# Assessing sequence quality in GalaxyTrakr V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bdvfi63n

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

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ABSTRACT

**PURPOSE:** Step-by-step instructions for checking WGS sequence quality. The MicroRunQC workflow, implimented 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 isoalte. 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:

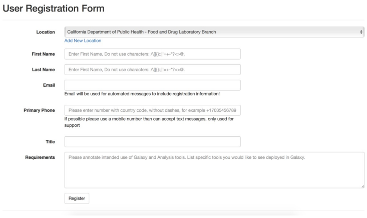
1. set up an account in GalaxyTrakr
2. Create a new history/workspace
3. Upload data
4. Execute the MicroRunQC workflow
5. Interpret the results

EXTERNAL LINK

https://galaxytrakr.org

## Account set up

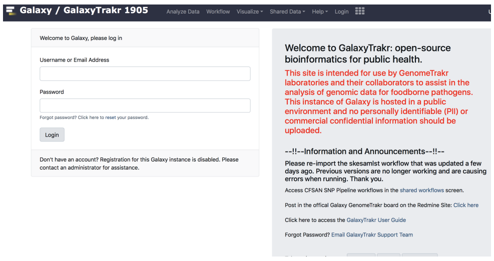
1 1. Create a GalaxyTrakr account here: https://account.galaxytrakr.org/Account/Register



User Registration Form

Location: California Department of Public Health - Food and Drug Laboratory Branch
Add New Location
First Name: Enter First Name, Do not use characters: ^/[]{}:;|'=+-*?<>@.
Last Name: Enter First Name, Do not use characters: ^/[]{}:;|'=+-*?<>@.
Email:
Email will be used for automated messages to include registration information!
Primary Phone: Please enter number with country code, without dashes, for example +17035456789
If possible please use a mobile number than can accept text messages, only used for support
Title:
Requirements: Please annotate intended use of Galaxy and Analysis tools. List specific tools you would like to see deployed in Galaxy.

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



Welcome to Galaxy, please log in

Username or Email Address

Password

Forgot password? Click here to reset your password.

Don't have an account? Registration for this Galaxy instance is disabled. Please contact an administrator for assistance.

Welcome to GalaxyTrakr: open-source bioinformatics for public health.

This site is intended for use by GenomeTrakr laboratories and their collaborators to assist in the analysis of genomic data for foodborne pathogens. This instance of Galaxy is hosted in a public environment and no personally identifiable (PII) or commercial confidential information should be uploaded.

--!!--Information and Announcements--!!--

Please re-import the skesamlst workflow that was updated a few days ago. Previous versions are no longer working and are causing errors when running. Thank you.

Access CFSAN SNP Pipeline workflows in the shared workflows screen.

Post in the offical Galaxy GenomeTrakr board on the Redmine Site: Click here

Click here to access the GalaxyTrakr User Guide

Forgot Password? Email GalaxyTrakr Support Team

**Citation:** Ruth Timme, Sai Laxmi Gubbala Venkata, Maria Balkey, Robyn Randolph, William Wolfgang, Errol Strain (03/18/2020). Assessing sequence quality in GalaxyTrakr. https://dx.doi.org/10.17504/protocols.io.bdvfi63n

Create a new history

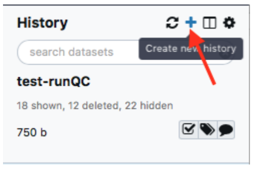
2 **Create a new history.**

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

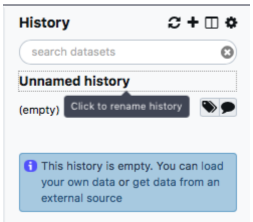
Save your MicroRunQC output here and any other relevant analyses, like serotyping, or AMR detection.

After all the analysis output from this run is saved to your internal data network or computer, older history's 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.

