# ARGA pipeline user guide

## http://mem.rcees.ac.cn:8083/

#### 1. Get Data

## <u>Get Data</u>

<u>Upload File</u> from your computer

1.1 Click the <u>Upload File</u>, then click the <u>Choose local file</u> at the pop-up window, select the object files to upload, Click the <u>Start</u>, then click the <u>Close</u> to close the pop-up window.

ownload from web	or upload fro	m disk				
Regular Composite	<u>Collection</u>					
	You added 1	file(s) to the queue. Ac	dd more files or click 'Sta	rt' to proceed.		
Name	Size	Туре	Genome	Settings	Status	
💻 tetA_primers.txt	<b>1.6</b> KB	Auto-de 🔻 <b>Q</b>	Additional S 🔻	•	0%	Ŵ
Type (set all):	Auto-d	etect 💌 <b>Q</b>	Genome (set al	l): Add	litional Species 🔹	,
	🖵 Choose local	file 🕞 Choose FTP 1	file 🕼 Paste/Fetch d	ata Pause	Reset Start	Close

1.2 The uploaded data would display in the right of the website.

History	C	۰.	
search datasets		8	
<b>Unnamed history</b> 4 shown			
5.22 КВ			
<u> </u>	ø	×	 Prepare to Upload
<u>3: tetB primers.txt</u>	ð	×	Uploading
2: tetA primers.txt	ø	×	Successfully Uploaded

1.3 Once the file is successfully uploaded, the dataset can be viewed, saved and deleted.



#### 2. Primer Assessment

#### Primer Assessment

<u>Primer Coverage</u> BLASTn program to calculate the number of matched sequences in targeted database

<u>Primer Specificity</u> BLASTn program to calculate the number of matched sequences in targeted database

2.1 Prepare the tab-delimited txt file as requested, and upload it to the pipeline.



Example File:

tetG_1F	CATCAGCGCCGGTCTTATG	1	
tetG_1R	CCCCATGTAGCCGAACCA	1	
tetG_2F	GCAGAGCAGGTCGCTGG	2	
tetG_2R	CCYGCAAGAGAAGCCAGAAG	2<	Degenerate Prime

2.2 Select the <u>Primer sequence file</u> to do primer coverage calculation, and then choose the corresponding database (<u>both database already integrated in</u> <u>SDARG or own database is acceptable</u>) to do primer coverage calculation. When calculating the primer coverage, <u>0 or 1 Mismatch</u> is allowed, the <u>Paired</u> <u>Calculating</u> is optional, <u>paired</u> is proper for primer pairs, this option would output both the coverage of single primer and coverage of primer pairs, while the <u>unpaired</u> only output the coverage of each primer. Click <u>Execute</u>.

Primer Coverage BLASTn program to calculate the number of matched sequences in targeted database (Galaxy Version	▼ Options
1.0.0)	
Primer sequence file(txt)	
1     1       1     1       1     1   30: tetG_primers_test.txt	•
Database in SDARG or other database	
Tetracycline	•
Tetracycline groups	
tetG	•
Mismatch	
0 (Mismatch not allowed)	•
Paired Calculating	
Paired	•
✓ Execute	

Example of Database in SDARG or other database:

If the primers are targeted <u>tetG</u> gene (Tetracycline resistance gene), we can firstly select the AR group - <u>Tetracycline</u> from the first option, then select the ARG type – <u>tetG</u> from the second option, chose 0 mismatch and paired calculation, execute.

Primer Coverage BLASTn program to calculate the number of matched sequences in targeted database (Galaxy Version 1.0.0)	<ul> <li>✓ Options</li> </ul>
Primer sequence file(txt)	
1     1       1     30: tetG_primers_test.txt	•
Database in SDARG or other database	
Tetracycline	•
	Q
Multidrug	
Polymycin	
Sulfonamide	
Tetracenomycin	
Tetracycline	
Thimethoprim	
Thiopeptide	
J Tunicamycin	
1 Oneomycin	-

_			
Pi	Primer Coverage BLASTn program to calculate the number o	of matched sequences in targeted database (Galaxy Version	▼ Options
1.	1.0.0)		
P	Primer sequence file(txt)		
	30: tetG_primers_test.txt		•
D	Database in SDARG or other database		
	Tetracycline		-
ſ	Tetracycline groups		
T	tetG		•
м	Mi		Q
	d tetA		<u>^</u>
P	Pé tetB		
F	P tetC		
ſ	tetD		
_	tetE		
Inp	pr tetG		
The	he tetH		
Prin	im tet)		-

There are two output file, one is the BLASTn result, and the other is the

coverage calculation result in txt file.

