment results

Bacteria (superkingdom)	240
Terrabacteria group (clade)	240
Firmicutes (phylum)	240
Bacilli (class)	240
Lactobacillales (order)	240
Streptococcaceae (family)	240
Streptococcus (genus)	240
Streptococcus pyogenes (species)	240

(4) Amplicon Details

This section presents primer target sequence amplicon lengths, gene annotations, and positional information.

Amplicon Details			NCBI species sequence alignment	
Amplicon Length	Gene annotation	Position	Length of species sequences (median)	Number of species sequences
98	-	intergenic	1849988.5	512
121	gyrA	exonic	1849988.5	512
106	coaA;rpsT	downstream|dist=629	1849988.5	512
113	covS	exonic	1849988.5	512
72	gyrA	exonic	1849988.5	512
139	gyrA	exonic	1849988.5	512
107	-	intergenic	1849988.5	512
624	covR	exonic	1849988.5	512
846	covS	exonic	1849988.5	512
1126	rgg2;shp2	exonic	1849988.5	512

(5) NCBI species sequence alignment

This section records the number of sequences of the queried species and the median sequence length of the species.

(6) Coverage

Primer coverage refers to the proportion of target sequences in the available database that

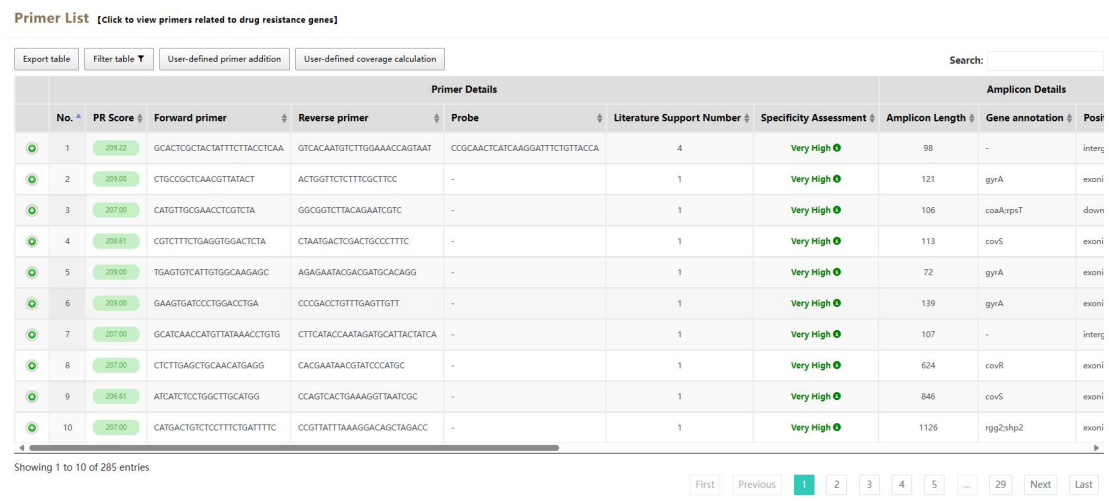
are trapped by the primer (full-length sequence). -1bp-2bp-3bp means fine-tuning the primer sequence, that is, shortening the length of the primers by 1bp, 2bp and 3bp separately.

Coverage (complete alignment)		Coverage-1bp		Coverage-2bp		Coverage-3bp		User-defined	
Forward	Reverse	Forward	Reverse	Forward	Reverse	Forward	Reverse	Add to Favorites	Notes
98.44%	99.61%	99.22%	99.61%	99.22%	99.61%	99.22%	99.61%	★	
100%	100%	100%	100%	100%	100%	100%	100%	★	
100%	96.88%	100%	100%	100%	100%	100%	100%	★	
99.61%	99.61%	99.61%	99.61%	99.61%	99.61%	99.61%	99.61%	★	
100%	100%	100%	100%	100%	100%	100%	100%	★	
100%	99.61%	100%	100%	100%	100%	100%	100%	★	
99.61%	99.22%	100%	100%	100%	100%	100%	100%	★	
99.61%	100%	100%	100%	100%	100%	100%	100%	★	
99.61%	99.22%	99.61%	100%	99.61%	100%	99.61%	100%	★	
100%	91.21%	100%	100%	100%	100%	100%	100%	★	

（7）User-defined

This section allows the user to label the primers according to their needs.