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



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

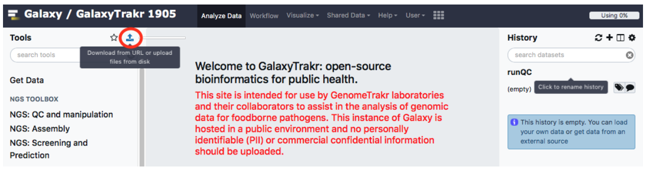


This history is empty. You can load your own data or get data from an external source

Upload data

3 **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.

3.1 Click on the upload/download icon on the top of the left web page to start an upload process.



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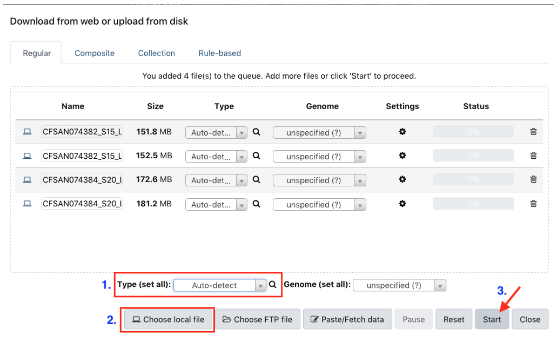
This site is intended for use by GenomeTrakr laboratories and their collaborators to assist in the analysis of genomic data for foodborne pathogens. This instance of Galaxy is hosted in a public environment and no personally identifiable (PII) or commercial confidential information should be uploaded.

This history is empty. You can load your own data or get data from an external source

**Citation:** Ruth Timme, Sai Laxmi Gubbala Venkata, Maria Balkey, Robyn Randolph, William Wolfgang, Errol Strain (03/18/2020). Assessing sequence quality in GalaxyTrakr. https://dx.doi.org/10.17504/protocols.io.bdvfi63n

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



Download from web or upload from disk

You added 4 file(s) to the queue. Add more files or click 'Start' to proceed.

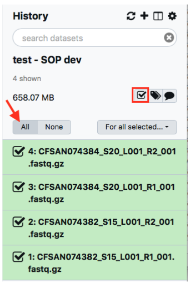
| Name | Size | Type | Genome | Settings | Status |
|---|---|---|---|---|---|
| CFSAN074382_S15_L | 151.8 MB | Auto-det... | unspecified (?) | | 0% |
| CFSAN074382_S15_L | 152.5 MB | Auto-det... | unspecified (?) | | 0% |
| CFSAN074384_S20_l | 172.6 MB | Auto-det... | unspecified (?) | | 0% |
| CFSAN074384_S20_l | 181.2 MB | Auto-det... | unspecified (?) | | 0% |

1. Type (set all): Auto-detect  Genome (set all): unspecified (?)

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

3.3 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 with each sample/isolate. GalaxyTrakr does this by creating a **List of Dataset Pairs.**

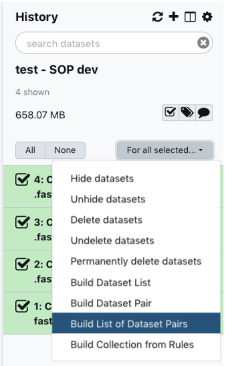
Within your newly created History panel, click the "check box," then select all the files you just uploaded by clicking "All" or by individually selecting the ones you want to pair.



☑ 4: CFSAN074384_S20_L001_R2_001.fastq.gz
☑ 3: CFSAN074384_S20_L001_R1_001.fastq.gz
☑ 2: CFSAN074382_S15_L001_R2_001.fastq.gz
☑ 1: CFSAN074382_S15_L001_R1_001.fastq.gz

**Screenshot of History panal 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.

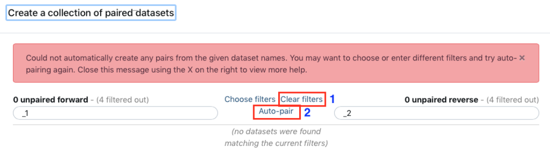
3.4 Click "For all selected" and choose "Build List of Dataset Pairs"



**Citation:** Ruth Timme, Sai Laxmi Gubbala Venkata, Maria Balkey, Robyn Randolph, William Wolfgang, Errol Strain (03/18/2020). Assessing sequence quality in GalaxyTrakr. https://dx.doi.org/10.17504/protocols.io.bdvfi63n