2: (	<u>Coverage.txt</u>	۲	ø	×	
31:	BLASTn.txt	۲	ø	×	
<pre># BLAST # Query # Datab # Field # 18 hi tetG_2F 37.4 tetG_2F 37.4 tetG_2F 37.4 tetG_2F 37.4</pre>	IN 2.2.31+ y: tetG_2R_1_Length_20_Degenera base: mergedatabase ds: query id. subject id. % identit ter found & 1_ength_20_Degenerate_1_Po R_1_ength_20_Degenerate_1_Po R_1_ength_20_Degenerate_1_Po	te_1_P y, alignu sition_: sition_: sition_:	osition, nent le: L_FR_: L_FR_: L_FR_:	1F ngth, 1 2 2 2	3_2 Aismatch ARID91 ARID91 ARID91 ARID91
tetG_2F 31.9 tetG_2F 31.9 tetG_2F 31.9 tetG_2F	A_iength_20Uegenerate_1_Po A1ength_20Degenerate_1_Po A_1ength_20Degenerate_1_Po A_1ength_20Degenerate_1_Po	sition sition sition sition	IFK: IFR: IFR: IFR:	2 2 2 2	ARID91 ARID91 ARID91 ARID91
31.9 tetG_2F 31.9 tetG_2F tetG_2F 31.9	R_1ength_20_Degenerate_1_Po R_1ength_20_Degenerate_1_Po R_1_ength_20_Degenerate_1_Po	sition	L_FR_: L_FR_: L_FR_:	2	ARID86 ARID91 ARID91
tetG_2H 31.9 tetG_2H 31.9 tetG_2H 31.9	R_1_Length_20_Degenerate_1_Po R_1_iength_20_Degenerate_1_Po R_1_iength_20_Degenerate_1_Po	sition sition sition	lFR: lFR: lFR:	2 2 2	ARID91 ARID91 ARID91
tetG_2H 31.9 tetG_2H 31.9 tetG_2H 31.9 tetG_2H 31.9	& _1ength_20Degenerate_1_Po & _1ength_20Degenerate_1_Po & _1ength_20Degenerate_1_Po & _1ength_20Degenerate_1_Po	sition sition sition sition	lFR: lFR: lFR: lFR:	2 2 2 2	ARID91 ARID91 ARID91 ARID91
31.9 tetG_2H 31.9 # BLAST # Query # Datab # Field	R. 1ength_20_Degenerate_1_Po IN 2.2 31+ v: tet52R_2_Length_20_Degenera bese: hergedatabase de query id. subject id. % identit	sition_: te_1_P y, alignu	l_FR_: osition	2 1F ngth, 1	ARID91 R_2 nismatch
# 18 hi tetG_2F 37.4 tetG_2F	itis folmd R_2_length_20_Degenerate_1_Po R_2_length_20_Degenerate_1_Po	sition	lFR: lFR:	2 2	ARID91 ARID91

31: BLASTn.txt The

file contains the detailed information of the

sequences matched to each primer sequence. In the example file, tetG\_2R (CCYGCAAGAGAAGCCAGAAG) is a degenerate primer, correspond to two traditional

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primers (CCCGCAAGAGAAGCCAGAAG and CCTGCAAGAGAAGCCAGAAG), hence in the

BLASTn result, there are two tetG\_2R primers displayed.

