Instruction manual of MiPRIME platform

1. Introduction

MiPRIME(<u>https://www.ai-bt.com</u>), a real-time Microbial Primer Mining platform for primer/probe sequences extraction of pathogenic microorganisms, reducing time and trial-anderror 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.

ft Home	Primer/Probe intelligent mining	Drug resistance gene primer search
	Miprimer	
* (Groups of 2-20 letters and numbers, such a	as abcd, abc123, etc
	Account name	
* (5-20 characters	
	Password	
	Confirm password	
	Sign up	
	sign up	
	Already have an account?	Sign in

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.

🔒 Home	Primer/Probe intelligent mining	Drug resistance gene primer search	
	Miprimer		
	= Account name		
	Password		
	Sign in		
	Don't have an account? Sig	jn up	

3. Main Functions of MiPRIME

3.1 Home Page

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

🚯 MiPRIME 🕇 Home 🗐 Primer/Probe	intelligent mining 🛛 🔠 Drug resistance gene	primer search				💄 Sign in 🛛 🛛 Help
Th pa th the	MiPRIME e design of primers and probes is essenti thogenic microorgasisms. The MPRIME d converient tool for literature-derived p and trial and-error costs, and filling a d of literature-sourced primer platforms.	ol to accurately detect Jatform is a powerful rimer mining, reducing app in the pan-microbial		*	·X•	
	The pip	variate data with literature, s	mining from Li pecies, ARGs/VFs, etc.	teratures	arpus	
	8.9 million anticles from PMC; Parsing Xmi files and tables; Store the information in Elasticsearch.	viruses, 24,784 bacteria, 55,881 (ungl. etc. from Taxob database: reference genome sequences from Ensembl/ NCBI	5 1/U anticide-resistant genes from CARDITIS2 VFs from 32 geners in the VFDB database	1358 focket corps with 1358 primers, 135 primers, 135 primers, 107 probes, 39 spe	vericus age. 8 reverse acles	
	NER: support two entity types		RE: corrected by sec	uences	1	
	Entity type Method Species GNormPlus & dictionary-based Primers string matching	Accuracy RE type 98.83% Species-Prim 98.02% Gene/ARGs/VFs-F	Methor er word co-occurrences; corrected by Primer of species OR sequences	f reference genome sequences of genes/ARGs/VFs	Accuracy 99.03% 98.45%	
	Application: Auto ranks and re	Countered high-quantity pr	Imers via PRscore.	berahure supported	1 1111111 2 1111111 3 1111111	

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.

🔥 Miprime	🔒 Home	Primer/Probe intelligent mining	👤 tom	Sign out	Help
	C.	Primer/Probe Intelligent Mining			
	• e.	Please input organism name or TaxID Q Search Task List g. Streptococcus pyogenes, Candida albicans			

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.

ort tal	ble Refresh table				Search:	
•	TaxID \$	Organism Name	\$ Rank 🍦	Submission time	\$ Task Status	\$
	5482	Candida tropicalis	species	2024-04-03 16:22:39	View Results	
2	83558	Chlamydia pneumoniae	species	2024-04-03 16:22:03	View Results	
	9606	Homo sapiens	species	2024-03-30 13:59:30	Running	
	1156769	Porcine kobuvirus	no rank	2024-03-24 15:33:44	View Results	
	10407	Hepatitis B virus	species	2024-02-21 13:53:00	View Results	

