## **ASIST**

A webserver for <u>Antimicrobial Susceptibility standards</u>

## **User Manual version 1.0.0**



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#### Introduction

Antimicrobial susceptibility testing (AST) is used to determine susceptibility of organisms to antibiotics. Determination of susceptibility is based on MIC breakpoints and are provided by EUCAST and CLSI. Likewise, phenotypic classification criteria are developed by CDC/ECDC and standards for assessing quality of whole genome sequence for reporting AST by EUCAST. In this study, these three standards were applied to Acinetobacter baumannii (AB) clinical isolates, as it is the most critical priority pathogen identified by WHO, to identify gaps in implementation of these standards in various studies. Public databases are used to obtain AB genomes and their susceptibility patterns. These datasets are then evaluated based on standards definition. Three standards are tested on 4776 strains of AB in a systematic fashion and an automated pipeline "ASIST" is developed on the basis of these global standards. We converged three global standards including whole genome sequence (WGS) inferred AST on clinical isolates of AB and have designed an automated pipeline that allows for characterization of clinical isolates based on these standards. From over four thousand genomes, only 788 (~16%) are associated with metadata that allows for interpretation of AST in globally acceptable standards. Also, in literature more than 70% studies do not reference any of the standards. It is imperative that standards are followed for reporting AST but there exists several gaps and challenges. It is recommended that a centralized repository and a structured metadata architecture is developed to provide a single globally acceptable framework to report AST.

The workflow system is implemented using the open-source workflow architecture, Galaxy. ASIST is freely available and can be accessed at <u>https://ab-openlab.csir.res.in/asist/static/</u>.

There are three modules in ASIST, described in this manual. All the modules are accessible without registering to the system. However, for maintaining user-sessions it is recommended that anyone who is interested in creating data or task intensive workflow should register (**Figure 1**). The benefit of registration includes user sessions, saved histories, visualization, generation and execution of workflow and many others.

	Login	
Salaxy ASIST Analyz	e Data Workflow Visualize - Shared Data - Help - Login or Register 📕	Using 0 bytes
	Welcome to Galaxy, please log in	
	Public name or Email Address	
	Password	
	Forgot password? Click here to reset your password.	
	Login	
	Don't have an account? Register here.	

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#### Figure 1: ASIST Login Page

All the modules of ASIST and some default modules by Galaxy are accessible through a webbased interface which has following components (**Figure 2**).

- a. Navigation Panel: It provides the links to major components of the server like Tools Page (Analyse Data), Workflow System, Shared Libraries, Visualization, Help Section and User Login/Registration.
- **b.** Tool Panel: This panel lists all the tools available in ASIST along with default utilities in Galaxy.
- c. Detail Panel (Canvas): This panel displays the interface of all the tools along with input parameters required to run a tool. It also provides help and examples to run a tool. This panel also displays the output of a tool after its execution when user clicks on the eye sicon show in **History Panel**.
- **d. History Panel:** This panel shows the information about the tools which are executed by a user. The information can include result after completion of a tool execution or error generated while running the tool. The workflow(s) are generated by extracting tasks from history panel.

Tool panel	Navigation panel	History panel
🐞 Galaxy ASIST	Analyze Data Workflow Visualize T Shared Data T Admin Help T User T	Using 47.5 MB
Tools 🕸 🛓 search tools Co Get Data	ASIST: A webserver for <u>A</u> ntimicrobial <u>S</u> uscept <u>i</u> b <u>st</u> andards	ility C + C + C + C + C + C + C + C + C + C
Send Data Collection Operations Text Manipulation	Home » ASIST Shared Data » ASIST Example Workflow » ASIST User Manual Details pan	el 24.33 MB
Convert Formats Filter and Sort	Overview of ASIST (Canvas) ASIST is an automated pipeline that can be used for the characterization of clinical isolates in of their acquired resistance profile. The acquired resistance profile of isolates can be analyzed three plobal standards is deterministing of suscentibility based on MIC breakmoints provided	36: ASIST on data 2 3 2 X
Statistics Graph/Display Data	EUCAST and CLSI, phenotypic classification criteria developed by CDC/ECDC and standards for assessing quality of whole genome sequence for reporting AST by EUCAST. In this study, pub databases and literature were used to obtain AB genomes and their susceptibility patterns. TH datasets were then evaluated based on these standards definition. Three standards were test	rr s lic licse 31: Quast on data 8 and
AMR TOOLS Phenotype Association	4776 strains of AB. From over four thousand genomes, only 788 (~16%) are associated with metadata that allows for interpretation of AST in globally acceptable standards. Also, in literat more than 70% studies do not reference any of the standards.	30: Quast on data 8 and

Figure 2: ASIST Homepage

#### **I.** Navigation Panel

#### 1. Analyze Data

The data analysis page is where everything happens. There, you can run any available tools on the data, run complete workflows, browse or download a result, and share files with other users. It is the default page when you open Galaxy in your browser, but you can also access it any time by clicking on "Analyze Data" in the Navigation Panel.