3.3.2.2 Six functional modules of Primer List



（1）Function 1 Forms Download

Users can click on Export table , which enables the form to be downloaded.

（2）Function 2 Filter Table

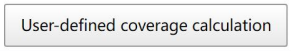
By clicking on Filter table , users can filter the primers according to its properties, such as amplicon length, literatures support number, availability of probe sequence (yes or no), and specificity assessment.

（3）Function 3 User-defined primer addition

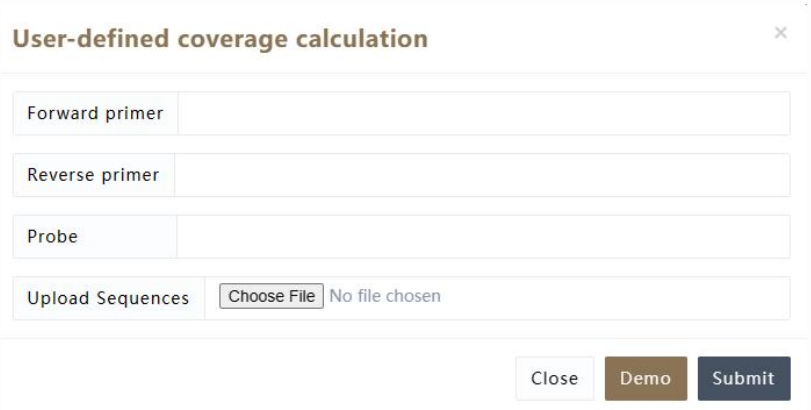
By clicking 

, users can add primers and probes in a user-defined way. When the input sequence is incorrect, a prompt will be given: Adding failed, please check if the sequence format is incorrect.

（4）Function 4 User-defined coverage calculation

Users can click a button 

to perform primer coverage calculations. Support users to enter forward primer, reverse primer and probe in the entry box. Or upload related sequence (fasta format files).



（5）Function 5 Search Box

The search box

allows the user to search for primers, probes, etc.

（6）Function 6 Additional information about primers

Users can click on the Expand button  on the primer sheet to obtain some additional information about that primer, including the position of the primer on the Reference genome, amplicon details, the supporting reference for the primer and primer assessment. Click on the title of the reference (in blue), which jumps to the full text and automatically tags the primer and

probe location in the article. The primer assessment section provides a detailed record of probable hairpin structures, self-dimers, cross-dimers, etc. for both forward and reverse primers. The ΔG of hairpins and dimers are calculated by MFEprimer.

1. Primer Position

Reference genome: GCF_900475035.1
Forward primer position: NZ_LS483338.1:818695-818720
Reverse primer position: NZ_LS483338.1:818623-818648

2. Amplicon Details

Amplicon position: NZ_LS483338.1:818623-818720
Amplicon length: 98
Gene annotation: amaP;truB [location: intergenic|dist=2589;dist=6981]
Amplicon sequence:

```
GTCACAATGTCTTGGAAACCAGTAATAGTAGTCAAAAATTGGTAACAGAAATCCTTGATGAGTTGCGGAAATTTGAGGTAAGAAATAGTAGCGAGTGC
```

3. References (4 in total)

1. Real-time polymerase chain reaction for microbiological diagnosis of parapneumonic effusions in Canadian children.
   Published Date: 2014-05 Journal: Can J Infect Dis Med Microbiol Impact Factor: 2.585
2. Identification of Streptococcus suis Meningitis by Direct Triplex Real-Time PCR, Burkina Faso.
   Published Date: 2020-09 Journal: Emerg Infect Dis Impact Factor: 16.126
3. Molecular Detection of Streptococcus pyogenes by Strand Invasion Based Amplification Assay.
   Published Date: 2018-10 Journal: Mol Diagn Ther Impact Factor: 4.476
4. Performance of the Biomark HD real-time qPCR System (Fluidigm) for the detection of nasopharyngeal bacterial pathogens and Streptococcus pneumoniae typing.
   Published Date: 2019-04-24 Journal: Sci Rep Impact Factor: 4.996