3.3 Platform results interface

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

			Sti	reptococcus pyogenes			Q Search	Task List		
itre	ptoc	occus p	vogenes							
ame	strept	ococcus pyo	genes): Bacteria	Taxib: 13	(Genus): Streptococcus		Kank: species			
	.97 (
rim	er List	t (Click to vie	w primers related to drug resista	ance genes]						
Export	table	Filter table T	User-defined primer addition	User-defined coverage calculation				Sea	rch:	
				Pr	imer Details				Amplicon Details	
	No. *	PR Score 0	Forward primer 0	Reverse primer \$	Probe \$	Literature Support Number (Specificity Assessment \$	Amplicon Length	Gene annotation 0	Positic
0	1	209.22	GCACTOGCTACTATTTCTTACCTCAA	GTCACAATGTCTTGGAAACCAGTAAT	CCGCAACTCATCAAGGATTTCTGTTACCA	4	Very High O	98		intergen
0	2	209.00	CTGCCGCTCAACGTTATACT	ACTGGTTCTCTTTCGCTTCC		1	Very High O	121	gyrA	exonic
0	3	207.00	CATGTTGCGAACCTCGTCTA	GGOGGTCTTACAGAATOGTC		1	Very High O	106	coaArrpsT	downstr
0	4	208.61	CGTCTTTCTGAGGTGGACTCTA	CTAATGACTCGACTGCCCTTTC		1	Very High O	113	covS	exonic
0	5	00.005	TGAGTGTCATTGTGGCAAGAGC	AGAGAATACGACGATGCACAGG		1	Very High O	72	gyrA	exonic
0	6	209.00	GAAGTGATCCCTGGACCTGA	CCCGACCTGTTTGAGTTGTT		1	Very High O	139	gyrA	exonic
	7	207.00	GONTGANCCATGUTATAAACCUGIG	CTICATACCAATAGATGCATTACTATCA		1	Very High O	107		internor
-	,	207.00					they may be			
	0	20100	CICITISAGEIGLARCAIGAGG	CACGAGINACGINICCCATOL			very high O	024	covs	exonic
•	9	10.005	AICAICICEIGGCIIGCAIGG	CCAGICACIGAAAGGI IAAICGC		10	Very High O	846	covs	exonic
•	10	207.00	CATGACTGTCTCCTTTCTGATTTTC	CCGTTATTTAAAGGACAGCTAGACC	-	1	Very High O	1126	rgg2;shp2	exonic
iene	Stati	stics			Sauch		Yewlous 1 2	3 4 5 .		Last
export	teole		a la compositione de la composition de		search:	speB				
0	Gene na	ama	were location	÷	total number of primers	covS	spern			
0	covs		NZ (\$483338.1:1460642-14621	45	12	emm	coaA rost	- spe8		
0	covR		NZ L5483338.1:1462150-14628	37	10	hasA	rgg2			
0	emm		NZ_L5483338.1:1590910-15921	88	9	ropB	nuvX- maB- mG-	- covs		
0	enn		NZ_LS483338.1:1589564-15907	01	8	gyrA csr			ovR.	
0	hasA		NZ_LS483338.1:1726111-17273	71	8	sdaß has				
0	rop8		NZ_LS483338.1:1609145-16099	88	5	and the second	nga smc		WT1	
	scpA/8		NZ_LS483338.1:1585681-15892	30	4	nra speG	ene- rpli-	enn		
0	smeZ		NZ_LS483338.1:1573936-15746	38	4	eno	speg rpoBL	hasA		
0			N7 15483338.1-905280-907767		4	and the	sdaB			
0	gyrA	_				smc	smeZ			

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*,

<u>https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1314</u>), which provides detailed information about the species.

Streptococcus pyogenes		
Name: Streptococcus pyogenes Taxology (superkingdom): Bacteria	Tax ID: 1314 Taxology (Genus): Streptococcus	Rank: 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 \$			
0	1	209.22	GCACTCGCTACTATTTCTTACCTCAA	GTCACAATGTCTTGGAAACCAGTAAT	CCGCAACTCATCAAGGATTTCTGTTACC	A 4	Very High 🕄			
0	2	209.00	CTGCCGCTCAACGTTATACT	ACTGGTTCTCTTTCGCTTCC	-	1	Very High 🕄			
0	3	207.00	CATGTTGCGAACCTCGTCTA	GGCGGTCTTACAGAATCGTC		1	Very High 🕄			
0	4	208.61	CGTCTTTCTGAGGTGGACTCTA	CTAATGACTCGACTGCCCTTTC	-	1	Very High 3			
0	5	209.00	TGAGTGTCATTGTGGCAAGAGC	AGAGAATACGACGATGCACAGG	а.	1	Very High			
0	6	209.00	GAAGTGATCCCTGGACCTGA	CCCGACCTGTTTGAGTTGTT	-	1	Very High 3			
0	7	207.00	GCATCAACCATGTTATAAACCTGTG	CTTCATACCAATAGATGCATTACTATCA		1	Very High			
0	8	207.00	CTCTTGAGCTGCAACATGAGG	CACGAATAACGTATCCCATGC	-	1	Very High 3			
0	9	206.61	ATCATCTCCTGGCTTGCATGG	CCAGTCACTGAAAGGTTAATCGC	а.	1	Very High			
0	10	207.00	CATGACTGTCTCCTTTCTGATTTTC	CCGTTATTTAAAGGACAGCTAGACC	-	1	Very High 3			