6									
Primer Coverage	,								
tetG_IF 0.23	e.,	bmittad D	imor Soquona		t cin	alo r	arimor		
tetG 2F 0.16	Ju	Difficted Fi	inter sequent	e Resul	ι – 5π	gie p	Jillei		
tetG 2R 0.17									
-									
PRIMER Pair	Coverage			_					
1 0.19	l Su	bmitted P	rimer Sequenc	ce Resul	t – pri	mer	pairs		
2 0.16		_					1 de 1		
Primer Database		R	ecommended	l Primer	Pairs				
Primer Names	Fermand Driner	G 0. F				<b>.</b> .	A		
	rorward rrimer	Loverage of For	ward Primer (%) Spe	cificity of	Forward H	Primer	(%)	Reverse Primer	Coverage of
Reverse Primer (	(%) Specifi	city of Reverse	ward Primer (%) Spe Primer (%) Cov	cificity of erage of Pri	Forward B imer Pair:	Primer s (%)	(%) Specifi	Reverse Primer icity of Primer	Coverage of Pairs (%)
Reverse Primer ( tetG_1 GCTCGGTG	(%) Specifi GTATCTCTGCTC	city of Reverse 70.00 100.00	ward Primer (%) Spe Primer (%) Cov AGCAACAGAATCGGGAACA	cificity of erage of Pri C 67.00	Forward I imer Pair: 100.00	Primer s (%) 67.00	(%) Specifi 100.00	Reverse Primer icity of Primer	Coverage of Pairs (%)
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 ICAACCAT	(%) Specifi GTATCTCTGCTC	Coverage of For city of Reverse 70.00 100.00 23.00 100.00	<pre>cward Primer (%) Spe Primer (%) Cov AGCAACAGAATCGGGAACA TGGCCCGGCAATCGGGAACA TGGCCCGGCAATCGGGAACA</pre>	cificity of erage of Pri C 67.00 22.00	Forward I imer Pair: 100.00 91.07	Primer s (%) 67.00 21.00	(%) Specifi 100.00	Reverse Primer icity of Primer tetG Primer	Coverage of Pairs (%) database
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 ICAACCA1 tetG_3 CATCAGCG	(%) Specifi GTATCTCTGCTC IGCCGATICGA CCCGGTCTTATG	coverage of For city of Reverse 70.00 100.00 23.00 100.00 23.00 100.00	ward Primer (%) Spe Primer (%) Cov AGCAACAGAATCGGGAACA IGGCCCGGCAATCATG CCCCATGTAGCCGAACCA	cificity of rerage of Pri C 67.00 22.00 19.00	Forward I imer Pair: 100.00 91.07 100.00	Primer s (%) 67.00 21.00 19.00	(%) Specifi 100.00 100.00 100.00	Reverse Primer icity of Primer tetG Primer	Coverage of Pairs (%) database
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 ICARCCAT tetG_3 CATCAGCG tetG_4 GCAGAGCA	(%) Specifi GTATCTCTGCTC IGCUGATICGA CCGGTCTTATG GGTCGCTGG	Coverage of For city of Reverse 70.00 100.00 23.00 100.00 23.00 100.00 16.00 100.00	ward Primer (%) Spe Primer (%) Cov AGCAACAGAATCGGGAACA IGUCCGGCAATCATG CCCCATGTAGCCGAACCA CCYGCAAGAGAAGCCAGAA	cificity of rerage of Pri C 67.00 22.00 19.00 G 17.00	Forward I imer Pair: 100.00 91.67 100.00 100.00	Primer s (%) 67.00 21.00 19.00 16.00	(%) Specifi 100.00 100.00 100.00 100.00	Reverse Primer icity of Primer tetG Primer	Coverage of Pairs (%) database
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 ICAACCAI tetG_3 CATCAGCG tetG_4 GCAGAGCA tetG_5 ITTCGGAI	(%) Specifi GTATCTCTGCTC TGCCGATTCGA CCGGTCTTATG GGTCGCTGG TCTTACGGTC	Coverage of Point           city of Reverse           70.00         100.00           23.00         100.00           23.00         100.00           16.00         100.00           15.00         100.00	ward Frimer (%) Spe Primer (%) Cov AGCAACAGAATCGGGAACA IGGCCGGGCAATCATG CCCCGGCAATGAGCCGAACCA CCYGCAAGAGAAGCCAGAA TCCTGCGATAGAGCTTAGA	cificity of erage of Pri C 67.00 22.00 19.00 G 17.00 . 15.00	Forward I imer Pair: 100.00 91.07 100.00 100.00 100.00	Primer s (%) 67.00 21.00 19.00 16.00 15.00	(%) Specifi 100.00 100.00 100.00 100.00	Reverse Primer icity of Primer tetG Primer	Coverage of Pairs (%) database