4. Primer Assessment

	Primer/Probe Sequence	Sequence Length (bp)	GC (%)	Tm (°C)
Forward primer	GCACTCGCTACTATTTCTTACCTCAA	26	42.31	65.99
Reverse primer	GTCACAATGTCTTGGAAACCAGTAAT	26	38.46	65.15

```
Primer F hairpin【ΔG = 9.88 kcal/mol】
/-/-/-------\-\-\--------
GCACTCGCTACTATTTCTTACCTCAA

Primer R hairpin【ΔG = 6.89 kcal/mol】
------//--//--------\\--\\
GTCACAATGTCTTGGAAACCAGTAAT

Primer F self-dimer:【Score: 2, ΔG = -1.04 kcal/mol】
                     GCACTCGCTACTATTTCTTACCTCAA
                     ::
AACTCCATTCTTTATCATCGCTCACG

Primer R self-dimer:【Score: 3, ΔG = 0.85 kcal/mol】
                    GTCACAATGTCTTGGAAACCAGTAAT
                    ::.::
TAATGACCAAAGGTTCTGTAACACTG

Cross-dimer (Primer F × Primer R):【Score: 4, ΔG = 1.33 kcal/mol】
GCACTCGCTACTATTTCTTACCTCAA
               ::::
              TAATGACCAAAGGTTCTGTAACACTG
```

TABLE 1

Real-time polymerase chain reaction 5′ exonuclease assays used for pleural fluid specimen testing

Organism	Target gene	Primer (5′ – 3′) Forward	Reverse	Probe
Streptococcus pneumoniae	lytA	ACGCAATCTAGCAGATGAAGCA	TCGTGCGTTTTAATTCCAGCT	AACGCTTGATACAGGGAG[*]
Streptococcus pyogenes	spy	GCACTCGCTACTATTTCTTACCTCAA	GTCACAATGTCTTGGAAACCAGTAAT	CCGCAACTCATCAAGGATTTCTGT
Haemophilus influenzae	hpd	GGTTAAATATGCCGATGGTGTTG	TGCATCTTTACGCACGGTGTA	TTGTGTACACTCCGTTGGT[*]
Staphylococcus aureus	nuc	AAATTACATAAAGAACCTGCGACA	GAATGTCATTGGTTGACCTTTGTA	AATTTAACCGTATCACCATCAATCC
Streptococcus intermedius/Streptococcus constellatus	16S rRNA	TGCAAGTAGAACGCACAGGATG	TGCAGTAAATGTTCTTATGCGGTATTAGCGCGTAGGTAACCTGCCT[‡]	
Bacillus atrophaeus (positive control)	atpD	TTGTCTGTGAATCGGATCTTTCTC	CACTTCATTTAGGCGACGATACT	TCCCAATGTTACATTACC[*]

All assays were labelled with fluorescein amidite.