(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:

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

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°C, 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 lever! 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 whether the ΔG of hairpin calculated 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: \geq 90. (3) Moderate: \geq 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 \ge 90. c) Moderate: the specificity score \ge 50. d) Low: the specificity score <50.

Primer specificity assessment

Result of primer specificity assessment

Forward primer	GCACTCGCTACTATTTCTTACCTCAA	Reverse primer	GTCACAATGTCTTGGAAACCAGTAAT
Primer specificity score ③	100	Primer specificity grade ③	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) $\mbox{$$$$$$$$$$$$$$$$}$	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)		t) Covera	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%	*	D	
100%	100%	100%	100%	100%	100%	100%	100%	*	E2	
100%	96.88%	100%	100%	100%	100%	100%	100%	*	D	
99.61%	99.61%	99.61%	99.61%	99.61%	99.61%	99.61%	99.61%	*	E2	
100%	100%	100%	100%	100%	100%	100%	100%	*	D	
100%	99.61%	100%	100%	100%	100%	100%	100%	*	E2	
99.61%	99.22%	100%	100%	100%	100%	100%	100%	*	D	
99.61%	100%	100%	100%	100%	100%	100%	100%	*	D	
99.61%	99.22%	99.61%	100%	99.61%	100%	99.61%	100%	*	D	
100%	91.21%	100%	100%	100%	100%	100%	100%	*	E2	

(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

xport	table	Filter table T	User-defined primer addition	User-defined coverage calculation				Search	h:	
				Pr	imer Details				Amplicon Details	
	No. *	PR Score \$	Forward primer \$	Reverse primer \$	Probe \$	Literature Support Number \$	Specificity Assessment \$	Amplicon Length \$	Gene annotation \$	Posi
0	1	209.22	GCACTCGCTACTATTTCTTACCTCAA	GTCACAATGTCTTGGAAACCAGTAAT	CCGCAACTCATCAAGGATTTCTGTTACCA	4	Very High O	98		interg
0	2	209.00	CTGCCGCTCAACGTTATACT	ACTGGTTCTCTTTCGCTTCC	2	1	Very High O	121	gyrA	exoni
0	3	207.00	CATGTTGCGAACCTCGTCTA	GGCGGTCTTACAGAATCGTC	-	1	Very High	106	coaA;rpsT	down
0	4	208.61	CGTCTTTCTGAGGTGGACTCTA	CTAATGACTCGACTGCCCTTTC	2	1	Very High	113	covS	exoni
0	5	209.00	TGAGTGTCATTGTGGCAAGAGC	AGAGAATACGACGATGCACAGG	-	1	Very High	72	gyrA	exoni
0	6	209.00	GAAGTGATCCCTGGACCTGA	CCCGACCTGTTTGAGTTGTT		1	Very High	139	дугА	exoni
0	7	207.00	GCATCAACCATGTTATAAACCTGTG	CTTCATACCAATAGATGCATTACTATCA		1	Very High O	107		interg
0	8	207.00	CTCTTGAGCTGCAACATGAGG	CACGAATAACGTATCCCATGC		ï	Very High O	624	covR	exoni
0	9	206.61	ATCATCTCCTGGCTTGCATGG	CCAGTCACTGAAAGGTTAATCGC		1	Very High	846	covS	exoni
0	10	207.00	CATGACTGTCTCCTTTCTGATTTTC	CCGTTATTTAAAGGACAGCTAGACC		1	Very High O	1126	rgg2;shp2	exoni

(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 **T**, 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.

