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Quality control assessment for microbial genomes: GalaxyTrakr MicroRunQC workflow V.6

Candace Hope Bias[1], Ruth Timme[1], Yesha Shrestha[2], Tina Lusk Pfefer[3], Paul Morin[4], Maria Balkey[3], Errol Strain[3]

[1]US Food and Drug Administration;
[2]Center for Veterinary Medicine, US Food and Drug Administration;
[3]Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland, USA;
[4]U.S. Food and Drug Administration, Jamaica, New York, USA

GenomeTrakr
Tech. support email: **genomeTrakr@fda.hhs.gov**

Ruth Timme
US Food and Drug Administration

DISCLAIMER

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We use this protocol and it's working

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ABSTRACT

PURPOSE: Step-by-step instructions for checking WGS sequence quality for bacterial pathogens. The MicroRunQC workflow, implemented in a custom Galaxy instance, will produce quality assessments for raw reads (Illumina paired-end fastq files) and draft de novo assemblies, along with reporting the sequence type for each isolate. This workflow will work on most microbial pathogens, so we advise laboratories to upload their entire MiSeq/NextSeq run through this workflow.

SCOPE: This protocol covers the following tasks:

1. Quick access to GenomeTrakr sequence quality thresholds by organism
2. Create a GalaxyTrakr account
3. Set up an account in GalaxyTrakr
4. Create a new history/workspace
5. Upload data
6. Execute the MicroRunQC workflow
7. Interpret the results - check against GenomeTrakr QC thresholds

Version updates:
V6: Minor edits, including section reorganization and addition of clarifying notes
V5: New column in the output table to capture additional mlst data fields when available in Sequence Type definition files (not available for all species)
V4: MicroRunQC updated to V1.1 Includes updates to skeza and mlst methods, as well as adjusted assembly QC thresholds for E.coli. Added *Enterobacter* QC thresholds to threshold table.
V3: updated with *Cronobacter* thresholds

Quick Access to QC Benchmarks

1 This protocol will walk the user through various aspects of the quality assessment of bacterial genome sequences, from setting up a GalaxyTrakr account to the quality control (QC) benchmarks GenomeTrakr uses for its sequencing efforts. For quick access, GenomeTrakr QC benchmarks are included in the table below.

These are also relevant for NARMS and VetLIRN contributors.

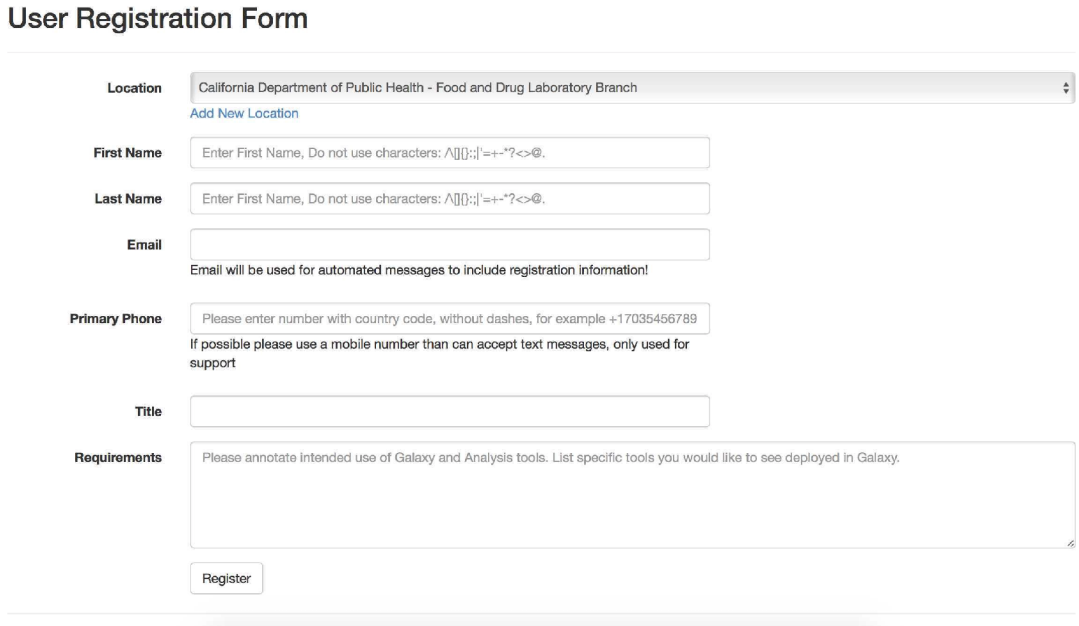
*MicroRunQC users should follow QC threshold guidelines established by their respective surveillance coordinating body(s).