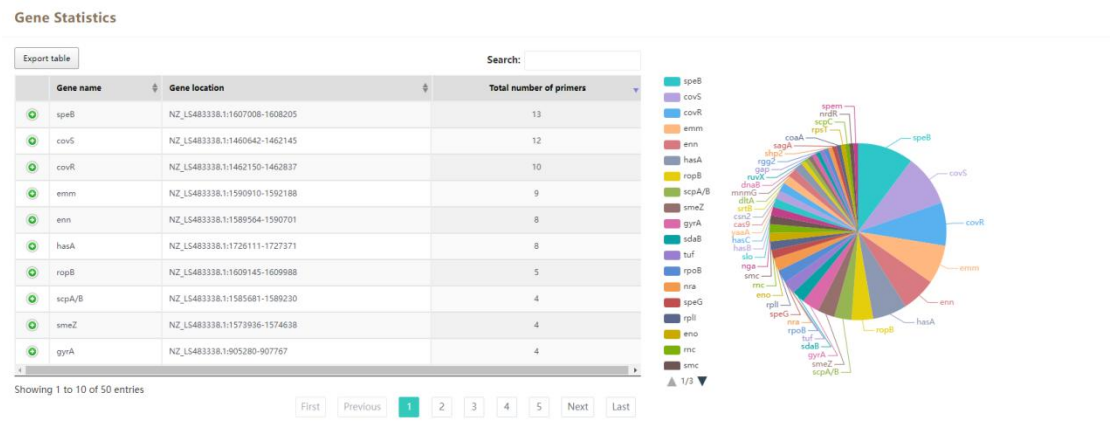
Probe modified from published with use of minor groove binder (MGB);

ZEN internal quencher (Integrated DNA Technologies, USA) used;

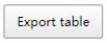
MGB probe used in place of dual fluorescence resonance energy transfer probes, forward and reverse primers lengthened at the 3′ end to increase melting temperatures. Ref Reference; rRNA Ribosomal RNA

3.3.3 The hotspots for gene amplification by primers in literature sources (Gene Statistics)

We use tables and pie charts to represent the association of literature source primers with species genes. The table details the gene name, the location of the gene in the reference genome, and the number of primers. The pie chart shows the proportion of primer numbers for each gene.



(1) Forms Download

Users can click on 

, which enables the form to be downloaded.

(2) Search Box

The search box

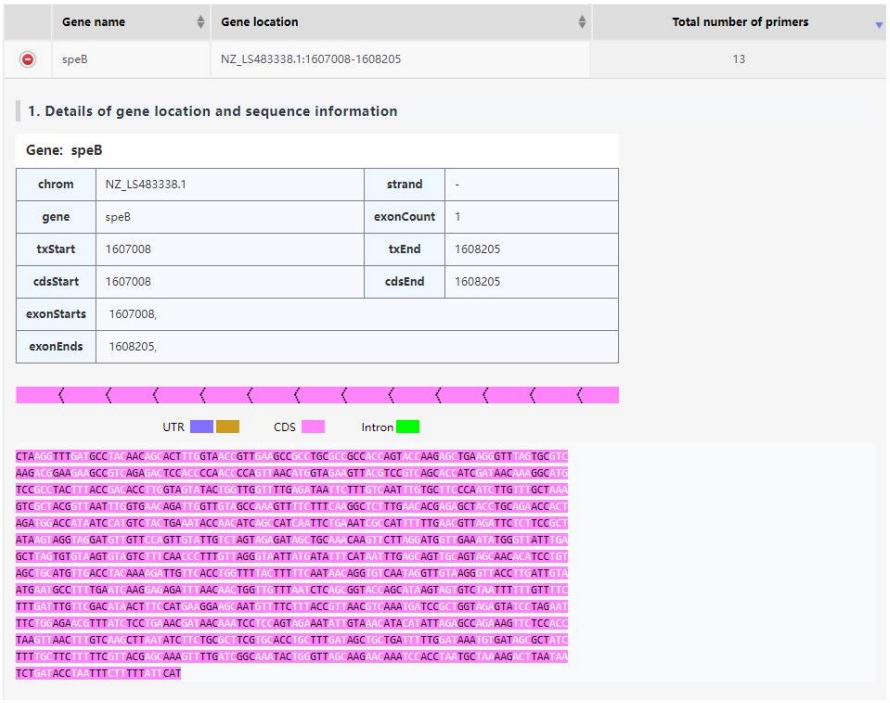
allows the user to search for gene name, etc.

(3) Details of primer-gene associations

Users can click on the Expand button  on the primer sheet to obtain details of primer-gene associations.