Figure 3: Analyze Data view and Tools.

#### 2. Workflow

Workflows are analysis that are intended to be executed (one or more times) with different user-provided input datasets. Workflow can be reused over and over, not only reducing tedious work, but enhancing reproducibility by applying the same exact methods to all of your data. Workflow is nothing but creating pipeline, user can use it again and again or user can publish it.

Workflow can be created through navigation panel or from tool panel. In workflow section user can create workflow or can upload or import the workflow. The canvas is where inputs, tools, and noodles are added and connected as you build and modify your workflow (Figure 3). Selecting Edit opens the workflow editor view (Figure 2). The navigator provides a full view of your workflow in a condensed format (Figure 3). Accessed by clicking on the

gear icon on the right side of the center workflow canvas upper bar, the workflow editor menu (Figure 3) is for global editor actions. It consists of Save, Run, Edit Attributes, auto re-layout, close.

				Workflow		Create wo	rkflow	Imp	oort workflow	
🐞 Galaxy ASI	ST			Analyze Data Workflow Visualize -	Shared Data ▼ Admin H	elp 🕶 User 🕶 🚺				Using 47.5 MB
Tools search tools	습	± O	Search Workflows		Ĵ		+ Create	↓ ≛ Import	History search datasets	2+⊡¢ 0
Get Data Send Data			Name	Saved workflows	0 Tags	Created in 5 minut	Bookman	*	workflow_ASIST_June	_14_21_fini
Collection Operations Text Manipulation			ASIST	rom history 'workflow ASIST June 14 21	finished	4 days ag			24.33 MB	<b>*</b>
Convert Formats Filter and Sort Join, Subtract and Gro	up		Workflow constructed f	rom history 'workflow_finished'		4 days ag	• •		II 40: ASIST on data 39 II 39: CLSI Profile on d ata 38	• # X • # X
Statistics Graph/Display Data			Edit Copy Download	Click on the edit o	ption to see	the workfl	ow		<ul> <li>38: ASIST on data 37</li> <li>36: ASIST on data 2</li> </ul>	• # X • # X
AMR TOOLS Phenotype Association	n		Rename Share View	Options					32: Quast on data 8 and data 5: Unaligned conti gs	● # ×
Genomics	ation		Delete						31: Quast on data 8 and data 5: Misassemblies	● # ×
ASIST TOOLS									30: Quast on data 8 and data 5: Log	● # ×
Workflows All workflows									29: Quast on data 8 and data 5: PDF report	• / ×

#### Figure 4: Options for workflow.

The following example of workflow shows the "prediction of acquired resistance profile using ASIST tools" Figure 5.



Figure 5: Workflow overview

#### 3. Shared data library

Data libraries are collections of Datasets that are accessible from within a Galaxy instance. Libraries are designed for sharing datasets in between users or groups. The data library of ASIST consists of MIC breakpoints and resistance profile of AB according to the CLSI breakpoints. Some of the actions that can be performed on data libraries are accessed by clicking the pop-up menu icon just right of the data library name.

- **View Information** –Shows the information about dataset.
- Import this dataset into your current history this creates an item in your current history on which you can perform analysis. The item is a pointer to the library dataset disk file, so the file is not copied on disk.
- > **Download this dataset** this allows you to download a local copy of the dataset.