	A	B	C	D	E	F	G	H	I	J
	Quality metric	***Salmonella***	***Listeria***	***E. coli***	***Shigella***	***Campylobacter***	***Vibrio para.***	***Cronobacter***	***Enterococcus faecium***	***Enterococcus faecalis***
	Average read quality Q score for R1 and R2	>=30	>=30	>=30	>=30	>=30	>=30	>=30	>=30	>=30
	Average coverage	>=30X	>=20 X	>=40X	>=40 X	>=20X	>=40X	>=20X	>=50X	>=40X
	De novo assembly: Seq. length (Mbp)	~4.3-5.2	~2.7-3.2	~4.5-5.9	~4.0-5.0	~1.5-1.9	~4.8-5.5	~4-5	~2.5-3.5	~2.5-3.25
	De novo assembly: no. contigs	<=300	<=300	<=400	<=550	<=300	<=300	<=500	<=350	<=200

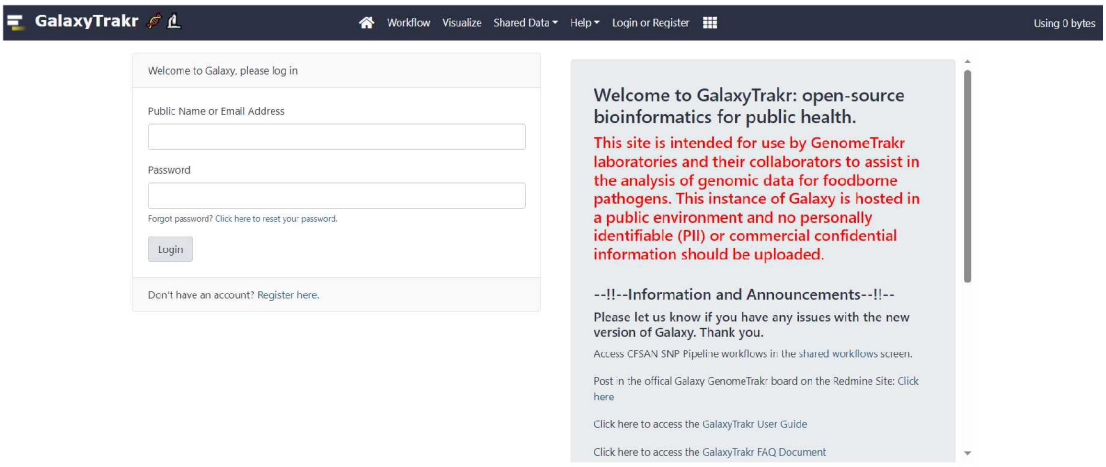
Account set up

2 1. Create a GalaxyTrakr account here: https://account.galaxytrakr.org/Account/Register

> **Note**
>
> This is a more detailed form than what is available by clicking "Register here" on the genometrakr.org main page. Please use the form linked here when creating a new account.



2.1 Log into your GalaxyTrakr account: https://galaxytrakr.org



Create a new history

3 **Create a new history.**

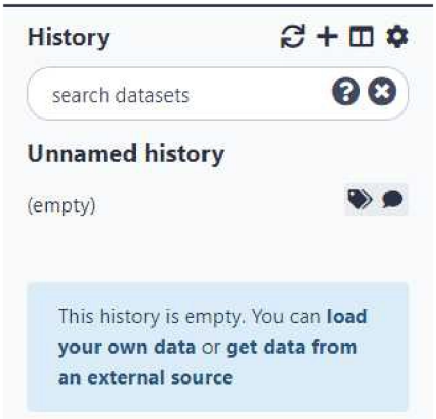
We recommend creating a new history for each new MiSeq Run and including the flow-cell ID and date in the history name.

Save your MicroRunQC output here and any other relevant analyses, like serotyping, or AMR detection.

After all the analysis output from this run is saved to your internal data network or computer, older histories should be purged/deleted so as not to occupy the limited storage space in your account. In some cases it may be useful to save, for a limited time, multiple histories or to run analyses concurrently in multiple histories. In these cases you need to pay attention to your % usage bar (shows % used of allocated storage space) in the upper right corner of the GalaxyTrakr page. If you need additional space you can contact galaxytrakrsupport@fda.hhs.gov and request additional storage.

3.1 Click on the + icon in the upper right History panel

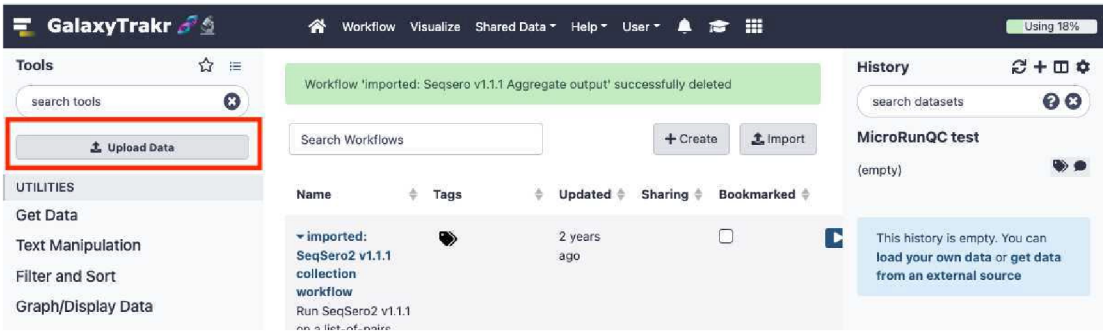
3.2 Name your new History by clicking on the “Unnamed history" text, type in desired name, and hit Enter. We recommend including the run cell ID and the date the run was started.