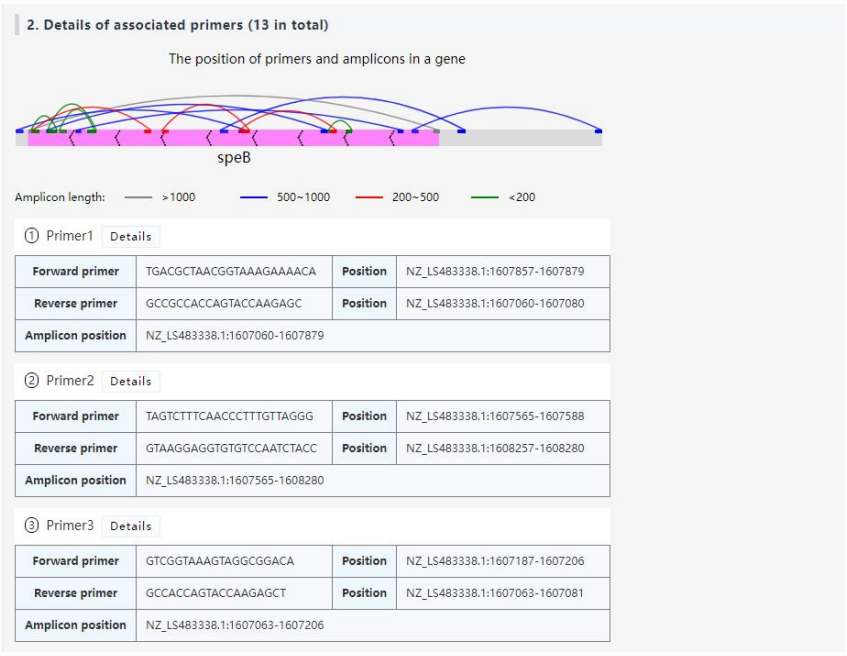
Details of gene location and sequence information

This section details chromosomes (chrom), positive and negative strands (strands), gene name (gene), exon numbers (exonCount), start and end positions of transcripts (txStart / txEnd), start and end positions of Coding DNA sequences (cdsStart / cdsEnd), start and end positions of exons (exonStarts / exonEnds), and gene sequences. We label sequences with different background colors for UTR, CDS, Intron, etc.



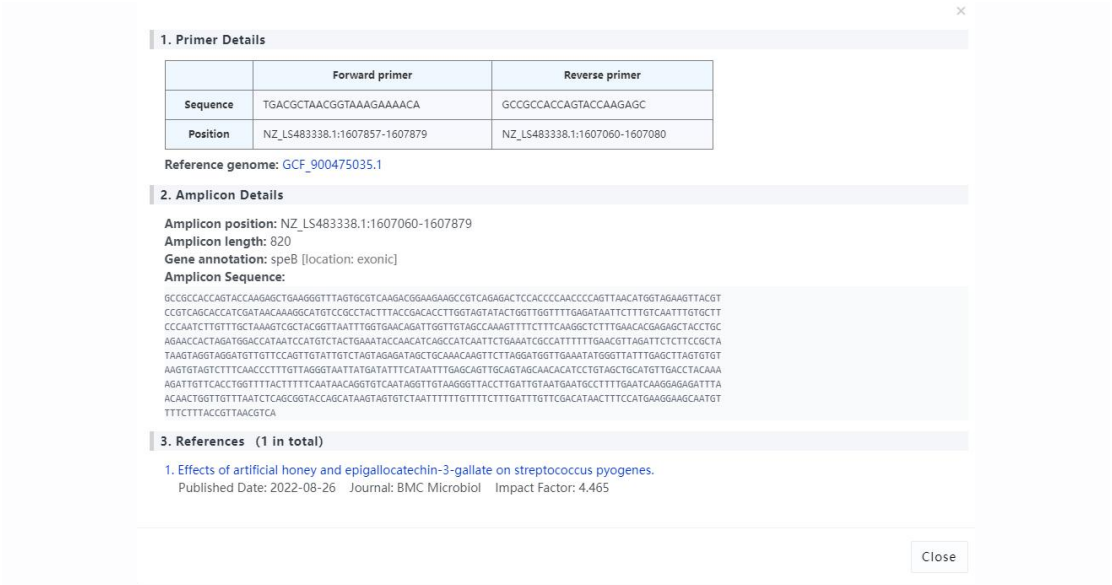
Details of associated primers

Here we plot a graph of the positions of primers and amplicons in a gene, with different colored curves indicating different length ranges of amplicons, and horizontal lines at the beginning and end of the curves indicating forward and reverse primers. This section lists all primers in a gene, listing forward and reverse primer sequences and positions, amplicon positions, etc.