3.5 A new window will open to help you pair the fastq files properly. Note how your paired reads are named (_R1 and _R2 in the example above)

Select **Clear filters**, then click **Auto-pair.**



Create a collection of paired datasets

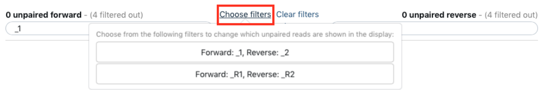
Could not automatically create any pairs from the given dataset names. You may want to choose or enter different filters and try auto-pairing again. Close this message using the X on the right to view more help.

**0 unpaired forward** - (4 filtered out) _1

**0 unpaired reverse** - (4 filtered out) _2

*(no datasets were found matching the current filters)*

Alternatively, instead of autopairing you can click "choose filters" and select the appropriate filter for the pairing:



**0 unpaired forward** - (4 filtered out) _1

**0 unpaired reverse** - (4 filtered out)

Choose from the following filters to change which unpaired reads are shown in the display:

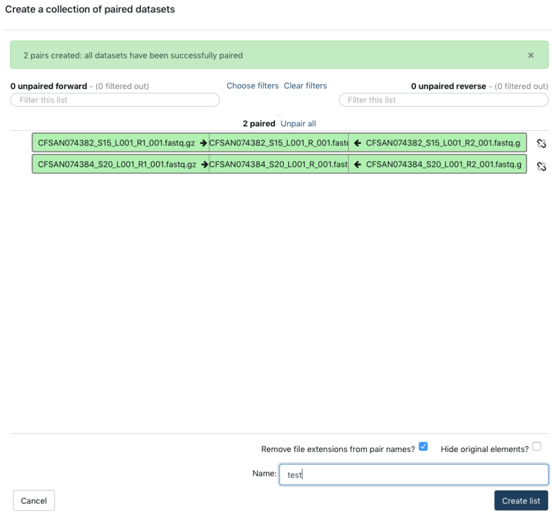
Forward: _1, Reverse: _2

Forward: _R1, Reverse: _R2

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

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

Click **Create list.**



Create a collection of paired datasets

2 pairs created: all datasets have been successfully paired

**0 unpaired forward** - (0 filtered out)

**0 unpaired reverse** - (0 filtered out)

**2 paired** Unpair all

CFSAN074382_S15_L001_R1_001.fastq.gz → CFSAN074382_S15_L001_R_001.fastq ← CFSAN074382_S15_L001_R2_001.fastq.g

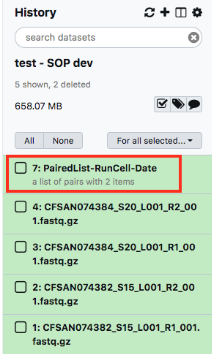
CFSAN074384_S20_L001_R1_001.fastq.gz → CFSAN074384_S20_L001_R_001.fast ← CFSAN074384_S20_L001_R2_001.fastq.g

Remove file extensions from pair names? ☑ Hide original elements? □

Name: test

**Citation:** Ruth Timme, Sai Laxmi Gubbala Venkata, Maria Balkey, Robyn Randolph, William Wolfgang, Errol Strain (03/18/2020). Assessing sequence quality in GalaxyTrakr. https://dx.doi.org/10.17504/protocols.io.bdvfi63n

3.7 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.