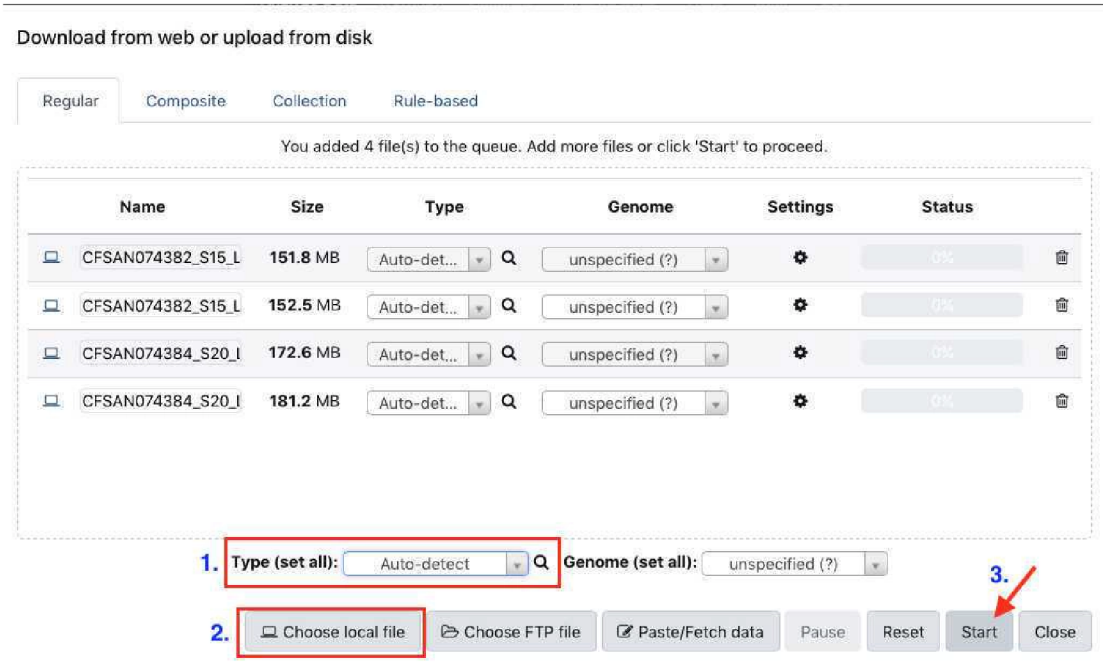
Upload data

4 **This section will describe the process for uploading raw fastq files into your active History panel.** After the files have been uploaded they will stay in your account until they are deleted.

4.1 Click on the Upload Data icon on the top of the left web page to start an upload process.



4.2 Select "**Type (set all):auto-detect.**" Click "**Choose local files**" button and navigate to the desired fastq files, then click "**Start**" to upload files. These files should be paired (two per sample/isolate).



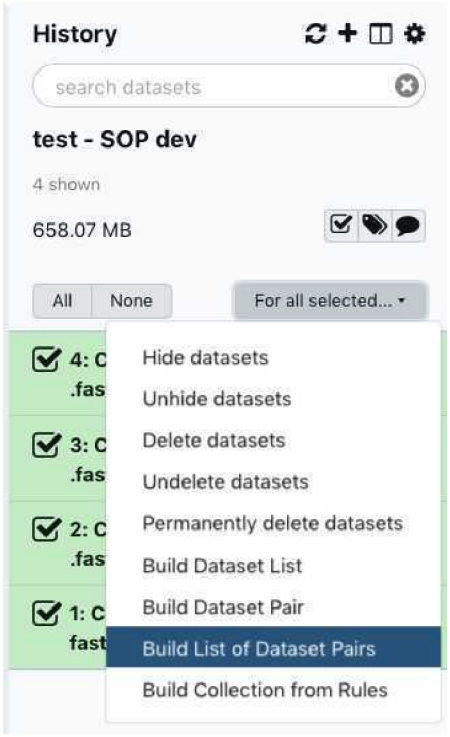
As the file uploads complete, each row will turn green. Samples in yellow are still in process.

4.3 You have just upload a set of forward and reverse reads. For further analysis, these files need to be paired properly so the platform knows which R1 and R2 files go together. GalaxyTrakr does this by creating a **List of Dataset Pairs.**

Within your newly created History panel, click the check box, then select all the files you just uploaded by clicking "All" or by individually selecting the ones you want to pair.