Additional information can be found at the click of a button Details .

Again, information on the sequence and position of the forward and reverse primers, the position and length of the amplicons, gene annotations, amplicon sequences, and related references are given.



1. Primer Details

	Forward primer	Reverse primer
Sequence	TGACGCTAACGGTAAAGAAAACA	GCCGCCACCAGTACCAAGAGC
Position	NZ_LS483338.1:1607857-1607879	NZ_LS483338.1:1607060-1607080

Reference genome: GCF_900475035.1

2. Amplicon Details

Amplicon position: NZ_LS483338.1:1607060-1607879
Amplicon length: 820
Gene annotation: speB [location: exonic]
Amplicon Sequence:

GCCGCCACCAGTACCAAGAGCTGAAGGGTTTAGTGCGTCAAGACGGAAGAAGCCGTCAGAGACTCCACCCCAACCCCAGTTAACATGGTAGAAGTTACGT
CCGTCAGCACCATCGATAACAAAGGCATGTCCGCCTACTTTACCGACACCTTGGTAGTATACTGGTTGGTTTTGAGATAATTCTTTGTCAATTTGTGCTT
CCCAATCTTGTTTGCTAAAGTCGCTACGGTTAATTTGGTGAACAGATTGGTTGTAGCCAAAGTTTTCTTTCAAGGCTCTTTGAACACGAGAGCTACCTGC
AGAACCACTAGATGGACCATAATCCATGTCTACTGAAATACCAACATCAGCCATCAATTCTGAAATCGCCATTTTTTGAACGTTAGATTCTCTTCCGCTA
TAAGTAGGTAGGATGTTGTTCCAGTTGTATTGTCTAGTAGAGATAGCTGCAAACAAGTTCTTAGGATGGTTGAAATATGGGTTATTTGAGCTTAGTGTGT
AAGTGTAGTCTTTCAACCCTTTGTTAGGGTAATTATGATATTTCATAATTTGAGCAGTTGCAGTAGCAACACATCCTGTAGCTGCATGTTGACCTACAAA
AGATTGTTCACCTGGTTTTACTTTTTCAATAACAGGTGTCAATAGGTTGTAAGGGTTACCTTGATTGTAATGAATGCCTTTTGAATCAAGGAGAGATTTA
ACAACTGGTTGTTTAATCTCAGCGGTACCAGCATAAGTAGTGTCTAATTTTTTGTTTTCTTTGATTTGTTCGACATAACTTTCCATGAAGGAAGCAATGT
TTTCTTTACCGTTAACGTCA

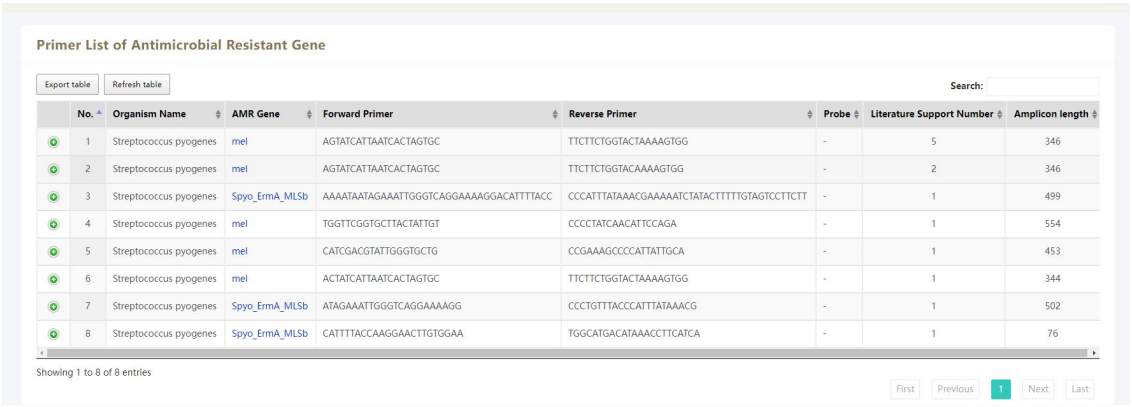
3. References (1 in total)