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 ICARCCAT tetG_3 ICARCAGC tetG_4 GCAGCAGCA tetG_5 TITCGGAT tetG_6 CAGCTITC	(%) Specifi GGTATCTCTGCTC IGCCGATICGA CCCGGTCTTATG GGTCGCTGG CTCTTACGGTC CGGATTCTTACGG	Coverage of Poincity of Reverse           70.00         100.00           23.00         100.00           23.00         100.00           16.00         100.00           15.00         100.00	ward Primer (%) Spe Primer (%) Cov ACCAACAGAATCGGGAACA IGGCCGGCAATCATG CCCCGGCAAGAGCCGGAAC TCCTGCGATAGAGCTTACA GATTGGTGAGGCTCGTTAG	cificity of erage of Pri C 67.00 22.00 19.00 G 17.00 C 15.00 C 15.00	Forward I imer Pair: 100.00 91.07 100.00 100.00 100.00 100.00	Primer s (%) 67.00 21.00 19.00 16.00 15.00 15.00	(%) Specifi 100.00 100.00 100.00 100.00 100.00 100.00	Reverse Primer icity of Primer tetG Primer Integrated	Coverage of Pairs (%) database Primer
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 CARCCAT tetG_3 CARCAGCA tetG_4 GCAGAGCA tetG_5 TITCGGAT tetG_6 CAGCTTC tetG_7 GCTCGGTG	(%) Specifi GGATCTCTGCTC IGCCGATICGA GGCGCGCTGTATG GGCGCGCGG ITCTTACGGTC IGGATCTTACGG GGATCTTTACGG GGATCTCTGCTC	Coverage of Poi           city of Reverse           70.00         100.00           23.00         100.00           23.00         100.00           16.00         100.00           15.00         100.00           70.00         100.00	<pre>(ward Primer (%) Spe Primer (%) Cov AGCAACAGAATCGGGAACA CCCCGCGCATCCATC CCCCATGGCGAACA CCYGCAAGAGAAGCCAGAA CCYGCGATAGAGCTACA GATTGGTGAGGCTCGTTAG CAAAGCCCCTTGCTTAGTTA</pre>	cificity of rerage of Pri C 67.00 19.00 G 17.00 G 15.00 C 15.00 C 15.00	Forward I imer Pairs 100.00 91.07 100.00 100.00 100.00 100.00 100.00	Primer s (%) 67.00 21.00 19.00 16.00 15.00 15.00 15.00	(%) Specifi 100.00 100.00 100.00 100.00 100.00 100.00 100.00	Reverse Primer icity of Primer tetG Primer Integrated	Coverage of Pairs (%) database Primer
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 ICARCCAT tetG_3 CATCARCG tetG_4 GCAGAGCA tetG_5 TITCGGAT tetG_6 CAGCTTC tetG_7 GCTCGGTG tetG_8 GGTCCTC	(%) Specifi GTATCTCTGCTC CCCGGTCTTATG GGTCGCTGG CTCTTACGGTC GGTTCTTACGGTC GGTATCTTACGG GGTATCTTACGG GGTATCTTGCTC IGGCTTCTCTTG	Coverage of Poi           city of Reverse           70.00         100.00           23.00         100.00           23.00         100.00           16.00         100.00           15.00         100.00           15.00         100.00           3.00         100.00           3.00         100.00	<pre>(ward Primer (%) Spe Primer (%) Cov ACCAACAGAATCGGGAACA IGCCCGGCAATCAGGGAACA CCCCGCAAGAGAAGCCAGAA CCCGGCAAGAGAAGCCTAGA GATGGTGAGGCCTCGTTAG CAAAGGCTCGTGGTGAGGCACCTAC</pre>	cificity of rerage of Pri C 67.00 22.00 19.00 G 17.00 G 15.00 C 15.00 C 15.00 C 15.00	Forward I imer Pair: 100.00 91.07 100.00 100.00 100.00 100.00 100.00 100.00	Primer s (%) 67.00 19.00 16.00 15.00 15.00 15.00 1.00	(%) Specifi 100.00 100.00 100.00 100.00 100.00 100.00 100.00	Reverse Primer icity of Primer tetG Primer Integrated Ouality R	Coverage of Pairs (%) database Primer eport
Reverse Primer ( tetG_1 GCTCGGTG tetG_2 ICARCCAN tetG_3 CATCAGCG tetG_4 GCACAGCA tetG_5 ITICGGAT tetG_6 CAGCTITC tetG_7 GCTCGGTG tetG_8 GGTGCTC tetG_8 CATTGCCC	<pre>Provide Filmet GTATCTCTGCTC GTATCTCTGCTC CCCGGTCTTATG GGCTCGTGG CTCTTACGGTC GGATCTCTGCG GGATCTCTGCTG CTGGCTTCTCTGCTG CTGCTGATCG</pre>	Coverage of Four           city of Reverse           70.00         100.00           23.00         100.00           100.00         100.00           15.00         100.00           15.00         100.00           15.00         100.00           3.00         100.00           7.00         70.00	<pre>(ward Primer (%) Spe AGCAACAGAATCGGGAACA AGCAACAGAATCGGGAACA CCCCCGCTATCATC CCCCCATGAGCGAACCA CCTGGCAAGAAGCCAGAA TCCTGGCAAGGCTCAGAA CATGGTGAGGCCCCTTGCTAGCA CAATGGTGAGGCCTGCTAAGC TTGGTGAGGCTGTAAGC</pre>	cificity of rerage of Pri C 67.00 22.00 19.00 G 17.00 C 15.00 C 15.00 C 15.00 A 7.00 2.00	Forward I imer Pair: 100.00 91.07 100.00 100.00 100.00 100.00 100.00 100.00 100.00	Primer s (%) 67.00 21.00 19.00 16.00 15.00 15.00 15.00 1.00 1.00	(%) Specifi 100.00 100.00 100.00 100.00 100.00 100.00 100.00 100.00 100.00 100.00	Reverse Primer icity of Primer tetG Primer Integrated Quality R	Coverage of Pairs (%) database Primer eport