History

search datasets

test - SOP dev

5 shown, 2 deleted

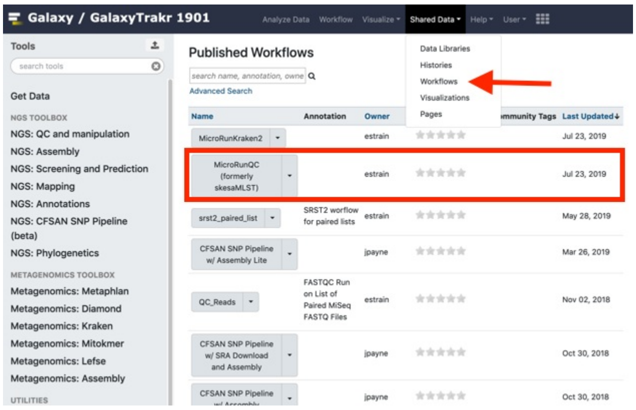
658.07 MB

- □ 7: PairedList-RunCell-Date
  a list of pairs with 2 items
- □ 4: CFSAN074384_S20_L001_R2_001.fastq.gz
- □ 3: CFSAN074384_S20_L001_R1_001.fastq.gz
- □ 2: CFSAN074382_S15_L001_R2_001.fastq.gz
- □ 1: CFSAN074382_S15_L001_R1_001.fastq.gz

Run the MicroRunQC workflow

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

4.1 Navigate to the “Shared Data" drop down menu, choose workflows and from the MicroRunQC drop down menu select import.



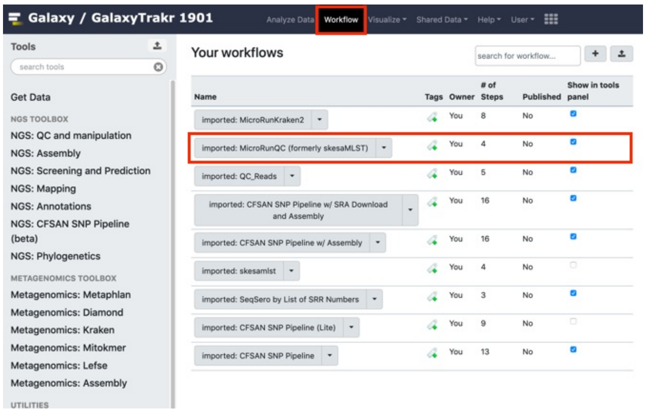
Published Workflows

search name, annotation, owne

Advanced Search

| Name | Annotation | Owner | mmunity Tags | Last Updated |
|---|---|---|---|---|
| MicroRunKraken2 | | estrain | ☆☆☆☆☆ | Jul 23, 2019 |
| MicroRunQC (formerly skesaMLST) | | estrain | ☆☆☆☆☆ | Jul 23, 2019 |
| srst2_paired_list | SRST2 worflow for paired lists | estrain | ☆☆☆☆☆ | May 28, 2019 |
| CFSAN SNP Pipeline w/ Assembly Lite | | jpayne | ☆☆☆☆☆ | Mar 26, 2019 |
| QC_Reads | FASTQC Run on List of Paired MiSeq FASTQ Files | estrain | ☆☆☆☆☆ | Nov 02, 2018 |
| CFSAN SNP Pipeline w/ SRA Download and Assembly | | jpayne | ☆☆☆☆☆ | Oct 30, 2018 |
| CFSAN SNP Pipeline w/ Assembly | | jpayne | ☆☆☆☆☆ | Oct 30, 2018 |

4.2 To see the new workflow in your “Workflows” tools panel on the left, open the Workflow tab and check “show in tools panel” for the workflow of interest.



Your workflows

search for workflow...

| Name | Tags | Owner | # of Steps | Published | Show in tools panel |
|---|---|---|---|---|---|
| imported: MicroRunKraken2 | | You | 8 | No | ☑ |
| imported: MicroRunQC (formerly skesaMLST) | | You | 4 | No | ☑ |
| imported: QC_Reads | | You | 5 | No | ☑ |
| imported: CFSAN SNP Pipeline w/ SRA Download and Assembly | | You | 16 | No | ☑ |
| imported: CFSAN SNP Pipeline w/ Assembly | | You | 16 | No | ☑ |
| imported: skesamlst | | You | 4 | No | □ |
| imported: SeqSero by List of SRR Numbers | | You | 3 | No | ☑ |
| imported: CFSAN SNP Pipeline (Lite) | | You | 9 | No | □ |
| imported: CFSAN SNP Pipeline | | You | 13 | No | ☑ |