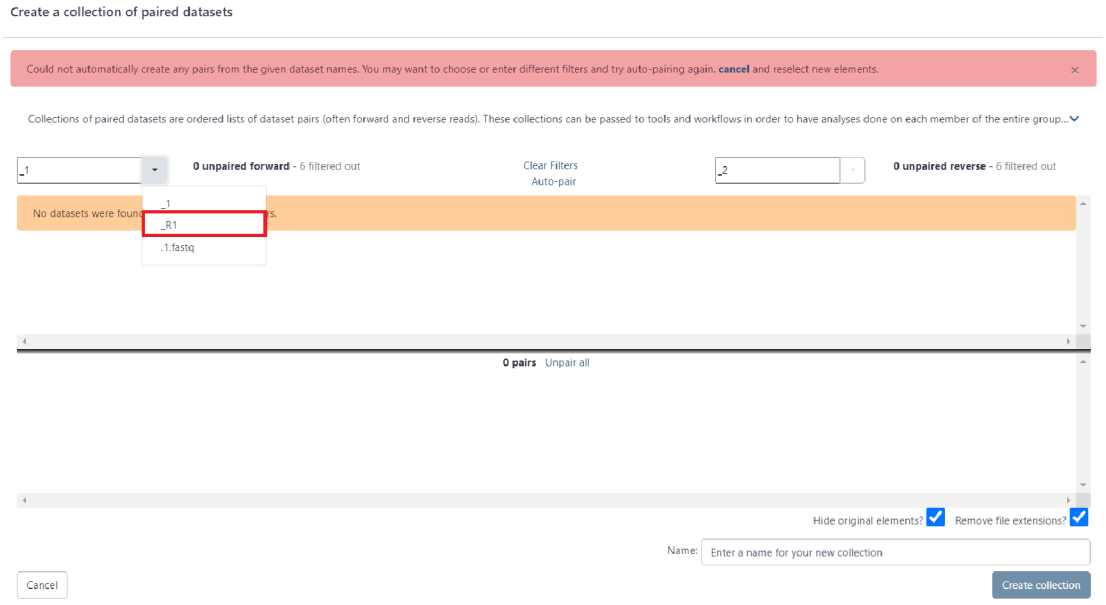
Screenshot of History panel showing recently uploaded files. Note the way the files are named, using R1 and R2 to identify the paired reads. This will be important in the next step. Some naming conventions can be slightly different.

4.4 Click "For all selected" and choose "Build List of Dataset Pairs"

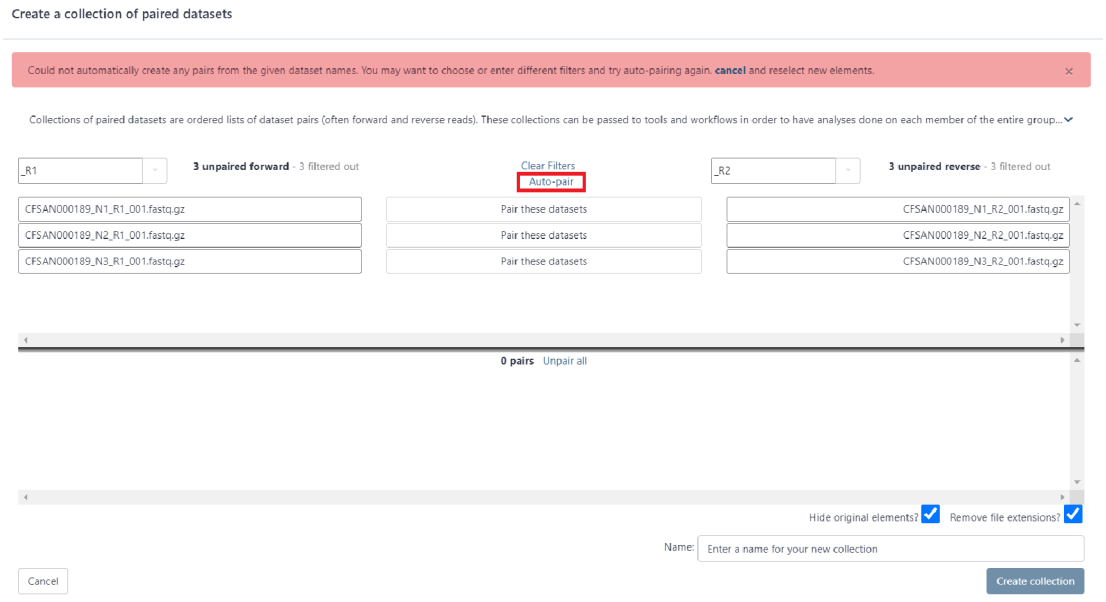


4.5 A new window will open to help you pair the fastq files properly. Note how your paired reads are named.

First, click on the drop down arrow and choose "_R1," if that is the naming convention your files follow. This automatically populates the corresponding "_R2" in the next box.