Specificity Assessment	No limit			~
Availability of probe sequence	No Limit			~
Filter by literature support number	0	to	max	
riter by amplicon length	0	to	max	

(3) Function 3 User-defined primer addition

By clicking User-defined primer addition, 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.

Forward primer	
Reverse primer	
Probe	

(4) Function 4 User-defined coverage calculation

Users can click a button User-defined coverage calculation 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).

Forward primer			
Reverse primer			
Probe			
Upload Sequence	s Choose File No file	e chosen	

(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

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 [AG = 9.88 kcal/mol] /-/-/-----GCACTCGCTACTATTTCTTACCTCAA

Primer R hairpin [&G = 6.89 kcal/mol]

Primer F self-dimer: [Score: 2, Δ6 = -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

		Primer (5' - 3')		
	Targe	t		
Organism	gene	Forward	Reverse	Probe
Streptococcus pneumoniae	lytA	ACGCAATCTAGCAGATGAAGCA	TCGTGCGTTTTAATTCCAGCT	AACGCTTGATACAGGGAG[*]
Streptococcus pyogenes	spy	Geaenceenachannechtacenca	AGTCACAATGTCTTGGAAACCAGTAAT	CCGCAACTCATCAAGGATTTCTGT
Haemophilus influenzae	hpd	GGTTAAATATGCCGATGGTGTTG	TGCATCTTTACGCACGGTGTA	TTGTGTACACTCCGTTGGT[*]
Staphylococcus aureus	nuc	AAATTACATAAAGAACCTGCGACA	GAATGTCATTGGTTGACCTTTGTA	AATTTAACCGTATCACCATCAATC
Streptococcus	16S	TGCAAGTAGAACGCACAGGATG	TGCAGTAAATGTTCTTATGCGGTATTA	GCGCGTAGGTAACCTGCCT[‡]
intermedius/Streptococcus constellatus	rRNA			
Bacillus atrophaeus	atpD	TTGTCTGTGAATCGGATCTTTCTC	CACTTCATTTAGGCGACGATACT	TCCCAATGTTACATTACC[*]
(positive control)				
All assays were labelled wit	h fluo	rescein amidite. ≓		
Probe modified from publis	shed v	vith use of minor groove binder (N	1GB); ≓	
ZEN internal quencher (Int	egrate	ed DNA Technologie <mark>s, USA)</mark> used;	4	
MGB probe used in place of	f dual merat	fluorescence resonance energy tra ures. Ref. Reference: rRNA Ribosor	ansfer probes, forward and reverse p nal RNA 🔁	rimers lengthened at the 3'

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.

21	t table			Search:		
	Gene name	¢	Gene location	\$ Total number of primers	speB	
	speB		NZ_LS483338.1:1607008-1608205	13	covR	spem
0	covS		NZ_LS483338.1:1460642-1462145	12	enn	sagA speB
0	covR		NZ_LS483338.1:1462150-1462837	10	ropB	rigg2 gapcovS
0	emm		NZ_LS483338.1:1590910-1592188	9	scpA/B	dna8- mnmG- dttA-
0	enn		NZ_LS483338.1:1589564-1590701	8	gyrA	csn2 can9
0	hasA		NZ_LS483338.1:1726111-1727371	8	sdaB	hasC hasB
0	горВ		NZ_LS483338.1:1609145-1609988	5	rpoB	nga smc er
)	scpA/B		NZ_LS483338.1:1585681-1589230	4	nra	eno- rpli-
	smeZ		NZ_LS483338.1:1573936-1574638	4	rpli	speg- nra- rpo8-
)	gyrA		NZ_L5483338.1:905280-907767	4	rnc	sdaB gyrA

(1) Forms Download

Users can click on Export table, 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.