					S	hared dat	a	Shar libi	ed data raries				
🔊 Galaxy ASIST			Analyze Data	Workflow	visualize <del>-</del>	Shared Data 🗸	Admin	Help 👻 Öser 👻					Using 47.5
# Search	+ Folder	+ Datasets -	Export to History 🔻	🖺 Downlo	ad 🔻 🛛	cess published resourc	es 🔺	) include deleted					
Libraries / CLSI MIC breakpoi	nts for bacter	ria				Workflows							
□ Name ↓ <sup>A</sup> <sub>Z</sub>			Description			Visualizations Pages		pe Size	Date Upd	ated (UTC)	State		
-	<u> ¬</u> Г		_										
AST_file_example.csv		Shared	uploaded csv file				CSV	1.8 KB	2021-06-1	0 10:50 AM	setting_metadata	0	📽 Manage
Clsi_profile_2020.csv		data	CLSI MIC breakpo	int 2020 csv fi	ile		CSV	731 bytes	2021-06-1	0 10:51 AM	setting_metadata	0	🔮 Manage
			« 0	1 2	» 15		$\langle \cdot \rangle$	per page, 2 total					

Figure 6: Data library view.

#### 4 Help

The help section of Galaxy consists of Support, Search, Mailing List, Videos, Wiki and How to cite Galaxy and interactive tours. User can fine user manual in help section.

#### 5. User

Login option and register option can get in user section. It is recommended that user register their account before using framework. Although unregistered users have access to tools available but their history is stored temporarily. On the other hand, registered users can save and retrieve their results in history panel later too.

### II. Tool Panel – ASIST tool

The user can get tools in tool panel. There are two categories of tools galaxy inbuilt tool and ASIST tools. Galaxy tools consists of Data importing, Manipulation, Filtering, Sorting, Format conversion etc. ASIST tools are specific for antimicrobial susceptibility profiling of bacteria.

#### 1. Importing data to the ASIST

A user can upload the data using the Galaxy tool **Get Data.** If the data is not available in appropriate form, file type option can be used to get the appropriate file type and click on the srat button. The following figure shows the file upload method.



Figure 7: Get Data screen.

#### 2. QUAST tool for genome quality analysis

The QUAST tool was used to calculate the genome fraction, NGA50 and number of contigs was used to identify good quality genomes for AST. Standards for assessing quality of whole genome sequence for reporting antimicrobial susceptibility i.e., Genome fraction=75%, NGA50=20000 and number of contigs=500 was used as per EUCAST criteria. The output file will be generated in html, pdf and text format which can be extracted later.

1. Search Quast			
🐞 Galaxy ASIST	Analyze Data 🛛 Workflow Visualize 🕶 Shared Data 👻 Admin Help 🕶 User 🕶 🌉		Using 47.5 MB
Tools     ☆ ±       quast     ♥	Quast Genome assembly Quality (Galaxy Version 5.0.2+galaxy1) 🗘 Favorite VOptions	History search datasets	2+0¢ 8
Genomics Quast Genome assembly Quality	Use customized names for the Input files? No, use dataset names  They will be used in reports, plots and logs	workflow_ASIST_June shed 26 shown, 16 deleted	e_14_21_fini
All workflows	Contigs/scaffolds file            ege Db          fis. ATCC_17978_mff.fasta         7: DU202.contig	24.33 MB	• • × ^
quast after uploading contigs	6: BJA0068.contig 5: BAL062.contig 4: AYP-A2.contig	<ul> <li>41: CLSI Profile on d ata 40</li> <li>40: ASIST on data 39</li> </ul>	• # ×
or WGS file using get data option	Type of assembly Genome Use a reference genome?	II 39: CLSI Profile on d ata 38	• # X
3. Select	No Many metrics can't be evaluated without a reference. If this is omitted, QUAST will only report the metrics that can be evaluated without a	36: ASIST on data 2	• # X
sequence	reference. Estimated reference genome size (in bp) for computing NGx statistics	32: Quast on data 8 and data 5: Unaligned conti gs	• # ×

Figure 8: QUAST tool for genome quality analysis.