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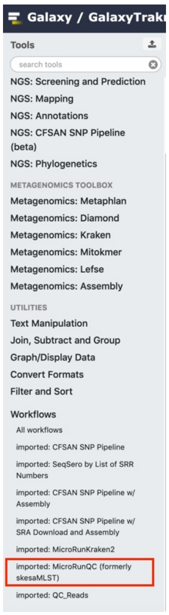
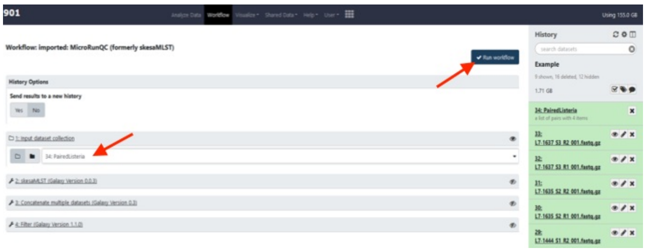
4.3 From the workflow menus select MicroRunQC



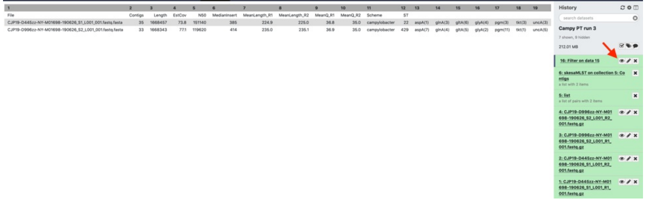
4.4 Select paired list dataset you created earlier.

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.


901
Analyze Data
Workflow
Visualize
Shared Data
Help
User
Using 155.0 GB
Workflow: imported: MicroRunQC (formerly skesaMLST)
Run workflow
History Options
Send results to a new history
Yes
No
1: Input dataset collection
34: PairedListeria
2: skesaMLST (Galaxy Version 0.0.3)
3: Concatenate multiple datasets (Galaxy Version 0.3)
4: Filter (Galaxy Version 1.1.0)
History
search datasets
Example
9 shown, 16 deleted, 12 hidden
1.71 GB
34: PairedListeria
a list of pairs with 4 items
33: L7-1637 S3 R2 001.fastq.gz
32: L7-1637 S3 R1 001.fastq.gz
31: L7-1635 S2 R2 001.fastq.gz
30: L7-1635 S2 R1 001.fastq.gz
29: L7-1444 S1 R2 001.fastq.gz

4.5 Upon completion of the pipeline all tiles in the history bar will be green.

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



| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| File | Contigs | Length | EstCov | N50 | MedianInsert | MeanLength_R1 | MeanLength_R2 | MeanQ_R1 | MeanQ_R2 | Scheme | ST |  |  |  |  |  |  |  |
| CJP19-D445zz-NY-M01698-190626_S1_L001_001.fastq.fasta | 35 | 1668457 | 73.8 | 151140 | 385 | 224.9 | 225.0 | 36.8 | 35.0 | campylobacter | 22 | aspA(1) | glnA(3) | gltA(6) | glyA(4) | pgm(3) | tkt(3) | uncA(3) |
| CJP19-D996zz-NY-M01698-190626_S2_L001_001.fastq.fasta | 33 | 1668343 | 77.1 | 119620 | 414 | 235.0 | 235.1 | 36.9 | 35.0 | campylobacter | 429 | aspA(7) | glnA(4) | gltA(5) | glyA(2) | pgm(11) | tkt(1) | uncA(5) |

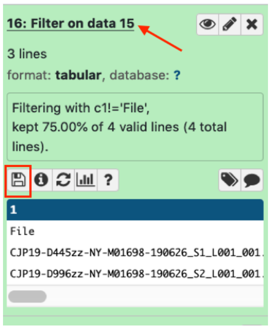
History
search datasets
Campy PT run 3
7 shown, 9 hidden
212.01 MB
16: Filter on data 15
6: skesaMLST on collection 5: Contigs
a list with 2 items
5: list
a list of pairs with 2 items
4: CJP19-D996zz-NY-M01698-190626_S2_L001_R2_001.fastq.gz
3: CJP19-D996zz-NY-M01698-190626_S2_L001_R1_001.fastq.gz
2: CJP19-D445zz-NY-M01698-190626_S1_L001_R2_001.fastq.gz
1: CJP19-D445zz-NY-M01698-190626_S1_L001_R1_001.fastq.gz