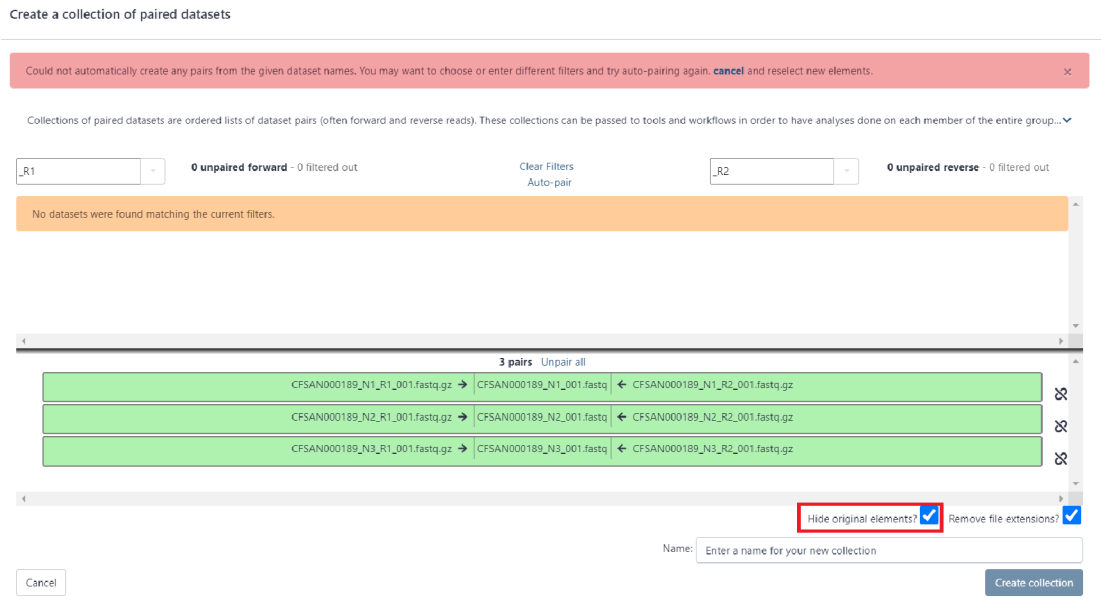


Click **Auto-pair.**



Paired reads will pair in the middle column and turn green.

Unselect "**Hide original elements,"** which is the default setting and undesired here.

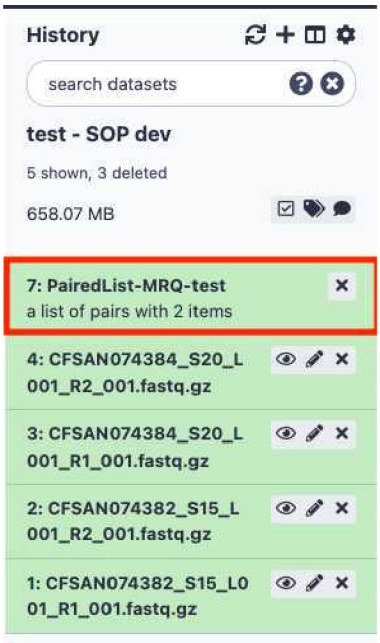


Clear the checkmark by "Hide original elements?" seen here by clicking on it.

Name your dataset: Example, "pairedSet-<FlowCell>-<date>"

Click **Create list.**

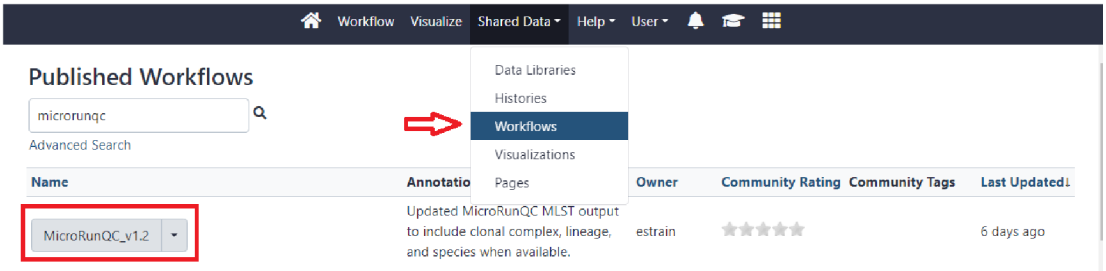
4.6 This paired dataset will now be available for analysis in your history panel. You can run multiple analyses on the same dataset in a history rather than upload the same sequence data to a new history to perform additional analyses. This will help you use your allocated storage space efficiently.



Run the MicroRunQC workflow

5 **Add the MicroRunQC workflow to your own "Workflows" panel.** You only have to do this step once for each new workflow you need.

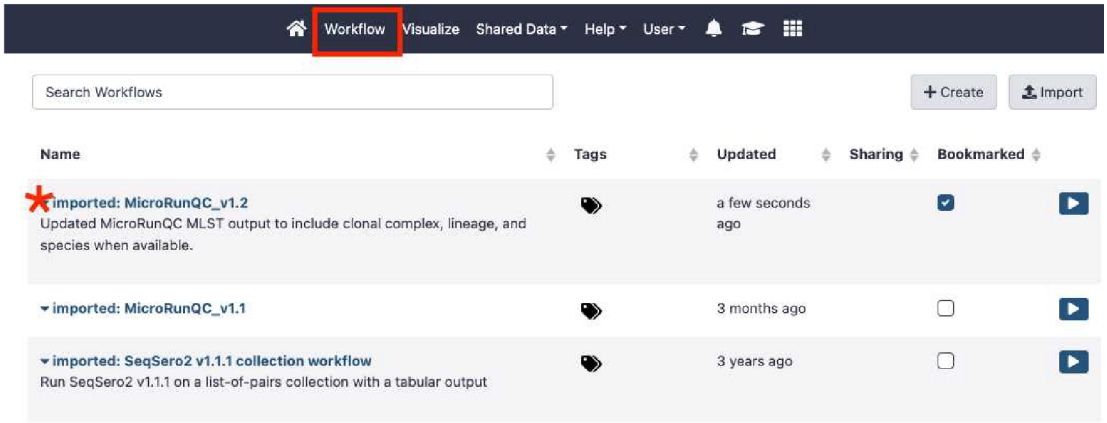
5.1 Navigate to the "**Shared Data**" drop down menu, choose "**Workflows,**" and search for "MicroRunQC_v1.2."