spe8 NZ_L548338.1:1607008-1608205 13 I Serie: spe8 I Strand gene spe8 strand - gene spe8 strand - txStart 1607008 1608205 - ito5tatt 1607008 ito8205 - ito5tatt 1607008 ito8205 - ito5tatt 1607008 - - ito67008 ito50. - - ito67008 - - - ito69205, - - -	spe8 NZ_LS483338.1:1607008-1608205 13 1. Details of gene location and sequence information Image: Spe8		name 4	Gene loc	ation			÷		Total number of primers
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A BOTTTON GCC //AAAC COACTT GTARCEGT COACCCC TGC COCCGAGE/COAGA CTAAAGAC TGA/ACGTT /ACTGCT GTCGAAAC GCCT AAAC COACTT CAAC CCCA TTAAC IN GTAAA GTT /CTCCT CAGCAC ATC AN AAC AGGCT G GTCGAAAC GCCT AAAC TTGT COACTT AAC IN GTAAA GTT /CTCCT CAGCAC ATC AN AAC AGGCT G CCCTACTT ACCCAM ACCC TCGTACT /TAC INT TTG ATA TTTT /CAAT INT GGCT CACT CAAC AGGCT G CCCTACTT ACCCAM ACCC TCGTACT /TAC AGCCATTT TTT /CAAT INT GGCT CACT ACCT ACCT ACCT ACCT ACCT ACCT AC	A CHING COLUMANC, SHUTT CHAIN CHING TO COLUMANCE COLUMN AND TAKE TO A CHING CHING COLUMN AND TAKE TO A CHING	AGGTITGATG AGGTITGATG AGGATAG AGGAAAAG GCTACTIA GCTACTIA GCTACTIA AGGGCTTA AGGGCTTA AGGGCTTT AGTGTCA AGGGCTTT AGTGTCA AGGGCTTT	160/005, 1608205, UUR UUR CCGT AACGACTICC CCGT AACGACACTICC CCGT AACGACACTICC CCGT AACGACACTICC CCGT AACGACTICC GCGT AACGACTICC GCGT AACGACTICC GCGT AACGACTICC GGAT AACGAC GGAT CC GGAT AACGAC GGAT	STALCOST GAN CAACCCA TT TAC IGGTIG TT STT GT AGC (AA CCAACAT CCA CTAACT GT STT GT AGC (AA CTG GT TT STA AGC (AA CTG GT SGA AGC AGA TT SGA AGC AGA TT	CDS GCCSCCTGCCCC AACATCGTATAC TITGAGTAATTC GTHCTTTAC GATACTGCATA GATACTGCATA GATACTGCATA TITHCAATAATTC TITHTCATCTCCGCG TICTTTACCTTA	Intron	GE TGAAGGETTI IA CCAATGATAACAA TECCAATGATGATGAT GE GTAACTGCI GE ATAACTACI GE ATAACTACI GE ATAACAACTA GE GTCI AACTTI GE GTCI AACTTI GE GTCI AACTTI GE GCCAAAGAAG	CTGC TIG AGGC TIG AGGC TIG CCTTAAA AACC ACT TICC GCT TICC GCT CTAG AAT CTAG AAT CTAG AAT		
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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.

	speB	2 (
n <mark>plicon length:</mark> —	— >1000 <u>—</u> 500~1000		200~500 <200	
1) Primer1 Deta	ils			
Forward primer	TGACGCTAACGGTAAAGAAAACA	Position	NZ_LS483338.1:1607857-1607879	7
Reverse primer	GCCGCCACCAGTACCAAGAGC	Position	NZ_LS483338.1:1607060-1607080	-
Amplicon position	NZ_LS483338.1:1607060-1607879			-
 Primer2 Deta 	ils			-
Forward primer	TAGTCTTTCAACCCTTTGTTAGGG	Position	NZ_LS483338.1:1607565-1607588	
Reverse primer	GTAAGGAGGTGTGTCCAATCTACC	Position	NZ_LS483338.1:1608257-1608280	-
Amplicon position	NZ_LS483338.1:1607565-1608280		1	
3 Primer3 Deta	ils			7
3) Primer3 Deta Forward primer	GTCGGTAAAGTAGGCGGACA	Position	NZ_LS483338.1:1607187-1607206	

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.