1. Effects of artificial honey and epigallocatechin-3-gallate on streptococcus pyogenes.
Published Date: 2022-08-26 Journal: BMC Microbiol Impact Factor: 4.465

Close

3.3.4 The submodule of MiPRIME for resistance and toxin gene detection

When the user clicks the button **[Click to view primers related to drug resistance genes]**, a list of resistance genes and their primers and probes are available, which are associated with the species being queried. The table details organism name, AMR gene (Blue font with link to CARD at https://card.mcmaster.ca/), forward / reverse primer sequences, probes, literature support numbers, and amplicon lengths.



Primer List of Antimicrobial Resistant Gene

Export table | Refresh table | Search:

	No.	Organism Name	AMR Gene	Forward Primer	Reverse Primer	Probe	Literature Support Number	Amplicon length
⊕	1	Streptococcus pyogenes	mel	AGTATCATTAATCACTAGTGC	TTCTTCTGGTACTAAAAGTGG	-	5	346
⊕	2	Streptococcus pyogenes	mel	AGTATCATTAATCACTAGTGC	TTCTTCTGGTACAAAAGTGG	-	2	346
⊕	3	Streptococcus pyogenes	Spyo_ErmA_MLSb	AAAATAATAGAAATTGGGTCAGGAAAAGGACATTTTACC	CCCATTTATAAACGAAAAATCTATACTTTTTGTAGTCCTTCTT	-	1	499
⊕	4	Streptococcus pyogenes	mel	TGGTTCGGTGCTTACTATTGT	CCCCTATCAACATTCCAGA	-	1	554
⊕	5	Streptococcus pyogenes	mel	CATCGACGTATTGGGTGCTG	CCGAAAGCCCCATTATTGCA	-	1	453
⊕	6	Streptococcus pyogenes	mel	ACTATCATTAATCACTAGTGC	TTCTTCTGGTACTAAAAGTGG	-	1	344
⊕	7	Streptococcus pyogenes	Spyo_ErmA_MLSb	ATAGAAATTGGGTCAGGAAAAGG	CCCTGTTTACCCATTTATAAACG	-	1	502
⊕	8	Streptococcus pyogenes	Spyo_ErmA_MLSb	CATTTTACCAAGGAACTTGTGGAA	TGGCATGACATAAACCTTCATCA	-	1	76

Showing 1 to 8 of 8 entries

First | Previous | 1 | Next | Last

(1) Function 1 Forms Download

Users can click on **Export table**, which enables the form to be downloaded.

(2) Function 2 Search Box

The search box **Search:** allows the user to search for AMR gene, forward / reverse primer, etc.

(3) Function 3 Details of amplicon and References

Users can click on the Expand button ⊕ on the sheet to obtain details of amplicon and

references.

No.	Organism Name	AMR Gene	Forward Primer	Reverse Primer	Probe	Literature Support Number	Amplicon length
1	Streptococcus pyogenes	mel	AGTATCATTAATCACTAGTGC	TTCTTCTGGTACTAAAAGTGG	-	5	346

1. Amplicon Details

Amplicon length: 346
Amplicon Sequence:

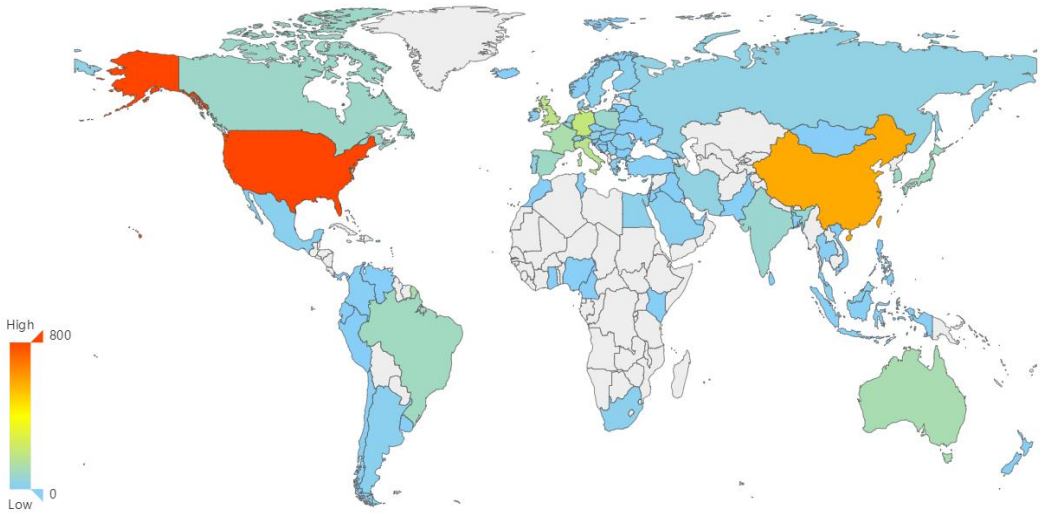
AGTATCATTAATCACTAGTGCCATCTTGCAAATGGCGATTATTTTTTACCTTACAGAAAAAACTGGATCCGCGATGGTCTTGTCTATGGCTTCACTATTA
GGTTTTTTACCCTATGCGGTCTTTGGACCTGCAATTGGTGTGCTAGTGGATCGTCATGATAGGAAGAAGATAATGATTGGTGCTGATTTAATTATCGCAG
CAGCTGGTTCGGTGCTTACTATTGTTGCATTCTATATGGAGCTACCTGTCTGGATGGTTATGATAGTATTGTTTATCCGTAGCATTGGAACAGCTTTTCA
CACCCCGGCTCTCAATGCGGTTACGCCACTTTTAGTACCAGAAGAA

2. References (5 in total)

1. Emergence of the M phenotype of erythromycin-resistant pneumococci in South Africa.
   Published Date: 1998 Apr-Jun Journal: Emerg Infect Dis Impact Factor: 16.126
2. High Frequency of Macrolide-Resistant *Streptococcus pneumoniae* Colonization in Respiratory Tract of Healthy Children in Ardabil, Iran.
   Published Date: 2019-02 Journal: Tanaffos
3. Molecular characterization, antibiotic resistance pattern and capsular types of invasive Streptococcus pneumoniae isolated from clinical samples in Tehran, Iran.
   Published Date: 2020-06-16 Journal: BMC Microbiol Impact Factor: 4.465
4. Aquaculture can promote the presence and spread of antibiotic-resistant Enterococci in marine sediments.
   Published Date: 2013-04-26 Journal: PLoS One Impact Factor: 3.752
5. Characterization of Staphylococci and Streptococci Isolated from Milk of Bovides with Mastitis in Egypt.
   Published Date: 2020-05-15 Journal: Pathogens

(4) Function 4 AMR gene global distribution

Here is an example of anti-microbial resistance gene, mel. The result can be accessed at http://106.37.92.187:1234/literature/aqe.php?db=cnki;pubmed&query=%22mel%20AND%20(resistance%20gene)%22&simultaneous=false. The user needs to click on the Statistics overview button in order to access the world map and distribution map of the queried gene. On the world map, red color indicates a higher quantity, while blue color indicates a lower quantity based on literature number.



4. Notes about use

1. Please keep your account number and password properly without disclosing them to others to avoid unnecessary losses.
2. If you encounter any problems or questions, please feel free to contact the site's support staff (zhangzhim@coyotebio.com), we will be happy to serve you.