32: Coverage.txt file displays both the coverage of the input

primer pairs of *tetG* gene, and the coverage of the previous collected *tetG* gene

primers, in this case, the users can choose the primers with higher coverage.

Example of Database in SDARG or other database:

If the primers are targeted *tetG* gene (Tetracycline resistance gene), we can

either select database as above, or we can firstly upload own database



The

, and select Others (own database) option, then select

the uploaded database as the targeted database in the primer coverage

calculation, execute.

Primer Coverage BLASTn program to calculate the number of matched sequences in targeted database (Galaxy Version 1.0.0)	▼ Options
Primer sequence file(txt)	
🕒 🛍 🗀 30: tetG_primers_test.txt	•
Database in SDARG or other database	
Others (own database)	•
	Q
Tetracenomycin	•
Tetracycline	
Thimethoprim	
Thiopeptide	_
Tunicamycin	
Vancomycin	
Others (own database)	<b>•</b>

Primer Coverage BLASTn program to calculate the number of matched sequences in targeted database (Galaxy Version 1.0.0)	▼ Options
Primer sequence file(txt)	
🕒 🖄 🗀 30: tetG_primers_test.txt	•
Database in SDARG or other database	
Others (own database)	•
Database(FASTA) If the primers target ARGs, there is no need to select your own database	
🕒 🛍 🗅 33: tetG_nucl_database.fasta	•
Mismatch	
0 (Mismatch not allowed)	•
Paired Calculating	
Paired	•
✓ Execute	

There are also two output files as above, one is the BLASTn result, and the

35: Coverage.txt	
34: BLASTn.txt	
Primer Coverage tetG_1F 0.23 tetG_1R 0.19 tetG_2F 0.16 tetG_2R 0.17 PRIMER Pair Coverage 1 0.19 2 0.16	
The difference is that in the	le, only the

other is the coverage calculation result in txt file.

The difference is that in the

coverage of the submitted primers are displayed in the result.

2.3 The primer specificity calculation is similar to the primer coverage calculation.

### 3. Sequence Annotation

#### Sequence Annotation

<u>Nucleotide Sequence</u>
Annotation Blastn program to
query nucleotide sequences in
SDARG
Protein Sequence Annotation
HMMsearch program to search
protein sequences against HMM
models of SDARG

3.1 Prepare the FASTA format file as requested, and upload it to the pipeline.

Example File:

>ARID822840 dihydropteroate synthase [Escherichia coli]
ATCCCCATCGTGGAAGTCGCTGCGGAGGCCGACGCCATGGTGACGGTGTTCGGCATTCTGAATCTCACCGAGGACTCCTTCTTCGATGAGAGCCCGGCGGCTAGACCCCGGCGGCGGCTGTCACCGCGGCGGC
ATCGAAATGCTGCGAGTCGGATCAGACGTCGTGGATGTCGGACCGGCCGCCAGCCA
CAGATGCACCGTGTTTCAATCGACAGCTTCCAACCGGAAACCCCAGCGCTATGCGCTCAAGCGCGGCGGCGGGGGCTACCCGAACGATATTCCTGACCGCTCTGCCCCGCTCTATCCCGATATTGCTGAG
GCGGACTGCAGGCTGGTGGTTATGCACTCAGCGCAGCGGGATGGCATCGCCACCCGCACCGGTCACCTTCGACCCGAAGACGCGCTCGACGAGATTGTGCGGTTCTTCGAGGCGCGGGTTTCCCGCCTTG
CGACGGAGGGGGTCGCTGCCGACCGGCTCATCCTCGATCCGGGGATGGGATTTTTTTT
CCGCTATTGGTCTCGGTGTCGCGGAAATCCTTCTTGGGCCGCCACCGTTGGCCTTCCTGTAAAGGATCTGGGTCCAGCGAGCCTTGCGGCGGAACTTCACGCGATCGGCAATGGCGCTGACTACGTCCGC
ACCCACGCGCCTGGAGATCTGCGAAGCGCAATCACCTTCTCGGAAAACCCTCGCGAAATTTCGCAGTCGCGACGCCAGAGACCGAGGGTTAGATCATGCCTAG
>ARID822880 dihydropteroate synthase [Enterobacter cloacae]
ATCCCCATCGTGGAAGTCGCTGCGGAGGCCGACGCCATGGTGACGGTGTTCGGCATTCTGAATCTCACCGAGGACTCCTTCTTCGATGAGAGCCCGGCGGCTAGACCCCGCCGGCGGCTGTCACCGCGGCGG
ATCGAAATGCTGCGAGTCGGAGTCAGACGTCGTGGATGTCGGACCGGCCGCCAGCCA
CAGATGCACCGTGTTTCAATCGACAGCTTCCAACCGGAAACCCCAGCGCTATGCGCTCAAGCGCGGCGGCGGGGGCTACCCTGAACGATATTCCTGACCGCTCTGCCCCTGCGCTCTATCCCGACGGTATTGCTGAG
GCGGACTGCAGGCTGGTGGTTATGCACTCAGCGCAGCGGGATGGCATCGCCACCCGCACCGGTCACCTTCGACCCGAAGACGCGCTCGACGAGATTGTGCGGTTCTTCGAGGCGCGGGTTTCCCGCCTTG
CGACGGAGGGGGTCGCTGCCGACCGGCTCATCCTCGATCCGGGGATGGGATTTTTTTT
CCGCTATTGGTCTCGGTGTCGCGGAAATCCTTCTTGGGCCCCACCGTTGGCCTTCCTGTAAAGGATCTGGGTCCAGCGAGCCTTGCGGCGGAACTTCACGCGATCGGCAATGGCGCTGACTACGTCCGC
ACCCACGCGCCTGGAGATCTGCGAAGCGCAATCACCTTCTCGGAAACCCTCGCGAAATTTCGCAGTCGCGACGCCAGAGACCGAGGGTTAGATCATGCCTAG

3.2 Select the nucleotide sequence file to do BLASTn with the integrated SDARG

nucleotide sequence database, chose the proper E-value (better  $\leq$  10-2), and

Execute.

Nucleotide Sequence Annotation Blastn program to query nucleotide sequences in SDARG (Galaxy Version 1.0.0)	▼ Options
nucleotide sequence file(FASTA)	
4	•
E-value	
0.0001	
✓ Execute	

The result is as following, the test example sequence should be annotated as

sull gene:

Query= ARID822840 dihydropteroate synthase [Escherichia coli]		
Length=876		
	Score	Е
Sequences producing significant alignments:	(Bits)	Value
aill99867132 ab ABF67777 1  D0517526 sull High 3 00F−164 Sull (	1581	0.0
gi  90265407 emb CAT77089.1  CT025832 sull High 3.00E-164 sulld	1581	0.0
gill90265368[emb]CAT77050.1] CT025832 sull High 3.00E-164 sulld	1581	0.0
gill85721150/gb/ABC76093.11 CP000252 sull Figh 3.00E-164 dibydr	1581	0.0
gi 820773496 gb AKG90356.1  KP742988 sull High 3.00E-164 dihvd	1581	0.0
gi 766547723 gb ATS16031.1 KP116299 sull High 3.00E-164 dihyd	1581	0.0
gi  765526620 gb ATS10216.1  KP453775 sull High 3.00E-164 dihyd	1581	0.0
gi 762229514 dbj BAQ55583.1 LC030435 sull High gene 3.00E-164	1581	0.0
gi 700587998 gb AIU94568.1 KM649682 sull High 3.00E-164 dihyd	1581	0.0
gi 693581793 dbj BAP68573.1 AP014650 sull High 3.00E-164 dihy	1581	0.0
gi 693581787 dbj BAP68567.1  AP014650 sull High 3.00E-164 dihy	1581	0.0
gi  690385531 gb AIR72316.1  KM023773 sull High 3.00E-164 dihyd	1581	0.0
gi 641398833 gb AIA58914.1  KJ510410 sull Middle 3.00E-164 sul	1581	0.0
gi  633259752 dbj BA079444.1  AB926431 sull High 3.00E-164 sulf	1581	0.0
gi  593804565 gb AHL68236.1  KF017283 sull High 3.00E-164 dihyd	1581	0.0
gi  575870568 emb CDF66134.1  HG003695 sull High_gene 3.00E-164	1581	0.0
gi  572099405 dbj BA027817.1  AB898038 sul1 High 3.00E-164 sulf	1581	0.0
gi  546201898 dbj BAN87141.1  AP013357 sull High_gene 3.00E-164	1581	0.0
gi  537705814 dbj BAN81907.1  AB846838 sull High 3.00E-164 dihy	1581	0.0
gi  528172304 gb AGS27666.1  CP006631 sull High 3.00E-164 Dihyd	1581	0.0
gi  514883720 gb AGO62374.1  JX442974 sull High_gene 3.00E-164	1581	0.0
gi  452916843 gb AGG19217.1  KC189475 sull High_gene 3.00E-164	1581	0.0
gi  444506920 gb AGE11239.1  JQ996150 sull High 3.00E-164 dihyd	1581	0.0
gi  443417939 emb CCO61950.1  HF546976 sull High_gene 3.00E-164	1581	0.0
gi  429345522 gb AFZ84491.1  JX988621 sull High_gene 3.00E-164	1581	0.0
gi  410066879 gb AFV58080.1  JX517203 sull High 3.00E-164 dihyd	1581	0.0
gi  407804968 gb EKF76221.1  JH930402 sull High_gene 3.00E-164	1581	0.0
gi  394349075 gb AFN35097.1  CP003684 sull High 3.00E-164 Dihyd	1581	0.0
gi  394348984 gb AFN35006.1  CP003684 sull High 3.00E-164 Dihyd	1581	0.0
gi  385721348 gb AFI72853.1  JQ364967 sull High_gene 3.00E-164	1581	0.0
g1  380714704 gb AFE02726.1  JQ639792 sull High_gene 3.00E-164	1581	0.0
g1  377806718 gb AFB76403.1  JN676148 sull High 3.00E-164 Dihyd	1581	0.0
g1  3/4413623 gb AEZ49746.1  JN687470 sull High_gene 3.00E-164	1581	0.0
g1  374413610 gb AEZ49733.1  JN687470 sull High_gene 3.00E-164	1581	0.0
g1  3/4413594 gb AEZ49717.1  JN687470 sull High_gene 3.00E-164	1581	0.0
giji365819152[gb]AEXUU942.1] JF274992 sull High_gene 3.00E-164	1581	0.0

3.3 Select the protein sequence file to do HMMsearch with the integrated SDARG

Hidden Markov Models database, and Execute.

Protein Sequence Annotation HMMsearch program to search protein sequences against HMM models of SDARG (Galaxy Version 1.0.0)	▼ Options
Protein Sequence file(FASTA)	
1     1       1 <th>•</th>	•
✓ Execute	

#### Example File:



Output result:

Scores for complete sequences (score includes all domains):
full sequence best 1 domain#dom-
[No hits detected that satisfy reporting thresholds]
Domain annotation for each sequence (and alignments):
[No towards detected that actively use while the shall de]
[Mo cargets detected that satisfy reporting thresholds]
Internal pipeline statistics summary:
Query model(s): 1 (207 nodes)
Target sequences: 3 (413 residues searched)
Passed MSV filter: 0 (0); expected 0.1 (0.02)
Passed bias filter: 0 (0); expected 0.1 (0.02)
Passed Vit filter: 0 (0); expected 0.0 (0.001)
Passed Fwd filter: 0 (0); expected 0.0 (1e-05)
Initial search space (Z): 3 [actual number of targets]
Domain search space (domZ): 0 [number of targets reported over threshold]
# CPU time: 0.01% 0.01% 00:00:00.02 Hispsed: 00:00:00.09
# MC/SEC: 0.90
Query: aac2ia [M=178]
Scores for complete sequences (score includes all domains):
full sequence best 1 domain#dom-
E-value score blas E-value score blas exp N Sequence Description
[No hits detected that satisfy reporting thresholds]
Domain annotation for each sequence (and alignments):
[No targets detected that satisfy reporting thresholds]

```
Query:
           sull [M=283]
 cores for complete sequences (score includes all domains)
     - full sequence
                           best 1 domain
                                               -#dom
                         E-value
   E-value
                  bias
                                 score
                                       bias
                                                    N
                                                      Sequence
                                                                                    Description
           score
                                               exp
                         1.2e-82 265.2
                                       0.0
                                              1.0 1 ARID823108 sul1 Sul1,
   1.1e-82 265.4
                  0.0
                                                                                     partial [Pseudomonas aeruginosa]
                                                      ARID823346 |sul1 |dihydropteroate synthase type 1, partial [Salmon
ARID823383 |sul1 |dihydropteroate synthase, partial (plasmid) [Pro
     3e-64 204.8
                   0.0
                         3.4e-64 204.6
                                         0.0
                                               1.0 1
   1.8e-57
           182.6
                  0.0
                         1.9e-57
                                 182.5
                                         0.0
                                               1.0 1
Domain annotation for each sequence (and alignments):
>> ARID823108 |sul1 |Sul1, partial [Pseudomonas aeruginosa]
                                                      alifrom ali to
       score bias c-Evalue i-Evalue hmmfrom hmm to
                                                                       envfrom env to
  #
                                                                                          acc
  1
      265.2
              0.0
                   1.2e-82
                             1.2e-82
                                        124
                                               283 .]
                                                           1
                                                                 160 []
                                                                            1
                                                                                  160 [] 1.00
 Alignments for each domain:
   domain 1 score: 265.2 bits; conditional E-value: 1.2e-82
                 suli 124 crlvvmrsaqrdGiatrtGhLRpedaldEivrffearvsalrrsgvaadrlildpGmgfflsdapetslhvlsnlqklksalg1rllv 211
                         crlvvm+sagrdGiatrtGhLRpedaldEivrffearvsalrrszvaadrlildpGmzffls+apetslhvlsnlgklksalzl+llv
 ARID823108 | sul1 | Sul1, 1 CRLVVMHSAQRDGIATRTGHLRPEDALDEIVRFFEARVSALRRSGVAADRLILDPGMGFFLSPAPETSLHVLSNLQKLKSALGLPLLV 88
                         sull 212 svsrksflgatvglpvkdlGeaslaaelhaigngadyvrvhakgdlrsaitfseilakfrsrdardrvldea 283
                         svsrksflgatvglpvkdlG+aslaaelhaigngadyvr+ha+gdlrsaitfse+lakfrsrdardr+ld+a
 ARID823108 sul1 [sul1, 89 SVSRKSFLGATVGLPVKDLGPASLAAELHAIGNGADVVRTHAPGDLRSAITFSETLAKFRSRDARDRGLDHA 160
                         ARID823346|sul1|dihydropteroate synthase type 1, partial [Salmonella enterica subsp. enterica serovar Pullorum]
                                                   alifrom ali to envfrom env to
      score bias c-Evalue i-Evalue hmmfrom hmm to
                                                                                      acc
  #
      204.6 0.0 3.4e-64 3.4e-64
                                       124
                                               250 ..
                                                           1
                                                                127 [.
                                                                            1
                                                                                  128 [] 0.99
  1 !
 Alignments for each domain:
   = domain 1 score: 204.6 bits;
                               conditional E-value: 3.4e-64
                          sull 124 orlvvmrsaqrdGiatrtGhLRpedaldEivrffearvsalrrsgvaadrlildpGmgfflsdapetslhvlsnlqkl 201
                                   crlvv++saqrdGiatrtGhLRpe+aldEivrff+arvsal+rsgvaadrlil+pGmgffls+apetslhvlsnlqkl
 ARID823346 |sul1 |dihydropteroate 1 CRLVVIHSAQRDGIATRTGHLRPENALDEIVRFFKARVSALQRSGVAADRLILNPGMGFFLSPAPETSLHVISNLQKL 78
                                  suli 202 ksalgirilvsvsrksfigatvgipvkdlGeaslaaelhaigngadyvr 250
 ksa gl+llvsvsrksflgatvglpvkdlG+aslaaelhaigngady+r
ARID823346 |sul1 |dihydropteroate 79 KSAWGLPLLVSVSRKSFLGATVGLPVKDLGPASLAAELHAIGNGADYFR 127
                                  ARID823383 |sul1 | dihydropteroate synthase, partial (plasmid) [Proteus mirabilis]
```

The output file of protein sequence annotation displays the result of the submitted sequence to each ARG type in SDARG, and part of the results are as above, we can annotate this test example sequence as *sul1* gene related protein sequences.

#### **Example Dataset:**

If there is any problem with the format of uploading file, please see example dataset

in the 2019 Example Dataset folder from the Shared Data in pipeline:

http://mem.rcees.ac.cn:8083/library/list#folders/F40876639881ca029.