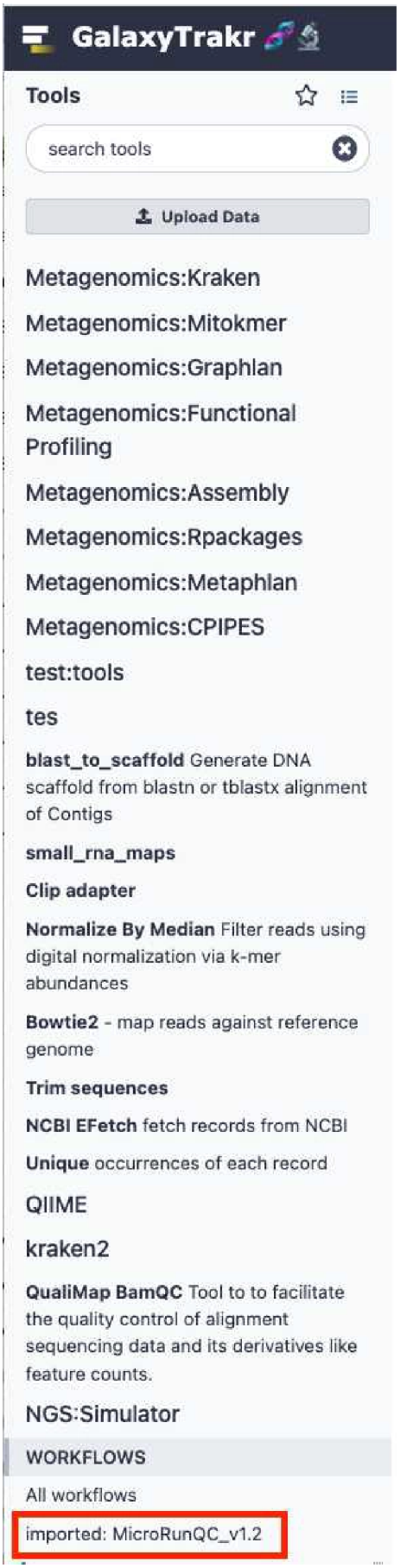
From the dropdown menu by the MicroRunQC title, select "**Import.**"

5.2 To see the new imported workflow, click the "**Workflow**" tab on the top panel.

Click the box under "**Bookmarked**" to make it available in the left panel under "**Workflows**" when MicroRunQC is searched for.



5.3 From the Workflow menu on the left panel, select **MicroRunQC_v1.2**


GalaxyTrakr
Tools
search tools
Upload Data
Metagenomics:Kraken
Metagenomics:Mitokmer
Metagenomics:Graphlan
Metagenomics:Functional Profiling
Metagenomics:Assembly
Metagenomics:Rpackages
Metagenomics:Metaphlan
Metagenomics:CPIPES
test:tools
tes
blast_to_scaffold Generate DNA scaffold from blastn or tblastx alignment of Contigs
small_rna_maps
Clip adapter
Normalize By Median Filter reads using digital normalization via k-mer abundances
Bowtie2 - map reads against reference genome
Trim sequences
NCBI EFetch fetch records from NCBI
Unique occurrences of each record
QIIME
kraken2
QualiMap BamQC Tool to to facilitate the quality control of alignment sequencing data and its derivatives like feature counts.
NGS:Simulator
WORKFLOWS
All workflows
imported: MicroRunQC_v1.2

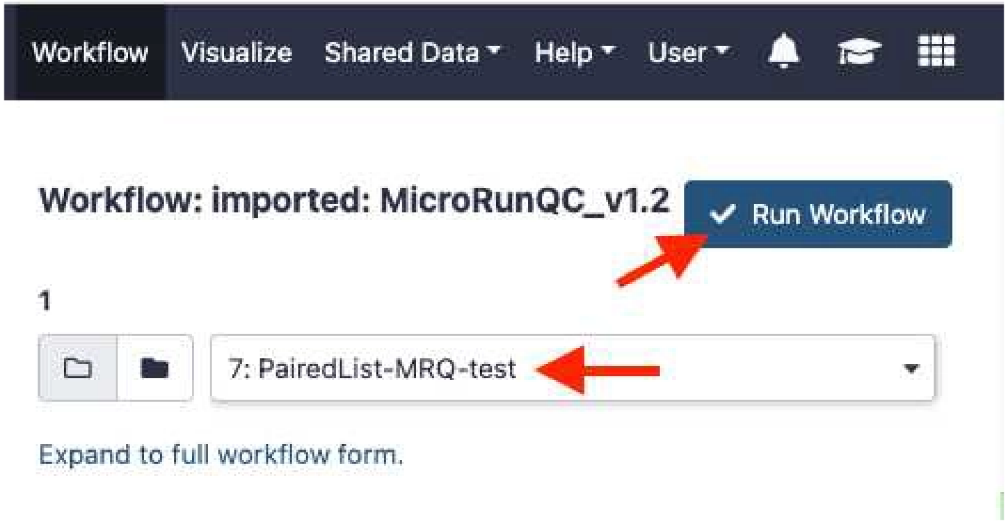
5.4 Select paired list dataset you created earlier.