3. ASIST for genome quality using QUAST tool and acquired resistance prediction using ASIST tool

🐞 Galaxy ASIST	Analyze Data Workflow Visualize • Shared Data • Admin Help • User • 🏥	Using 50.0 MB
Tools 🏠 📩	CLSI Profile CLSI MIC breakpoints profile (Galaxy Version 1.0.0)	History C + 🗆 🌣
search tools		search datasets 😢
Collection Operations	Input file of Antimicrobial Susceptibility Testing (AST) MIC values for strains	workflow_ASIST_June_14_21_fini
Text Manipulation	□ @ □ 1: MIC_ASIST_example_2.csv 2. Select input file in csv format • ▷	shed
Convert Formats	Input AST file in CSV format (Please see example given below)	35 shown, 16 deleted
Filter and Sort	Choose MIC Breakpoints	26.86 MB
Join, Subtract and Group	CLSI MIC breakpoints 3. Select CLSI breakpoints	51: CLSI Profile on data (9) A Y
Statistics	CSV File	1
Graph/Display Data	CLSI MIC breakpoints for Bacteria (2020)	5 lines, 1 comments
	Email notification	format: csv, database: ?
AMR TOOLS		₿ Ø C Ш ?
Phenotype Association	Yes No	1.Strain name 2.Amika
NGS: QC and manipulation	Send an email notification when the job completes.	Strain name Amikaci
Genomics	✓ Execute 4. Execute	Acinetobacter baumannii AB307-0294 Suscept
ASIST TOOLS		Acinetobacter baumannii AB5075-UW Resista
ASIST Tools	<b>O USAGE:</b> Please provide the input CSV file containing AST MIC values for the tested strains.	<
CLSI Profile CLSI MIC breakpoints profile	Input AST file example:	50: CLSI Profile on data 🛛 🕐 🖋 🗙 1
ASIST Antimicrobial Susceptibility	Acinetobacter baumannii A85,Colistin,0.1,mg/L	5 lines, 1 comments
standards based phenotypes	Acinetobacter baumannii AB307-0294,Imipenem,1,mg/L	format: csv, database: ?
1. Select CLSI profile		

Figure 9: Usage of CLSI MIC breakpoints tools for resistance profile of bacteria.

ools 🏠 📩		History	C+⊡¢
search tools	ASIST Antimicrobial Susceptibility standards based phenotypes (Galaxy Version 1.0.0)  ☆ Favorite  ◆ Options	search datasets	0
ollection Operations	Input file in CSV format	workflow_ASIST_June	e_14_21_fini
ext Manipulation	🗅 😢 🗅 2: CLSI Profile on data 1 2. Select MIC breakpoints 🔹 🕞	shed	
onvert Formats	Use CSV format. values	36 shown, 16 deleted	
Iter and Sort	Email notification	26.86 MB	
in, Subtract and Group	Yes No	52: ASIST on data 2	. / X
atistics	Send an email notification when the job completes.		
raph/Display Data	3. Select MIC breakpoints values	1	• / ×
MR TOOLS		5 lines, 1 comments	
nenotype Association	<b>1</b> USAGE: Please provide the input CSV file containing AST results as Suseptible(S) and Resistant(R) for the tested strains.	format: csv, database: ?	
GS: OC and manipulation	Input file:	BOCM ?	**
enomics	Strain name, "Amikacin, Tobramycin, Gentamycin, Imipenem, Meropenam, Doripenem, Ciprofloxacin, Levofloxacin, Piperacillin/tazobactam, Ticarcillin/c	1.Strain name Strain name	2.Amika Amikaci
	,Resistance_phenotype,Aminoglycoside,Aminoglycoside,Aminoglycoside,Beta-lactams-Carbapenems,Beta-lactams-Carbapenems,Carbapenem ,Fluoroqu. Acinetobacter baumannii strain FDA-CDC-AR_0288,,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resi	Acinetobacter baumannii A85	NA
SIST Tools	Acinetobacter baumannii strain FDA-CDC-AR_0303,,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant Acinetobacter baumannii strain FDA-CDC-AR_0304.,NA.Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,NA.Res	Acinetobacter baumannii AB30 Acinetobacter baumannii AB50	17-0294 Suscept 175-UW Resista
CLSI Profile CLSI MIC breakpoints	Acinetobacter baumannii strain FOA-CDC-AM_0308,,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant Acinetobacter baumannii strain FOA-CDC-AM_0309,,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant,Resistant, <	Acinetobacter baumannii AC30	NA >
SIST Antimicrobial Susceptibility	Output file:	50: CLSI Profile on data 1	• / ×

Figure 10: Usage of ASIST tools for acquired resistance profile of bacteria.

#### III. Detail Panel

This panel displays the interface of all the tools along with Input Parameters required to run a tool. It also provides help and examples to run a tool. This panel also displays the Output of a tool after its execution when user clicks on the eye icon show in **History Panel**.

#### **IV. History Panel**

When data is uploaded from your computer or analysis is done on existing data using Galaxy, each output from those steps generates a dataset. These datasets (and the output datasets from later analysis on them) are stored by Galaxy in **Histories**.

Users that have registered an account and logged in can have many histories and the history panel allows switching between them and creating new ones.



11. History Panel view