Sequence Position		Reverse primer	
Position	TGACGCTAACGGTAAAGAAAACA	GCCGCCACCAGTACCAAGAGC	
	NZ_LS483338.1:1607857-1607879	NZ_LS483338.1:1607060-1607080	
Reference ge	enome: GCF_900475035.1		
2. Amplicon	Details		
GCCGCCACCAGTAC CCGTCAGCACCATC CCCAATCTTGTTTC AGAACCACTAGATC TAAGTAGGTAGGAT AAGTGTAGTCTTTC AGATTGTTCACCTT	CCAAGAGCTGAAGGGTTAGTGCGTCAAGACGGAAGAAGCC CGATAACAAGGCATGTCGCGCTACTTTACCGACACCTTGG CGATAACAAGGCATGTCGCGCTACTTTACCGACACCTGG SGACCATAATCCATGGTTAATTGGTGAACAGATGGTGTG SGACCATAATCCATGTCTATGTGAAGATAGCTGCAAG GGTGTGTCCAGGTGATTATGTATTTCCTAATTGGA SGACCTTTGTCAGGTGATATTGATTTCTAATTTGAA SGTTTTACTGACGGTGATCAGGTGTCAATTGGTTGAGG	GTCAG46ACTCCACCCCAACTCCAGTTAACATGGTAGAAGTTACG TAGTATACTGGTIGTTTTGAAGTAATTCTTTGTCAATTGGTGT TAGTATACTGGTIGTTTTGAAGTATTCTTGTCAATTGGGTA AGCCAAAGTTTGTGAAGTGGGTAGATTGAACGAG46ACTACCTGC CAATTCGAAATGGCCAATTTTTGAACGAG4AGCTAAGTGGT AGATTCGTAGGTAGGTGAAATGGGTATTTGAACTGAGGAGAGAGTTA GTTACCTGGATGGAAGGAGCGCTTTGGATGAAGCAAGTG TTTCTTTGGTTGTTCGACGACTACTTTCCATGAAGGAGGAGATTA	
ACAACTGGTTGTTT TTTCTTTACCGTTA	AACGTCA		
ACAACTGGTTGTTT TTTCTTTACCGTTA	AACGTCA s (1 in total)		

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.

ort 1	able	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

(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	φ	Reverse Primer	4	Probe (Literature Support Number \$	Amplicon length
•	1	Streptococcus pyogenes	mel	AGTATCATTAATCACTAGTGC		TTCTTCTGGTACTAAAAGTGG		-	5	346
	1. A	mplicon Details								
	Amp	licon length: 346								
	AGTA GGTT CAGC	TCATTAATCACTAGTGCCATCTTGCAAA TTTTAATCACTAGTGCCATCTTGCAAA TTTTACCCTATGCGGTCTTTGGACCTGC TGGTTCGGTGCTTACTATTGTTGCATTC	IGGCGATTATTITTACCTTA VATTGGTGTGCTAGTGGATCG IATATGGAGCTACCTGTCTGG	CAGAAAAAACTGGATCCGCGATGGTCTTGTCTATGGCTTCACTA TCATGATAGGAAGAAGATAATGATTGGTGCTGATTTAATTATCC ATGGTTATGATAGTATTGTTTATCCGTAGCATTGGAACAGCTTI	ATTA ICAG ITCA					
	2. Re	eferences (5 in total)								
	1. Ei	mergence of the M phenoty	pe of erythromycin-r	resistant pneumococci in South Africa.						
	2. H	igh Frequency of Macrolide-	Resistant Streptocod	ccus pneumoniae Colonization in Respirator	y Tract of Healt	hy Children in Ardabil, Iran.				
	3. N P	lolecular characterization, ar ublished Date: 2020-06-16	itibiotic resistance pa Journal: BMC Micro	attern and capsular types of invasive Strept biol Impact Factor: 4.465	ococcus pneum	oniae isolated from clinical samples in Tehra	an, Iran.			
	4. A P	quaculture can promote the ublished Date: 2013-04-26	presence and spread Journal: PLoS One	d of antibiotic-resistant Enterococci in mari Impact Factor: 3.752	ne sediments.					
	5. C	haracterization of Staphyloco ublished Date: 2020-05-15	Journal: Pathogens	i Isolated from Milk of Bovides with Mastiti	s in Egypt.					

(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(re

sistance%20gene)%22&simultaneous=false. The user needs to click on the

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.