Click **Run Workflow**. This can take some time depending on the number of samples you are analyzing. If you choose to you can log out of GalaxyTrakr and log back in at a later time to see if the job is completed.

Note

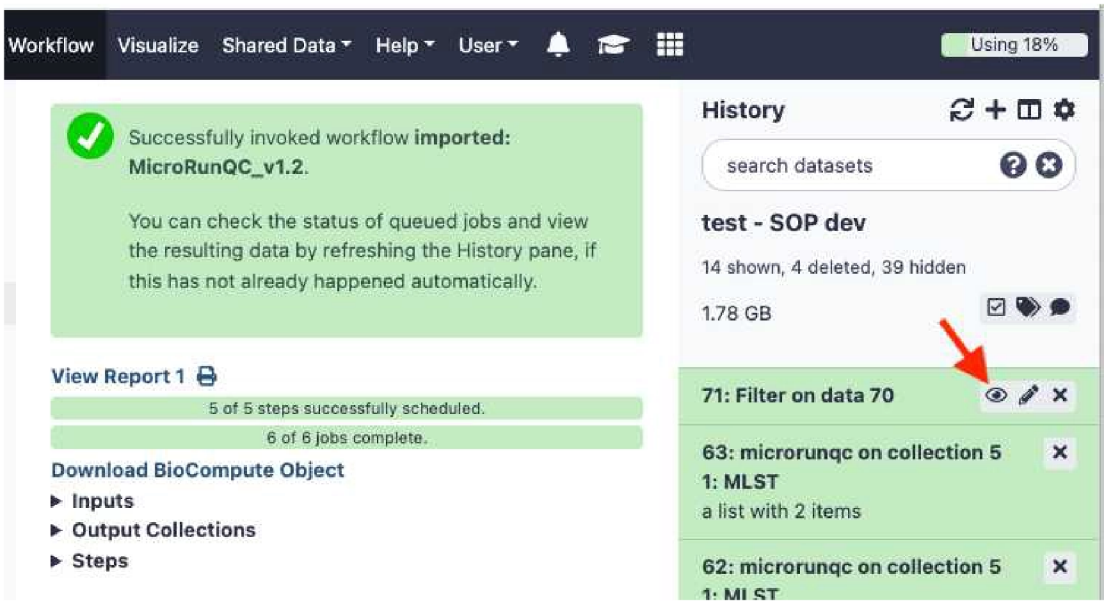
If you see an orange box at the top of the workflow before running it that states that the tool is out of date or contains errors, please go back to **Step 4.1** of this protocol and import the version of the tool with the most current "Last Updated" date. The version number on the MicroRunQC worflow does not increment when only the MLST database is updated, meaning that though the version number of the workflow may remain the same, re-import may be necessary for the most up-to-date analysis of your genomes.

Example of warning box



5.5 Upon completion of the pipeline all tiles in the History pane will be green.

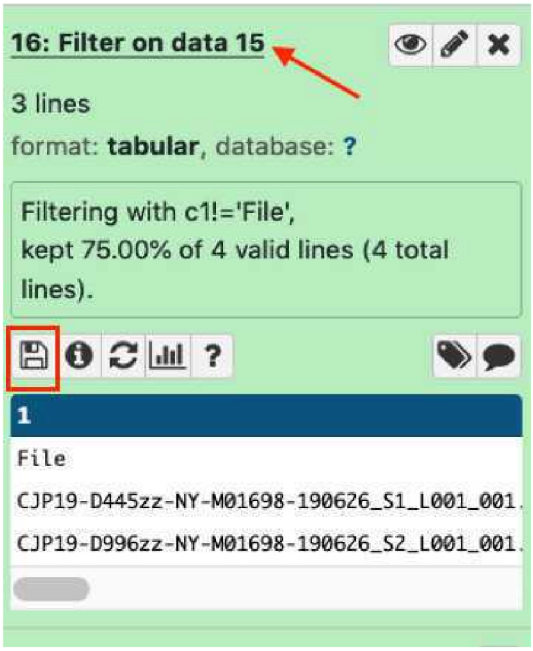
In the "**Filter on Data ##**" tile, click on the "Eye" icon to view the output table in the GalaxyTrakr window.