Interpret the results

5 **Download and interpret the results:**

**Citation:** Ruth Timme, Sai Laxmi Gubbala Venkata, Maria Balkey, Robyn Randolph, William Wolfgang, Errol Strain (03/18/2020). Assessing sequence quality in GalaxyTrakr. https://dx.doi.org/10.17504/protocols.io.bdvfi63n

5.1 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 that can be opened on excel.



**16: Filter on data 15**

3 lines

format: **tabular**, database: ?

Filtering with c1!='File',
kept 75.00% of 4 valid lines (4 total lines).

```
1
File
CJP19-D445zz-NY-M01698-190626_S1_L001_001.
CJP19-D996zz-NY-M01698-190626_S2_L001_001.
```

5.2 The MicroRunQC output file includes the following metrics:

| ***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 (pubmlst.org) database scheme (e.g. senterica for Salmonella enterica) |
| **ST** | Assembly | Sequence Type |
| **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.

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

5.3 Example output for 4 Listeria samples run through the MicroRunQC workflow:

| File name | Contigs | Length | EstCov | N50 | MedianInsert | MeanLength_R1 | MeanLength_R2 | MeanQ_R1 | MeanQ_R2 | Scheme | ST | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FSL-R9-8346 | 14 | 2876874 | 87.8 | 512255 | 408 | 147.6 | 147.7 | 33.1 | 32.7 | lmonocytogenes | 389 | abcZ(52) | bglA(1) | cat(12) | dapE(71) | dat(2) | ldh(1) | lhk |
| FSL-R9-8348 | 11 | 2832172 | 84.2 | 1464158 | 388 | 147.9 | 147.9 | 33.2 | 32.9 | lmonocytogenes | 795 | abcZ(7) | bglA(10) | cat(18) | dapE(6) | dat(5) | ldh(7) | lhk |
| FSL-R9-8350 | 14 | 2884629 | 64.2 | 450082 | 390 | 147.2 | 147.2 | 33.1 | 32.7 | lmonocytogenes | 37 | abcZ(5) | bglA(7) | cat(3) | dapE(5) | dat(1) | ldh(8) | lhk |
| FSL-R9-8352 | 12 | 2902520 | 85.9 | 1460419 | 390 | 148.1 | 148.2 | 33.1 | 32.8 | lmonocytogenes | 391 | abcZ(7) | bglA(6) | cat(62) | dapE(28) | dat(5) | ldh(2) | lhk |

Spreadsheet showing example output for 5 *Listeria monocytogenes* samples from a NextSeq sequencing run.

5.4 Quality control threshold guidelines for the GenomeTrakr surveillance network. These are also relevant for NARMS and VetLIRN contributors.

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

| **Quality metric** | ***Salmonella*** | ***Listeria*** | ***E. coli*** | ***Shigella*** | ***Campylobacter*** | ***Vibrio para.*** |
|---|---|---|---|---|---|---|
| Average read quality Q score for R1 and R2 | >=30 | >=30 | >=30 | >=30 | >=30 | >=30 |
| Average coverage | >=30X | >=20X | >=40X | >=40X | >=20X | >=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 |
| *De novo* assembly: no. contigs | <=300 | <=300 | <=500 | <=650 | <=300 | <=300 |

**Quality control threshold guidelines estabolished for the GenomeTrakr surveillance network.**

**Citation:** Ruth Timme, Sai Laxmi Gubbala Venkata, Maria Balkey, Robyn Randolph, William Wolfgang, Errol Strain (03/18/2020). Assessing sequence quality in GalaxyTrakr. https://dx.doi.org/10.17504/protocols.io.bdvfi63n