Interpret the results

6 **Download and interpret the results:**

6.1 Click **Filter on data ##** and then the floppy disc icon. The tabular file can be opened in a text reader or converted to a format (.txt) that can be opened in Excel.



6.2 The MicroRunQC output file includes the following columns:

	A	B	C
	Parameter	***Input***	***Description***
	Contigs	Assembly	Number of contigs in the de-novo SKESA assembly. Contigs smaller than 200 base-pairs (bp) are not counted.
	Length	Assembly	Total length of all contigs > 200bp. This should approximate the size of the genome for the target organism.
	EstCov	Assembly	Mean coverage for contigs in the SKESA assembly.
	N50	Assembly	Sequence length of the shortest contig at 50% of the total genome length
	MedianInsert	Read	Distance between forward and reverse reads. Calculated by mapping reads to SKESA assembly using bwa.
	MeanLength_R1	Read	Mean length of forward read
	MeanLength_R2	Read	Mean length of reverse read
	MeanQ_R1	Read	Mean Q-score of forward read
	MeanQ_R2	Read	Mean Q-score of reverse read
	Scheme	Assembly	PubMLST scheme name (output from mlst application that scans contig files against traditional PubMLST typing schemes.
	ST	Assembly	Sequence Type
	MLST extra	Assembly	e.g. Listeria clonal complex info

	A	B	C
	Loci	Assembly	gene (allele number) – for example aroC(118)

MicroRunQC output table headers. This table lists the summary metrics for sequence quality, number of contigs, and estimated genome size, along with other common metrics for reads (Median Insert Size and Mean Length) and assemblies (N50). Additionally, if the Multi-Locus Sequence Type (MLST) for the isolate is available from pubmlst, the workflow also reports Sequence Type (ST) and the associated alleles.

***MLST extra:** Additional data fields reported when available in Sequence Type definition files (not available for all species)
1. clonal_complex – sequences grouped by similarity to central allelic profile (e.g., *Campylobacter* ST-21 complex)
2. CC – clonal_complex – Abbreviation used for organism like *Listeria*, ST profiles are maintained by different groups
3. Lineage – *Listeria monocytogenes* lineage (I,II,III, and IV), Listeria species also reported here (e.g.L.innocua)
4. species – e.g., *Vibrio alginolyticus*

**This output should be saved either to your LIMS or to a spreadsheet linked to the sequencing run and samples.

6.3

Example output for 1 *Salmonella* and 5 *Listeria* isolates.

	A	B
	Srain ID	**Lab Confirmation**
	FDA1216271-C001-001	Listeria mono
	FDA817806-S073-001	Listeria mono
	FDA746634	Listeria mono
	FDA1213377-C001-002	Listeria grayi
	FDA933376-S060-005	Listeria innocua
	FDA1213835-C001-001	Salmonella

Lab confirmed IDs for 6 isolates

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
	File	**Contigs**	**Length**	**EstCov**	**N50**	**Median Insert**	**Mean Length_R1**	**Mean Length_R2**	**Mean Q_R1**	**Mean Q_R2**	**Scheme**	ST	MLST extra							

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
	FDA121627-1-C001-001	16	29119499	36.7	476210	321	148.4	148.4	36.4	34.6	listeria_2	5	CC=CC5,Lineage=I	abcZ(2)	bglA(1)	cat(11)	dapE(3)	dat(3)	ldh(1)	lhkA(7)
	FDA817806-S073-001	20	30683534	179.6	525438	329	234.7	235.2	36.7	31.9	listeria_2	321	CC=CC321,Lineage=II	abcZ(5)	bglA(6)	cat(8)	dapE(62)	dat(6)	ldh(7)	lhkA(34)
	FDA746634	30	30528888	41.4	293947	320	148.4	148.4	36.5	36	listeria_2	-		abcZ(2)	bglA(1)	cat(11)	dapE(3)	dat(3)	ldh(1)	lhkA(~7)
	FDA121337 7-C001-002	20	26721800	155.1	473181	270	147.3	147.3	37.2	36.1	-	-								
	FDA933376-S060-005	9	28818689	213	14987900	303	232.1	232.2	37	36.2	listeria_2	1489	CC=CC1489,Lineage=L. innocua	abcZ(250)	bglA(21)	cat(83)	dapE(298)	dat(20)	ldh(458)	lhkA(216)
	FDA121383 5-C001-001	37	48323655	34.4	294936	354	149	149	36.6	35.7	senterica_achtman_2	214		aroC(14)	dnaN(72)	hemD(21)	hisD(12)	purE(6)	sucA(19)	thrA(15)

MicroRunQC example report showing mlst ST results for different *Listeria* species.

The mlst *Listeria* database includes multiple species, including *Listeria monocytogenes* and *L. innocua*. When available, the *Listeria* clonal complex (CC) or *L. monocytogenes* lineage is listed alongside the ST.

6.4 For quality control threshold guidelines for the GenomeTrakr surveillance network, go to step #1 These are also relevant for NARMS and VetLIRN contributors.

*MicroRunQC users should follow QC threshold guidelines established by their respective surveillance coordinating body(s).